POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - I

Course: BOHCT 1.1

(Biology and Diversity of Virus, Bacteria and Fungi)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

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Director's Note

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance mode offered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials have been composed by distinguished faculty from reputed institutions, utilizing data from e-books, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 1.1

Biology and Diversity of Virus, Bacteria and Fungi
(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 1.1	Biology and Diversity of Virus	**Unit 1. Introduction, nomenclature and classification of viruses**	1. Nature and origin of virion. 2. Nomenclature and classification, distinctive properties of viruses, morphology (symmetry) and a general account on different types of viruses, Viral genome.	1
		Unit 2. Structural and molecular organisation of virion and its identification techniques	3. Structure & chemistry of viruses-capsid and their arrangements, types of envelopes and their composition, Molecular organization of virion with special reference to TMV and HIV. 4. Isolation, purification and identification of viruses based on chemical, physical and immunological techniques.	1
		Unit 3. Modern classification of viruses and its transmission	5. Transmission of plant viruses, genetic basis of cell to cell movement of plant viruses. 6. Management of plant viruses following classical and modern technique.	1
		Unit 4. Life cycle of viruses and sub viral agents	7. Molecular basis of Lytic and Lysogenic cycle. 8. Prions, viroids, virusoids, Satellite virus.	1
	Biology and Diversity of Bacteria	**Unit 5. Structure, taxonomy, and phylogeny of bacteria**	1. Microbial taxonomy and phylogeny of Bacteria. 2. Ultra structure of Gram positive and Gram negative bacteria. 3. Bacterial motility, bacterial sporulation. 4. Bacterial growth kinetics, factors affecting growth.	1
		Unit 6. Nutritional types and reproduction of bacteria	5. Photolithotrophs, chemolithotrophs, photoorganotrophs & chemoorganotrophs, Mixotroph. 6. Organization and replication of genetic material in bacteria. Genetic recombination (conjugation, transformation and transduction) in bacteria.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 1.1	Biology and Diversity of Bacteria	**Unit 7. Microbial ecology**	7. Concept of microbial ecology with reference to air, water and soil. 8. Microbes associated with food, food-borne infections and intoxications; preservation of food. 9. Air, water, and soil-borne disease–causal organism, symptoms, control.	1
		Unit 8. Immunology and Indutrial Microbiology	10. Cells and organ of immune system, antigen (chemical nature and types), immunoglobulins (structure and types), brief idea about hypersensitivity and vaccine. 11. Industrial production of ethanol, penicillin, vitamin B12. 12. Cosmetic microbiology-current trends.	1
	Biology and Diversity of Fungi and their allies	**Unit 9. Classification and cell wall structure of fungi**	1. Distinctive features of fungi to form a separate kingdom; modern trends in classification 2. The architecture of fungal cell, cell wall, cell membrane, cell organelles, cytoskeleton, and protoplast technology; translocation in mycelia	1
		Unit 10. Genome organization and reproduction of fungi	3. Genome organization in fungi; extra chromosomal and transposable genetic elements in fungi 4. Somatic recombination in fungi: heterothallism; heterokaryosis and parasexuality	1
		Unit 11. Reproduction patterns	5. Diversity of somatic, reproductive and fruiting structures in different groups: Myxomycota, Oomycota, Chytridiomycota, Zygomycota, Ascomycota, 6. Fungal spores: types, dispersal, dormancy and germination	1
		Unit 12. Diversity & reprodution	5. Diversity of somatic, reproductive and fruiting structures in different groups: Basidiomycota, Deuteromycota	1

Content

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Unit 5.	Structure, taxonomy, and phylogeny of bacteria	36-64
Unit 6.	Nutritional types and reproduction of bacteria	65-84
Unit 7.	Microbial ecology	85-102, 115-125
Unit 8.	Immunology and Indutrial Microbiology	103-114, 125-129
Unit 9.	Classification and cell wall structure of fungi	131-144
Unit 10.	Genome organization and reproduction of fungi	145-154
Unit 11.	Reproduction patterns	155-227
Unit 12.	Diversity & reprodution	227-236

COURSE – BOHCT1.1

Biology and Diversity of Virus, Bacteria and Fungi

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group – A (Biology and Diversity of Virus)

Content Structure

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1. Introdution
2. Objetives
3. Nature and origin of virion

4. Nomenclature and classification; Distinctive properties of virus; Morphology (symmetry) and a general account on different kinds of viruses; Viral genome

5. Structure, chemistry of viruses-capsid and their arrangements; Types of envelops and their composition; molecular organization of virion with special reference to TMV and HIV

6. Isolation, purification and identification of viruses

7. Transmission of plant viruses; genetic basis of cell to cell movement of plant viruses

8. Management of plant viruses; Satellite virus

9. Viral replication : Lytic and lysogenic cycles

10. Subviral particles-prions, viroids, virusoids

11. Let's sum up

12. Suggest reading

13. Assignment

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1. Introduction

These are infectious agents with fairly simple, acellular organization. They possess only one type of nucleic acid, either DNA or RNA, and only reproduce within living cells. Clearly viruses are quite different from procaryotic and eucaryotic microorganisms, and are studied by **virologists.** Despite their simplicity in comparison with cellular organisms, viruses are extremely important and deserving of close attention. The study of viruses has contributed significantly to the

discipline of molecular biology. Many human viral diseases are already known and more are discovered or arise every year, as demonstrated by the recent appearance of AIDS. The whole field of genetic engineering is based in large part upon discoveries in virology. Thus it is easy to understand why **virology** (the study of viruses) is such a significant part of microbiology. Viruses have had enormous impact on humans and other organisms, yet very little was known about their nature until fairly recently. Shortly after the turn of the century, Vilhelm Ellermann and Oluf Bang in Copenhagen reported that leukemia could be transmitted between chickens by cell-free filtrates and was probably caused by a virus. Three years later in 1911, Peyton Rous from the

Rockefeller Institute in New York City reported that a virus was responsible for a malignant muscle tumor in chickens. These studies established that at least some malignancies were caused by viruses. It was soon discovered that bacteria themselves also could be attacked by viruses. The first published observation suggesting that this might be the case was made in 1915 by Frederick W. Twort. Twort isolated bacterial viruses that could attack and destroy micrococci and intestinal bacilli. Although he speculated that his preparations might contain viruses, Twort did not follow up on these observations. It remained for Felix d'Herelle to establish decisively the existence of bacterial viruses. D'Herelle isolated bacterial viruses from patients with dysentery, probably caused by *Shigella dysenteriae.* A short time later Frederick C. Bawden and Norman W. Pirie managed to separate the TMV virus particles into protein and nucleic acid. Thus by the late 1930s it was becoming clear that viruses were complexes of nucleic acids and proteins able to reproduce only in living cells.

2. Course Objectives

- The objective of this module is not only to develop betterment towards the knowledge of virus and subviral particle.

- On completion of study of this course you will be able to

  ***** Know the nature, origin and characteristics of virion
  *****Isolation ,purification and identification of virus particles
  *****Gather knowledge of various subviral particles
  *****Acquire knowledge and develop skills for management of virues

3. Nature and Origin of Virion

Introduction

A virus is a small infectious agent that replicates only inside the living cells of other organisms. Viruses can infect all types of life forms, from animals and plants to microorganisms, including bacteria and archaea. Since Dmitri Ivanovsky's (1892) article describing a non-bacterial pathogen infecting tobacco plants, and the discovery of the tobacco mosaic virus by Martinus Beijerinck (1898), about 5,000 virus species have been described in detail, although there are millions of types. Viruses are found in almost every ecosystem on Earth and are the most abundant type of biological entity. The study of viruses is known as virology.

Origin of virus

The origin of viruses remains unclear because they do not form fossils, so molecular techniques have been the most useful means of hypothesising how they arose. However, these techniques rely on the availability of ancient viral DNA or RNA but most of the viruses that have been preserved and stored in laboratories are less than 90 years old. Molecular methods have only been successful in tracing the ancestry of viruses that evolved in the 20th century. Three main theories speculate on the origins of viruses:

Regressive theory: Viruses may have once been small cells that parasitised larger cells. Over time, genes not required by their parasitism were lost. The bacteria Rickettsia and Chlamydia are living cells that, like viruses, can reproduce only inside host cells. They lend credence to this theory, as their dependence on parasitism is likely to have caused the loss of genes that enabled them to survive outside a cell.

Cellular origin theory: Some viruses may have evolved from bits of DNA or RNA that "escaped" from the genes of a larger organism. The escaped DNA could have come from plasmids-pieces of DNA that can move between cells-while others may have evolved from bacteria.

Coevolution theory: Viruses may have evolved from complex molecules of protein and DNA at the same time as cells first appeared on earth and would have depended on cellular life for many millions of years.

4. Nomenclature and classification; Distinctive properties of virus; Morphology (symmetry) and a general account on different kinds of viruses; Viral genome

Nature of Viruses:

Viruses are infective microorganisms. They show several differences from typical bacterial cells.

1. Size: On the whole viruses are much smaller than bacteria. Most animal and plant viruses are invisible under the light microscope. Some of smaller viruses are only 200Å in diameter.

2. No independent metabolism:

Viruses cannot multiply outside a living cell. No virus has been cultivated in a cell-free medium. Viruses do not have an independent metabolism. They are metabolically inactive outside the host cell because they do not possess enzyme systems and protein synthesis machinery. Thus viruses are obligatory intracellular parasites.

3. Simple structure:

Viruses have a very simple structure. They consist of a nucleic acid core surrounded by a protein coat. In this respect they differ from typical cells which are made up of proteins, carbohydrates, lipids and nucleic acids. Myxoviruses have a membranous envelope consisting of proteins, carbohydrate and lipid outside the usual protein coat which derived from the host cell.

4. Absence of cellular structure:

Viruses do not have any cytoplasm, and thus cytoplasmic organelles like mitochondria, Golgi complexes, ribosomes, lysosomes etc. are absent.

5. Nucleic acids:

Viruses usually have only one nucleic acid, either DNA or RNA. Rous Sarcoma Virus (RSV), producing certain cancer, is the only virus having both DNA and RNA.

6. Crystallization:

Many of the smaller viruses can be crystallized, and thus behave like chemicals.

7. No growth and division:

Viruses do not have the power of growth and division. The genetic material of virus reproduces only in a host cell. Thus viruses do not show all the characteristics of typical living organisms. They, however, possess two fundamental characteristics of living systems. Firstly, they contain nucleic acid as their genetic material. The nucleic acid contains all the instructions

for the structure and the function of the virus. Secondly, they can reproduce themselves, even if only by using the host cells's synthesis machinery.

Structure of Viruses:

(a)Size: Variable. Most viruses are much smaller than bacteria. The size ranges in between 100A to 250 mu. Some viruses are larger than bacteria, for example the psittacos is a virus measuring 0.75 mu in diameter.

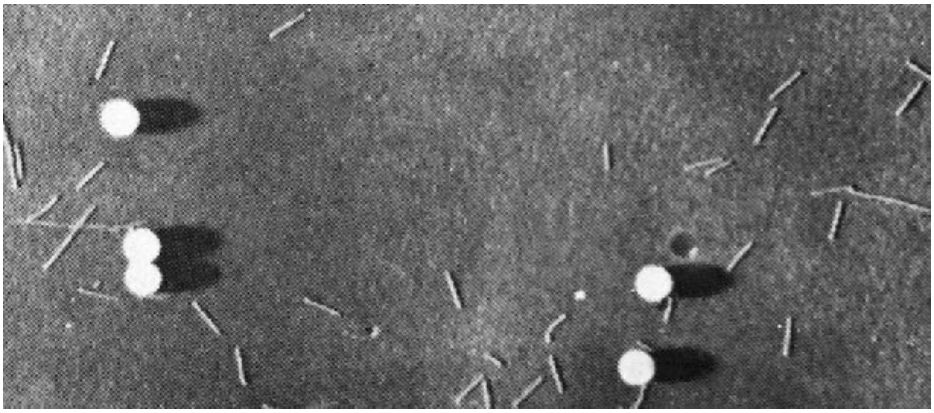

Figure Tobacco Mosaic Virus (Source: Book – Microbiology; L.M. Prescott)

(b) Symmetry: Viruses occur in three main shapes. They are spherical (Cubical or polyhydral), helical (Cylinderical or rod-like) and complex. Cubical viruses may be tetrahydral (4 faces) < dodecahedral (12 faces) or icosahedral (20 faces). The Herpes virus is dodecahedral. The Tobacco mosaic virus (TMV) and the bacteriophage are, respectively, helical and complex.

1. Spherical / Cubical: PhI X 174, Herpes virus, Tipula virus, Polyoma virus.

2. Helical / Cylinderical: Tobacco Mosaic virus, Influenza virus Mumps virus.

3. Complex: Vaccinia virus, ORF virus, Vesicular Stomatitis virus.

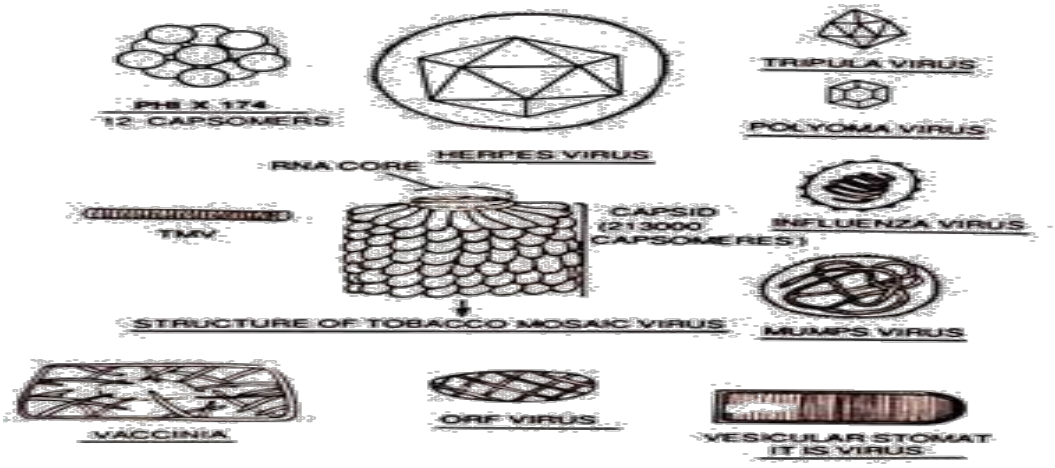

Figure 1: Different structure of Virus

(c) Morphology:

Morphologically a virus is a core of nucleic acid (DNA or RNA) surrounded by a protein shell. An intact virus unit is known as **virion**. Its protein coat is called capsid. The capsid is composed of a number of subunits of a particular shape. These sub-units are known as **capsomeres**. The capsid protects the nucleic acid against the action of nuclease enzyme. Some proteins of capsid help in binding the virus to the surface of host cells. Some surface proteins act as enzyme and dissolve the surface layer of host cell and thus help in penetration of its nucleic acid into the host cell.

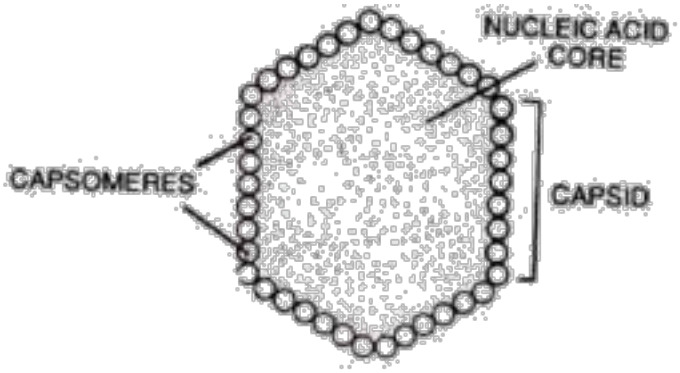

Figure 2: General diagram of virion

The **polio virus** (Poliomyelitis) is a most extensively studied animal virus. It has a very simple organization. It consists of a protein coat built up out of 60 structurally equivalent, asymmetric protein subunits of approximately 60 Å in diameter. The spherical protein coat has a diameter about 300Å. It encloses the genetic material, RNA. The protein coat contains about 49, 600 amino acids and RNA contains about 5200 nucleotides. The single-stranded RNA of poliovirus, thus, has triplet codes for 1700 amino acids. During infection, it alters cell metabolism drastically and leads quick death of host cell. **Tobacco mosaic virus** is the most extensively studied plant virus. It is a helically symmetrical, rod-shaped virus having the length of 3000Å and diameter of 180A. It RNA is a single stranded spirally coiled molecule formed of 6500 nucleotides. The capsid is formed of 2130 capsomeres, each with a molecular weight of 18,000. The capsomeres are elliptical and remain arranged helically around to form capsid.

Classification of Viruses:

Viruses may be classified according to the type of the host and genetic material.

On the basis of type of host, viruses are:

1. Animal Viruses:

They live inside animal cells including man. On entering the cell, these disturb the metabolism of the host cell and cause various diseases. The common animal viruses are small pox virus, influenza virus, mumps virus, polio virus and herpes virus. In many animal viruses an extra envelope surrounds their protein coat. The membrane consists of proteins, lipids and carbohydrates and is derived from the host plasma membrane. Inside the host cell they may multiply and form numerous new viral particles. Usually, animal viruses release from the host cells by the rupturing and subsequent death of the host cells.

2. Plant Viruses:

They are parasites of plant cells. Their genetic material is RNA which remains enclosed in the protein coat. The most important plant viruses are tobacco mosaic virus (TMV), tobacco rattle virus (TRV), potato virus (PV), southern bean mosaic virus (SBMV), beet yellow virus (BYV) and turnip yellow virus (TYV).

3. Bacterial virus:

They are parasitic on bacteria and so also called **bacteriophages**. There are many varieties of bacteriophages. Their size and shape varies from species to species. Some phages are spherical, some comma-shaped whereas majority of them have tadpole-like appearance.

On the basis of nucleic acids, viruses are:

1. DNA viruses:

These viruses possess DNA as the genetic material. On replication this DNA produces new DNA. DNA transmits information for protein synthesis through RNA. (DNA → RNA → Protein).

2. RNA viruses:

These viruses possess RNA as the genetic material. The RNA replicates directly to produce new RNA. Information for protein synthesis passes from RNA to protein without involment of DNA. (RNA → RNA → Protein).

3. DNA – RNA viruses:

In a group of RNA tumour viruses called leuko viruses or rous viruses the genetic material is alternately DNA and RNA. In addition to the normal mode of transfer found in DNA viruses (DNA → RNA → Protein) the rous viruses also transfer information from RNA to DNA (RNA-DNA→RNA →Protein).

With respect to number of strands, four types of nucleic acids have been found in viruses:

1. Double stranded DNA:

Double stranded DNA has been reported in pox viruses, the bacteriophages T 2, T 4, T 6, T 3, T 7 and lamda, herpes viruses, adeno viruses, polyoma virus SV-40 and papilloma viruses.

2. Single stranded DNA:

Single stranded DNA is found in the bacteriophages phi X 174 and M-13 and is cyclic.

3. Double stranded RNA:

Double stranded RNA has been found within viral capsid in the reoviruses of animals and in the wound tumour virus and rice dwarf viruses of plants.

4. Single stranded RNA:

Single stranded RNA is found in most of RNA viruses e.g. Tobacco mosaic virus, influenza virus, poliomylitis bacteriophage MS – 2, F – 2, Coliophage R 17 and the avian leukemia virus.

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5. Structure, chemistry of viruses-capsid and their arrangements; Types of envelops and their composition; molecular organization of virion with special reference to TMV and HIV.

All virions, even if they possess other constituents, are constructed around a **nucleocapsid** core (indeed, some viruses consist only of a nucleocapsid). The nucleocapsid is composed of a nucleic acid, either DNA or RNA, held within a protein coat called the **capsid,** which protects viral genetic material and aids in its transfer between host cells.

There are four general morphological types of capsids and virion structure.

1. Some capsids are **icosahedral** in shape. An icosahedron is a regular polyhedron with 20 equilateral triangular faces and 12 vertices

2. Capsids are **helical** and shaped like hollow protein cylinders, which may be either rigid or flexible
3. Many viruses have an **envelope,** an outer membranous layer surrounding the nucleocapsid. Somewhat variable shape even though their nucleocapsid can be either icosahedral or helical
4. Complex viruses have capsid symmetry that is neither purely icosahedral nor helical

Both helical and icosahedral capsids are large macromolecular structures constructed from many copies of one or a few types of protein subunits or protomers. Probably the most important advantage of this design strategy is that the information stored in viral genetic material is used with maximum efficiency. For example, the tobacco mosaic virus (TMV) capsid contains a single type of small subunit possessing 158 amino acids. Only about 474 nucleotides out of 6,000 in the virus RNA are required to code for coat protein amino acids. Unless the same protein is used many times in capsid construction, a large nucleic acid, such as the TMV RNA, cannot be enclosed in a protein coat without using much or all of the available genetic material to code for capsid proteins. If the TMV capsid were composed of six different protomers of the same size as the TMV subunit, about 2,900 of the 6,000 nucleotides would be required for its construction, and much less genetic material would be available for other purposes.

Helical Capsids

Helical capsids are shaped much like hollow tubes with protein walls. The tobacco mosaic virus provides a well-studied example of helical capsid structure. A single type of protomer associates together in a helical or spiral arrangement to produce a long, rigid tube, 15 to 18 nm in diameter by 300 nm long. The RNA genetic material is wound in a spiral and positioned toward the inside of the capsid where it lies within a groove formed by the protein subunits. Not all helical capsids are as rigid as the TMV capsid. Influenza virus RNAs are enclosed in thin, flexible helical capsids folded within an envelope. The size of a helical capsid is influenced by both its protomers and the nucleic acid enclosed within the capsid. The diameter of the capsid is a function of the size, shape, and interactions of the protomers. The nucleic acid determines helical capsid length because the capsid does not seem to extend much beyond the end of the DNA or RNA.

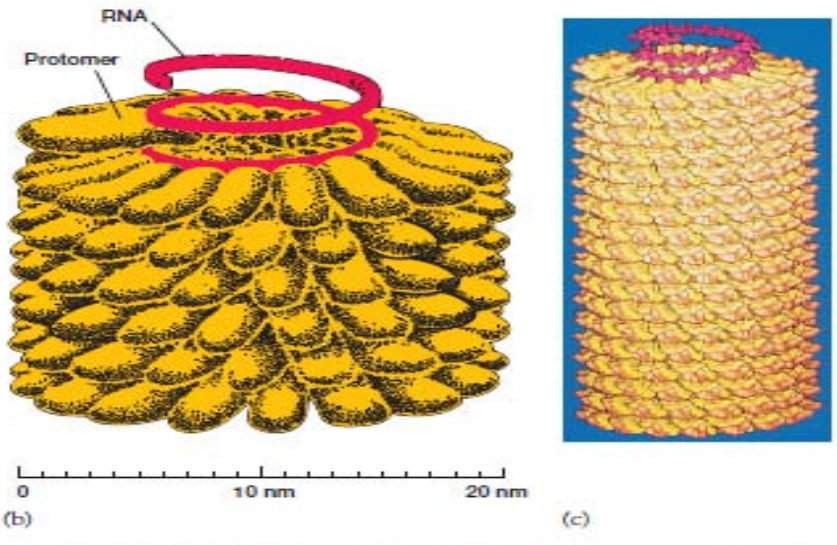

Figure 3: a) An electron micrograph of the negatively stained helical capsid (X400,000) B)TMV structure (Source: Book – Microbiology; L.M. Prescott)

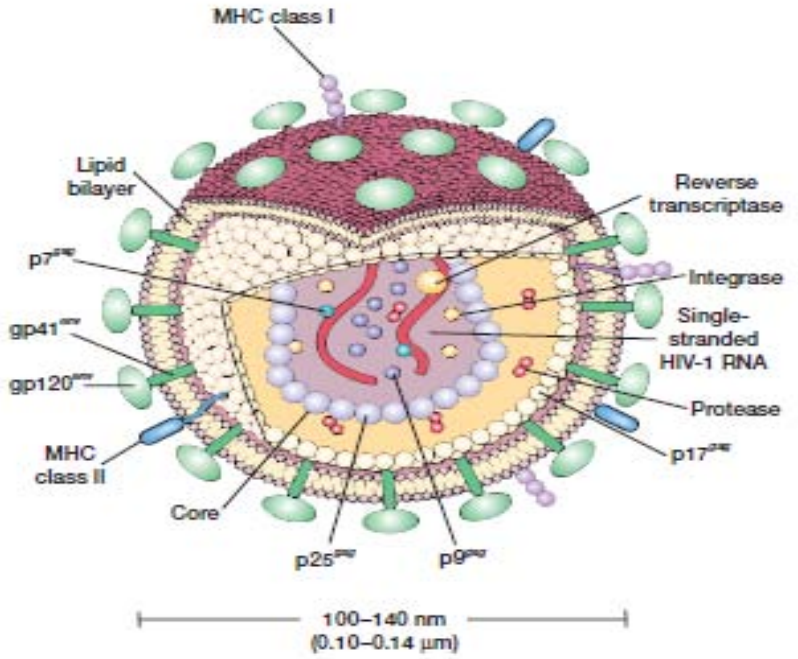

Figure 4: Schematic diagram of the HIV-1 virion. (Source: Book – Microbiology; L.M. Prescott)

Retroviruses use the enzyme **reverse transcriptase** to carry out this unusual process. Retroviruses were the first viruses shown to cause cancer, and the **human immunodeficiency virus** (HIV) is a retrovirus that causes **acquired immunodeficiency syndrome** (AIDS). Retroviruses are enveloped viruses that carry several enzymes in the virion. These include reverse transcriptase, integrase, and a retroviral-specific protease. The genome of the retrovirus is unique and consists of two identical single-stranded RNAs of the plus sense. The genome contains the genes ***gag*** (structural proteins), ***pol*** (reverse transcriptase and integrase), and ***env*** (envelope proteins). At each end of the retrovirus genome are repeated sequences that are essential for viral replication.

Viruses with Capsids of Complex Symmetry**:** Although most viruses have either icosahedral or helical capsids, many viruses do not fit into either category. The poxviruses and large bacteriophages are two important examples. The poxviruses are the largest of the animal viruses (about 400 x 240 x 200 nm in size) and can even be seen with a phase contrast microscope or in stained preparations. They possess an exceptionally complex internal structure with an ovoid- to brickshaped exterior. The double-stranded DNA is associated with proteins and contained in the nucleoid, a central structure shaped like a biconcave disk and surrounded by a membrane.

Two elliptical or lateral bodies lie between the nucleoid and its outer envelope, a membrane and a thick layer covered by an array of tubules or fibers. Some large bacteriophages are even more elaborate than the poxviruses. The T2, T4, and T6 phages that infect *E. coli* have been studied.

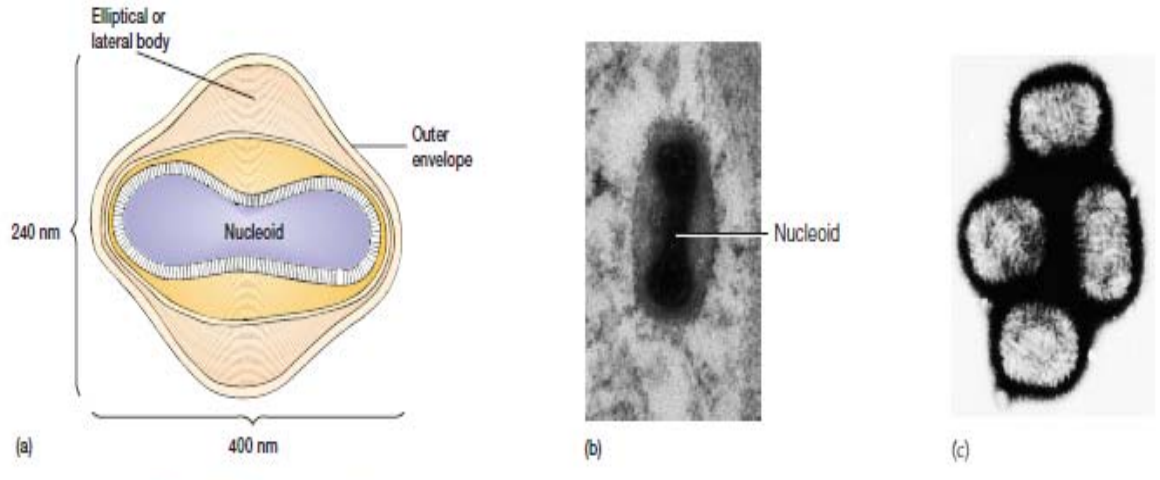

Figure 5: a) Diagram of vaccinia structure b) Virion clearly showing the nucleoid c) An electron micrograph of four virions.(Source: Book – Microbiology; L.M. Prescott)

Their head resembles an icosahedron elongated by one or two rows of hexamers in the middle and contains the DNA genome. The tail is composed of a collar joining it to the head, a central hollow tube, a sheath surrounding the tube, and a complex baseplate. The sheath is made of 144 copies of the gp18 protein arranged in 24 rings, each containing six copies. In T-even phages, the baseplate is hexagonal and has a pin and a jointed tail fiber at each corner. The tail fibers are responsible for virus attachment to the proper site on the bacterial surface.

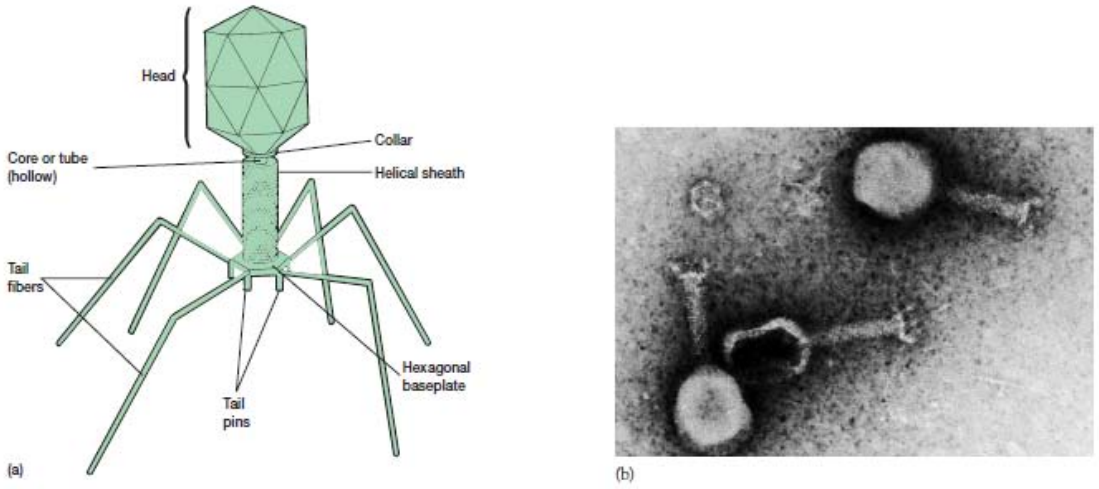

Figure 6: Structure of the T4 bacteriophage b) The micrograph shows the phage before injection of its DNA (Source: Book – Microbiology; L.M. Prescott)

6. Isolation, purification and identification of viruses

Viruses can exist in two phases: extracellular and intracellular. Virions, the extracellular phase, possess few if any enzymes and cannot reproduce independent of living cells. In the intracellular phase, viruses exist primarily as replicating nucleic acids that induce host metabolism to synthesize virion components; eventually complete virus particles or virions are released.

***Methods of Purification**

- Method vary from virus to virus or even strains, For isometric viruses, rod shaped viruses, flexuous viruses

  i)Carbon tetra chloride base method

  ii) N-butanol based method

iii) Calcium phosphate based method

Iv) Cavileer buffer based method

***Isolation**

First step in purification and characterization of plant viruses

Choice of host:

- Propagative host
- Assay host: LL

Preparation of sap extract

- Use of additives or stabilizing agents

Substances protecting against phenolics

- cysteine hydrochloride, sodium sulphite: prevent action of phenol oxidases.
- PVP-polyvinyl pyrrolidine, PEG- Polyehtylene glycol: reduces binding of virus with phenols

Additives that removes plant protein and ribosomes

- Mg bentonite-reduces contamination of virus extract with nucleases and ribosomes (mainly 19s protein)
- Charcoal: adsorb host pigments
- NaEDTA-ethylene diamine tetracetic acid at 0.01M, ph 7.4

Enzymes

- eg. Pectinase is used to degrade mucilage in sap of cocoa leaves prior to precipitation of CSSC, Trypsin -TuMv

Detergents & other additives

- Non-toxic detergents like Triton X-100 or Tween 80-used in extraction medium help in release of virus particles from cell components

Purification

Clarification:

- Removal of host constituents only
- Extracting medium supplemented with antioxidanats or reducing agents (2-mercaptaethanol, thioglycolic acid, Sodium sulphite) & chelating agents (EDTA ethylene diamine tetracetic acid) i)Potyviruses in alkaline medium ii)Isometric viruses in acidic medium

- Centrifugation at 1000 to 10,000 rpm for 5- 15 min
- The host constituents settle as pellet no the virus particles

Concentration

- Concentration: commonly used method i) High speed centrifugation or ii)Ultra centrifugation
- Done for 1-2 hrs at 35,000 to 60,000 rpm
- In this aqueous phase is discarded and pellet containing virus particles is resuspended in buffer.
- To increase the purity of the virus preparation the suspension may be subjected to alternate cycles of low and high speed centrifugation called differential centrifugation.

Final purification

- Density gradient centrifugation (rate zonal centrifugation):
- Involves high speed centrifugation of 50,000 to 70,000 rpm
- Uses some dense substances sucrose/ CsC_{l2} to create different densities
- Components of virus suspension are separated according to size, shape and density (Sedimentation coefficient)
- Testing of purity
- Other methods of final purification are i) Gel electrophoresis ii) Gel chromatography
- Storage of purified preparations

  i) At-20^{o}C by adding few drops of chlorobutanol, sodium azide, etc. to prevent growth of microbes and stabilize the virus

  ii)In liquid nitrogen by adding equal vol. of glycerol in final preparation. iii) As lyophilized

Virus concentration

- US absorption spectrum

  i)Virus concentration in the purified preparations analyzed by measuring the absorption spectrum of the virus particles at 260/280 nm ratio under UV spectrophotometer.

ii)Values of A min/A max, A260/A280 calculated to know the approximate percentage of necleo-protein by using data processor yielding spectral curves (absorbance vs. wavelength).

- The UV-absorption of the purified virus preparations show optical density (OD) value of i) 0.29 to 0.31 at 260 nm ii) 0.861 to 1.013 at 280nm

7. Transmission of plant viruses; genetic basis of cell to cell movement of plant viruses

Since plant cells are protected by cell walls, plant viruses have a considerable obstacle to overcome when trying to establish themselves in a host. TMV and a few other viruses may be carried by the wind or animals and then enter when leaves are mechanically damaged. Some plant viruses are transmitted through contaminated contaminated seeds, tubers, or pollen. Soil nematodes can transmit viruses (e.g., the tobacco ring spot virus) while feeding on roots. Tobacco necrosis virus is transmitted by parasitic fungi. However, the most important agents of transmission are insects that feed on plants, particularly sucking insects such as aphids and leafhoppers.

Insects transmit viruses in several ways. They may simply pick up viruses on their mouth parts while feeding on an infected plant, then directly transfer the viruses to the next plant they visit.

Viruses may be stored in an aphid's foregut; the aphid will infect plants when regurgitating while it is feeding. Several plant viruses for example, the wound tumor virus-can multiply in Leaf hopper tissues before reaching the salivary glands and being inoculated into plants (i.e., it uses both insects and plants as hosts).

Once inside, viroids move from cell to cell via plasmodesmata, which are the thin strands of cytoplasm that link plant cells. These are given below-

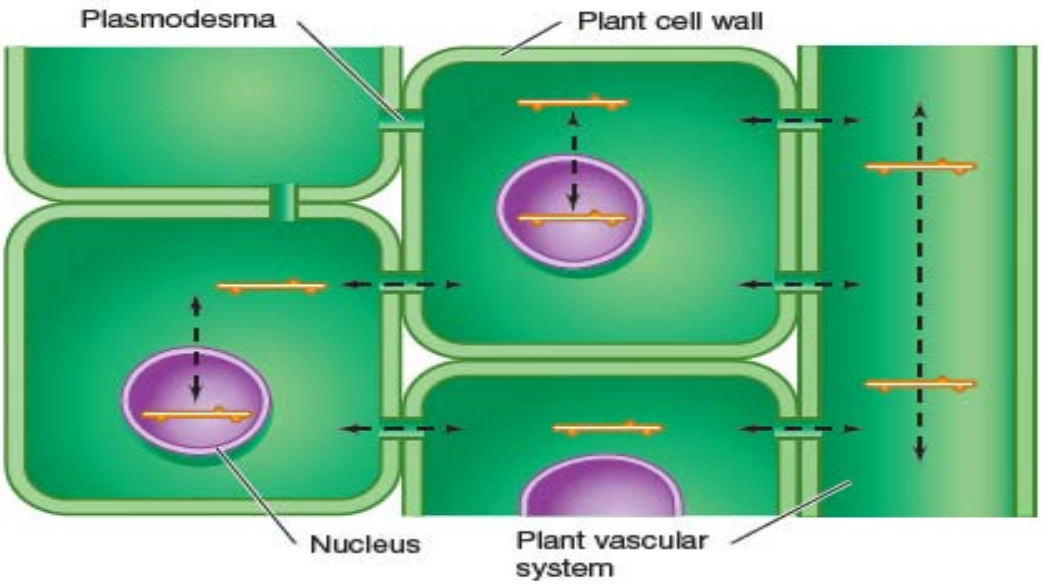

Figure 7: Viroid movement inside plant cell (Sources – Brock Biology of Microorganism; Madigan et.al.; 14th edition)

8. Management of plant viruses following classical and modern technique.

Incidence of virus diseases and their damage caused to economically important crops are reportedly increasing every day particularly in the countries embedding tropical and subtropical conditions. Integrated management approaches involving utilization of virus resistant crops and efficient management of insect vectors can reduce this disastrous problem. However, in developing countries such strategies are rarely applied due to lack of farmer's knowledge about plant virus diseases. Intensifying spread and prevalence of virus disease epidemics have been reported in many studies. It is difficult for farmers to identify virus diseases because viral disease symptoms such as leaf streaking, distortion, stunting, vein clearing, mosaic and mottle can be similar in appearance to those caused by abiotic stresses, herbicidal injuries or variation in nutritional levels. Plant infecting viruses show greater variation in their genetic make up, transmission and disease symptoms.

The best way is to avoid the disease.

1. If the plant viruses are prevailing in an area continuously; farmers just need to apply crop rotation to avoid the availability of same host.
2. Selection of seed should be done from credible sources ensuring virus free tags. This may include Cuttings, bulls, rhizomes, tubers and seeds.
3. Eradicate the diseased plant from the field which will eliminate the inoculum from the field.
4. Insect vectors are the active transmitters of the viruses from weeds and other plant sources. These must be efficiently managed though eradication of weeds which harbor them and via sowing of trap crops. e.g. Cotton reddening for white flies in bhendi. Similarly soil fumigation can be applied against in nematode transmitted viruses to control nematodes.
5. Understanding the non crop plants which are active hosts and harbor's of plant viruses is also important as they are the virus factories which must be terminated through cleaning of farm sides.
6. Selection of virus tolerant verities can be very effective. e.g. Parbhani Kranti against yellow vein mosaic of the bhendi.
7. Hot water treatment can be effective against some viruses. e.g. Sugarcane mosaic can be reduced by such treatment at 52^0 C for 30 minutes.
8. Lastly and most importantly; the education to all.

9. Molecular basis of Lytic and Lysogenic cycle.

The life cycle of bacteriophages has been a good model for understanding how viruses affect the cells they infect, since similar processes have been observed for eukaryotic viruses, which can cause immediate death of the cell or establish a latent or chronic infection. **Virulent phages** typically lead to the death of the cell through cell lysis. **Temperate phages**, on the other hand, can become part of a host chromosome and are replicated with the cell genome until such time as they are induced to make newly assembled viruses, or progeny viruses.

The Lytic Cycle:

During the **lytic cycle** of virulent phage, the bacteriophage takes over the cell, reproduces new phages, and destroys the cell. T-even phage is a good example of a well-characterized class of virulent phages. There are five stages in the bacteriophage lytic cycle. **Attachment** is the first stage in the infection process in which the phage interacts with specific bacterial surface receptors (e.g., lipopolysaccharides and OmpC protein on host surfaces). Most phages have a narrow host range and may infect one species of bacteria or one strain within a species. This unique recognition can be exploited for targeted treatment of bacterial infection by phage therapy or for phage typing to identify unique bacterial subspecies or strains. The second stage of infection is entry or **penetration**. This occurs through contraction of the tail sheath, which acts like a hypodermic needle to inject the viral genome through the cell wall and membrane. The phage head and remaining components remain outside the bacteria.

The third stage of infection is **biosynthesis** of new viral components. After entering the host cell, the virus synthesizes virus-encoded endonucleases to degrade the bacterial chromosome. It then hijacks the host cell to replicate, transcribe, and translate the necessary viral components (capsomeres, sheath, base plates, tail fibers, and viral enzymes) for the assembly of new viruses. Polymerase genes are usually expressed early in the cycle, while capsid and tail proteins are expressed later. During the **maturation** phase, new virions are created. To liberate free phages, the bacterial cell wall is disrupted by phage proteins such as holin or lysozyme. The final stage is release. Mature viruses burst out of the host cell in a process called **lysis** and the progeny viruses are liberated into the environment to infect new cells.

The Lysogenic Cycle:

In a **lysogenic cycle**, the phage genome also enters the cell through attachment and penetration. A prime example of a phage with this type of life cycle is the lambda phage. During the lysogenic cycle, instead of killing the host, the phage genome integrates into the bacterial chromosome and becomes part of the host. The integrated phage genome is called a **prophage**. A bacterial host with a prophage is called a **lysogen**. The process in which a bacterium is infected by a temperate phage is called **lysogeny**. It is typical of temperate phages to be latent or inactive within the cell. As the bacterium replicates its chromosome, it also replicates the phage's DNA and passes it on to new daughter cells during reproduction. The presence of the phage may

alter the phenotype of the bacterium, since it can bring in extra genes (e.g., toxin genes that can increase bacterial virulence). This change in the host phenotype is called **lysogenic conversion** or **phage conversion**. Some bacteria, such as *Vibrio cholerae* and *Clostridium botulinum*, are less virulent in the absence of the prophage. The phages infecting these bacteria carry the toxin genes in their genome and enhance the virulence of the host when the toxin genes are expressed. In the case of *V. cholera*, phage encoded toxin can cause severe diarrhea; in *C.botulinum*, the toxin can cause paralysis. During lysogeny, the prophage will persist in the host chromosome until **induction**, which results in the excision of the viral genome from the host chromosome. After induction has occurred the temperate phage can proceed through a lytic cycle and then undergo lysogeny in a newly infected cell.

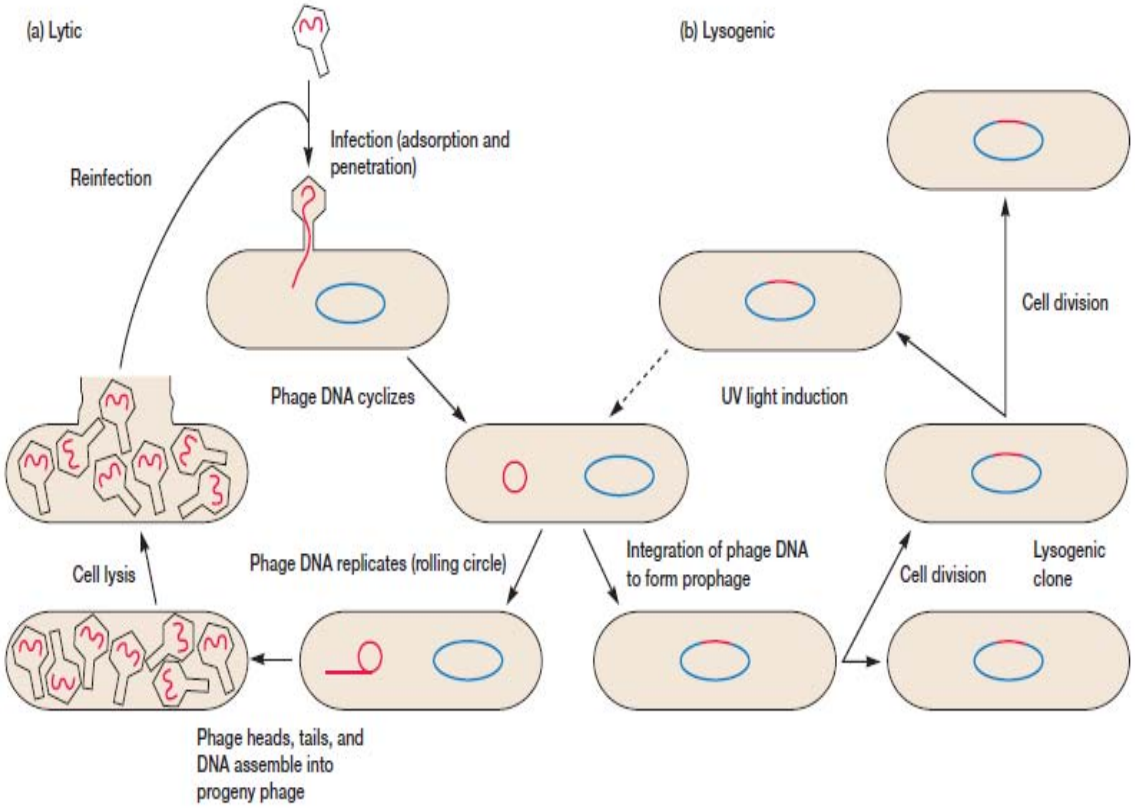

Figure 8: Lytic versus Lysogenic cycle in Phage lambda (Source – Book Microbiology by L.M. Prescott)

Regulation of Lysis or Lysogeny cycle:

Whether lysis or lysogeny occurs in a lambda infection depends in large part on the levels of two key repressor proteins that can accumulate in the cell following infection: the *lambda repressor*, also called the ***cI*** protein, and a second repressor called ***Cro***. In a nutshell, the first repressor to accumulate will control the outcome of the infection.

If genes encoding the cI protein are rapidly transcribed following infection and cI accumulates, it represses the transcription of all other lambda-encoded genes, including *cro*. When this happens, the lambda genome integrates into the host's genome and becomes a prophage. Cro, on the other hand, represses expression of a protein called cII whose function is to activate the synthesis of cI. Hence, following infection, if cI is present at insufficient levels to repress expression of phage-specific genes, Cro can accumulate in the cell; if this happens, lambda travels the lytic pathway. Control of these alternative lifestyles-lysis or lysogeny-of lambda has been likened to a "genetic switch," where a defined series of events must occur for one pathway to be favored over the other.

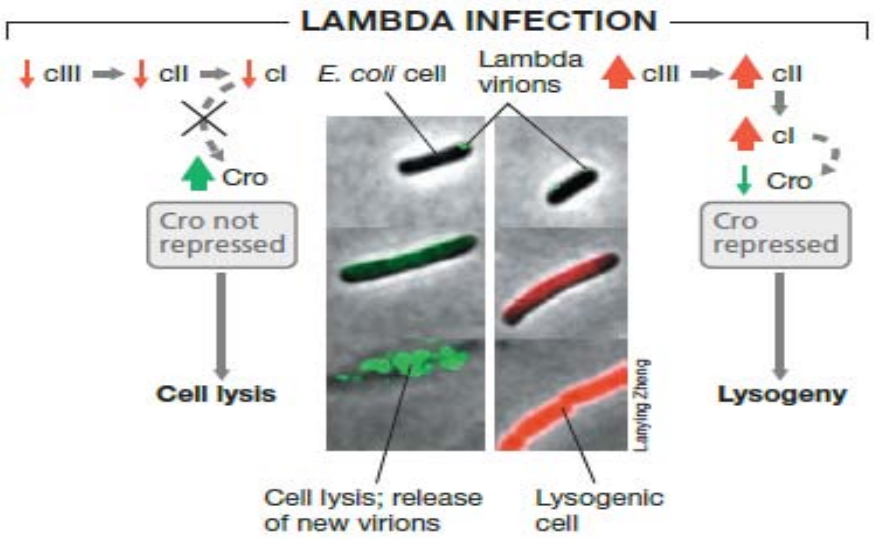

Figure 9: Regulation of lytic and lysogenic cycle in lambda phage (Sources – Brock Biology of Microorganism; Madigan et.al.; 14th edition)

10. Prions, viroids, virusoids, Satellite virus.

A) Prions

Prions are infectious agents composed entirely of a protein material that can fold in multiple, structurally abstract ways, at least one of which is transmissible to other prion proteins, leading to disease in a manner that is epidemiologically comparable to the spread of viral infection. Prions composed of the prion protein (**PrP**) are believed to be the cause of transmissible spongiform encephalopathies (TSEs) among other diseases.

Prions were initially identified as the causative agent in animal TSEs derived from scrapie in sheep and later bovine spongiform encephalopathy (BSE) - known popularly as "mad cow disease. Human diseases are Crudzefelt Jacob disease (CJD), Fatal familial imsonia and Kuru. Several yeast proteins have also been identified as having prionogenic properties.

A protein as a stand-alone infectious agent stands in contrast to all other known infectious agents such as viruses, bacteria, fungi, and parasites, all of which contain nucleic acids (DNA, RNA, or both). For this reason, a minority of researchers still consider the prion/TSE hypothesis unproven. Prions may propagate by transmitting their misfolded protein state. When a prion enters a healthy organism, it induces existing, properly folded proteins to convert into the misfolded prion form. In this way, the prion acts as a template to guide the misfolding of more proteins into prion form. In yeast, this refolding is assisted by chaperone proteins such as Hsp104. These refolded prions can then go on to convert more proteins themselves, leading to a chain reaction resulting in large amounts of the prion form. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicate when breakage causes two growing ends to become four growing ends. The incubation period of prion diseases is determined by the exponential growth rate associated with prion replication, which is a balance between the linear growth and the breakage of aggregates. The propagation of the prion depends on the presence of normally folded protein in which the prion can induce misfolding; animals that do not express the normal form of the prion protein can neither develop nor transmit the disease.

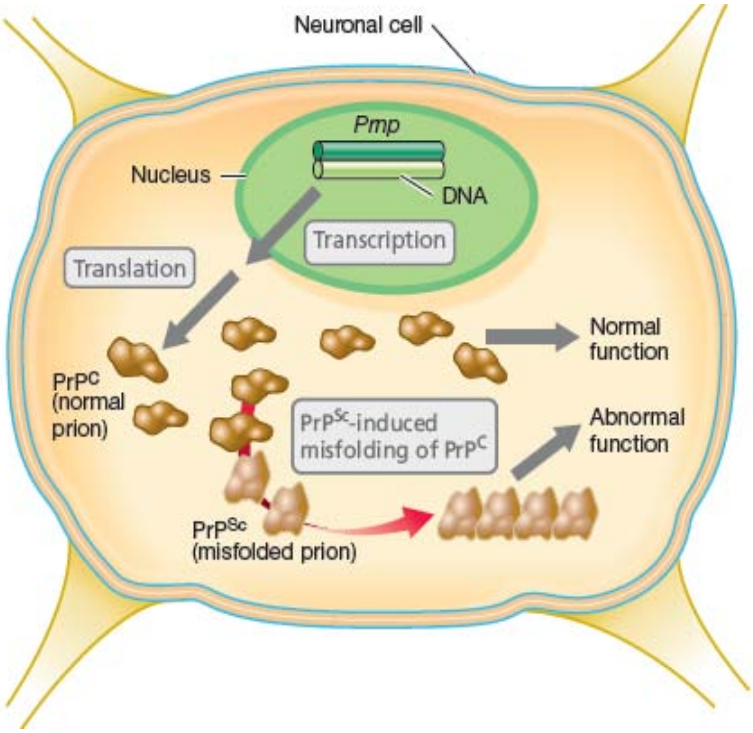

Figure 10: Mechanism of prion misfolding in Neuronal Cell (Sources – Brock Biology of Microorganism; Madigan et.al.; 14th edition)

Prion aggregates are extremely stable and accumulate in infected tissue, causing tissue damage and cell death. This structural stability means that prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. Prion structure varies slightly between species, but nonetheless prion replication is subject to epimutation and natural selection just like other forms of replication.

B) Viroids

Viroids are the smallest infectious pathogens known. They are composed solely of a short strand of circular, single-stranded RNA without protein coat. All known viroids are inhabitants of higher plants, in which most cause diseases, some of which are of slight to catastrophic economic importance. The first recognized viroid, the pathogenic agent of the potato spindle tuber disease, was discovered, initially molecularly characterized, and named by Theodor Otto Diener, plant pathologist at the U.S Department of Agriculture's Research Center in Beltsville, Maryland, in 1971.

Discovery of the viroid triggered the third major extension of the biosphere in history to include smaller lifelike entities—after the discovery of the "subvisible" microorganisms by Antonie van Leeuwenhoek in 1675 and the "submicroscopic" viruses by Dmitri Iosifovich Ivanovsky in 1892. The unique properties of viroids have been recognized by the International Committee for Virus Taxonomy with the creation of a new order of subviral agents.

In a year 2000 compilation of the most important Millennial Milestones in Plant Pathology, the American Phytopathological Society has ranked the 1971 discovery of the viroid as one of the Millennium's ten most important pathogen discoveries. It is in this framework where viroids represent the frontier of life (246 to 467nt), an aspect that should attract the attention of anybody interested in biology. Although viroids are composed of nucleic acid, they do not code for any protein. The viroid's replication mechanism uses RNA polymerase II, a host cell enzyme normally associated with synthesis of messenger RNA from DNA, which instead catalyzes "rolling circle" synthesis of new RNA using the viroid's RNA as a template. Some viroids are ribozymes, havingcatalytic properties which allow self-cleavage and ligation of unit-size genomes from larger replication intermediates.

With Diener's 1989 hypothesis that viroids may represent "living relics" from the widely assumed, ancient, and non-cellular RNA world—extant before the evolution of DNA or proteins—viroids have assumed significance beyond plant pathology to evolutionary science, by representing the most plausible RNAs capable of performing crucial steps in abiogenesis, the evolution of life from inanimate matter. The human pathogen hepatitis D virus is a "defective" RNA virus similar to a viroid.

Figure 11: Viroid structure consist of single stranded circular circular RNA (Sources – Brock Biology of Microorganism; Madigan et.al.; 14th edition)

C) Virusoids

Virusoids are circular single-stranded RNAs dependent on plant viruses for replication and encapsidation. The genome of virusoid consist of several hundred nucleotides and does not code for any proteins. Virusoids are essentially viroids that have been encapsulated by a helper virus coat protein. They are thus similar to viroids in their means of replication (rolling circle replication), but they differ in that viroids do not possess a protein coat.

Virusoids, while being studied in virology, are subviral particles rather than viruses. Since they depend on helper viruses, they are classified as satellites. In the virological taxonomy they appear as Satellites/Satellite nucleic acids/Subgroup 3: Circular satellite RNAs. The term *virusoid* is also sometimes used more generally to refer to all satellites.

Satellite viruses:

A satellite is a subviral agent composed of nucleic acid that depends on the co-infection of a host cell with a helper virus for its replication. These are most commonly associated with plants, but are also found in mammals, arthropods, and bacteria, have the components to make their own protein shell to enclose their genetic material, but rely on a helper virus to replicate. Most viruses have the capability to use host enzymes or their own replication machinery to independently replicate their own viral RNA. Satellite viruses in contrast, are completely dependent on a helper virus for replication. The symbiotic relationship between a satellite and a helper virus to catalyze the replication of a satellite viral genome is also dependent on the host to provide components like replicases to carry out replication. A satellite virus of mama virus that inhibits the replication of its host has been termed a virophage. However, the usage of this term remains controversial due to the lack of fundamental differences between virophages and classical satellite viruses.

Categories of satellite viruses

1. Chronic bee-paralysis virus-associated satellite virus
2. Satellites that resemble tobacco necrosis satellite virus
3. Nodavirus-associated satellite virus
4. Adenovirus-associated satellite virus (*Dependovirus*)
5. Mimivirus-associated satellite virus (Sputnik, virophage)

Virus-dependent nucleic acids:

a) Single stranded DNAsa.Alphasatellites (encoding a replication initiator protein)
b) Betasatellites (encoding a pathogenicity determinant)
c) Double stranded RNAs

d) Single stranded RNAs
   a. Large linear single stranded satellite RNAs
   b. Small linear single stranded satellite and satellite-like RNAs
   c. Small circular single stranded satellite RNAs
   d. Hepadnavirus-associated satellite-like RNAs (*Deltavirus*)
   e. Polerovirus-associated RNAs

Structure of satellite virus

Satellite viruses are those that are dependent for their own replication on some (catalytic) activity encoded in another "helper" virus that coinfects the host cell. The structures of three plant ssRNA satellite viruses represent some of the highest resolutions known and have been comparatively reviewed. The structures of satellite tobacco mosaic virus (STMV), satellite tobacco necrosis virus (STNV), and satellite panicum mosaic virus (SPMV) have T=1 capsids composed of 60 identical copies of unembellished jelly-roll β barrels constructed of only 155 to 195 amino acids. What is remarkable is how little the assembly context of these domains is conserved. The same end always points toward the 5-fold axis, but the domains are rotated to different extents around the 5-fold axis. Furthermore, between STNV and the others, there is a 70° rotation of the barrel about its long axis. Contacts across the dimer interface are different: end-to-end (with respect to the barrel in STNV), compared with side-to-side in SPMV and STMV, involving only loops in STMV, but also βB in SPMV. Such disparity may be an evolutionary consequence of these viruses being satellites, perhaps with the opportunity to exchange genetic information with their (quite different) helper viruses (Ban *et al*., 1995).

11. Let's sum up

This part about the biology and diversity of virus which contribute morphological and structural organization of viruses. Genome organisation of some specified viruses and its molecular evolution. Different techniques of isolation, purification and cultivation of viruses are described in details. Management of viruses in respect of agricultural prospect and its disease control also have been included.

12. Suggested reading

1. Brock biology of microorganism, 10th edition Madigan,Martinko,Bender, Buckley and Stahl. Pearson, New York, San francisco USA.
2. Microbiology, 5th edition, Lancin M. Prescott, ISBN-0-07-282905-2
3. Microbiology, 5th edition, M. Pelczar, ISBN: 9780074623206, 0074623206
4. https://www.wikipedia.org/

13. Assignment

1. Mention the important properties of virus.
2. Describe the structure of viral genome
3. Differentiate between lytic cycle and lysogenic cycle
4. Describe briefly the transmission of plant virus.
5. Write short notes on : Prion, viroid
6. Regulation of lytic and lysogenic cycle

COURSE – BOHCT1.1

Biology and Diversity of Virus, Bacteria and Fungi

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group – B (Biology and Diversity of Bacteria)

Content Structure

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1. Introduction
2. Objectives
3. Microbial taxonomy and phylogeny, major groups of Bacteria.
4. Ultra structure of Gram positive and Gram negative bacteria.
5. Bacterial motility, bacterial sporulation.
6. Bacterial growth kinetics, factors affecting growth.
7. Photolithotrophs, chemolithotrophs, photoorganotrophs & chemoorganotrophs, Mixotroph.
8. Organization and replication of genetic material in bacteria. Genetic recombination (conjugation, transformation and transduction) in bacteria.
9. Concept of microbial ecology with reference to air, water and soil.
10. Microbes associated with food, food-borne infections and intoxications; preservation of food.
11. Cells and organ of immune system, antigen (chemical nature and types), immunoglobulins (structure and types), brief idea about hypersensitivity and vaccine.
12. Air, water, and soil-borne disease – causal organism, symptoms, control.
13. Industrial production of ethanol, penicillin and vitamin B12.
14. Cosmetic microbiology-current trends.
15. Let’s sum up
16. Suggested Reading
17. Assignment

1. Introduction

Bacteria are a type of biologicalcell.They constitute large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria have a number of shapes, ranging from spheres to rods and spirals. Bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. Most bacteria have not been characterised, and only about half of the bacterial phyla have species that can be grown in the laboratory. The study of bacteria is known as bacteriology, a branch of microbiology.

2. Course Objectives

The objective of this module is not only to develop a better apttitude towards the knowledge of bacteria and their application.

On completion of study of this course you will be able to
*Know the nature, origin and characteristics of bacteria
* Isolation, purification and identification of bacteria
*Gather knowledge of various applications of bacteria in agricultural purpose

3. Microbial taxonomy and phylogeny, major groups of Bacteria.

Introduction

Living organisms are fascinating by its diversity whether it is plants, animals or microbes. A handful of soil is populated with more than the human population on earth. They play important essential roles in nature. So if we arrange these microbes in order or hierarchy by based on its similarity or differences in any characteristics, we can easily get to know and get easy access to all the microbes. So it is desirable to determine the classification. Greek Philosopher Aristotle who is the one classified the living things as plants and animals around 2000 years ago. So in this lecture, we will learn about taxonomy, how is it classifified? What methods are available to classify them? And then brief description about microbial evolution and diversity and its phylogeny.

Taxonomy

Taxonomy [Greek *taxis,* arrangement, and *nomos,* law, or *nemein,* to distribute] is defined as the science of biological classification. In simple term, taxonomy is orderly arranging organisms under study into groups of larger units.

It consists of *three* interrelated parts namely. **Classification** is the arrangement of organisms into groups or taxa (s., taxon) based on mutual similarity or evolutionary relatedness. **Nomenclature** is concerned with the assignment of names to taxonomic groups in agreement with published rules. **Identification** is the practical side of taxonomy, the process of determining that a particular isolate belongs to a recognized taxon. (So in short Identify-Naming them and classify them)

Classification

It is bringing order to the diverse variety of organisms present in nature. So there are two general ways the classification can be constructed. First one is based on the morphological characters (phenetic classification) and second is based on evolutionary relationship (phylogenetic classification)

Phenetic classification - Grouping organisms together based on the mutual similarity of their phenotypic characteristics. It does not provide information about phylogenetic relations.

Phylogenetic classification - These are systems based on evolutionary relationships rather than external appearance (the term phylogeny [Greek *phylon,* tribe or race, and *genesis,* generation or

origin] refers to the evolutionary development of a species). It is based on the direct comparison of genetic materials and/or gene product.

Nomenclature (Binomial system)

Biologists in the middle ages used to follow polynomial system, i.e naming organisms with many names (*poly* -many, *nomo* - name). For example name for the European honeybee, was *Apis pubescens, thorace subgriseo, abdomine fusco, pedibus posticis glabris utrinque margine ciliatis* (just for example no need to be memorized). Later Binomial systems were developed by Swedish biologist Carolus Linnaeus (1707–1778) based on the anatomical characteristics of plants and animals. Nomenclature in microbiology is developed based on the principals established for the plant and Animal kingdom by Linnaeus. The first word in the binomial is the genus name and is always capitalized. The second word is species name and never capitalized. For example honeybee, *Apis mellifera*

Taxonomic ranks:

In prokaryotic taxonomy the most commonly used levels or ranks (in ascending order) are species, genera, families, orders, classes, phyla, kingdom or domain. In order to remember the seven categories of the taxonomic hierarchy in their proper order, it may be useful to memorize a **O**rder – **f**amily – **g**enus – **s**pecies). The basic taxonomic group in microbial taxonomy is the species. A **species** is a collection of strains that have a similar G+C composition and 70% or greater similarity as judged by DNA hybridization. Ideally a species also should be phenotypically distinguishable from other similar species. An example of hierarchy in taxonomy is given below.

Rank	Example
Domain	*Bacteria*
Phylum	*Proteobacteria*
Class	*γ- Proteobacteria*
Order	*Enterobacteriales*
Family	*Enterobactericeae*
Genus	*Shigella*
Species	*S.dysentriae*

A strain is a population of organisms that is distinguishable from at least some other populations within a particular taxonomic category. It is considered to have descended from a single organism or pure culture isolate. Strains within a species may differ slightly from one another in in many ways. Biovars are variant prokaryotic strains characterized by biochemical or physiological differences, morphovars differ morphologically, and serovars have distinctive antigenic properties. One strain of a species is designated as the type strain. It is usually one of the first strains studied and often is more fully characterized than other strains; however, it does not have to be the most representative member but this strain can be considered as reference strain and can be compared with other strains. Each species is assigned to a genus, the next rank in the taxonomic hierarchy. A genus is a well-defined group of one or more species that is clearly separate from other genera.

Techniques for identifying or determining taxonomical characters

In order to identify and classify microorganisms, we need to know about their characteristics. There are two ways to determine the taxonomical characters; classical and molecular characters

Classical characteristics:- This approach uses morphological, biochemical, physiological, ecological and genetic characteristics. It is mainly used in microbial taxonomy.

1. *Morphology:-* Morphology is the one which can be easily studied and analyzed. Structural features (*cell shape, size, colony morphology, appendages, and etc.*) depend on the expression of many genes, are usually genetically stable.

2. *Physiology and metabolism:-* Organisms are classified based on the requirements for growth characters like carbon and nitrogen sources, cell wall constituents, general nutritional type, energy sources, optimum growth temperature, Motility.

3. *Ecology:-* These are taxonomically valuable because even very closely related microorganisms can differ considerably with respect to ecological characteristics. The ability to cause disease in a particular host; and habitat preferences such as requirements for temperature, pH, oxygen, and osmotic concentration are examples of ecological characteristics.

***4. Genetic analysis*:-** The study of chromosomal gene exchange between species through transformation and conjugation (in Enteric bacteria) is sometimes useful in their classification. Most bacteria are harboring plasmids, classification based on plasmid is also an important part of classification.

Molecular characteristics: This is the most powerful approaches to study taxonomy by analyzing proteins and nucleic acids. Because these are either direct gene products or the genes themselves, comparisons of proteins and nucleic acids yield considerable information about true relatedness.

1. ***Comparing amino acid sequences:-*** Comparison of amino acid sequences of proteins from different organisms reveals its taxonomic relations. The most direct approach is to determine the amino acid sequence of proteins with the same function. If the sequences of proteins with the same function are similar, the organisms possessing them are probably closely related. The electrophoretic mobility of proteins is useful in studying relationships at the species and subspecies levels. Antibodies can discriminate between very similar proteins, and immunologic techniques are used to compare proteins from different microorganisms.

2. ***Nucleic acid composition:-*** By direct comparison of microbial genomes and based on the G+C content of different organisms (*Escherichia coli* 48-52 %). And genomic fingerprinting (RFLP, AFLP) reveals its relatedness with others.

3. ***Nucleic acid hybridization:-*** It uses the property of complementarities in double stranded DNA. More distantly related organism can be identified based on DNA-RNA hybridization

4. ***Nucleic acid sequencing:-*** Techniques are now available to sequence both DNA and RNA. 5S and 16S RNA (prokaryotes), 18S (fungi) analysis of microorganisms can reveal their relatedness because of its functional role is same in all ribosomes and slow structural changes with time.

Microbial evolution and Diversity

It has been estimated that our planet is about 4.6 billion years old. Around 3.5 to 3.8 billion years old fossilized remains of prokaryotic cells have been discovered in sedimentary rocks. Thus earlier prokaryotes were anaerobic and arose shortly after the earth cooled. Cyanobacteria and oxygen-producing photosynthesis probably developed 2.5 to 3.0 billion or more years ago. It appears likely that modern eukaryotic cells arose from prokaryotes about 1.4 billion years ago.

Two hypotheses for the evolution of eukaryotic cells

1. Organelles arose within prokaryotes from the invagination of the plasma membrane

2. *Endosymbiotic hypothesis* - Fusion of ancient true bacteria and archaea to form a nucleus. They proposed that the eukaryotic line diverged from the *Archaea* and then the nucleus formed, possibly from the Golgi apparatus Mitochondria and chloroplasts develop later from a permanent symbiotic relationship with other bacteria, e.g., cyanelle (cyanobacterium) living

inside the protist *Cyanophora paradoxa*. Cyanobacteria have been considered the most likely ancestors of chloroplasts. More recently*Prochloron* has become the favorite candidate. The existence of this bacterium suggests that chloroplasts arose from a common ancestor of prochlorophytes and cyanobacteria. Mitochondria arose from an endosymbiotic relationship between the free-living primitive eukaryotic and bacteria with aerobic respiration (possibly an ancestor of three modern groups: *Agrobacterium, Rhizobium,*and *Rickettsia*).

Divisions of Life

Kingdom systems of classification

- **Five-kingdom system** (Whittaker, 1960s) - based upon cell type, organization, and the means of nutrient acquisition (Monera, Protista, Fungi, Plantae, Animalia)
- **Six-kingdom system** - differs from five-kingdom system by dividing prokaryotes into bacteria and archaea (Bacteria, Archaea, Protista, Fungi, Plantae, Animalia)
- **Eight-kingdom system** (Cavalier-Smith) - further division of the protists using rRNA data and grouping organisms into two empires (Eucaryota and Bacteria) containing a total of eight kingdoms [(Bacteria, Archaea), (Archezoa, Protista, Plantae, Chromista, Fungi, Animalia)]

Domains

Advances in genomic DNA sequencing of the microorganisms, biologists are increasingly adapting the classification of living organisms that recognizes three **domains**, a taxonomic level higher than kingdom. Archaebacteria are in one domain, eubacteria in a second, and eukaryotes in the third. Domain Eukarya is subdivided into four kingdoms plants, animals, fungi, protists.

Domain- Archaebacteria

The term *archaebacteria* (Greek, *archaio,* ancient) refers to the ancient origin of this group of bacteria, which seem to have diverged very early from the eubacteria. They are inhabited mostly in extreme environments. The archaebacteria are grouped (based primarily on the environments in which they live) into three general categories methanogens, extremophiles and non extreme Archaebacteria.

Domain- Bacteria

The Eubacteria are the most abundant organisms on earth. It plays critical roles like cycling carbon and sulfur. Much of the world's photosynthesis is carried out by eubacteria. However, certain groups of eubacteria are also responsible for many forms of disease.

Domain- Eukarya

It consists of four kingdoms. The first of which is protista, mostly unicellular organism like amoeba. The other three kingdoms are plants, fungi, animals. Multicellularity and sexuality are the two unique characters that differentiate from prokaryote and eukaryotes.

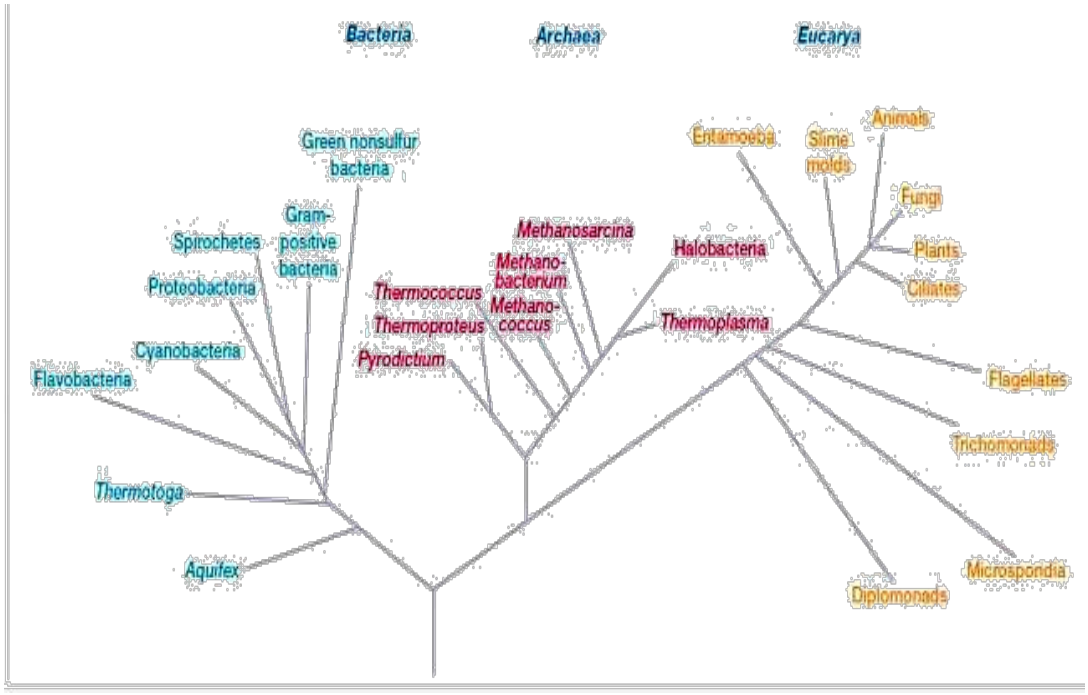


Fig. 1 Phylogenetic position of bacteria on the basis of 16S rRNA sequence analysis. (Sources – Brock Biology of Microorganism; Madigan et.al.; 14th edition)

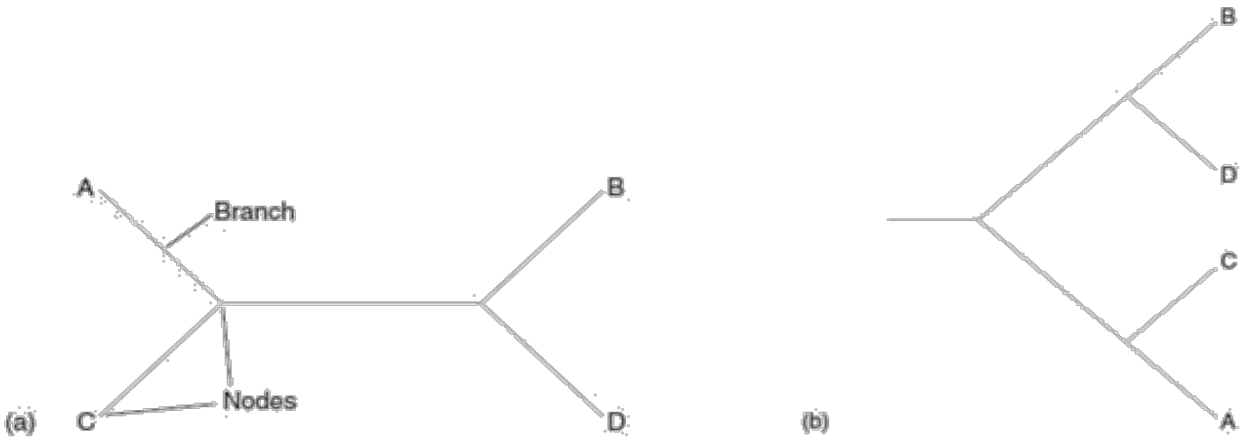


Fig. 2 . Phylogenetic tree. a) unrooted tree, b) rooted tree.

Molecular chronometers

This concept, first suggested by Zuckerkandl and Pauling (1965), which is based on thought that the sequences of many rRNAs and proteins gradually change over time without destroying or severelyaltering their functions. Changes increases with time linearly. If sequences of similar molecules from two organisms differs, it means that they diverged very long time ago.

Phylogenetic tree: Phylogenetic relationships are illustrated in the form of branched diagrams or trees (denrograms). A phylogenetic tree is a graph made of branches that connect nodes. The nodes represent taxonomic units such as species or genes; the external nodes, those at the end of the branches, represent living organisms. The tree may have a time scale, or the length of the branches may represent the number of molecular changes that have taken place between the two nodes. Finally, a tree may be unrooted or rooted. An unrooted tree simply represents phylogenetic relationships but does not provide an evolutionary path. Figure 2. a. shows that A is more closely related to C than it is to either B or D, but does not specify the common ancestor for the four species or the direction of change. In contrast, the rooted tree Figure 2. b. does give a node that serves as the common ancestor and shows the development of the four species from this root.

Parsimony analysis Phylogenetic relationships also can be estimated by techniques such as parsimony analysis. In this approach, relationships are determined by estimating the minimum number of sequence changes required to give the final sequences being compared. It is presumed that evolutionary change occurs along the shortest pathway with the fewest changes or steps from an ancestor to the organism in question.

Oligonucleotide signature sequences

The 16S rRNA of most major phylogenetic groups has one or more characteristic nucleotide sequences called oligonucleotide signatures. **Oligonucleotide signature sequences** are specific oligonucleotide sequences that occur in most or all members of a particular phylogenetic group. They are rarely or never present in other groups, even closely related ones. Thus signature sequences can be used to place microorganisms in the proper group.

Polyphasic taxonomy Studying phylogeny based on both genotypic and phenotypic information ranging from molecular characteristics to ecological characters.

Numerical Taxonomy Computer based approaches of grouping organisms is called Numerical taxonomy which is based on presence or absence of selected characters in the group of organisms. It is method of estimating percent similarity (ratio between the number of characters

same and total number of characters organisms having). This method has great practical usefulness as well as being relatively unbiased in its approach. It has high degree of stability and predictability

Major groups of bacteria

1. Bacteria archeota: a) Crenarcheota is a phylum under the domain Archae. This particular phylum is said to not belong to the main lineage of the other archaeal group because of their 16S rRNA. These organisms are said to be found in hydrothermal environments, high temperatures. In addition, these organisms are found in small group or by themselves. Also, a new phylum therefore many of the characteristics of these organisms are still being discovered. Euryarchaeotes: b) Euryarchaeota is a phylum located in the Archae domain. The majority of organisms within this phylum are methanogen, produce methane as a metabolic outcome, but they can also be halophiles or thermoacidophiles. In addition, these organisms are typically found in the intestines and can survive in extreme salt concentrations. Also, these organisms can range from anaerobes, living without oxygen, to aerobes, living with oxygen. One of the main characteristics of this group is it rRNA sequences. Crenarchaeotes: Crenarchaeotes is a phylum under the domain Archae. The organisms in this group are hyperthermophiles, can survive in high temperature conditions. In addition, the environments that these organisms live in are very acidic with a pH as low as one. Also, these organisms use hydrogen as a basis of electrons to reduce sulfur; the reduce sulfur allows these organisms to get the energy they need to synthesize food from carbon dioxide. Moreover, these organisms lack histones, differ in rRNA sequencing and are found in the marine which are all major characteristics of these organisms.

c) Nanoarchaeotes: Nanoarchaeotes is a phylum off of the domain Archae. The organisms that make up this phylum consist of organisms that can survive in high temperatures. In addition, these organisms are small and known as the smallest organism in the world. Also, these organisms are made up of only around a thousand based pairs of DNA. Moreover, a specific way of identifying these organisms is by their specific rRNA sequence.

2. Cyanobacteria: Cyanobacteria is a phylum in the Bacteria kingdom; many time these organisms are referred to blue-green algae. All of these organisms obtain their energy through photosynthesis; however, these organisms did not always complete the process of photosynthesis but rather developed the system through evolution. Also, since these organism use photosynthesis, they have chloroplast to help with this process. In addition, these organisms complete the process of nitrogen fixation and the use of the thick-walled heterocysts that hold

enzyme nitogenase. Moreover, these organisms are very diverse and live in a variety of environments throughout the Earth.

3. Spirochetes: Spirochetes are a phylum that is a part of the Bacteria kingdom. This phylum's organisms shape is long, helically coiled; the lengths of these organisms range from five to two hundred and fifty μm and a diameter of one tenth to six tenth μm. In addition, the location of the flagella "runs lengthwise between the bacterial inner membrane and outer membrane", is one of the most important characteristics of this phylum. Also, these organisms reproduce through binary fission. In addition, the majority of the organisms in this phylum are free-living and anaerobic, but there are many exceptions. Proteotic Bacteria: Proteotic Bacteria is a phylum that is a part of the Bacteria kingdom. This phylum is rather large and contains organism that are grand negative. One of the interesting facts about this phylum is that the organisms can be anaerobic, survive living in oxygen free environments, or aerobic, live in environments with oxygen. Many of these organisms are free-living and parasitic; therefore, many of these organisms cause diseases, such as *E.coli*. Chlamydias: Chlamydias is a phylum that is a part of the Bacteria kingdom. Also, these organisms are parasitic and can only survive in animal cells; these organisms depend on the host ATP to survive. Another characteristic that is a part of this phylum is the lack of peptidoglycan which could result from the gram- negative walls.

4. Gram-Positive Bacteria: Gram-Positive Bacteria is a phylum of the Bacteria kingdom.Some of the species in this phylum are capable of forming colonies, such as actinomycetes. Also, many of these organisms are free-living. In addition, many times these organisms are tiny and have few genes. Moreover, many of the organisms that havecell walls which encompass teichoic acids and peptidogly can which causes the organisms to look violet or blue. However, notall of the organisms in this phylum have a cell wall. Other characteristics that make up this phylum include cytoplasmic lipid membrane, a thick peptidoglycan layer, a capsule of polysaccharides, and flagella.

5. Gram-negative bacteria are bacteria that do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation. They are characterized by their cell envelopes, which are composed of a thin peptidoglycan cell wall sandwiched between an inner cytoplasmiccell membrane and a bacterial outer membrane.

Gram-negative bacteria are found everywhere, in virtually all environments on Earth that support life. The gram-negative bacteria include the model organism *Escherichia coli*, as well as many pathogenic bacteria, such as *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Chlamydia*

trachomatis, and *Yersinia pestis*. They are an important medical challenge, as their outer membrane protects them from many antibiotics (including penicillin); detergents that would normally damage the peptidoglycans of the (inner) cell membrane; and lysozyme, an antimicrobial enzyme produced by animals that forms part of the innate immune system.

4. Ultra structure of Gram positive and Gram negative bacteria.

Cell Wall

The cell walls of bacteria deserve special attention for several reasons:

- They are an essential structure for viability, as described above.
- They are composed of unique components found nowhere else in nature.
- They are one of the most important sites for attack by antibiotics.
- They provide ligands for adherence and receptor sites for drugs or viruses.
- They cause symptoms of disease in animals.
- They provide for immunological distinction and immunological variation among strains of bacteria.

Most procaryotes have a rigid **cell wall**. The cell wall is an essential structure that protects the cell protoplast from mechanical damage and from osmotic rupture or **lysis**. Procaryotes usually live in relatively dilute environments such that the accumulation of solutes inside the procaryotic cell cytoplasm greatly exceeds the total solute concentration in the outside environment. Thus, the osmotic pressure against the inside of the plasma membrane may be the equivalent of 10-25 atm. Since the membrane is a delicate, plastic structure, it must be restrained by an outside wall made of porous, rigid material that has high tensile strength. Such a material is **murein**, the ubiquitous component of bacterial cell walls. Murein is a unique type of **peptidoglycan**, a polymer of disaccharides (glycan) cross-linked by short chains of amino acids (peptide). Many types of peptidoglycan exist. All Bacterial peptidoglycans contain N-acetylmuramic acid, which is the definitive component of murein. The cell walls of Archaea may be composed of protein, polysaccharides, or peptidoglycan-like molecules, but never do they contain murein. This feature distinguishes the Bacteria from the Archaea.

In the **Gram-positive Bacteria** (those that retain the purple crystal violet dye when subjected to the Gram-staining procedure), the cell wall consists of several layers of peptidoglycan. Running

perpendicular to the peptidoglycan sheets is a group of molecules called **teichoic acids** which are unique to the Gram-positive cell wall.

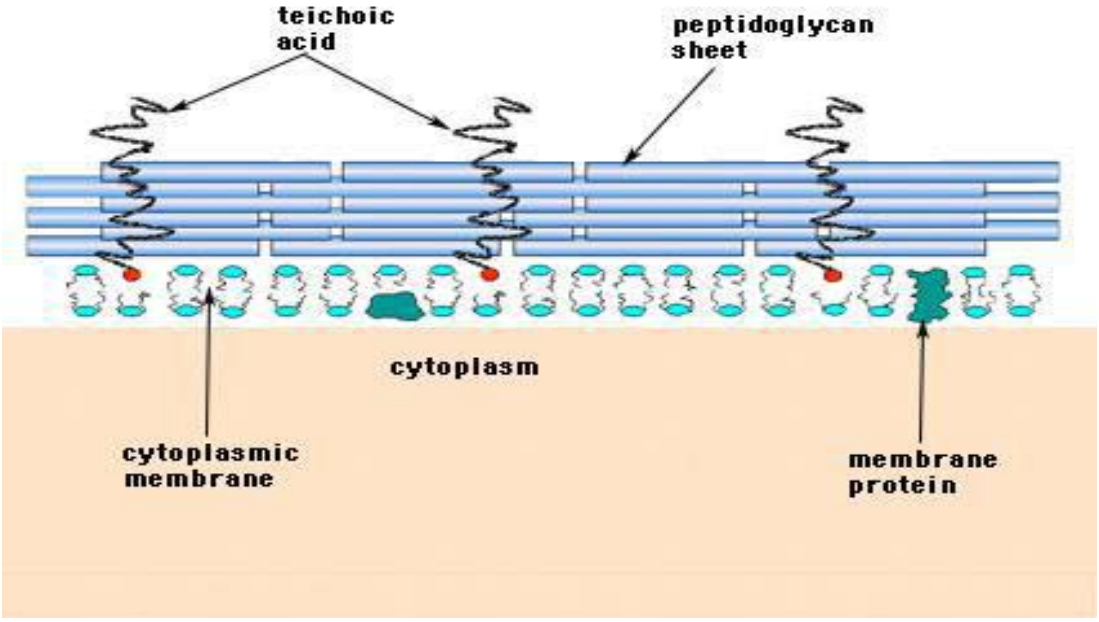


Figure 3. Structure of the Gram-positive bacterial cell wall.

In the **Gram-negative Bacteria** (which do not retain the crystal violet), the cell wall is composed of a single layer of peptidoglycan surrounded by a membranous structure called the **outer membrane**. The outer membrane of Gram-negative bacteria invariably contains a unique component, **lipopolysaccharide** (**LPS** or**endotoxin**), which is toxic to animals. In Gram-negative bacteria the outer membrane is usually thought of as part of the cell wall.

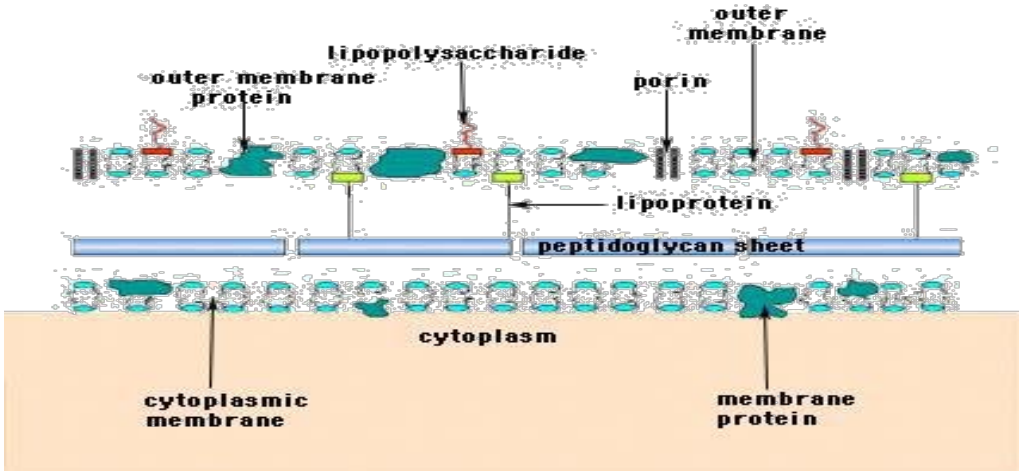


Figure 4: Structure of the Gram-negative cell wall.

In the Gram-positive Bacteria, the cell wall is thick (15-80 nanometers), consisting of several layers of peptidoglycan. In the Gram-negative Bacteria the cell wall is relatively thin (10 nanometers) and is composed of a single layer of peptidoglycan surrounded by an outer membrane. Peptidoglycan structure and arrangement in *E. coli* is representative of all*Enterobacteriaceae*, as well as many other Gram-negative bacteria. The glycan backbone is made up of alternating molecules of N-acetylglucosamine (G) and N-acetylmuramic acid (M) connected by a beta 1,4-glycoside bond. The 3-carbon of N-acetylmuramic acid (M) is substituted with a lactyl ether group derived from pyruvate. The lactyl ether connects the glycan backbone to a peptide side chain that contains L-alanine, (L-ala), D-glutamate (D-glu), Diaminopimelic acid (DAP), and D-alanine (D-ala). MurNAc is unique to bacterial cell walls, as is D-glu, DAP and D-ala. The muramic acid subunitof *E. coli* is shown in below.

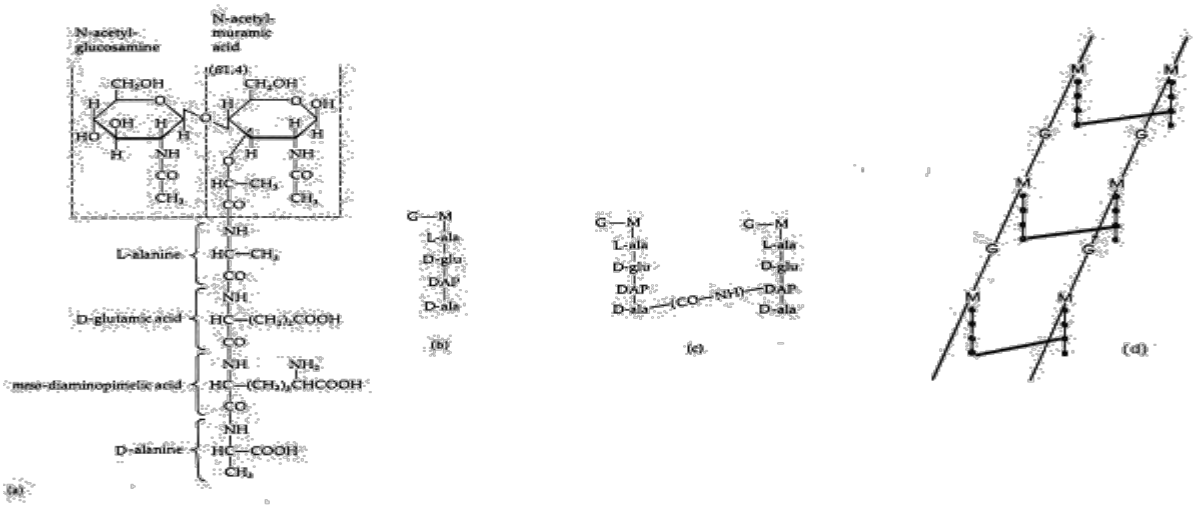


Figure 5. The structure of the muramic acid subunit of the peptidoglycan of *Escherichia coli*. This is the type of murein found in most Gram-negative bacteria. The glycan backbone is a repeat polymer of two amino sugars, N-acetylglucosamine (G) and N-acetylmuramic acid (M). Attached to the N-acetylmuramic acid is a tetrapeptide consisting of L-ala-D-glu-DAP-D-ala. b. Abbreviated structure of the muramic acid subunit. c. Nearby tetrapeptide side chains may be linked to one another by an interpeptide bond between DAP on one chain and D-ala on the other. d. The polymeric form of the molecule.

The assembly of peptidoglycan on the outside of the plasma membrane is mediated by a group of periplasmic enzymes, which are transglycosylases, transpeptidases and carboxypeptidases. The mechanism of action of penicillin and related beta-lactam antibiotics is to block transpeptidaseand carboxypeptidase enzymes during their assembly of the murein cell wall. Hence, the beta lactam antibiotics are said to "block cell wall synthesis" in the bacteria.

The glycan backbone of the peptidoglycan molecule can be cleaved by an enzyme called lysozyme that is present in animal serum, tissues and secretions, and in the phagocytic lysosome. The function of lysozyme is to lyse bacterial cells as a constitutive defense against bacterial pathogens. Some Gram-positive bacteria are very sensitive to lysozyme and the enzyme is quite active at low concentrations. Lachrymal secretions (tears) can be diluted 1:40,000 and retain the ability to lyse certain bacterial cells. Gram-negative bacteria are less vulnerable to attack by lysozyme because their peptidoglycan is shielded by the outer membrane. The exact site of lysozymal cleavage is the beta 1,4 bond between N-acetylmuramic acid (M) and N-acetylglucosamine (G) , such that the muramic acid subunit is the result of the action of lysozyme on bacterial peptidoglycan.

In Gram-positive bacteria there are numerous different peptide arrangements among peptidoglycans. The best studied is the murein of *Staphylococcus aureus*shown in Figure 17 below. In place of DAP (in *E. coli*) is the diamino acid, L-lysine (L-lys), and in place of the interpeptide bond (in Gram-negatives) is an**interpeptide bridge** of amino acids that connects a free amino group on lysine to a free carboxy group on D-ala of a nearby tetrapeptide side chain. This arrangement apparently allows for more frequent cross-bonding between nearby tetrapeptide side chains. In *S. aureus*, the interpeptide bridge is a peptide consisting of 5 glycine molecules (called a **pentaglycine bridge**). Assembly of the interpeptide bridge in Gram-positive murein is inhibited by the beta lactam antibiotics in the same manner as the interpeptide bond in Gram-negative murein. Gram-positive bacteria are more sensitive to penicillin than Gram-negative bacteria because the peptidoglycan is not protected by an outer membrane and it is a more abundant molecule. In Gram-positive bacteria, peptidoglycans may vary in the amino acid in place of DAP or L-lys in position 3 of the tetrapeptide, and in the exact composition of the interpeptide bridge. At least eight different types of peptidoglycan exist in Gram-positive bacteria.

Gram-negative bacteria may contain a single monomolecular layer of murein in their cell walls while Gram-positive bacteria are thought to have several layers or "wraps" of peptidoglycan.

Closely associated with the layers of peptidoglycan in Gram-positive bacteria are a group of molecules called teichoic acids. **Teichoic acids** are linear polymers of polyglycerol or polyribitol substituted with phosphates and a few amino acids and sugars. The teichoic acid polymers are occasionally anchored to the plasma membrane (called **lipoteichoic acid**, **LTA**) apparently directed outward at right angles to the layers of peptidoglycan. The functions of teichoic acid are not known. They are essential to viability of Gram-positive bacteria in the wild. One idea is that they provide a channel of regularly-oriented negative charges for threading positively charged substances through the complicated peptidoglycan network. Another theory is that teichoic acids are in some way involved in the regulation and assembly of muramic acid subunits on the outside of the plasma membrane. There are instances, particularly in the streptococci, wherein teichoic acids have been implicated in the adherence of the bacteria to tissue surfaces.

5. Bacterial Motility, bacterial sporulation

Many but not all bacteria exhibit motility, i.e. self-propelled motion, under appropriate circumstances. Motion can be achieved by one of three mechanisms. Most motile bacteria move by the use of flagella, rigid structures 20 nm in diameter and 15-20 μm long which protrude from the cell surface. In some bacteria, there is only a single flagellum – such cells are called monotrichous. In these circumstances, the flagellum is usually located at one end of the cell (polar). Some bacteria have a single flagellum at both ends – amphitrichous. However, many bacteria have numerous flagella; if these are located as a tuft at one end of the cell, this is described as lophotrichous (e.g. *Chromatium*), if they are distributed all over the cell, as peritrichous.

Flagella consist of a hollow, rigid cylinder composed of a protein called flagellin, which forms a filament anchored to the cell by a curved structure called the hook, which is attached to the basal body. Flagellae are, in effect, rotary motors comprising a number of proteinaceous rings embedded in the cell wall. These molecular motors are powered by the phosphorylation cascade responsible for generating energy within the cell. In action, the filament rotates at speeds from 200 to more than 1,000 revolutions per second, driving the rotation of the flagellum. The organization of these structures is quite different from that of eukaryotic flagella. The direction of rotation determines the movement of the cell. Periodically the direction of rotation is briefly reversed, causing what is known as a “tumble”, and results in reorientation of the cell. When

anticlockwise rotation is resumed, the cell moves off in a new direction. Watch for the tumbles in this video. This allows bacteria to change direction. Bacteria can sense nutrients and move towards them – a process is known as chemotaxis. Additionally, they can also move away from harmful substances such as waste products and in response to temperature, light, gravity, etc. This apparently intelligent behavior is achieved by changes in the frequency of tumbles. When moving towards a favourable stimulus or away from an unfavourable one, the frequency of tumbles is low, thus the cell moves towards or away from the stimulus as appropriate. However, when swimming towards an unfavourable or away from a favourable stimulus, the frequency of tumbles increases, allowing the cell to reorient itself and move to a more suitable growth.

The second type of motility is shown by Spirochaetes, helical bacteria which have a specialized internal structure known as the axial filament which is responsible for rotation of the cell in a spiral fashion and consequent locomotion. The third mechanism is gliding motility. Gliding motility is the movement of cells over surfaces without the aid of flagella, a trait common to many bacteria. Gliding bacteria all secrete copious slime, but the exact mechanism which propels the cells is not known. The gliding motility apparatus which propels the cells involves a complex of proteins, yet the full nature of this "motor" and how the components interact is not understood. Under the microscope, motile bacteria seem to move in a purposeful way, though they may frequently change direction. However, even dead cells, such as those in this video, move. Rapid movement is due to capillary action or convection currents on the microscope slide. However, the motion which causes most problems is Brownian motion, first observed in 1827 by the English botanist Robert Brown. This is due to random molecular bombardment of tiny bacterial cells by the molecules of the solvent.

The Mechanism of Flagellar Movement

Procaryotic flagella operate differently from eucaryotic flagella. The filament is in the shape of a rigid helix, and the bacterium moves when this helix rotates. The direction of flagellar rotation determines the nature of bacterial movement. Monotrichous, polar flagella rotate counterclockwise (when viewed from outside the cell) during normal forward movement, whereas the cell itself rotates slowly clockwise.

The rotating helical flagellar filament thrusts the cell forward in a run with the flagellum trailing behind. Monotrichous bacteria stop and tumble randomly by reversing the direction

of flagellar rotation. Peritrichously flagellated bacteria operate in a somewhat similar way. To move forward, the flagella rotate counterclockwise. As they do so, they bend at their hooks to form a rotating bundle that propels them forward. Clockwise rotation of the flagella disrupts the bundle and the cell tumbles.

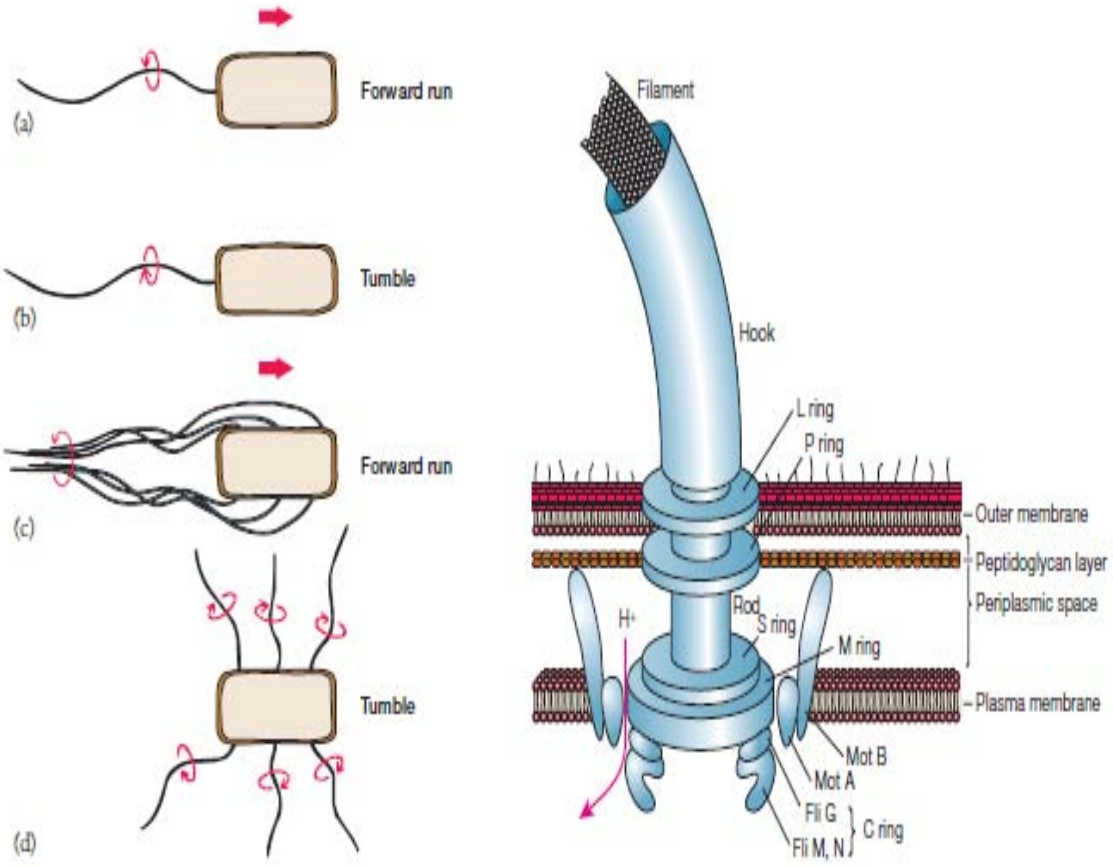


Figure. A) Flagellar Motility B)Mechanism of flagellar motility

Because bacteria swim through rotation of their rigid flagella, there must be some sort of motor at the base. A rod or shaft extends from the hook and ends in the M ring, which can rotate freely in the plasma membrane. It is believed that the S ring is attached to the cell wall in gram-positive cells and does not rotate. The P and L rings of gram-negative bacteria would act as bearings for the rotating rod. There is some evidence that the basal body is a passive structure and rotates within a membrane-embedded protein complex much like the rotor of an electrical motor turns in the center of a ring of electromagnets (the stator). The exact mechanism that drives basal body rotation still is not clear. The rotor portion of the motor seems to be made primarily of a rod, the M ring, and a C ring joined to it on the cytoplasmic side of the basal body. These two rings are

made of several proteins; Fli G is particularly important in generating flagellar rotation. The two most important proteins in the stator part of the motor are Mot A and Mot B. These form a proton channel through the plasma membrane, and Mot B also anchors the Mot complex to cell wall peptidoglycan. There is some evidence that Mot A and Fli G directly interact during flagellar rotation. This rotation is driven by proton or sodium gradients in procaryotes, not directly by ATP as is the case with eucaryotic flagella. The flagellum is a very effective swimming device. From the bacterium's point of view, swimming is quite a task because the surrounding water seems as thick and viscous as molasses. The cell must bore through the water with its helical or corkscrewshaped flagella, and if flagellar activity ceases, it stops almost instantly.

Bacteria can move by mechanisms other than flagellar rotation. Spirochetes are helical bacteria that travel through viscous substances such as mucus or mud by flexing and spinning movements caused by a special **axial filament** composed of periplasperiplasmic flagella. A very different type of motility, **gliding motility,** is employed by many bacteria: cyanobacteria, myxobacteria and cytophagas, and some mycoplasmas. Although there are no visible external structures associated with gliding motility, these bacteria can coast along solid surfaces at rates up to 3 _m/second.

Chemotaxis

Bacteria do not always swim aimlessly but are attracted by such nutrients as sugars and amino acids, and are repelled by many harmful substances and bacterial waste products. (Bacteria also can respond to other environmental cues such as temperature light, and gravity) Movement toward chemical attractants and away from repellents is known as **chemotaxis.** Such behavior is of obvious advantage to bacteria. Chemotaxis may be demonstrated by observing bacteria in the chemical gradient produced when a thin capillary tube is filled with an attractant and lowered into a bacterial suspension. As the attractant diffuses from the end of the capillary, bacteria collect and swim up the tube. The number of bacteria within the capillary after a short length of time reflects the strength of attraction and rate of chemotaxis.

A bacterium travels in a straight or slightly curved line, a **run,** for a few seconds; then it will stop and **tumble** or **twiddle** about. The tumble is followed by a run in a different direction. When the bacterium is exposed to an attractant gradient, it tumbles less frequently (or has longer runs)

when travelling up the gradient, but tumbles at normal frequency if moving down the gradient. Consequently the bacterium moves up the gradient.

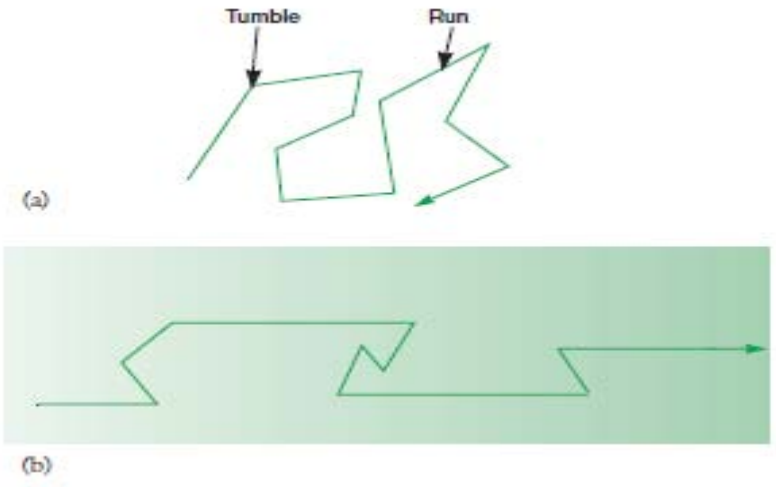


Figure. Directed movement in bacteria. A) Random movement B) Movement in an attachement. (Adopted from Prescott's book)

Behavior is shaped by temporal changes in chemical concentration: the bacterium compares its current environment with that experienced a few moments previously; if the attractant concentration is higher, tumbling is suppressed and the run is longer. The opposite response occurs with a repellent gradient. Tumbling frequency decreases (the run lengthens) when the bacterium moves down the gradient away from the repellent. Although bacterial chemotaxis appears to be deliberate, directed movement, it is important to keep in mind that this is not the case. When the environment is constant, bacteria tend to move in a random walk. That is, there is a random sequence of runs followed by tumbles. If a run is in the direction of improving conditions, tumbles are suppressed so that the cell tends to move in the preferred direction. This is said to be a biased random walk toward attractants and away from repellants. Individual cells do not choose a particular direction. Instead, they determine whether or not to continue in the same direction.

Endospore

An **endospore** is a dormant, tough, and non-reproductive structure produced by certain bacteria from the Firmicute phylum. The name "endospore" is suggestive of a spore or seed-like form (*endo* means within), but it is not a true spore (i.e., not an offspring). It is a stripped-down, dormant form to which the bacterium can reduce itself. Endospore formation is usually triggered by a lack of nutrients, and usually occurs in gram-positive bacteria. In endospore formation, the bacterium divides within its cell wall. One side then engulfs the other. Endospores enable bacteria to lie dormant for extended periods, even centuries. There are many reports of spores remaining viable over 10,000 years, and revival of spores millions of years old has been claimed. There is one report of viable spores of *Bacillus marismortui* in salt crystals approximately 250 million years old. When the environment becomes more favorable, the endospore can reactivate itself to the vegetative state. Most types of bacteria cannot change to the endospore form. Examples of bacteria that can form endospores include *Bacillus* and *Clostridium*.

The endospore consists of the bacterium's DNA, ribosomes and large amounts of dipicolinic acid. Dipicolinic acid is a spore-specific chemical that appears to help in the ability for endospores to maintain dormancy. This chemical accounts for up to 10% of the spore's dry weight. Endospores can survive without nutrients. They are resistant to ultraviolet radiation, desiccation, high temperature, extreme freezing and chemical disinfectants. Thermo-resistant endospores were first hypothesized by Ferdinand Cohn after studying *Bacillus subtilis* (pictured to the right) growth on cheese after boiling the cheese. Its spores being the reproductive mechanism for the growth was a large blow to the previous suggestions of spontaneous generation. Endospores are commonly found in soil and water, where they may survive for long periods of time. A variety of different microorganisms form "spores" or "cysts," but the endospores of low G+C gram-positive bacteria are by far the most resistant to harsh conditions.
Some classes of bacteria can turn into exospores, also known as microbial cysts, instead of endospores. Exospores and endospores are two kinds of "hibernating" or dormant stages seen in some classes of microorganisms.

Structure

The spore is sometimes surrounded by a thin covering known as the exosporium, which overlies the *spore coat*. The spore coat, which acts like a sieve that excludes large toxic molecules like lysozyme, is resistant to many toxic molecules and may also contain enzymes that are involved

in germination. It is composed of keratin and other core specific proteins, which makes the endospore extremely hardy. The *cortex* lies beneath the spore coat and consists of peptidoglycan. The *core wall* lies beneath the cortex and surrounds the protoplast or *core* of the endospore. The core contains the spore chromosomal DNA which is encased in chromatin-like proteins known as SASPs (small acid-soluble spore proteins), that protect the spore DNA from UV radiation and heat. The core also contains normal cell structures, such as ribosomes and other enzymes, but is not metabolically active.

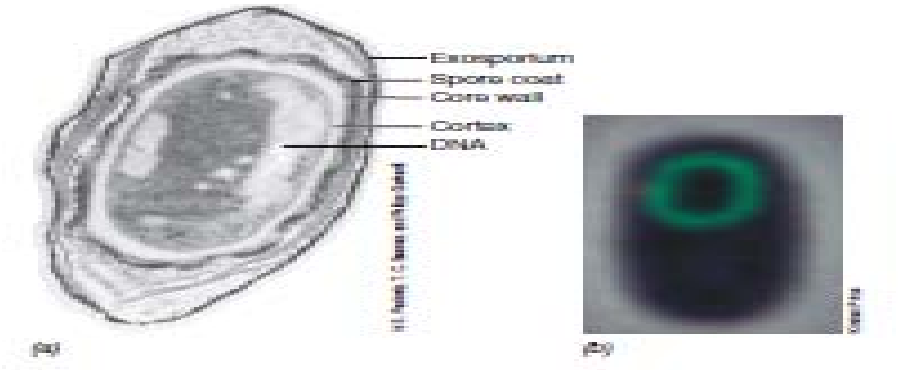


Figure: Structure of endospore

Up to 20% of the dry weight of the endospore consists of calcium dipicolinate within the core, which is thought to stabilize the DNA. Dipicolinic acid could be responsible for the heat resistance of the spore, and calcium may aid in resistance to heat and oxidizing agents. However, mutants resistant to heat but lacking dipicolinic acid have been isolated, suggesting other mechanisms contributing to heat resistance are also at work. Small acid-soluble proteins (SASPs) are found in endospores. These proteins tightly bind and condense the DNA, and are in part responsible for resistance to UV light and DNA-damaging chemicals.

Visualising endospores under light microscopy can be difficult due to the impermeability of the endospore wall to dyes and stains. While the rest of a bacterial cell may stain, the endospore is left colourless. To combat this, a special stain technique called a Moeller stain is used. That allows the endospore to show up as red, while the rest of the cell stains blue. Another staining technique for endospores is the Schaeffer-Fulton stain, which stains endospores green and bacterial bodies red. The arrangement of spore layers is as follows: Exosporium, Spore coat, Spore cortex, Core wall

Location

The position of the endospore differs among bacterial species and is useful in identification. The main types within the cell are terminal, sub terminal, and centrally placed endospores. Terminal

endospores are seen at the poles of cells, whereas central endospores are more or less in the middle. Subterminal endospores are those between these two extremes, usually seen far enough towards the poles but close enough to the center so as not to be considered either terminal or central. Lateral endospores are seen occasionally. Examples of bacteria having terminal endospores include *Clostridium tetani*, the pathogen that causes the disease tetanus. Bacteria having a centrally placed endospore include *Bacillus cereus*.

Endospore formation and cycle

Under conditions of starvation, especially the lack of carbon and nitrogen sources, a single endospore forms within some of the bacteria. The process is called sporulation. When a bacterium detects environmental conditions are becoming unfavourable it may start the process of endosporulation, which takes about eight hours. The DNA is replicated and a membrane wall known as a *spore septum* begins to form between it and the rest of the cell. The plasma membrane of the cell surrounds this wall and pinches off to leave a double membrane around the DNA, and the developing structure is now known as a forespore. Calcium dipicolinate, the calcium salt of dipicolinic acid, is incorporated into the forespore during this time. The dipicolinic acid helps stabilize the proteins and DNA in the endospore.

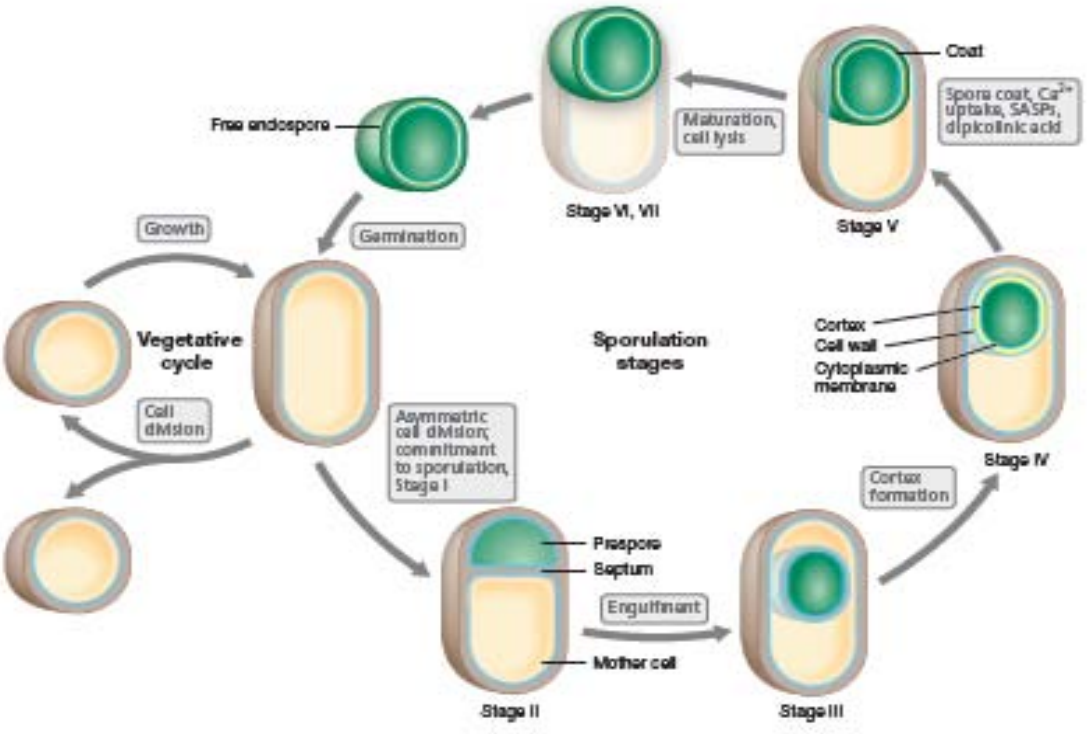


Figure 6. Different stages of endospore sporulation. (Adopted from Brocks biology)

Next the peptidoglycan cortex forms between the two layers and the bacterium adds a spore coat to the outside of the forespore. In the final stages of endospore formation the newly forming endospore is dehydrated and allowed to mature before being released from the mother cell. The cortex is what makes the endospore so resistant to temperature. The cortex contains an inner membrane known as the core. The inner membrane that surrounds this core leads to the endospore's resistance against UV light and harsh chemicals that would normally destroy microbes.

Sporulation is now complete, and the mature endospore will be released when the surrounding vegetative cell is degraded. Endospores are resistant to most agents that would normally kill the vegetative cells they formed from. Unlike persister cells, endospores are the result of a morphological differentiation process triggered by nutrient limitation (starvation) in the environment; endosporulation is initiated by quorum sensing within the "starving population. Most disinfectants such as household cleaning products, alcohols, quaternary ammonium compounds and detergents have little effect on endospores.

Endospores are able to survive at 100 °C for hours, although the longer the number of hours the fewer that will survive. An indirect way to destroy them is to place them in an environment that reactivates them to their vegetative state. They will germinate within a day or two with the right environmental conditions, and then the vegetative cells, not as hardy as endospores, can be straightforwardly destroyed. This indirect method is called Tyndallization. It was the usual method for a while in the late 19th century before the introduction of inexpensive autoclaves. Prolonged exposure to ionising radiation, such as x-rays and gamma rays, will also kill most endospores.

Bacterial endospores are resistant to antibiotics, most disinfectants, and physical agents such as radiation, boiling, and drying. The impermeability of the spore coat is thought to be responsible for the endospore's resistance to chemicals. The heat resistance of endospores is due to a variety of factors:

- Calcium dipicolinate, abundant within the endospore, may stabilize and protect the endospore's DNA.
- Small acid-soluble proteins (SASPs) saturate the endospore's DNA and protect it from heat, drying, chemicals, and radiation. They also function as a carbon and energy source for the development of a vegetative bacterium during germination.

- The cortex may osmotically remove water from the interior of the endospore and the dehydration that results is thought to be very important in the endospore's resistance to heat and radiation.
- Finally, DNA repair enzymes contained within the endospore are able to repair damaged DNA during germination.

Importance

As a simplified model for cellular differentiation, the molecular details of endospore formation have been extensively studied, specifically in the model organism *Bacillus subtilis*. These studies have contributed much to our understanding of the regulation of gene expression, transcription factors, and the sigma factor subunits of RNA polymerase. Endospores of the bacterium *Bacillus anthracis* were used in the 2001 anthrax attacks. The powder found in contaminated postal letters was composed of extracellular anthrax endospores. This intentional distribution lead to 22 known cases of anthrax (11 inhalation and 11 cutaneous) making the case fatality rate among patients with inhalation anthrax 45% (5/11). The six other individuals with inhalation anthrax and all the individuals with cutaneous anthrax recovered. Had it not been for antibiotic therapy many more might have been stricken.

According to WHO veterinary documents, *B. anthracis* sporulates when it sees oxygen instead of the carbon dioxide present in mammal blood; this signals to the bacteria that it has reached the end of the animal, and an inactive dispersable morphology is useful. Sporulation requires the presence of free oxygen. in the natural situation, this means the vegetative cycles occur within the low oxygen environment of the infected host and, within the host, the organism is exclusively in the vegetative form. once outside the host, sporulation commences upon exposure to the air and the spore forms are essentially the exclusive phase in the environment.

6. Bacterial growth: kinetics, growth curve, factors affecting growth

Bacterial growth is the asexual reproduction, or cell division, of a bacterium into two daughter cells, in a process called binary fission. Providing no mutational event occurs, the resulting daughter cells are genetically identical to the original cell. Hence, bacterial growth occurs. Both daughter cells from the division do not necessarily survive. However, if the number surviving exceeds unity on average, the bacterial population undergoes exponential growth. The measurement of an exponential bacterial growth curve in batch culture was tra dgc

poditikmmuuuugd xjonally a part of the training of all microbiologists; the basic means requires bacterial enumeration (cell counting) by direct and individual (microscopic, flow cytometry), direct and bulk (biomass), indirect and individual (colony counting), or indirect and bulk (most probable number, turbidity, nutrient uptake) methods. Models reconcile theory with the measurement.

Different phases

Bacterial growth curve:

In autecological studies, the growth of bacteria (or other microorganisms, as protozoa, microalgae or yeasts) in batch culture can be modeled with four different phases: (A) **lag phase**, (B) **log phase** or **exponential phase** (C) **stationary phase** and (D) **death phase.**

During **lag phase**, bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide. During the lag phase of the bacterial growth cycle, synthesis of RNA, enzymes and other molecules occurs. During the lag phase cells change very little because the cells do not immediately reproduce in a new medium. This period of little to no cell division is called the lag phase and can last for 1 hour to several days. During this phase cells are not dormant. The **log phase** (sometimes called the logarithmic phase or the exponential phase) is a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of **exponential growth**, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time. The actual rate of this growth (i.e. the slope of the line in the figure) depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The **stationary phase** is often due to a growth-limiting factor such as the depletion of an essential nutrient, and/or the formation of an inhibitory product such as an organic acid. Stationary phase results from a situation in which growth rate and death rate are equal. The number of new cells created is limited by the growth factor and as a result the rate of cell growth

matches the rate of cell death. The result is a “smooth,” horizontal linear part of the curve during the stationary phase. Mutations can occur during stationary phase.

At **death phase** (decline phase), bacteria die. This could be caused by lack of nutrients, environmental temperature above or below the tolerance band for the species, or other injurious conditions.

This basic batch culture growth model draws out and emphasizes aspects of bacterial growth In reality, even in batch culture, the four phases are not well defined. The cells do not reproduce in synchrony without explicit and continual prompting (as in experiments with stalked bacteria) and their exponential phase growth is often not ever a constant rate, but instead a slowly decaying rate, a constant stochastic response to pressures both to reproduce and to go dormant in the face of declining nutrient concentrations and increasing waste concentrations. Near the end of the logarithmic phase of a batch culture, competence for natural genetic transformation may be induced, as in *Bacillus subtilis* and in other bacteria. Natural genetic transformation is a form of DNA transfer that appears to be an adaptation for repairing DNA damages. Batch culture is the most common laboratory growth method in which bacterial growth is studied, but it is only one of many. It is ideally spatially unstructured and temporally structured. The bacterial culture is incubated in a closed vessel with a single batch of medium. In some experimental regimes, some of the bacterial culture is periodically removed and added to fresh sterile medium. In the extreme case, this leads to the continual renewal of the nutrients. This is a chemostat, also known as continuous culture. It is ideally spatially unstructured and temporally unstructured, in a steady state defined by the rates of nutrient supply and bacterial growth. In comparison to batch culture, bacteria are maintained in exponential growth phase, and the growth rate of the bacteria is known. Related devices include turbidostats and auxostats. When *Escherichia coli* is growing very slowly with a doubling time of 16 hours in a chemostat most cells have a single chromosome. Bacterial growth can be suppressed with bacteriostats, without necessarily killing the bacteria. In a synecological, true-to-nature situation in which more than one bacterial species is present, the growth of microbes is more dynamic and continual. Liquid is not the only laboratory environment for bacterial growth. Spatially structured environments such as biofilms or agar surfaces present additional complex growth models.

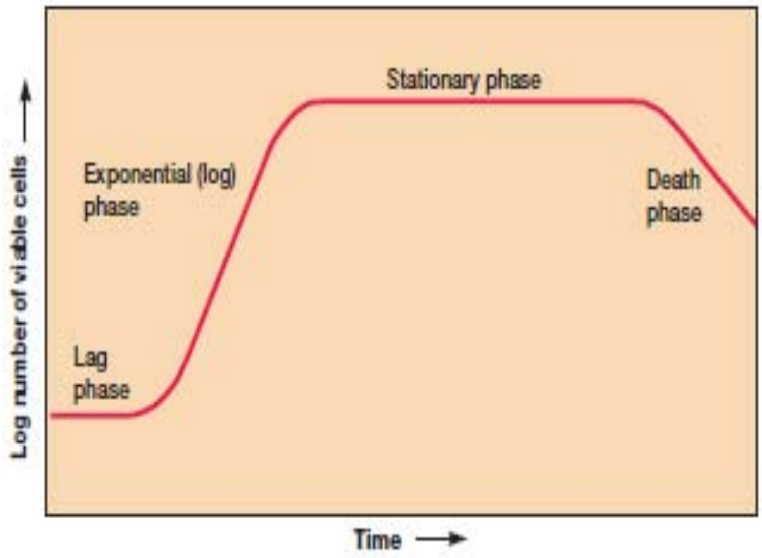


Figure: Microbial growth curve in closed system

The Mathematics of Growth

Knowledge of microbial growth rates during the exponential phase is indispensable to microbiologists. Growth rate studies contribute to basic physiological and ecological research and the solution of applied problems in industry. Therefore the quantitative aspects of exponential phase growth will be discussed.

During the exponential phase each microorganism is dividing at constant intervals. Thus the population will double in number during a specific length of time called the **generation time** or **doubling time.** This situation can be illustrated with a simple example. Suppose that a culture tube is inoculated with one cell that divides every 20 minutes. The population will be 2 cells after 20 minutes, 4 cells after 40 minutes, and so forth. Because the population is doubling every generation, the increase in population is always 2^n where n is the number of generations. The resulting population increase is exponential or logarithmic.

These observations can be expressed as equations for the generation time.

Let N_0 = the initial population number

N_t = the population at time t

n = the number of generations in time t

Then inspection of the results in table 6.1 will show that

$$N_t = N_0 \times 2^n.$$

Solving for n, the number of generations, where all logarithms are to the base 10,

$$\log N_t = \log N_0 + n \cdot \log 2, \text{ and}$$

$$n = \frac{\log N_t - \log N_0}{\log 2} = \frac{\log N_t - \log N_0}{0.301}$$

The rate of growth during the exponential phase in a batch culture can be expressed in terms of the **mean growth rate constant** ***(k)***. This is the number of generations per unit time, often expressed as the generations per hour.

$$k = \frac{n}{t} = \frac{\log N_t - \log N_0}{0.301t}$$

The time it takes a population to double in size—that is, the **mean generation time** or mean doubling time (g), can now be calculated. If the population doubles ($t = g$), then

$$N_t = 2\,N_0.$$

Substitute $2N_0$ into the mean growth rate equation and solve for k.

$$k = \frac{\log (2N_0) - \log N_0}{0.301g} = \frac{\log 2 + \log N_0 - \log N_0}{0.301g}$$

$$k = \frac{1}{g}$$

The mean generation time is the reciprocal of the mean growth rate constant.

$$g = \frac{1}{k}$$

The mean generation time (g) can be determined directly from a semilogarithmic plot of the growth data (**figure 6.4**) and the growth rate constant calculated from the g value. The generation time also may be calculated directly from the previous equations. For example, suppose that a bacterial population increases from 10^3 cells to 10^9 cells in 10 hours.

$$k = \frac{\log 10^9 - \log 10^3}{(0.301)(10\text{ hr})} = \frac{9-3}{3.01\text{ hr}} = 2.0 \text{ generations/hr}$$

$$g = \frac{1}{2.0\text{ gen./hr}} = 0.5 \text{ hr/gen. or } 30 \text{ min/gen.}$$

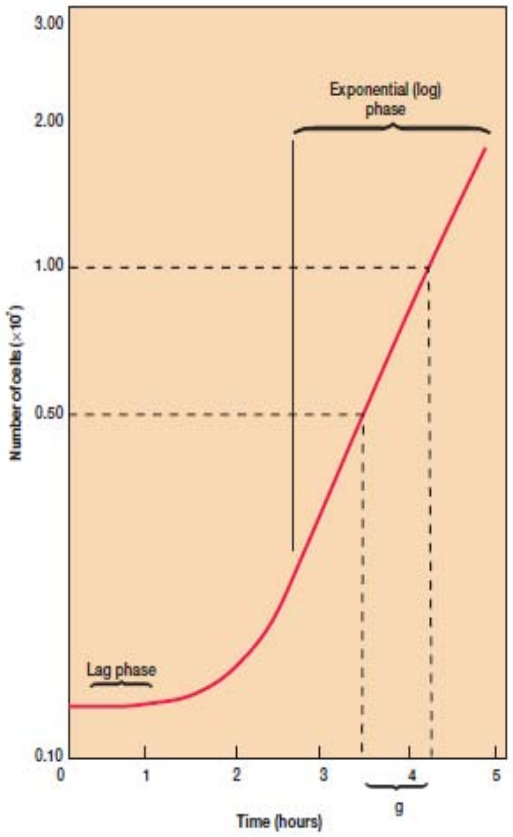


Figure 6.4 Generation Time Determination. The generation time can be determined from a microbial growth curve. The population data are plotted with the logarithmic axis used for the number of cells. The time to double the population number is then read directly from the plot. The log of the population number can also be plotted against time on regular axes.

Generation times vary markedly with the species of microorganism and environmental conditions. They range from less than 10 minutes (0.17 hours) for a few bacteria to several days with some eucaryotic microorganisms. Generation times in nature are usually much longer than in culture.

Factors affecting growth

Environmental factors influence rate of bacterial growth such as acidity (pH), temperature, water activity, macro and micro nutrients, oxygen levels, and toxins. Conditions tend to be relatively consistent between bacteria with the exception of extremophiles. Bacterium have optimal growth conditions under which they thrive, but once outside of those conditions the stress can result in either reduced or stalled growth, dormancy (formation spores), or death.

Temperature

Low temperatures tend to reduce growth rates which have led to refrigeration being instrumental in food preservation. Depending on temperature, bacteria can be classified as:

Psychrophiles

Psychrophiles are extremophilic cold-loving bacteria or archaea with an optimal temperature for growth at about 15°C or lower (maximal temperature for growth at 20°C, minimal temperature for growth at 0°C or lower). Psychrophiles are typically found in Earth's extremely cold ecosystems, such as polar ice-cap regions, permafrost, polar surface, and deep oceans.

Mesophiles

Mesophiles are bacteria that thrive at moderate temperatures, growing best between 20° and 45 °C. These temperatures align with the natural body temperatures of humans, which is why many human pathogens are mesophiles.

Thermophiles

Survive under temperatures of 45° - 60°C

Acidity

Optimal acidity for bacteria tends to be around pH 6.5 to 7.0 with the exception of acidophiles. Some bacteria can change the pH such as by excreting acid resulting in sub-optimal conditions.

Oxygen

Bacteria can be aerobes or anaerobes.

Micronutrients

Ample nutrients

Toxins

Toxins such as ethanol can hinder or kill bacterial growth. This is used beneficially for disinfection and in food preservation.

7. Photolithotrophs, chemolithotrophs, photoorganotrophs and chemoorganotrophs, mixotroph.

Autotrophs

Autotrophs are bacteria which obtain their nutrition from inorganic compounds. Carbon dioxide is typically the sole source of cellular carbon. Autotrophs will use hydrogen sulfide, ammonia or hydrogen gas to reduce carbon into necessary sugars. Nitrifying bacteria, which oxidize ammonia to create nitrites and nitrates, are an example of bacteria which use autotrophic nutrition.

Heterotrophs

Bacteria that require organic sources of carbon such as sugars, fats and amino acids are termed heterotrophs. Saprophytic bacteria are an example. They attain their nutrition from dead organic matter. Using enzymes, these bacteria will breakd own complex compounds and use the nutrients to release energy. Saprophytic bacteria are essentially decomposers and play an important role in ecosystem by releasing simpler products which plants and animals can use.

Phototrophs

Phototrophic bacteria absorb light energy, then utilize this in photosynthesis to create cellular energy. There are two types of phototrophs; those which do not produce oxygen as a byproduct are termed anaerobic phototrophs, while those which do produce oxygen are termed aerobic phototrophs. Both autotrophs and heterotrophs can be phototrophs. Cyanobacteria are an example of bacteria which execute photoautotrophic nutrition.

Chemotrophs

These bacteria obtain chemical energy from their surroundings and convert it into adenosine triphosphate (ATP) for cellular use. Chemotrophs attain energy from oxidation-reduction reactions of inorganic compounds such ammonia, hydrogen sulfide and iron. For instance, sulfur bacteria is a chemoautotroph which produces energy by oxidizing hydrogen sulfide into sulfur and water.

Lithotrophs

Lithotrophs are bacteria which use reduced inorganic compounds as the electron donor (H-donor) in anaerobic or aerobic respiration.

Table: Major nutritional types of microorganism

Major Nutritional Types[a]	Sources of Energy, Hydrogen/Electrons, and Carbon	Representative Microorganisms
Photolithotrophic autotrophy (Photolithoautotrophy)	Light energy Inorganic hydrogen/electron (H/e^-) donor CO_2 carbon source	Algae Purple and green sulfur bacteria Cyanobacteria
Photoorganotrophic heterotrophy (Photoorganoheterotrophy)	Light energy Organic H/e^- donor Organic carbon source (CO_2 may also be used)	Purple nonsulfur bacteria Green nonsulfur bacteria
Chemolithotrophic autotrophy (Chemolithoautotrophy)	Chemical energy source (inorganic) Inorganic H/e^- donor CO_2 carbon source	Sulfur-oxidizing bacteria Hydrogen bacteria Nitrifying bacteria Iron-oxidizing bacteria
Chemoorganotrophic heterotrophy (Chemoorganoheterotrophy)	Chemical energy source (organic) Organic H/e^- donor Organic carbon source	Protozoa Fungi Most nonphotosynthetic bacteria (including most pathogens)

8. Organization and replication of genetic material of bacteria, baterial chromosome, plasmid, recombination in bacteria (transformation, conjugation and transduction)

Bacterial chromosome and replication

A circular bacterial chromosome is a bacterial chromosome in the form of a molecule of circular DNA. Unlike the linear DNA of most eukaryotes, typical bacterial chromosomes are circular. Most bacterial chromosomes contain a circular DNA molecule – there are no free ends to the DNA. Free ends would otherwise create significant challenges to cells with respect to DNA replication and stability. Cells that do contain chromosomes with DNA ends, or telomeres (most eukaryotes), have acquired elaborate mechanisms to overcome these challenges. However, a circular chromosome can provide other challenges for cells. After replication, the two progeny circular chromosomes can sometimes remain interlinked or tangled, and they must be resolved so that each cell inherits one complete copy of the chromosome during cell division.

Replication of a circular bacterial chromosome

Bacterial chromosome replication is best understood in the well-studied bacteria *Escherichia coli* and *Bacillus subtilis*. *Chromosome* replication proceeds in three major stages: initiation, elongation and termination. The initiation stage starts with the ordered assembly of "initiator" proteins at the origin region of the chromosome, called oriC. These assembly stages are regulated to ensure that chromosome replication occurs only once in each cell cycle. During the elongation phase of replication, the enzymes that were assembled at oriC during initiation proceed along each arm ("replichore") of the chromosome, in opposite directions away from the oriC, replicating the DNA to create two identical copies. This process is known as bidirectional replication. The entire assembly of molecules involved in DNA replication on each arm is called a "replisome." At the forefront of the replisome is a DNA helicase that unwinds the two strands of DNA, creating a moving "replication fork". The two unwound single strands of DNA serve as templates for DNA polymerase, which moves with the helicase (together with other proteins) to synthesise a complementary copy of each strand. In this way, two identical copies of the original DNA are created. Eventually, the two replication forks moving around the circular chromosome meet in a specific zone of the chromosome, approximately opposite oriC, called the terminus region. The elongation enzymes then disassemble, and the two "daughter" chromosomes are resolved before cell division is completed.

Initiation

The *E. coli* bacterial replication origin, called *oriC* consists of DNA sequences that are recognised by the DnaA protein, which is highly conserved amongst different bacterial species. DnaA binding to the origin initiates the regulated recruitment of other enzymes and proteins that will eventually lead to the establishment of two complete replisomes for bidirectional replication. DNA sequence elements within *ori*C that are important for its function include DnaA boxes, a 9-mer repeat with a highly conserved consensus sequence 5'–TTATCCACA–3', that are recognized by the DnaA protein. DnaA protein plays a crucial role in the initiation of chromosomal DNA replication. Bound to ATP, and with the assistance of bacterial histone-like proteins [HU] DnaA then unwinds an AT-rich region near the left boundary of *oriC*, which carries three 13-mer motifs, and opens up the double-stranded DNA for entrance of other replication proteins.

This region also contains four "GATC" sequences that are recognized by DNA adenine methylase (Dam), an enzyme that modifies the adenine base when this sequence is unmethylated or hemimethylated. The methylation of adenines is important as it alters the conformation of DNA to promote strand separation, and it appears that this region of *ori*C has a natural tendency to unwind. DnaA then recruits the replicative helicase, DnaB, from the DnaB-DnaC complex to the unwound region to form the pre-priming complex.

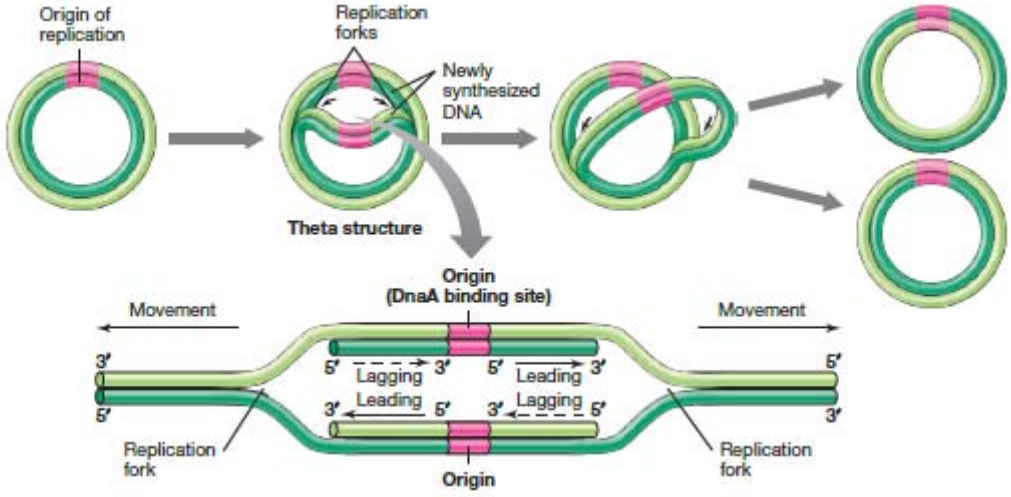


Figure: Replication process of prokaryotic system

After DnaB translocates to the apex of each replication fork, the helicase both unwinds the parental DNA and interacts momentarily with primase. In order for DNA replication to continue, single stranded binding proteins are needed to prevent the single strands of DNA from forming secondary structures and to prevent them from re-annealing. In addition, DNA gyrase is needed to relieve the topological stress created by the action of DnaB helicase.

Elongation

When the replication fork moves around the circle, a structure shaped like the Greek letter theta (Ɵ) is formed. John Cairnsdemonstrated the theta structure of E. coli chromosomal replication in 1963, using an innovative method to visualize DNA replication. In his experiment, he radioactively labeled the chromosome by growing his cultures in a medium containing 3H-thymidine. The nucleoside base was incorporated uniformly into the bacterial chromosome. He then isolated the chromosomes by lysing the cells gently and placed them on an electron micrograph (EM) grid which he exposed to X-ray film for two months. This Experiment clearly demonstrates the theta replication model of circular bacterial chromosomes. Bacterial chromosomal replication occurs in a bidirectional manner. This was first demonstrated by specifically labelling replicating bacterial chromosomes with radioactive isotopes. The regions of DNA undergoing replication during the experiment were then visualized by using autoradiography and examining the developed film microscopically. This allowed the researchers to see where replication was taking place. The first conclusive observations of bidirectional replication were from studies of *B. subtilis*. Shortly after, the *E. coli* chromosome was also shown to replicate bidirectionally.

Deoxynucleotides are then added to this primer by a single DNA polymerase III dimer, in an integrated complex with DnaB helicase. Leading strand synthesis then proceeds continuously, while the DNA is concurrently unwound at the replication fork. In contrast, lagging strand synthesis is accomplished in short Okazaki fragments. First, an RNA primer is synthesized by primase, and, like that in leading strand synthesis, DNA Pol III binds to the RNA primer and adds deoxyribonucleotides. When the synthesis of an Okazaki fragment has been completed, replication halts and the core subunits of DNA Pol III dissociates from the β sliding clamp [B sliding clap is the processivity subunit of DNA Pol III]. The RNA primer is removed and replaced with DNA by DNA polymerase I [which also possesses proofreading exonuclease activity] and the remaining nick is sealed by DNA ligase, which then ligates these fragments to form the lagging strand. A substantial proportion (10-15%) of the replication forks originating at

oriC encounter a DNA damage or strand break when cells are grown under normal laboratory conditions (without an exogenous DNA damaging treatment). The encountered DNA damages are ordinarily processed by recombinational repair enzymes to allow continued replication fork progression.

Termination

Most circular bacterial chromosomes are replicated bidirectionally, starting at one point of origin and replicating in two directions away from the origin. This results in semiconservative replication, in which each new identical DNA molecule contains one template strand from the original molecule, shown as the solid lines, and one new strand, shown as the dotted lines. Termination is the process of fusion of replication forks and disassembly of the replisomes to yield two separate and complete DNA molecules. It occurs in the terminus region, approximately opposite oriC on the chromosome. The terminus region contains several DNA replication terminator sites, or "Ter" sites. A special "replication terminator" protein must be bound at the Ter site for it to pause replication. Each Ter site has polarity of action, that is, it will arrest a replication fork approaching the Ter site from one direction, but will allow unimpeded fork movement through the Ter site from the other direction. The arrangement of the Ter sites forms two opposed groups that forces the two forks to meet each other within the region they span. This arrangement is called the "replication fork trap.

Replication of the DNA separating the opposing replication forks, leaves the completed chromosomes joined as ‘catenanes’ or topologically interlinked circles. The circles are not covalently linked, but cannot be separated because they are interwound and each is covalently closed. The catenated circles require the action of topoisomerases to separate the circles [decatanation]. In *E.coli*, DNA topoisomerase IV plays the major role in the separation of the catenated chromosomes, transiently breaking both DNA strands of one chromosome and allowing the other chromosome to pass through the break. There has been some confusion about the role DNA gyrase plays in decatenation. To define the nomenclature, there are two types of topoisomerases: type I produces transient single-strand breaks in DNA and types II produces transient double-strand breaks. As a result, the type I enzyme removes supercoils from DNA one at a time, whereas the type II enzyme removes supercoils two at a time. The topo I of both prokaryotes and eukaryotes are the type I topoisomerase. The eukaryotic topo II, bacterial gyrase, and bacterial topo IV belong to the type II.

We often forget that DNA gyrase does in fact have topoisomerase type II activity; thus, with it being a homologue of topoisomerase IV (also having topoisomerase II activity) we expect similarity in the two proteins' functions. DNA gyrase preliminary role is to introduce negative super coils into DNA, thereby relaxing positive supercoils that come into play during DNA replication. Topoisomerase IV also relaxes positive supercoils; therefore, DNA Gyrase and topoisomerase IV play an almost identical role in removing the positive supercoils ahead of a translocating DNA polymerase, allowing DNA replication to continue unhindered by topological strain.

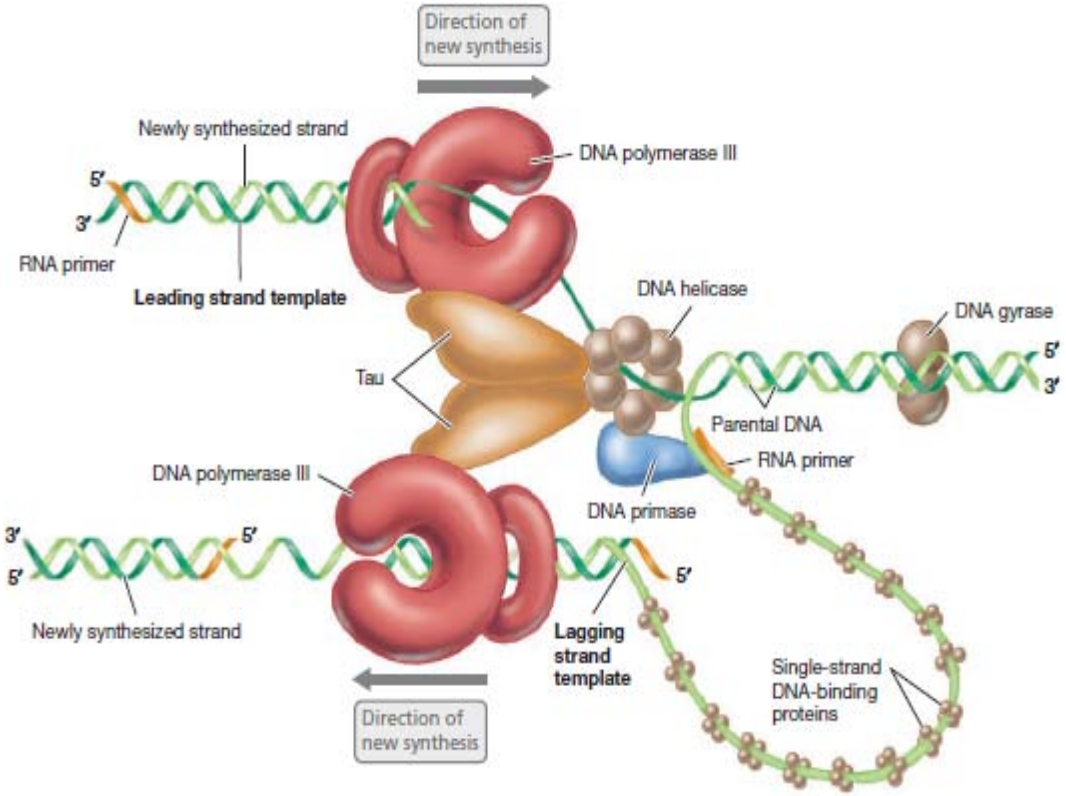


Figure: Replication process in *E.coli*

Confusion arises when some scientific literature state that DNA gyrase is the sole enzyme responsible for decatanation. In an experiment conducted by Zechiedrich, Khodursky and Cozzarelli in 1997, it was found that topoisomerase IV is the only important decatenase of DNA replication intermediates in bacteria. In this particular experiment, when DNA gyrase alone were inhibited, most of the catenanes were unlinked. However, when Topoisomerase IV alone was inhibited, decatenation was almost completely blocked. The results obtained suggest that

Topoisomerase IV is the primary decatenase *in vivo*, and although DNA gyrase does play a role in decatenation, its function is not as essential as topoisomerase IV in the decatentation of interlinked chromosomes.

Plasmids

A **plasmid** is a small DNA molecule within a cell that is physically separated from a chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms. In nature, plasmids often carry genes that may benefit the survival of the organism, for example antibiotic resistance. While the chromosomes are big and contain all the essential genetic information for living under normal conditions, plasmids usually are very small and contain only additional genes that may be useful to the organism under certain situations or particular conditions. Artificial plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms. In the laboratory, plasmids may be introduced into a cell via transformation.

Plasmids are considered *replicons*, units of DNA capable of replicating autonomously within a suitable host. However, plasmids, like viruses, are not generally classified as life. Plasmids are transmitted from one bacterium to another (even of another species) mostly through conjugation. This host-to-host transfer of genetic material is one mechanism of horizontal gene transfer, and plasmids are considered part of the mobilome. Unlike viruses (which encase their genetic material in a protective protein coat called a capsid), plasmids are "naked" DNA and do not encode genes necessary to encase the genetic material for transfer to a new host. However, some classes of plasmids encode the conjugative "sex" pilus necessary for their own transfer. The size of the plasmid varies from 1 to over 200 kbp, and the number of identical plasmids in a single cell can range anywhere from one to thousands under some circumstances.

The relationship between microbes and plasmid DNA is neither parasitic nor mutualistic, because each implies the presence of an independent species living in a detrimental or commensal state with the host organism. Rather, plasmids provide a mechanism for horizontal gene transfer within a population of microbes and typically provide a selective advantage under a given environmental state. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or the proteins produced may act as toxins under similar circumstances, or allow the organism to utilize particular organic compounds that would be advantageous when nutrients are scarce.

Classification and Types

Plasmids may be classified in a number of ways. Plasmids can be broadly classified into conjugative plasmids and non-conjugative plasmids. Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmid may be transferred from one bacterium to another via sex pili encoded by some of the *tra* genes. Non-conjugative plasmids are incapable of initiating conjugation; hence they can be transferred only with the assistance of conjugative plasmids. An intermediate class of plasmids are mobilizable, and carry only a subset of the genes required for transfer. They can parasitize a conjugative plasmid, transferring at high frequency only in its presence.

Plasmids can also be classified into incompatibility groups. A microbe can harbour different types of plasmids; however, different plasmids can only exist in a single bacterial cell if they are compatible. If two plasmids are not compatible, one or the other will be rapidly lost from the cell. Different plasmids may therefore be assigned to different incompatibility groups depending on whether they can coexist together. Incompatible plasmids (belonging to the same incompatibility group) normally share the same replication or partition mechanisms and can thus not be kept together in a single cell.

Another way to classify plasmids is by function. There are five main classes:

- Fertility F-plasmids, which contain *tra* genes. They are capable of conjugationand result in the expression of sex pili.
- Resistance (R) plasmids, which contain genes that provide resistance Against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- Col plasmids, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
- Degradative plasmids, which enable the digestion of unusual substances, e.g.toluene and salicylic acid.
- Virulence plasmids, which turn the bacterium into a pathogen.

Bacterial Genetics

Transformation in bacteria

Notani and Setlow (1974) have described the mechanism of bacterial transformation. Moreover, in *S. pneumoniae* the competent state is transient and persists only for a short period. The competent state is induced by the competence activator protein of molecular weight of 1,000 Dalton. It binds to the plasma membrane of receptor and triggers the synthesis of 10 new proteins within 10 minutes. The competence factor (CF) accelerates the process of transport or leakage of autolysin molecules into the periplasmic space. Moreover, in *H. influenzae* no competence factors have been reported.

Only Changes in cell envelope accompany the development of competence state. The cell envelope of competent cells contains increased level of polysacccharide as compared to the cells of log phase. Structural changes in competent cells induce numerous vesicles called transformosome buds on the surface that contains protein and mediates the uptake of transforming DNA. Transformation is accomplished in the following steps.

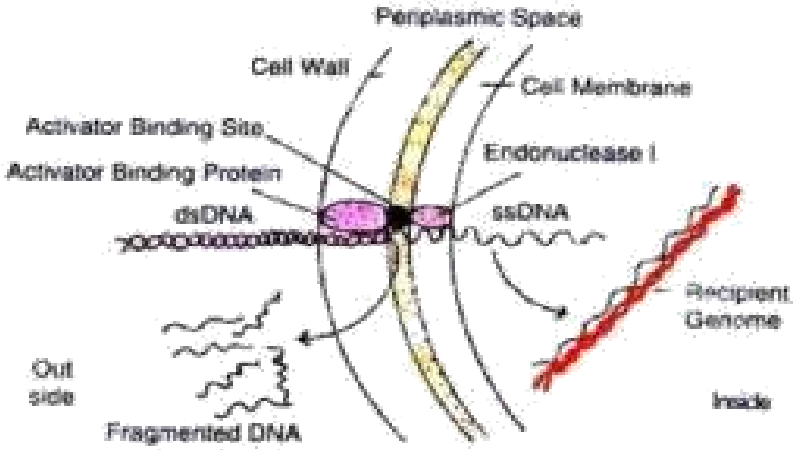


Figure : Diagrammatic presentation of transformation in streptococci.

(a) DNA binding:

As a result of random collision, DNA comes first in the contact of cell surface of competent bacteria (Figs. 8.4 and 8.5 A-B). First the DNA binding is reversible and lasts for about 4-5 seconds. Thereafter, it becomes irreversible permanently. For about 2 minutes it remains in non-transforming state. Thereafter, before 5 minutes it is converted into the transforming state.

The period (about 10 minutes) during which no transformation occurs in competent recipient cells is called eclipse. Both types of DNA, transforming and non-transforming, bind to the cell

surface where the receptor sites are located. In *B. subtilis* membrane vesicles in competent cells are found that bind to 20 mg of dsDNA/mg of membrane protein. The competent cells show six fold more DNA binding sites than the non-competent cells.

In *H influenzae* transformosome bud forms the surface and contains proteins that mediate DNA uptake. It binds with conserved sequence (5'AAGTGCGGTCA 3′) present at 4 kb interval on DNA. The DNA uptake site contains two proteins of 28 and 52 kilo-Daltons. After binding, the receptor proteins present the donor DNA to the membrane associated uptake sites.

In *S. pneumoniae* the CF induces the ability to bind DNA molecules.

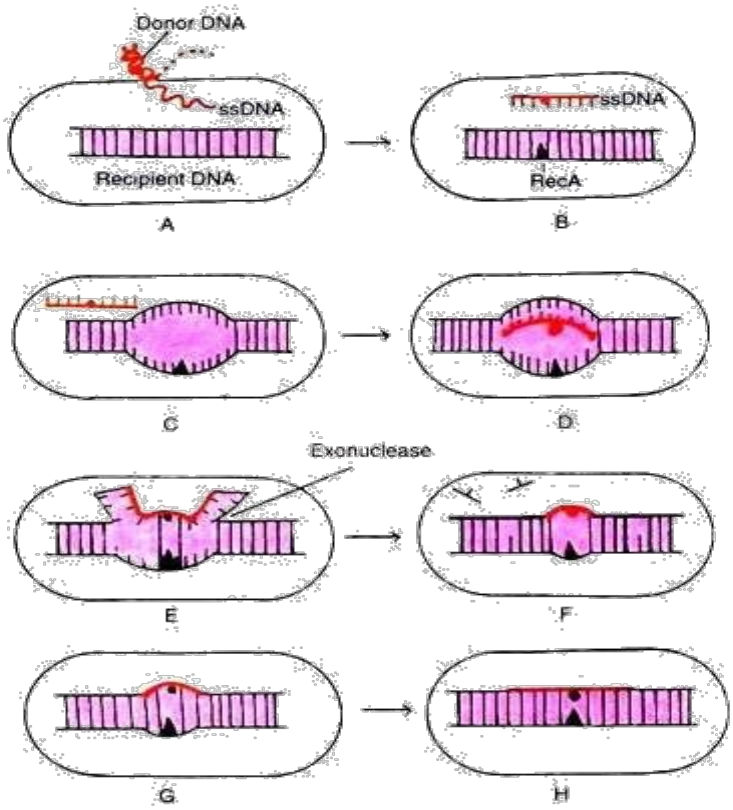


Fig. 8.5 : Mechanism of transformation. A, binding and penetration of donor DNA in competent recipient cell; B, binding of SSB to donor ssDNA and RecA to recipient DNA; C-D, synapsis and assimilation; E, nicking of recipient stand; F, branch migration, trimming and integration; G, sealing of nicks by DNA ligase; H, mismatch repair.

(b) Penetration:

The DNA molecules that bind permanently enter the competent recipient cells. DNA is also resistant to DNase degradation. The nucleolytic enzymes located at the surface of competent recipient cells act upon the donor DNA molecule when it binds the cell membrane. The endonuclease-1 of the recipient cells which is associated with cell membrane acts as DNA

translocase by attacking and degrading one strand of the dsDNA. Consequently only complementary single strand of DNA enters into the recipient cells.

It has been confirmed by performing the experiments with radiolabelling of donor DNA. The mutant cells of *S. pneumoniae* lack endonuclease-1, therefore, transformation does not occur. Interestingly in *B. subtilis* degradation of one strand is being delayed. Hence, both the strands enter the recipient cell. The upper limit of peneterating DNA into the recipient cell is about 750 base pairs.

The size of donor DNA affects transformation. Successful transformation occurs with the donor DN.A of molecular weight between 30,00,000 and 8 million Dalton. With increasing the concentration of donor DNA the number of competent cells increases. DNA uptake process is the energy requiring mechanism because it can be inhibited by the energy requiring inhibitors.

After penetration the donor DNA migrates from periphery of cell to the bacterial DNA. This movement in different bacteria differs. For example, in B. subtilis this movement occurs for about 16-60 minutes. During this movement, DNA is associated with mesosomes which possibly transport it to the bacterial DNA.

(c) Synapsis formation:

The single stranded DNA is coated with SSB proteins, which maintain, the single stranded region in a replication fork (Fig. 8.5B). The single strand of the donor DNA or portion of it is linearly inserted into the recipient DNA (Fig. 8.5 C-D). The bacterial protein like E. coli RecA protein probably facilitates the DNA pairing during recombination. It causes the local unwinding of dsDNA of the recipient cell from the 5′ end.

How the displaced single strand is cut, still not known? Base pairing i.e. synapsis occurs between the homologous donor ssDNA and the recipient DNA. Unwinding of the recipient DNA continues at the end of assimilated DNA and allows the fraction of invading DNA to increase base pairs. This process is called branch migration (F).

(d) Integration:

The endonuclease cuts the unpaired free end of donor DNA or the recipient DNA. This process is called trimming (Fig. 8.5E-F). The nick is sealed by DNA ligase (G). Consequently, a heteroduplex region containing mismatched base pairs is formed (H). Furthermore, in the progenies whether the donor marker is or is not recovered, depends on the occurrence of mismatch repair.

If the mismatch repair occurs again, it depends whether the unpaired base in the donor or recipient strand is removed. After replication the heteroduplex forms the homo-duplexes, one of these is of normal type and the second is transformed duplex. The normal duplex is from the recipient cell in origin, whereas the transformed duplex is from the donor genome.

The efficiency of integration of genetic markers into the genome of recipient cell varies with different genes that the recipient cell possesses. This genetic trait is called hex (high efficiency of integration). The hex system eliminates a large fraction of low efficiency (LE) markers and permits high efficiency (HE) markers to be integrated. Therefore, the hex function is a mismatch-base correction system.

The donor genes differing from the recipient genes by a single base pair create a mismatch when integrated initially. The hex mismatch repair system (with LE markers) can correct either of donor strands. Therefore, there is fifty-fifty chance for a given marker to be retained. The HE markers correct only the recipient strand. For the LE markers, hex mismatch repair system unusually removes the mismatched bases of the donor DNA and the cell retains the recipient genotype, whereas for HE markers the same system removes the mismatched bases of recipient DNA and the cell consists of donor genotype. In the later case, after replication of chromosome and cell division the one progeny cell contains the donor genotype and the other has the recipient genotype. These two types of cells can be differentiated through plating method by using the antibiotic markers. However, for pneumococci it is a general feature that all the strains discriminate between LH and HE markers when transformation has occurred with homologous DNA. The hexcells (mutant in hex function) fail to discriminate between the two markers and, therefore, integrate all markers with high efficiency, because one of the two daughter cells after cell division contains the genotype.

Bacterial conjugation

Bacterial conjugation is the transfer of genetic material between bacterial cells by direct cell-to-cell contact or by a bridge-like connection between two cells. It is a mechanism of horizontal gene transfer as are transformation and transductional though these two other mechanisms do not involve cell-to-cell contact. Bacterial conjugation is often regarded as the bacterial equivalent of sexual reproduction or mating since it involves the exchange of genetic material. However, it is not sexual reproduction, since no exchange of gamete occurs. The genetic information transferred is often beneficial to the recipient. Benefits may include antibiotic resistance, xenobiotic tolerance or the ability to use new metabolites. Such beneficial plasmids may be considered bacterial endosymbionts. Other elements, however, may be viewed as bacterial parasites and conjugation as a mechanism evolved by them to allow for their spread.

Mechanism

The F-plasmid is an episome (a plasmid that can integrate itself into the bacterial chromosome by homologous recombination) with a length of about 100 kb. It carries its own origin of replication, the *oriV*, and an origin of transfer, or *oriT*. There can only be one copy of the F-plasmid in a given bacterium, either free or integrated, and bacteria that possess a copy are called *F-positive* or *F-plus* (denoted F^+). Cells that lack F plasmids are called *F-negative* or *F-minus* (F^-) and as such can function as recipient cells.

Among other genetic information, the F-plasmid carries a *tra* and *trb* locus, which together are about 33 kb long and consist of about 40 genes. The *tra* locus includes the *pilin* gene and regulatory genes, which together form pili on the cell surface. The locus also includes the genes for the proteins that attach themselves to the surface of F^- bacteria and initiate conjugation. Though there is some debate on the exact mechanism of conjugation it seems that the pili are not the structures through which DNA exchange occurs. This has been shown in experiments where the pilus is allowed to make contact, but then is denatured with SDS and yet DNA transformation still proceeds. Several proteins coded for in the *tra* or*trb* locus seem to open a channel between the bacteria and it is thought that the traD enzyme, located at the base of the pilus, initiates membrane fusion.

When conjugation is initiated by a signal the **relaxase** enzyme creates a nick in one of the strands of the conjugative plasmid at the *oriT*. Relaxase may work alone or in a complex of over a dozen proteins known collectively as a **relaxosome**. In the F-plasmid system the relaxase enzyme is called TraI and the relaxosome consists of TraI, TraY, TraM and the integrated host factor IHF. The nicked strand, or *T-strand*, is then unwound from the unbroken strand and transferred to the recipient cell in a 5'-terminus to 3'-terminus direction. The remaining strand is replicated either independent of conjugative action (vegetative replication beginning at the *oriV*) or in concert with conjugation (conjugative replication similar to therolling circle replication of lambda phage). Conjugative replication may require a second nick before successful transfer can occur. A recent report claims to have inhibited conjugation with chemicals that mimic an intermediate step of this second nicking event.

1. The insertion sequences (yellow) on both the F factor plasmid and the chromosome have similar sequences, allowing the F factor to insert itself into the genome of the cell. This is calledhomologous recombination and creates an Hfr (high frequency of recombination) cell. **2.** The Hfr cell forms a pilus and attaches to a recipient F- cell. **3.** A nick in one strand of the Hfr cell's chromosome is created. **4.** DNA begins to be transferred from the Hfr cell to the recipient cell while the second strand of its chromosome is being replicated. **5.** The pilus detaches from the recipient cell and retracts. The Hfr cell ideally wants to transfer its entire genome to the recipient cell. However, due to its large size and inability to keep in contact with the recipient cell, it is not able to do so. **6.a.** The F- cell remains F- because the entire F factor sequence was not received. Since no homologous recombination occurred, the DNA that was transferred is degraded by enzymes. 6.**b.** In very rare cases, the F factor will be completely transferred and the F- cell will become an Hfr cell.

If the F-plasmid that is transferred has previously been integrated into the donor's genome (producing an Hfr strain [High Frequency of Recombination]) some of the donor's chromosomal DNA may also be transferred with the plasmid DNA. The amount of chromosomal DNA that is transferred depends on how long the two conjugating bacteria remain in contact. In common laboratory strains of *E. coli* the transfer of the entire bacterial chromosome takes about 100 minutes. The transferred DNA can then be integrated into the recipient genome via homologous recombination.

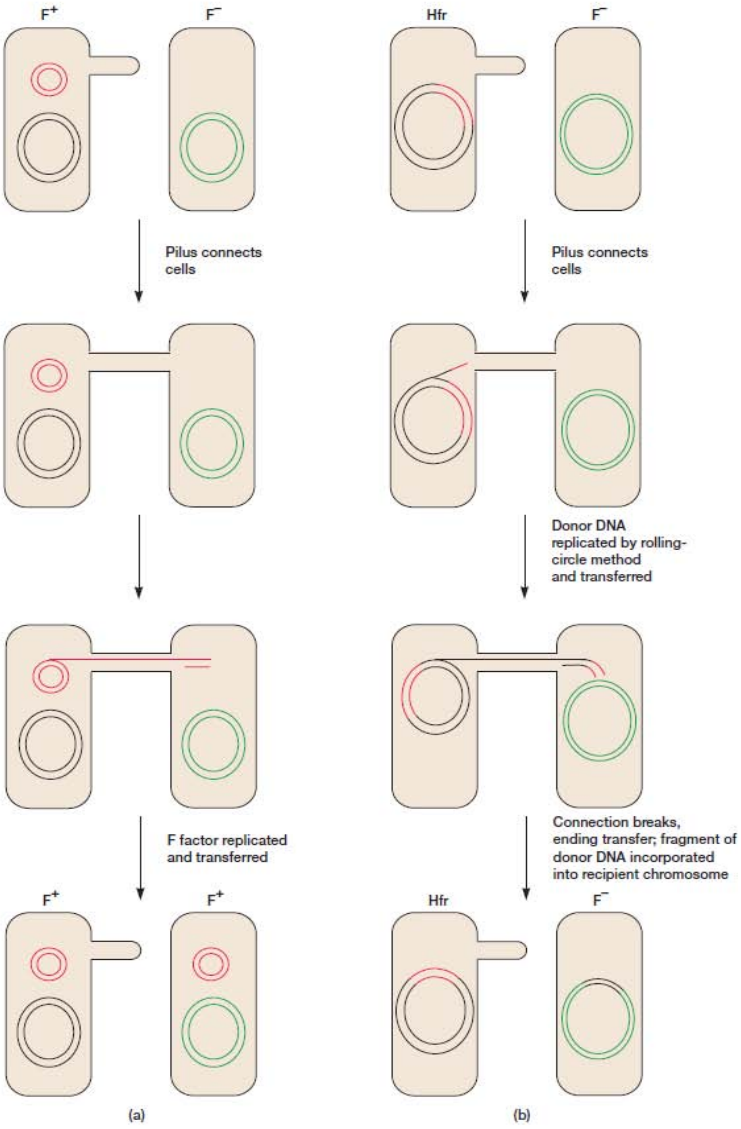


Figure: Bacterial conjugation

A cell culture that contains in its population cells with non-integrated F-plasmids usually also contains a few cells that have accidentally integrated their plasmids. It is these cells that are responsible for the low-frequency chromosomal gene transfers that occur in such cultures. Some strains of bacteria with an integrated F-plasmid can be isolated and grown in pure culture. Because such strains transfer chromosomal genes very efficiently they are called **Hfr** (**h**igh **f**requency of **r**ecombination). The *E. coli* genome was originally mapped by interrupted mating experiments in which various Hfr cells in the process of conjugation were sheared from

recipients after less than 100 minutes (initially using a Waring blender). The genes that were transferred were then investigated.

Since integration of the F-plasmid into the *E. coli* chromosome is a rare spontaneous occurrence, and since the numerous genes promoting DNA transfer are in the plasmid genome rather than in the bacterial genome, it has been argued that conjugative bacterial gene transfer, as it occurs in the *E. coli* Hfr system, is not an evolutionary adaptation of the bacterial host, nor is it likely ancestral to eukaryotic sex.

Bacterial Transduction

Transduction is the process by which foreign DNA is introduced into a cell by avirus or viral vector. An example is the viral transfer of DNA from one bacterium to another. Transduction does not require physical contact between the cell donating the DNA and the cell receiving the DNA (which occurs in conjugation), and it is DNase resistant (transformation is susceptible to DNase). Transduction is a common tool used by molecular biologists to stably introduce a foreign gene into a host cell's genome (both bacterial and mammalian cells). When viruses, including bacteriophages (viruses that infect bacteria), infect bacterial cells, their normal mode of reproduction is to harness the replicational, transcriptional, and translationmachinery of the host bacterial cell to make numerous virions, or complete viral particles, including the viral DNA or RNA and the protein coat.

Generalized transduction

Generalized transduction is the process by which any bacterial gene may be transferred to another bacterium via a bacteriophage, and very rarely a small number of phages carry the donor(bacterial genome)genome,(1 phage in 10.000 ones carry the donor genome).[5] In essence, this is the packaging of bacterial DNA into a viral envelope. This may occur in two main ways, recombination and headful packaging.

If bacteriophages undertake the lytic cycle of infection upon entering a bacterium, the virus will take control of the cell’s machinery for use in replicating its own viral DNA. If by chance bacterial chromosomal DNA is inserted into the viral capsid which is usually used to encapsulate the viral DNA, the mistake will lead to ***generalized transduction***.

If the virus replicates using 'headful packaging', it attempts to fill the nucleocapsid with genetic material. If the viral genome results in spare capacity, viral packaging mechanisms may incorporate bacterial genetic material into the new virion.

The new virus capsule now loaded with part bacterial DNA continues to infect another bacterial cell. This bacterial material may become recombined into another bacterium upon infection.

When the new DNA is inserted into this recipient cell it can fall to one of three fates

1. The DNA will be absorbed by the cell and be recycled for spare parts.
2. If the DNA was originally a plasmid, it will re-circularize inside the new cell and become a plasmid again.
3. If the new DNA matches with a homologous region of the recipient cell's chromosome, it will exchange DNA material similar to the actions in bacterial recombination.

Specialized transduction

Specialized transduction is the process by which a *restricted* set of bacterial genes is transferred to another bacterium. The genes that get transferred (donor genes) depend on where the phage genome is located on the chromosome. Specialized transduction occurs when the prophage excises imprecisely from the chromosome so that bacterial genes lying adjacent to the prophage are included in the excised DNA. The excised DNA is then packaged into a new virus particle, which then delivers the DNA to a new bacterium, where the donor genes can be inserted into the recipient chromosome or remain in the cytoplasm, depending on the nature of the bacteriophage.

When the partially encapsulated phage material infects another cell and becomes a "prophage" (is covalently bonded into the infected cell's chromosome), the partially coded prophage DNA is called a "heterogenote".

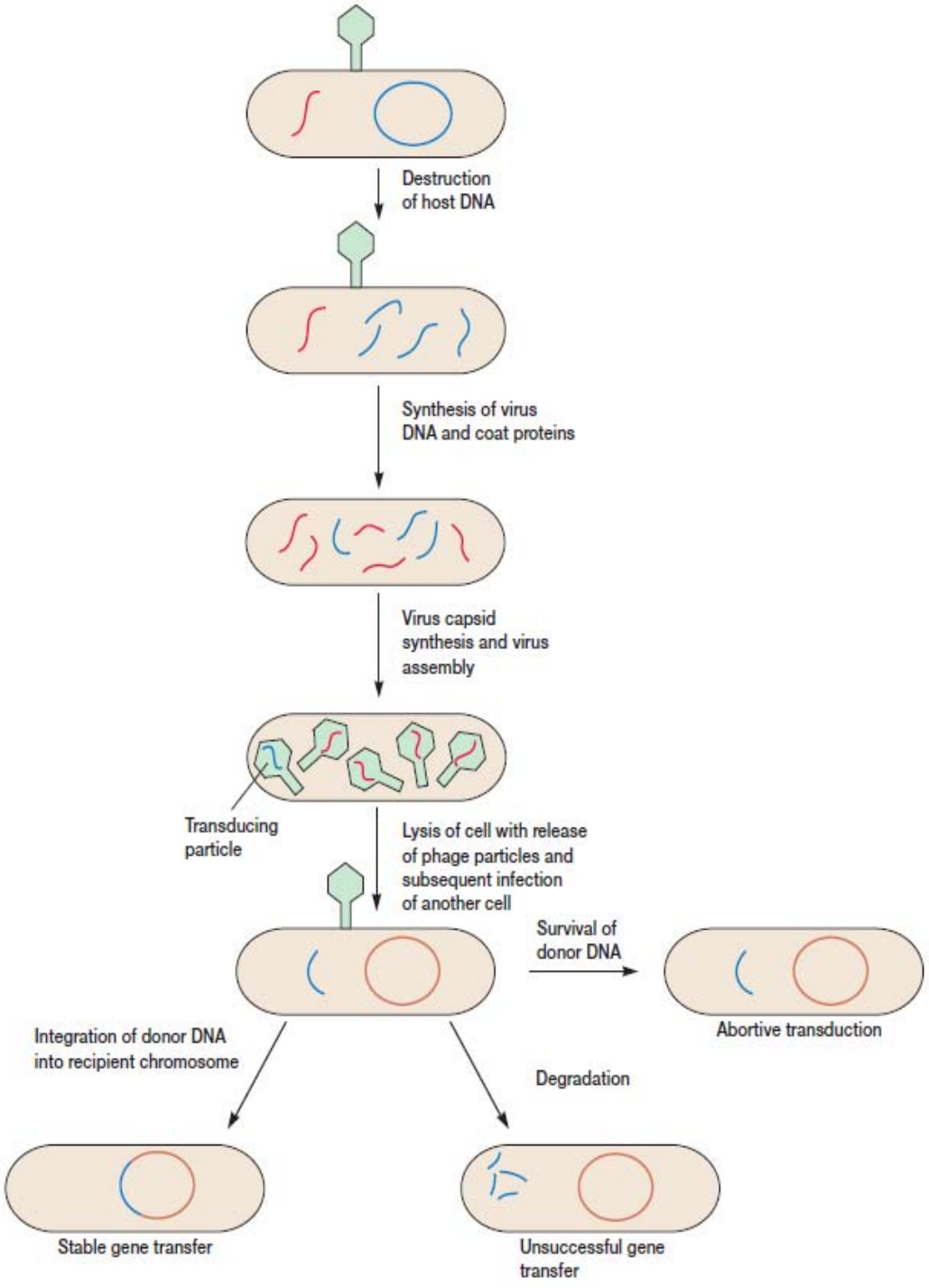


Figure: Generalized transduction of bacteria

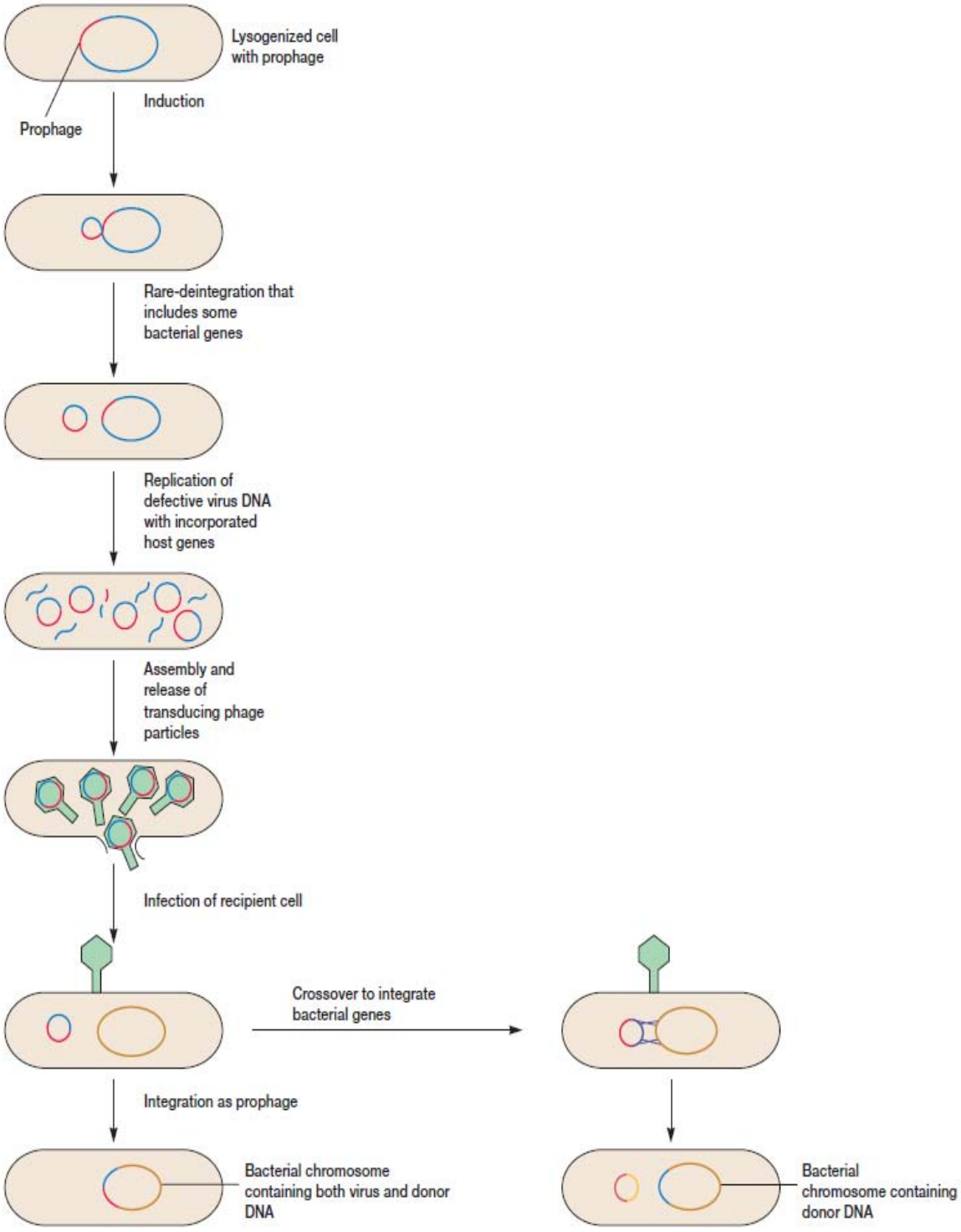


Figure: Specialized transduction by a temperate bacteriophage

9. Microbial ecology: concept of microbial ecology with reference to air, water and soil

Air Microbiology

Aeromicrobiology is the study of living microbes which are suspended in the air. These microbes are referred to as bioaerosols. Though there are significantly less atmospheric microorganisms than there are in oceans and in soil, there is still a large enough number that they can affect the atmosphere. Once suspended in the air column, these microbes have the opportunity to travel long distances with the help of wind and precipitation, increasing the occurrence of widespread disease by these microorganisms. These aerosols are ecologically significant because they can be associated with disease in humans, animals and plants. Typically microbes will be suspended in clouds, where they are able to perform processes that alter the chemical composition of the cloud, and may even induce precipitation.

Physical Environment

There are many factors within the physical environment that affect the launching, transport and deposition of bioaerosols. Particles which become suspended in the air column arise mainly from terrestrial and aquatic environments and are typically launched by air turbulence. Winds are the primary means of transport for bioaerosols. Bioaerosols can be deposited by a number of mechanisms, including gravity pulling them down, making contact with surfaces, or combining with rain which pulls the particles back down to earth's surface.

Atmosphere

Along with water droplets, dust particles and other matter, air contains microbes. Microbes follow a particular pathway in which they are suspended into the atmosphere. First they are launched into the air. The source of the launching of airborne microbes stems from humans, animals and vegetation. Then they are transported (by various methods including winds, machinery and people) and finally are deposited somewhere new. The atmosphere can have a variety of physical characteristics, and can be very extreme in terms of the relative humidity, temperature and radiation. These factors play a huge role in what kinds of microbes will survive in the atmosphere and how long they will stay alive.

Clouds

One area that bioaerosols can be found in is within clouds. Cloud water is a mixture of organic and inorganic compounds suspended within moisture (contribution of microbial activity of clouds). The conditions in clouds are not conducive to much life, as microbes present there must withstand freezing temperatures, the threat of desiccation, and extreme UV rays. Clouds are also an acidic environment, with a pH ranging from 3 to 7. Nevertheless, there are extremophile microbes which can withstand all of these environmental pressures. Clouds serve as a transport for these microbes, dispersing them over long distances.

Physical Environment Stresses

The atmosphere is a difficult place for a microbe to survive. Dessication is the primary stress that aeromicrobes face, and it limits the amount of time that they can survive while suspended in the air. Humidity within the air is a second factor which can affect the survival of organisms. Certain bacteria, including Gram + bacteria, are more tolerant of high humidity in the air, while others are more tolerant of dessication and dry conditions, such as Gram + cells. Temperature must be in an intermediate range, as too hot of temperatures can denature proteins, and too cold of temperatures can cause ice crystal formation. Finally, radiation poses a potential hazard for aeromicrobes, as it can damage DNA within the cells.

Microbial Communities

Many different microorganisms can be in aerosol form in the atmosphere, including viruses, bacteria, fungi, yeasts and protozoans. In order to survive in the atmosphere, it is important that these microbes adapt to some of the harsh climatic characteristics of the exterior world, including temperature, gasses and humidity. Many of the microbes that are capable of surviving harsh conditions can readily form endospores, which can withstand extreme conditions. Many of these microorganisms can be associated with specific and commonly known diseases.

Bacterial

One such bacterial microorganism that can resist environmental stresses is *Bacillus anthracis*. It is a gram positive rod shaped bacteria that utilizes spore formation to resist environmental stresses. The spore is a dehydrated cell with extremely thick cell walls which can remain inactive for many years. This spore makes *Bacillus anthracis* a highly resilient bacteria, allowing it can survive extreme temperatures, chemical contamination, and low nutrient environments. This bacteria is associated with Anthrax, which is a severe respiratory disease that infects humans.

Fungal

Another such microorganism that can resist environmental stresses is *Aspergillus fumigatus*, which is a major airborne fungal pathogen. This pathogen is capable of causing many human diseases when conidia are inhaled into the lungs. While *A. fumigatus* lacks virulence traits, it is very adaptable to changing environmental conditions and therefore is still capable of mass infection.

Viral

An example of a viral airborne pathogen is the Avian Influenza Virus, which is a single stranded RNA visur that can infect a broad range of animal species as well as humans and cause the Avian Influenza.

Microbial Processes

The figure on the bottom right depicts the processes that a microbe undergoes during its life cycle. The microbes undergo the emission process, in which they are emitted from surfaces such as water, soil or vegetation and become airborne and transported into the airstream. The red boxes indicate some of the harsh environmental conditions that the microbes must withstand while airborne. The microbes that are able to withstand and survive these environmental pressures are the more resistant varieties. The microbes make it into clouds, where they can begin the breakdown of organic compounds. Finally, the microbes are "rained" out of the clouds through wet deposition, and they begin colonization of their new location.

Droplet Formation

The emission process mentioned above, in which microbes are lifted in the air often involves microbes being suspended in droplets, which are large enough to keep the microbes hydrated and large enough to maintain a virulent amount of pathogen, but are still small enough to stay suspended in the air.

Water microbiology

Water microbiology is concerned with the microorganisms that live in water, or can be transported from one habitat to another by water. Water can support the growth of many types of microorganisms. This can be advantageous. For example, the chemical activities of certain strains of yeasts provide us with beer and bread. As well, the growth of some bacteria in contaminated water can help digest the poisons from the water. However, the presence of other disease causing microbes in water is unhealthy and even life threatening. For example, bacteria

that live in the intestinal tracts of humans and other warm blooded animals, such as *Escherichia coli*, *Salmonella*, *Shigella*, and *Vibrio*, can contaminate water if feces enter the water. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. The contamination of the municipal water supply of Walkerton, Ontario, Canada in the summer of 2000 by strain O157:H7 sickened 2,000 people and killed seven people.

The intestinal tract of warm-blooded animals also contains viruses that can contaminate water and cause disease. Examples include rotavirus, enteroviruses, and coxsackie virus.

Another group of microbes of concern in water microbiology are protozoa. The two protozoa of the most concern are *Giardia* and *Cryptosporidium*. They live normally in the intestinal tract of animals such as beaver and deer. *Giardia* and *Cryptosporidium* form dormant and hardy forms called cysts during their life cycles. The cyst forms are resistant to chlorine, which is the most popular form of drinking water disinfection, and can pass through the filters used in many water treatment plants. If ingested in drinking water they can cause debilitating and prolonged diarrhea in humans, and can be life threatening to those people with impaired immune systems. *Cryptosporidium* contamination of the drinking water of Milwaukee, Wisconsin with in 1993 sickened more than 400,000 people and killed 47 people.

Many microorganisms are found naturally in fresh and saltwater. These include bacteria, cyanobacteria, protozoa, algae, and tiny animals such as rotifers. These can be important in the food chain that forms the basis of life in the water. For example, the microbes called cyanobacteria can convert the energy of the sun into the energy it needs to live. The plentiful numbers of these organisms in turn are used as food for other life. The algae that thrive in water is also an important food source for other forms of life. A variety of microorganisms live in fresh water. The region of a water body near the shoreline (the littoral zone) is well lighted, shallow, and warmer than other regions of the water. Photosynthetic algae and bacteria that use light as energy thrive in this zone. Further away from the shore is the limnitic zone. Photosynthetic microbes also live here. As the water deepens, temperatures become colder and the oxygen concentration and light in the water decrease. Now, microbes that require oxygen do not thrive. Instead, purple and green sulfur bacteria, which can grow without oxygen, dominate. Finally, at the bottom of fresh waters (the benthic zone), few microbes survive. Bacteria that can survive in the absence of oxygen and sunlight, such as methane producing bacteria, thrive.

Saltwater presents a different environment to microorganisms. The higher salt concentration, higher pH, and lower nutrients, relative to freshwater, are lethal to many microorganisms. But, salt loving (halophilic) bacteria abound near the surface, and some bacteria that also live in freshwater are plentiful (i.e., *Pseudomonas* and *Vibrio*). Also, in 2001, researchers demonstrated that the ancient form of microbial life known as archaebacteria is one of the dominant forms of life in the ocean. The role of archaebacteria in the ocean food chain is not yet known, but must be of vital importance.

Another microorganism found in saltwater is a type of algae known as dinoflagellelates. The rapid growth and multiplication of dinoflagellates can turn the water red. This "red tide" depletes the water of nutrients and oxygen, which can cause many fish to die. As well, humans can become ill by eating contaminated fish. Water can also be an ideal means of transporting microorganisms from one place to another. For example, the water that is carried in the hulls of ships to stabilize the vessels during their ocean voyages is now known to be a means of transporting microorganisms around the globe. One of these organisms, a bacterium called *Vibrio cholerae*, causes life threatening diarrhea in humans.

Drinking water is usually treated to minimize the risk of microbial contamination. The importance of drinking water treatment has been known for centuries. For example, in pre-Christian times the storage of drinking water in jugs made of metal was practiced. Now, the anti-bacterial effect of some metals is known. Similarly, the boiling of drinking water, as a means of protection of water has long been known.

Chemicals such as chlorine or chlorine derivatives has been a popular means of killing bacteria such as *Escherichia coli* in water since the early decades of the twentieth century. Other bacteria-killing treatments that are increasingly becoming popular include the use of a gas called **ozone** and the disabling of the microbe's genetic material by the use of ultraviolet light. Microbes can also be physically excluded form the water by passing the water through a filter. Modern filters have holes in them that are so tiny that even particles as miniscule as viruses can be trapped.

An important aspect of water microbiology, particularly for drinking water, is the testing of the water to ensure that it is safe to drink. Water quality testing can de done in several ways. One popular test measures the turbidity of the water. Turbidity gives an indication of the amount of suspended material in the water. Typically, if material such as soil is present in the water then microorganisms will also be present. The presence of particles even as small as bacteria and

viruses can decrease the clarity of the water. Turbidity is a quick way of indicating if water quality is deteriorating, and so if action should be taken to correct the water problem.

In many countries, water microbiology is also the subject of legislation. Regulations specify how often water sources are sampled, how the sampling is done, how the analysis will be performed, what microbes are detected, and the acceptable limits for the target microorganisms in the water sample. Testing for microbes that cause disease (i.e., *Salmonella typhymurium* and *Vibrio cholerae*) can be expensive and, if the bacteria are present in low numbers, they may escape detection. Instead, other more numerous bacteria provide an indication of fecal **pollution** of the water. *Escherichia coli* has been used as an indicator of fecal pollution for decades. The bacterium is present in the intestinal tract in huge numbers, and is more numerous than the disease-causing bacteria and viruses. The chance of detecting *E. coli* is better than detecting the actual disease causing microorganisms. *E.coli* also had the advantage of not being capable of growing and reproducing in the water (except in the warm and food-laden waters of tropical countries). Thus, the presence of the bacterium in water is indicative of recent fecal pollution. Finally, *E.coli* can be detected easily and inexpensively.

Soil Microbiology

Soil microbiology is the study of organisms in soil, their functions, and how they affect soil properties. It is believed that between two and four billion years ago, the first ancient bacteria and microorganisms came about in Earth's oceans. These bacteria could fix nitrogen, in time multiplied and as a result released oxygen into the atmosphere. This led to more advanced microorganisms. Microorganisms in soil are important because they affect soil structure and fertility. Soil microorganisms can be classified as bacteria, actinomycetes, fungi, algae and protozoa. Each of these groups has characteristics that define them and their functions in soil.

Up to 10 billion bacterial cells inhabit each gram of soil in and around plant roots, a region known as the rhizosphere. In 2011, a team detected more than 33,000 bacterial and archaeal species on sugar beet roots. The composition of the rhizobiome can change rapidly in response to changes in the surrounding environment.

Bacteria

Bacteria and Archaea are the smallest organisms in soil apart from viruses. Bacteria and Archaea are prokaryotic. All of the *other* microorganisms are eukaryotic, which means they have

a more advanced cell structure with internal organelles and the ability to reproduce sexually. A prokaryote has a very simple cell structure with no internal organelles. Bacteria and archaea are the most abundant microorganisms in the soil, and serve many important purposes, including nitrogen fixation.

Biochemical processes

One of the most distinguished features of bacteria is their biochemical versatility. A bacterial genus called *Pseudomonas* can metabolize a wide range of chemicals and fertilizers. In contrast, another genus known as *Nitrobacter* can only derive its energy by turning nitrite into nitrate, which is also known as oxidation. The genus *Clostridium* is an example of bacterial versatility because it, unlike most species, can grow in the absence of oxygen, respiring anaerobically. Several species of *Pseudomonas*, such as *Pseudomonas aeruginosa* are able to respire both aerobically and anaerobically, using nitrate as the terminal electron acceptor.

Nitrogen fixation

Bacteria are responsible for the process of nitrogen fixation, which is the conversion of atmospheric nitrogen into nitrogen-containing compounds (such as ammonia) that can be used by plants. Autotrophic bacteria derive their energy by making their own food through oxidation, like the *Nitrobacters* species, rather than feeding on plants or other organisms. These bacteria are responsible for nitrogen fixation. The amount of autotrophic bacteria is small compared to heterotrophic bacteria (the opposite of autotrophic bacteria, heterotrophic bacteria acquire energy by consuming plants or other microorganisms), but are very important because almost every plant and organism requires nitrogen in some way.

Actinomycetes

Actinomycetes are soil microorganisms. They are a type of bacteria, but they share some characteristics with fungi that are most likely a result of convergent evolution due to a common habitat and lifestyle.

Similarities to fungi

Although they are members of the Bacteria kingdom, many actinomycetes share characteristics with fungi, including shape and branching properties, spore formation and secondary metabolite production. **a.** The mycelium branches in a manner similar to that of fungi **b.** They form aerial mycelium as well as conidia. **c.** Their growth in liquid culture occurs as distinct clumps or pellets, rather than as a uniform turbid suspension as in bacteria

Antibiotics

One of the most notable characteristics of the actinomycetes is their ability to produce antibiotics. Streptomycin, neomycin, erythromycin and tetracycline are only a few examples of these antibiotics. Streptomycin is used to treat tuberculosis and infections caused by certain bacteria and neomycin is used to reduce the risk of bacterial infection during surgery. Erythromycin is used to treat certain infections caused by bacteria, such as bronchitis, pertussis (whooping cough), pneumonia and ear, intestine, lung, urinary tract and skin infections.

Fungi

Fungi are abundant in soil, but bacteria are more abundant. Fungi are important in the soil as food sources for other, larger organisms, pathogens, beneficial symbiotic relationships with plants or other organisms and soil health. Fungi can be split into species based primarily on the size, shape and color of their reproductive spores, which are used to reproduce. Most of the environmental factors that influence the growth and distribution of bacteria and actinomycetes also influence fungi. The quality as well as quantity of organic matter in the soil has a direct correlation to the growth of fungi, because most fungi consume organic matter for nutrition. Fungi thrive in acidic environments, while bacteria and actinomycetes cannot survive in acid, which results in an abundance of fungi in acidic areas. Fungi also grow well in dry, arid soils because fungi are aerobic, or dependent on oxygen, and the higher the moisture content in the soil, the less oxygen is present for them.

Algae

Algae can make their own nutrients through photosynthesis. Photosynthesis converts light energy to chemical energy that can be stored as nutrients. For algae to grow, they must be exposed to light because photosynthesis requires light, so algae are typically distributed evenly wherever sunlight and moderate moisture is available. Algae do not have to be directly exposed to the Sun, but can live below the soil surface given uniform temperature and moisture conditions. Algae are also capable of performing nitrogen fixation.

Types

Algae can be split up into three main groups: the Cyanophyceae, the Chlorophyceae and the Bacillariaceae. The Cyanophyceae contain chlorophyll, which is the molecule that absorbs sunlight and uses that energy to make carbohydrates from carbon dioxide and water and also pigments that make it blue-green to violet in color. The Chlorophyceae usually only have

chlorophyll in them which makes them green, and the Bacillariaceae contain chlorophyll as well as pigments that make the algae brown in color.

Blue-green algae and nitrogen fixation

Blue-green algae, or Cyanophyceae, are responsible for nitrogen fixation. The amount of nitrogen they fix depends more on physiological and environmental factors rather than the organism's abilities. These factors include intensity of sunlight, concentration of inorganic and organic nitrogen sources and ambient temperature and stability.

Protozoa

Protozoa are eukaryotic organisms that were some of the first microorganisms to reproduce sexually, a significant evolutionary step from duplication of spores, like those that many other soil microorganisms depend on. Protozoa can be split up into three categories: flagellates, amoebae and ciliates.

Flagellates

Flagellates are the smallest members of the protozoa group, and can be divided further based on whether they can participate in photosynthesis. Nonchlorophyll-containing flagellates are not capable of photosynthesis because chlorophyll is the green pigment that absorbs sunlight. These flagellates are found mostly in soil. Flagellates that contain chlorophyll typically occur in aquatic conditions. Flagellates can be distinguished by their flagella, which is their means of movement. Some have several flagella, while other species only have one that resembles a long branch or appendage.

Amoebae

Amoebae are larger than flagellates and move in a different way. Amoebae can be distinguished from other protozoa by their slug-like properties and pseudopodia. A pseudopodium or "false foot" is a temporary obtrusion from the body of the amoeba that helps pull it along surfaces for movement or helps to pull in food. The amoeba does not have permanent appendages and the pseudopodium is more of a slime-like consistency than a flagellum.

Ciliates

Ciliates are the largest of the protozoa group, and move by means of short, numerous cilia that produce beating movements. Cilia resemble small, short hairs. They can move in different directions to move the organism, giving it more mobility than flagellates or amoebae.

10. Microbes associated with food, food-borne infections and intoxications; preservation of food.

Food microbiology is the study of the microorganisms that inhabit, create, orcontaminate **food**, including the study of microorganisms causing food spoilage, pathogens that may cause disease especially if food is improperly cooked or stored, those used to produce fermented foods such as cheese,yogurt, bread, beer, and wine, and those with other useful roles such as producing probiotics.

Preservation of food

Traditional techniques

Curing

Bag of Prague powder, also known as "curing salt" or "pink salt". It is typically a combination of salt and sodium nitrite, with the pink color added to distinguish it from ordinary salt. The earliest form of curing was dehydration or drying, used as early as 12,000 BC.Smoking and salting techniques improve on the drying process and add antimicrobial agents that aid in preservation. Smoke deposits a number of pyrolysis products onto the food, including the phenols syringol, guaiacol and catechol. Salt accelerates the drying process using osmosis and also inhibits the growth of several common strains of bacteria. More recently nitrites have been used to cure meat, contributing a characteristic pink colour.

Cooling

Cooling preserves food by slowing down the growth and reproduction of microorganisms and the action of enzymes that causes the food to rot. The introduction of commercial and domestic refrigerators drastically improved the diets of many in the Western world by allowing food such as fresh fruit, salads and dairy products to be stored safely for longer periods, particularly during warm weather.

Before the era of mechanical refrigeration, cooling for food storage occurred in the forms of root cellars and iceboxes. Rural people often did their own ice cutting, whereas town and city dwellers often relied on the ice trade. Today, root cellaring remains popular among people who value various goals, including local food, heirloom crops, traditional home cooking techniques, family farming, frugality, self-sufficiency, organic farming, and others.

Freezing

Freezing is also one of the most commonly used processes, both commercially and domestically, for preserving a very wide range of foods, including prepared foods that would not have required freezing in their unprepared state. For example, potato waffles are stored in the freezer, but potatoes themselves require only a cool dark place to ensure many months' storage. Cold stores provide large-volume, long-term storage for strategic food stocks held in case of national emergency in many countries.

Boiling

Boiling liquid food items can kill any existing microbes. Milk and water are often boiled to kill any harmful microbes that may be present in them.

Heating

Heating to temperatures which are sufficient to kill microorganisms inside the food is a method used with perpetual stews. Milk is also boiled before storing to kill many microorganisms.

Sugaring

The earliest cultures have used sugar as a preservative, and it was commonplace to store fruit in honey. Similar to pickled foods, sugar cane was brought to Europe through the trade routes. In northern climates without sufficient sun to dry foods, preserves are made by heating the fruit with sugar. "Sugar tends to draw water from the microbes (plasmolysis). This process leaves the microbial cells dehydrated, thus killing them. In this way, the food will remain safe from microbial spoilage." Sugar is used to preserve fruits, either in an antimicrobial syrup with fruit such as apples, pears, peaches, apricots, and plums, or in crystallized form where the preserved material is cooked in sugar to the point of crystallization and the resultant product is then stored dry. This method is used for the skins of citrus fruit (candied peel), angelica, and ginger. Also, sugaring can be used in the production of jam and jelly.

Pickling

Pickling is a method of preserving food in an edible, antimicrobial liquid. Pickling can be broadly classified into two categories: chemical pickling and fermentation pickling.

In chemical pickling, the food is placed in an edible liquid that inhibits or kills bacteria and other microorganisms. Typical pickling agents include brine (high in salt), vinegar, alcohol, and vegetable oil. Many chemical pickling processes also involve heating or boiling so that the food being preserved becomes saturated with the pickling agent. Common chemically pickled foods

include cucumbers, peppers, corned beef, herring, and eggs, as well as mixed vegetables such as piccalilli.

In fermentation pickling, bacteria in the liquid produce organic acids as preservation agents, typically by a process that produces lactic acid through the presence of lactobacillales. Fermented pickles include sauerkraut, nukazuke, kimchi, and surströmming.

Lye

Sodium hydroxide (lye) makes food too alkaline for bacterial growth. Lye will saponify fats in the food, which will change its flavor and texture. Lutefisk uses lye in its preparation, as do some olive recipes. Modern recipes for century eggs also call for lye.

Canning

Canning involves cooking food, sealing it in sterilized cans or jars, and boiling the containers to kill or weaken any remaining bacteria as a form of sterilization. It was invented by the French confectioner Nicolas Appert. By 1806, this process was used by the French Navy to preserve meat, fruit, vegetables, and even milk. Although Appert had discovered a new way of preservation, it wasn't understood until 1864 when Louis Pasteur found the relationship between microorganisms, food spoilage, and illness.

Foods have varying degrees of natural protection against spoilage and may require that the final step occur in a pressure cooker. High-acid fruits like strawberries require no preservatives to can and only a short boiling cycle, whereas marginal vegetables such as carrots require longer boiling and addition of other acidic elements. Low-acid foods, such as vegetables and meats, require pressure canning. Food preserved by canning or bottling is at immediate risk of spoilage once the can or bottle has been opened.

Lack of quality control in the canning process may allow ingress of water or micro-organisms. Most such failures are rapidly detected as decomposition within the can causes gas production and the can will swell or burst. However, there have been examples of poor manufacture (under processing) and poor hygiene allowing contamination of canned food by the obligateanaerobe *Clostridium botulinum*, which produces an acute toxin within the food, leading to severe illness or death. This organism produces no gas or obvious taste and remains undetected by taste or smell. Its toxin is denatured by cooking, however. Cooked mushrooms, handled poorly and then canned, can support the growth of *Staphylococcus aureus*, which produces a toxin that is not destroyed by canning or subsequent reheating.

Jellying

Food may be preserved by cooking in a material that solidifies to form a gel. Such materials include gelatin, agar, maizeflour, and arrowroot flour. Some foods naturally form a protein gel when cooked, such as eels and elvers, and sipunculidworms, which are a delicacy in Xiamen, in the Fujian province of the People's Republic of China. Jellied eels are a delicacy in the East End of London, where they are eaten with mashed potatoes. Potted meats in aspic (a gel made from gelatin and clarified meat broth) were a common way of serving meat off-cuts in the UK until the 1950s. Many jugged meats are also jellied.

A traditional British way of preserving meat (particularly shrimp) is by setting it in a pot and sealing it with a layer of fat. Also common is potted chicken liver; jellying is one of the steps in producing traditional pâtés.

Jugging

Meat can be preserved by jugging. Jugging is the process of stewing the meat (commonly game or fish) in a covered earthenware jug or casserole. The animal to be jugged is usually cut into pieces, placed into a tightly-sealed jug with brine or gravy, and stewed. Red wine and/or the animal's own blood is sometimes added to the cooking liquid. Jugging was a popular method of preserving meat up until the middle of the 20th century.

Burial

Burial of food can preserve it due to a variety of factors: lack of light, lack of oxygen, cool temperatures, pH level, or desiccants in the soil. Burial may be combined with other methods such as salting or fermentation. Most foods can be preserved in soil that is very dry and salty (thus a desiccant) such as sand, or soil that is frozen.

Many root vegetables are very resistant to spoilage and require no other preservation than storage in cool dark conditions, for example by burial in the ground, such as in a storage clamp. Century eggs are traditionally created by placing eggs in alkaline mud (or other alkaline substance), resulting in their "inorganic" fermentation through raised pH instead of spoiling. The fermentation preserves them and breaks down some of the complex, less flavorful proteins and fats into simpler, more flavorful ones. Cabbage was traditionally buried during Autumn in northern US farms for preservation. Some methods keep it crispy while other methods produce sauerkraut. A similar process is used in the traditional production of kimchi. Sometimes meat is buried under conditions that cause preservation. If buried on hot coals or ashes, the heat can kill

pathogens, the dry ash can desiccate, and the earth can block oxygen and further contamination. If buried where the earth is very cold, the earth acts like a refrigerator. Before burial, meat (pig/boar) can be fatted. The tallow of the animal is heated and poured over meat in a barrel. Once the fat hardens the barrel is sealed and buried in a cold cellar or ground.

In Orissa, India, it is practical to store rice by burying it underground. This method helps to store for three to six months during the dry season. Butter and similar substances have been preserved as bog butter in Irish peat bogs for centuries.

Fermentation

Some foods, such as many cheeses, wines, and beers, use specific micro-organisms that combat spoilage from other less-benign organisms. These micro-organisms keep pathogens in check by creating an environment toxic for themselves and other micro-organisms by producing acid or alcohol. Methods of fermentation include, but are not limited to, starter micro-organisms, salt, hops, controlled (usually cool) temperatures and controlled (usually low) levels of oxygen. These methods are used to create the specific controlled conditions that will support the desirable organisms that produce food fit for human consumption. Fermentation is the microbial conversion of starch and sugars into alcohol. Not only can fermentation produce alcohol, but it can also be a valuable preservation technique.

Fermentation can also make foods more nutritious and palatable. For example, drinking water in the Middle Ages was dangerous because it often contained pathogens that could spread disease. When the water is made into beer, the boiling during the brewing process kills any bacteria in the water that could make people sick. Additionally, the water now has the nutrients from the barley and other ingredients, and the microorganisms can also produce vitamins as they ferment irradiated for fruit fly quarantine.

Modern industrial techniques

Techniques of food preservation were developed in research laboratories for commercial applications.

Pasteurization

Pasteurization is a process for preservation of liquid food. It was originally applied to combat the souring of young local wines. Today, the process is mainly applied to dairy products. In this method, milk is heated at about 70°C (158°F) for 15–30 seconds to kill the bacteria present in it and cooling it quickly to 10°C (50 °F) to prevent the remaining bacteria from growing. The milk

is then stored in sterilized bottles or pouches in cold places. This method was invented by Louis Pasteur, a French chemist, in 1862.

Vacuum packing

Vacuum-packing stores food in a vacuum environment, usually in an air-tight bag or bottle. The vacuum environment strips bacteria of oxygen needed for survival. Vacuum-packing is commonly used for storing nuts to reduce loss of flavor from oxidization. A major drawback to vacuum packaging, at the consumer level, is that vacuum sealing can deform contents and rob certain foods, such as cheese, of its flavor.

Artificial food additives

Preservative food additives can be *antimicrobial* which inhibit the growth of bacteria or fungi, including mold-or*antioxidant*, such as oxygen absorbers, which inhibit the oxidation of food constituents. Common antimicrobial preservatives include calcium propionate, sodium nitrate, sodium nitrite, sulfites (sulfur dioxide, sodium bisulfite, potassium hydrogen sulfite, etc.).

Irradiation

Irradiation of food is the exposure of food to ionizing radiation. The two types of ionizing radiation used are beta particles (high-energy electrons) and gamma rays (emitted from radioactive sources such as cobalt-60 or cesium-137). Treatment effects include killing bacteria, molds, and insect pests, reducing the ripening and spoiling of fruits, and at higher doses inducing sterility. The technology may be compared to pasteurization; it is sometimes called "cold pasteurization", as the product is not heated.

The irradiation process is not directly related to nuclear energy, but does use radioactive isotopes produced in nuclear reactors. Cobalt-60, for example does not occur naturally and can only be produced through neutron bombardment ofcobalt-59. Ionizing radiation at high energy levels is hazardous to life (hence its usefulness in sterilisation); for this reason, irradiation facilities have a heavily shielded irradiation room where the process takes place. Radiation safety procedures are used to ensure that neither the workers in such facilities nor the environment receives any radiation dose above administrative limits. Irradiated food does not and cannot become radioactive, and national and international expert bodies have declared food irradiation as wholesome. However, the wholesomeness of consuming such food is disputed by opponents and consumer organizations. National and international expert bodies have declared food irradiation as "wholesome"; organizations of the United Nations, such as the World Health

Organization and Food and Agriculture Organization, endorse food irradiation. International legislation on whether food may be irradiated or not varies worldwide from no regulation to full banning. Irradiation may allow lower-quality or contaminated foods to be rendered marketable.

Modifying atmosphere is a way to preserve food by operating on the atmosphere around it. Salad crops that are notoriously difficult to preserve are now being packaged in sealed bags with an atmosphere modified to reduce the oxygen (O_2) concentration and increase the carbon dioxide (CO_2) concentration. There is concern that, although salad vegetables retain their appearance and texture in such conditions, this method of preservation may not retain nutrients, especially vitamins. There are two methods for preserving grains with carbon dioxide. One method is placing a block of dry ice in the bottom and filling the can with the grain. Another method is purging the container from the bottom by gaseous carbon dioxide from a cylinder or bulk supply vessel.

Carbon dioxide prevents insects and, depending on concentration, mold and oxidation from damaging the grain. Grain stored in this way can remain edible for approximately five years. Nitrogen gas (N_2) at concentrations of 98% or higher is also used effectively to kill insects in the grain through hypoxia. However, carbon dioxide has an advantage in this respect, as it kills organisms through hypercarbia and hypoxia (depending on concentration), but it requires concentrations of above 35%, or so. This makes carbon dioxide preferable for fumigation in situations where a hermetic seal cannot be maintained. Controlled Atmospheric Storage (CA): "CA storage is a non-chemical process. Oxygen levels in the sealed rooms are reduced, usually by the infusion of nitrogen gas, from the approximate 21 percent in the air we breathe to 1 percent or 2 percent. Temperatures are kept at a constant 0–2°C (32–36°F). Humidity is maintained at 95 percent and carbon dioxide levels are also controlled. Exact conditions in the rooms are set according to the apple variety. Researchers develop specific regimens for each variety to achieve the best quality. Computers help keep conditions constant." "Eastern Washington, where most of Washington’s apples are grown, has enough warehouse storage for 181 million boxes of fruit, according to a report done in 1997 by managers for the Washington State Department of Agriculture Plant Services Division. The storage capacity study shows that 67 percent of that space-enough for 121,008,000 boxes of apples-is CA storage."

Air-tight storage of grains (sometimes called hermetic storage) relies on the respiration of grain, insects, and fungi that can modify the enclosed atmosphere sufficiently to control insect pests.

This is a method of great antiquity, as well as having modern equivalents. The success of the method relies on having the correct mix of sealing, grain moisture, and temperature.

A patented process uses fuel cells to exhaust and automatically maintain the exhaustion of oxygen in a shipping container, containing, for example, fresh fish.

Nonthermal plasma

This process subjects the surface of food to a "flame" of ionized gas molecules, such as helium or nitrogen. This causes micro-organisms to die off on the surface.

High-pressure food preservation

High-pressure food preservation or pascalization refers to the use of a food preservation technique that makes use of high pressure. "Pressed inside a vessel exerting 70,000 pounds per square inch (480 MPa) or more, food can be processed so that it retains its fresh appearance, flavor, texture and nutrients while disabling harmful microorganisms and slowing spoilage." By 2005, the process was being used for products ranging from orange juice to guacamole to deli meats and widely sold.

Biopreservation

Biopreservation is the use of natural or controlled microbiota or antimicrobials as a way of preserving food and extending its shelf life. Beneficial bacteria or thefermentation products produced by these bacteria are used in biopreservation to control spoilage and render pathogens inactive in food. It is a benign ecological approach which is gaining increasing attention.

Of special interest are lactic acid bacteria (LAB). Lactic acid bacteria have antagonistic properties that make them particularly useful as biopreservatives. When LABs compete for nutrients, their metabolites often include active antimicrobials such as lactic acid, acetic acid, hydrogen peroxide, and peptide bacteriocins. Some LABs produce the antimicrobial nisin, which is a particularly effective preservative. These days, LAB bacteriocins are used as an integral part of hurdle technology. Using them in combination with other preservative techniques can effectively control spoilage bacteria and other pathogens, and can inhibit the activities of a wide spectrum of organisms, including inherently resistant Gram-negative bacteria.

Hurdle technology

Hurdle technology is a method of ensuring that pathogens in food products can be eliminated or controlled by combining more than one approach. These approaches can be thought of as "hurdles" the pathogen has to overcome if it is to remain active in the food. The right

combination of hurdles can ensure all pathogens are eliminated or rendered harmless in the final product. Hurdle technology has been defined by Leistner (2000) as an intelligent combination of hurdles that secures the microbialsafety and stability as well as the organoleptic and nutritional quality and the economic viability of food products. The organoleptic quality of the food refers to their sensory property that is its look, taste, smell, and texture. Examples of hurdles in a food system are high temperature during processing, low temperature during storage, increasing the acidity, lowering the water activity or redox potential, and the presence of preservatives or biopreservatives. According to the type of pathogens and how risky they are, the intensity of the hurdles can be adjusted individually to meet consumer preferences in an economical way, without sacrificing the safety of the product.

11. Cells and organ of immune system, antigen (chemical nature and types), immunoglobulins (structure and types), brief idea about hypersensitivity and vaccine.

The key primary lymphoid organs of the immune system include the thymus and bone marrow, as well as secondary lymphatic tissues including spleen, tonsils, lymph vessels, lymph nodes, adenoids, skin, and liver. The thymus "educates" T cells and provides an inductive environment for the development of T cells from hematopoietic progenitor cells. The thymus is largest and most active during the neonatal and pre-adolescent periods of development. By the early teens, the thymus begins to atrophy and thymic stroma is replaced by adipose tissue. Nevertheless, residual T-lymphopoiesis continues throughout adult life.

Bone marrow is the flexible tissue found in the interior of bones. In humans, red blood cells are produced in the heads of long bones. The red bone marrow is a key element of the lymphatic system, being one of the primary lymphoid organs that generate lymphocytes from immature hematopoietic progenitor cells. Bone marrow and thymus constitute the primary lymphoid tissues involved in the production and early selection of lymphocytes.

The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid, called lymph, unidirectionally towards the heart. The lymphatic system has multiple interrelated functions including the transportation of white blood cells to and from the lymph nodes into the bones, and the transportation of antigen - presenting cells (such as dendritic cells) to the lymph nodes where an immune response is stimulated. Lymphoid tissue is found in many organs, particularly the lymph nodes.

The Lymph Nodes and Lymph Vessels in Human Beings: The lymphatic system is a part of the circulatory system, comprising a network of conduits called lymphatic vessels that carry a clear fluid called lymph. The spleen is similar in structure to a large lymph node and acts primarily as a blood filter. It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. The palatine tonsils and the nasopharyngeal tonsil are lymphoepithelial tissues located near the oropharynx and nasopharynx. These immunocompetent tissues are the immune system's first line of defense against ingested or inhaled foreign pathogens. The fundamental immunological roles of tonsils aren't yet understood.

Lymph nodes are distributed widely throughout areas of the body, including the armpit and stomach, and linked by lymphatic vessels. Lymph nodes are garrisons of B, T and other immune

cells. Lymph nodes act as filters or traps for foreign particles and are important in the proper functioning of the immune system. They are packed tightly with the white blood cells, called lymphocytes and macrophages.

The skin is one of the most important parts of the body because it interfaces with the environment, and is the first line of defense from external factors, acting as an anatomical barrier from pathogens and damage between the internal and external environment in bodily defense. Langerhans cells in the skin are part of the adaptive immune system.

The liver has a wide range of functions, including immunological effects-the reticuloendothelial system of the liver contains many immunologically active cells, acting as a “sieve” for antigens carried to it via the portal system.

Immune System Cells

Leukocytes (white blood cells) are immune system cells involved in defending the body against infectious disease and foreign materials. Five different types of leukocytes exist, all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. The innate leukocytes include the phagocytes, mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens and are important mediators in the activation of the adaptive immune system.

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte. During the acute phase of inflammation neutrophils migrate toward the site of inflammation and are usually the first cells to arrive at the scene of infection. Macrophages reside within tissues and produce a wide array of chemicals. They also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system. Dendritic cells are phagocytes in tissues that are in contact with the external environment, and are located mainly in the skin, nose, lungs, stomach, and intestines. These cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T-cells, one of the key cell types of the adaptive immune system.

A Phagocyte in Action:

Neutrophil engulfing anthrax bacteria:

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis.

Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites, and play a role in allergic reactions, such as asthma.

Natural killer cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses. The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow.

Blood Cells: Red blood cells, several white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

T cells recognize a “non-self” target, such as a pathogen, only after antigens have been processed and presented in combination with a “self” receptor, called a major histocompatibility complex (MHC) molecule. There are two major subtypes of T cells: the killer T cell, which kills cells that are infected with viruses (and other pathogens) or are otherwise damaged or dysfunctional, and the helper T cell, which regulates both innate and adaptive immune responses and helps determine which immune responses the body makes to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly. A third, minor subtype are the γ T cells that recognize intact antigens not bound to MHC receptors.

In contrast, the B cell antigen-specific receptor is an antibody molecule on the B cell surface, which recognizes whole pathogens without any need for antigen processing. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture.

Antigen

In immunology, an **antigen** is a molecule capable of inducing an immune response (to produce an antibody) in the host organism. Sometimes antigens are part of the host itself in an autoimmune disease.

Antigens are "targeted" by antibodies. Each antibody (immune response) is specifically produced by the immune system to match an antigen after cells in the immune system come into contact with it; this allows a precise identification or matching of the antigen and the initiation of a tailored response. The antibody is said to "match" the antigen in the sense that it can bind to it due to an adaptation performed to a region of the antibody; because of this, many different antibodies are produced, each with specificity to bind a different antigen while sharing the same basic structure. In most cases, an adapted antibody can only react to and bind one specific antigen; in some instances, however, antibodies may cross-react to and bind more than one antigen.

Also, an antigen is a molecule that binds to Ag-specific receptors, but cannot necessarily induce an immune response in the body by itself. Antigens are usually peptides (amino acid chains), polysaccharides (chains of monosaccharides/simple sugars) or lipids. In general, saccharides and lipids (as opposed to peptides) qualify as antigens but not as immunogens since they cannot elicit an immune response on their own. Furthermore, for a peptide to induce an immune response (activation of T-cells by antigen-presenting cells) it must be a large enough size, since peptides too small will also not elicit an immune response. The term antigen originally described a structural molecule that binds specifically to an antibody. It was expanded to refer to any molecule or a linear molecular fragment that can be recognized by highly variable antigen receptors (B-cell receptor or T-cell receptor) of the adaptive immune system.

The antigen may originate from within the body ("self-antigen") or from the external environment ("non-self"). The immune system usually does not react to self-antigens under normal homeostatic conditions due to negative selection of T cells in the thymus and is supposed to identify and attack "non-self" invaders from the outside world or modified/harmful substances present in the body under distressed conditions.

Antigen presenting cells present antigens in the form of peptides on histocompatibility molecules. The T cell/T lymphocyte (a subtype of white blood cell), of the adaptive immune system, selectively recognize the antigens. Depending on the antigen and the type of the histocompatibility molecule, different types of T cells will be activated. For T-Cell Receptor (TCR)recognition, the peptide must be processed into small fragments inside the cell and presented by a major histocompatibility complex (MHC). The antigen cannot elicit the immune response without the help of an immunologic adjuvant. Similarly, the adjuvant component of vaccines plays an essential role in the activation of the innate immune system.

An immunogen is an antigen substance (or adduct) that is able to trigger a humoral (innate) or cell-mediated immune response. It first initiates an innate immune response, which then causes the activation of the adaptive immune response. An antigen binds the highly variable immunoreceptor products (B-cell receptor or T-cell receptor) once these have been generated. Immunogens are those antigens, termed immunogenic, capable of inducing an immune response.

At the molecular level, an antigen can be characterized by its ability to bind to an antibody's variable Fab region. Different antibodies have the potential to discriminate among specific epitopes present on the antigen surface. A hapten is a small molecule that changes the structure of an antigenic epitope. In order to induce an immune response, it needs to be attached to a large carrier molecule such as a protein (a complex of peptides). Antigens are usually carried by

proteins andpolysaccharides, and less frequently, lipids. This includes parts (coats, capsules, cell walls, flagella, fimbriae, and toxins) ofbacteria, viruses, and other microorganisms. Lipids and nucleic acids are antigenic only when combined with proteins and polysaccharides. Non-microbial non-self antigens can include pollen, egg white and proteins from transplanted tissues and organs or on the surface of transfused blood cells. Vaccines are examples of antigens in an immunogenic form, which are intentionally administered to a recipient to induce the memory function of adaptive immune system toward the antigens of the pathogen invading that recipient.

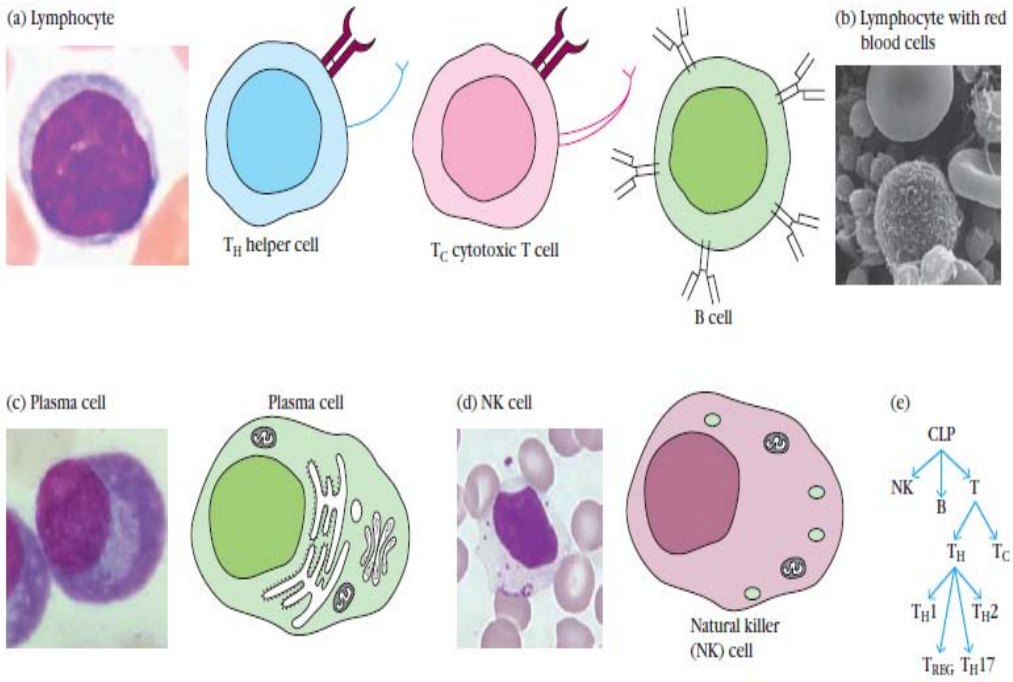


Figure. Example of differents types of Lymphocytes (Adopted from Kuby immunology)

Immunoglobulins:

An **antibody,** neutralize pathogens such as pathogenic bacteria and viruses. The antibody recognizes a unique molecule of the pathogen, called an antigen, via the Fab's variable region. Each tip of the "Y" of an antibody contains aparatope (analogous to a lock) that is specific for one particular epitope (similarly analogous to a key) on an antigen, allowing these two structures to bind together with precision. Using this binding mechanism, an antibody can*tag* a microbe or an infected cell for attack by other parts of the immune system, or can neutralize its target directly (for example, by blocking a part of a microbe that is essential for its invasion and survival). Depending on the antigen, the binding may impede the biological process causing the disease or may activate macrophages to destroy the foreign substance. The ability of an antibody

to communicate with the other components of the immune system is mediated via its Fc region (located at the base of the "Y"), which contains a conserved glycosylation site involved in these interactions. The production of antibodies is the main function of the humoral immune system.

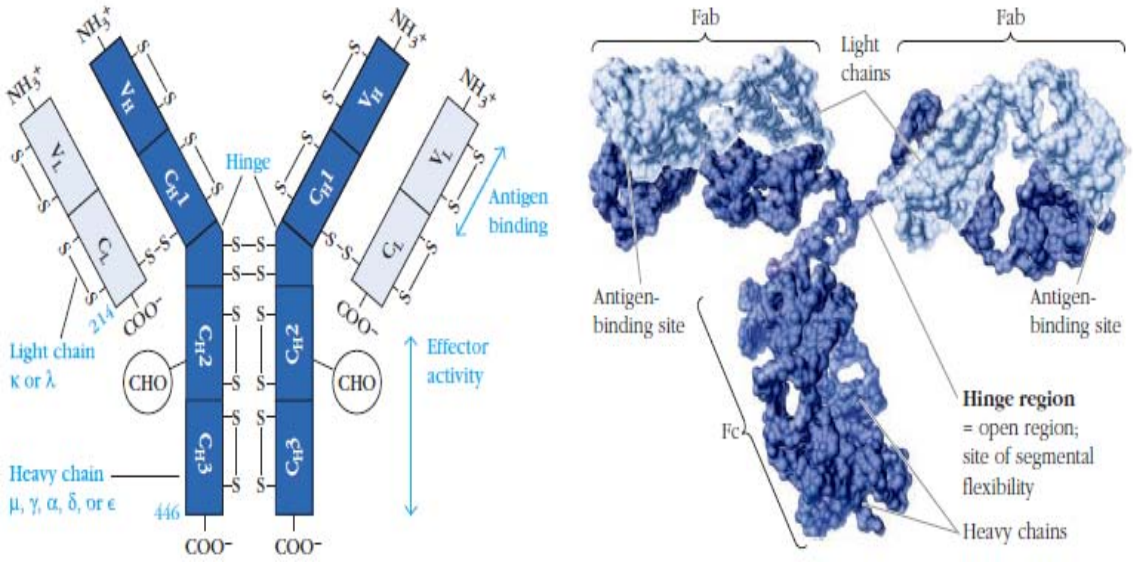


Figure. Schematic diagram of immunoglobulins (Adopted from Kuby,Immunology)

Antibodies are secreted by B cells of the adaptive immune system, mostly by differentiated B cells called plasma cells. Antibodies can occur in two physical forms, a soluble form that is secreted from the cell to be free in the blood plasma, and a membrane-bound form that is attached to the surface of a B cell and is referred to as the B-cell receptor (BCR). The BCR is found only on the surface of B cells and facilitates the activation of these cells and their subsequent differentiation into either antibody factories called plasma cells or memory B cells that will survive in the body and remember that same antigen so the B cells can respond faster upon future exposure.[6] In most cases, interaction of the B cell with a T helper cell is necessary to produce full activation of the B cell and, therefore, antibody generation following antigen binding. Soluble antibodies are released into the blood and tissue fluids, as well as many secretions to continue to survey for invading microorganisms.

Antibodies are glycoproteins belonging to the immunoglobulin superfamily. They constitute most of the gamma globulinfraction of the blood proteins. They are typically made of basic structural units-each with two large heavy chains and two small light chains. There are several

different types of antibody heavy chains that define the five different types of crystallisable fragments (Fc) that may be attached to the antigen-binding fragments. The five different types of Fc regions allow antibodies to be grouped into five *isotypes*. Each Fc region of a particular antibody isotype is able to bind to its specific Fc Receptor (except for IgD, which is essentially the BCR), thus allowing the antigen-antibody complex to mediate different roles depending on which FcR it binds. The ability of an antibody to bind to its corresponding FcR is further modulated by the structure of the glycan(s) present at conserved sites within its Fc region. The allergen-IgE-FcRε interaction mediates allergic signal transduction to induce conditions such as asthma. Though the general structure of all antibodies is very similar, a small region at the tip of the protein is extremely variable, allowing millions of antibodies with slightly different tip structures, or antigen-binding sites, to exist. This region is known as the *hypervariable region*. Each of these variants can bind to a different antigen. This enormous diversity of antibody paratopes on the antigen-binding fragments allows the immune system to recognize an equally wide variety of antigens. The large and diverse population of antibody paratope is generated by random recombination events of a set of genesegments that encode different antigen-binding sites (or *paratopes*), followed by random mutations in this area of the antibody gene, which create further diversity. This recombinational process that produces clonal antibody paratope diversity is called V(D)J or VJ recombination. Basically, the antibody paratope is polygenic, made up of three genes, V, D, and J. Each paratope locus is also polymorphic, such that during antibody production, one allele of V, one of D, and one of J is chosen. These gene segments are then joined together using random genetic recombination to produce the paratope. The regions where the genes are randomly recombined together is the hyper variable region used to recognise different antigens on a clonal basis. Antibody genes also re-organize in a process called class switching that changes the one type of heavy chain Fc fragment to another, creating a different isotype of the antibody that retains the antigen-specific variable region. This allows a single antibody to be used by different types of Fc receptors, expressed on different parts of the immune system.

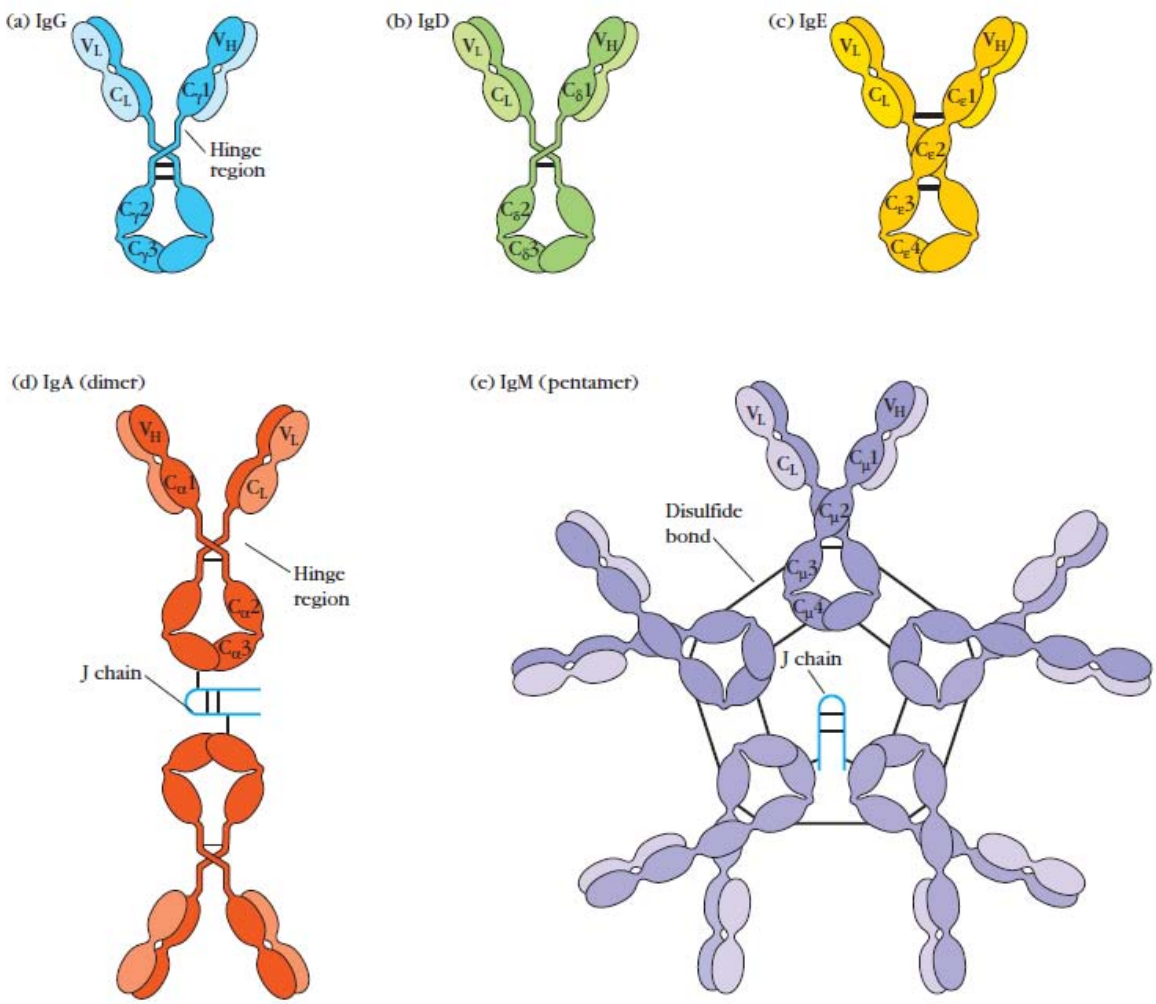


Figure. General structure of five classes of imunoglobulins

Hypersensityvity

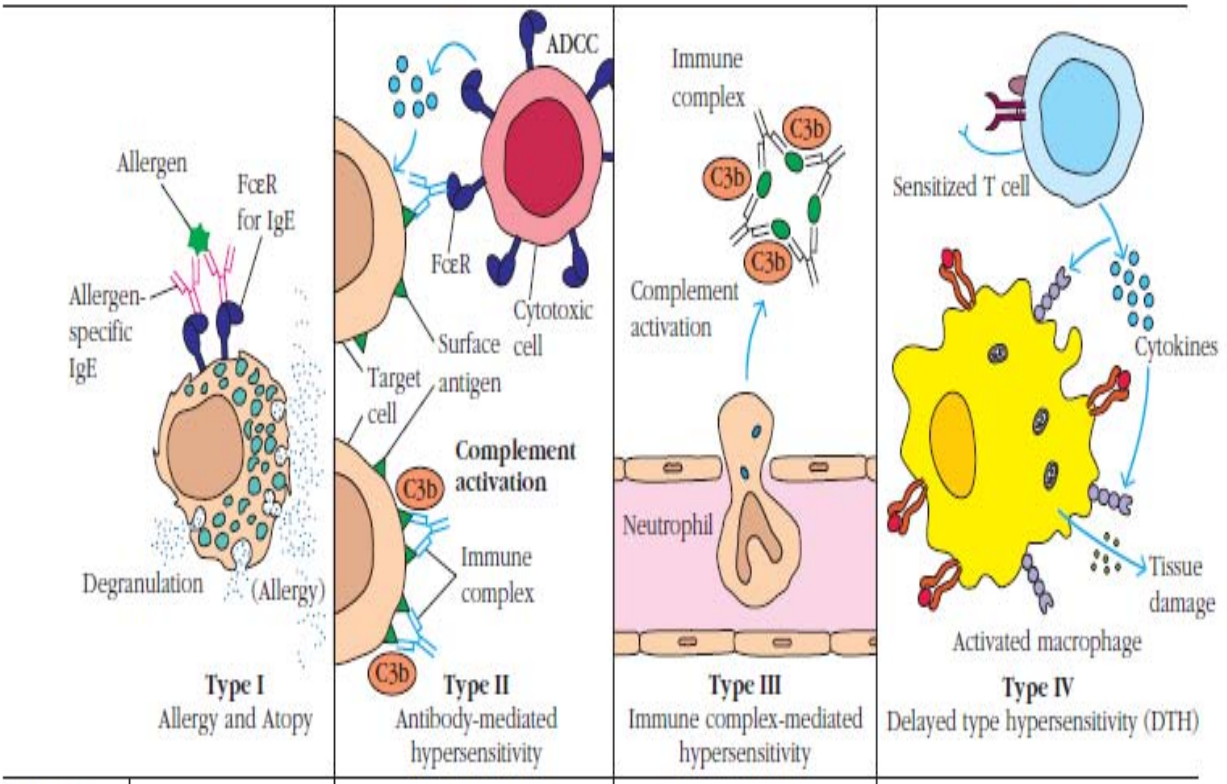
The immune response uses multiple strategies to reduce damage to self by turning off responses when pathogen is cleared and avoiding reactions to self antigens. However, these checks and balances can break down, leading to immune-mediated reactions that are more detrimental than protective. Some immune-mediated disorders are caused by a failure of immune tolerance.

Two French scientists, Paul Portier and Charles Richet, were the fi rst to recognize and describe hypersensitivities. In the early twentieth century, as part of their studies of the responses of bathers in the Mediterranean to the stings of Portuguese man-o'-war jellyfish (*Physalia physalis*), they demonstrated that the toxic agent in the sting was a small protein. They reasoned that eliciting an antibody response that could neutralize the toxin may serve to protect the host.

Since that time, immunologists have learned that there are multiple types of hypersensitivity reactions. **Immediate hypersensitivity** reactions result in symptoms that manifest themselves within very short time periods after the immune stimulus, like those described above. Other types of hypersensitivity reactions take hours or days to manifest themselves, and are referred to as ***d*elayed-*t*ype *h*ypersensitivity (DTH)** reactions. In general, immediate hypersensitivity reactions result from antibody-antigen reactions, whereas DTH is caused by T-cell reactions.

As it became clear that diff erent immune mechanisms give rise to distinct hypersensitivity reactions, two immunologists, P. G. H. Gell and R. R. A. Coombs, proposed a classifi cation scheme to discriminate among the various types of hypersensitivity.

Type I hypersensitivity reactions are mediated by IgE antibodies, and include many of the most common allergies to respiratory allergens, such as pollen and dust mites. **Type II hypersensitivity** reactions result from the binding of IgG or IgM to the surface of host cells, which are then destroyed by complement- or cell-mediated mechanisms. In **type III hypersensitivity** reactions, antigen-antibody complexes deposited on host cells induce complement fi xation and an ensuing infl ammatory response. **Type lV hypersensitivity** reactions result from inappropriate T-cell activation. It should be noted that, although this classifi cation method has proven to be a useful analytical and descriptive tool, many clinical hypersensitivity disorders include molecular and cellular contributions from components belonging to more than one of these categories. Th e subdivisions are not as frequently evoked in real-world clinical settings as they once were.



Immune mediator	IgE	IgG or IgM	Immune complexes	T cells
Mechanism	Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.	Ab directed against cell surface antigens mediates cell destruction via complement activation or ADCC.	Ag-Ab complexes deposited in various tissues induce complement activation and an ensuing inflammatory response mediated by massive infiltration of neutrophils.	Sensitized T cells (T_H1, T_H2 and others) release cytokines that activate macrophages or T_C cells which mediate direct cellular damage.
Typical manifestat-ions	Includes systemic anaphylaxis and localized anaphylaxis such as hay fever, asthma, hives, food allergies, and eczema.	Includes blood transfusion reactions, erythroblastosis fetalis, and autoimmune hemolytic anemia.	Includes localized Arthus reaction and generalized reactions such as serum sickness, necrotizing vasculitis, glomerulonephritis, rheumatoid arthritis, and systemic lupus erythematosus.	Includes contact dermatitis, tubercular lesions, and graft rejection.

Figure. The four types of hypersensitivity reaction. (Adopted from Kuby, immunology)

Vaccine

As previously noted, the diseases for which we already have vaccines are ones in which the infection is acute and either resolves in several weeks or causes death of the infected individual. In the absence of vaccination, many individuals would survive as a result of the immune system defeating the invader. For these diseases, the vaccine mimics the pathogen and causes an immune response similar to that raised by the pathogen. These vaccines have eradicated smallpox, pushed polio to the brink of extinction, and spared countless individuals from hepatitis A and B, measles, rotavirus disease, tetanus, typhus, and other dangerous diseases.

In contrast, successful vaccines have yet to be developed for too many deadly or debilitating diseases (AIDS, herpes, hepatitis C, malaria, tuberculosis) because they are due to chronic infections. In a chronic infection, the pathogen is able to evade the immune system or subvert it into making ineffective responses. Successful vaccines against these chronic diseases must be able to stimulate immune responses that are similar to those resulting from most natural exposures to the pathogen. To date, this has been an unsolvable challenge in medical immunology and vaccine development. In the remainder of this section, various approaches to the design of vaccines, both currently used vaccines and experimental ones, are described and examined in terms of their ability to induce humoral and cell-mediated immunity and the production of memory cells. **Vaccinomics,** the application of genomics and bioinformatics to vaccine development, is bringing a fresh approach to the Herculean problem of making vaccines against various microorganisms and parasites.

Whole-Organism Vaccines

Many of the current vaccines in use for humans that are effective against viral and bacterial diseases consist of whole microorganisms that are either inactivated (killed) or **attenuated** (live but avirulent). These are termed **whole-organism vaccines.** Inactivated vaccines are effective, but they often require several boosters and normally do not adequately stimulate cell-mediated immunity or secretory IgA production. In contrast, attenuated vaccines usually are given in a single dose and stimulate both humoral and cell-mediated immunity. Even though whole-organism vaccines are considered the “gold standard” of existing vaccines, they can be problematic in their own way. For example, whole-organism vaccines fail to shield against some diseases. Attenuated vaccines that do work can also cause full-blown illness in individuals whose immune system is compromised (AIDS patients, cancer patients undergoing chemotherapy, the elderly). These same individuals may also contract the disease from healthy people who have

been vaccinated recently. Moreover, attenuated viruses can at times mutate in ways that restore virulence, as has happened in some monkeys given an attenuated simian form of the AIDS virus. In the case of very lethal diseases, the risk of reversion to virulence is intolerable. Whole-organism vaccines, whether live or dead, have another big drawback. Since they are composed of complete pathogens, they retain molecules that are not involved in evoking immunity. These molecules, as well as contaminants that are unavoidable byproducts of the manufacturing process, can trigger allergic or other disruptive reactions.

Purified Macromolecules as Vaccines

A few of the common risks associated with whole-organism vaccines can be avoided by using only specific, purified macromolecules derived from pathogenic microorganisms. Currently, there are three general forms of **macromolecule vaccines:** (1) capsular polysaccharides, (2) recombinant surface antigens, and (3) inactivated exotoxins called **toxoids.**

Recombinant-Vector Vaccines

Genetic vaccines are quite different in structure from wholeorganism vaccines. It is now possible to isolate genes that encode major antigens from a pathogen and insert them into nonvirulent viruses or bacteria. The vaccines are usually delivered by needle injection or by a device called a gene gun. The attenuated microorganism serves as a vector, replicating within the host and expressing the gene product of the pathogen–encoded antigenic proteins. The antigens can elicit humoral immunity when they escape from the vector, and they can also elicit cellular immunity when they are broken down and properly displayed on the cell surface (just as occurs when cells harbor an active pathogen). Recently several microorganisms have been used in the production of these **recombinant-vector vaccines.** Examples include adenovirus, vaccinia virus, canarypox virus, attenuated poliovirus, and attenuated strains of *Salmonella* and *Mycobacterium.*

DNA Vaccines

A more complicated genetic vaccine to emerge in recent years is the DNA vaccine. A **DNA vaccine** elicits protective immunity against a microbial pathogen by activating both branches of the immune system: humoral and cellular. Long-lasting memory cells also are generated. The immunization procedure begins with the injection into muscle of a plasmid preparation that contains genes for pathogen antigens. The plasmids are taken up by muscle cells, enter the cell nuclei, and express their antigen genes. The muscle cells commence protein synthesis and produce the pathogen's antigenic proteins.

12. Air, water, and soil-borne disease – causal organism, symptoms, control.

Airborne diseases

An **airborne disease** is any disease that is caused by pathogens that can be transmitted through the air. Such diseases include many of considerable importance both in human and veterinary medicine. The relevant pathogens may be viruses, bacteria, or fungi, and they may be spread through breathing, talking, coughing, sneezing, raising of dust, spraying of liquids, toilet flushing or any activities which generates aerosol particles or droplets. Human airborne diseases do not include conditions caused by air pollution such as volatile organic compounds (VOCs), gasses and any airborne particles, though their study and prevention may help inform the science of airborne disease transmission.

Airborne diseases include any that are caused via transmission through the air. Many airborne diseases are of great medical importance. The pathogens transmitted may be any kind of microbe, and they may be spread in aerosols, dust or liquids. The aerosols might be generated from sources of infection such as the bodily secretions of an infected animal or person, or biological wastes such as accumulate in lofts, caves, garbage and the like. Such infected aerosols may stay suspended in air currents long enough to travel for considerable distances, though the rate of infection decreases sharply with the distance between the source and the organism infected.

Airborne pathogens or allergens often cause inflammation in the nose, throat, sinuses and the lungs. This is caused by the inhalation of these pathogens that affect a person's respiratory system or even the rest of the body. Sinus congestion, coughing and sore throats are examples of inflammation of the upper respiratory air way due to these airborne agents. Air pollution plays a significant role in airborne diseases which is linked to asthma. Pollutants are said to influence lung function by increasing air way inflammation.

Many common infections can spread by airborne transmission at least in some cases, including: Anthrax (inhalational),Chickenpox, Influenza, Measles, Smallpox, Cryptococcosis, and Tuberculosis.

Airborne diseases can also affect non-humans. For example, Newcastle disease is an avian disease that affects many types of domestic poultry worldwide which is transmitted via airborne contamination. Often, airborne pathogens or allergens cause inflammation in the nose, throat,

sinuses, and the upper airway lungs. Upper airway inflammation causes coughing congestion, and sore throat. This is caused by the inhalation of these pathogens that affect a person's respiratory system or even the rest of the body. Sinus congestion, coughing and sore throats are examples of inflammation of the upper respiratory air way due to these airborne agents.

Causes

An airborne disease can be caused by exposure to a source: an infected patient or animal, by being transferred from the infected person or animal's mouth, nose, cut, or needle puncture. People receive the disease through a portal of entry: mouth, nose, cut, or needle puncture.

Transmission

Airborne transmission of disease depends on several physical variables endemic to the infectious particle. Environmental factors influence the efficacy of airborne disease transmission; the most evident environmental conditions are temperature and relative humidity. The sum of all the factors that influence temperature and humidity, either meteorological (outdoor) or human (indoor), as well as other circumstances influencing the spread of the droplets containing the infectious particles, as winds, or human behavior, sum up the factors influencing the transmission of airborne diseases.

Climate, living area, Rainfall (number of rainy days being more important than total precipitation), mean of sunshine daily hours, latitude, altitude are characteristic agents to take in account when assessing the possibility of spread of any airborne infection. Furthermore, some infrequent or exceptional extreme events also influence the dissemination of airborne diseases, as tropical storms, hurricanes, typhoons, or monsoons. Climate conditions determine temperature, winds and relative humidity in any territory, either all year around or at isolated moments (days or weeks). Those are the main factors affecting the spread, duration and infectiousness of droplets containing infectious particles. For instance, influenza virus, is spread easily in northern countries (north hemisphere), because of climate conditions which favour the infectiousness of the virus but on the other hand, in those countries, lots of bacterial infections cannot spread outdoor most of the year, keeping in a latent stage.

UV is harmful to both viruses and bacteria. UV incidence can determine the survival of the infectious particles, so that in those territories with a higher average of sunshine daily hours, and closer to the equator, some particles lose their infectious ability. Infectious particles show and increased survival in the presence of UV light at higher relative humidity levels. It is thought to be due to the protective effect of larger particle sizes, as evaporation would be less at these higher RH levels, showing a protective effect of a thicker water coat.

After isolated events, as tropical storms, has been determined that firstly the quantity of fungal spores is decreased, but a few days later, an exponentially increased number of spores is found, compared to normal conditions.

Socioeconomics and living conditions: They have a minor role in airborne diseases transmission, but they also have to be taken in consideration. Dwelling is an important aspect. In cities the spread of diseases is faster than in rural areas and outskirts. Normally, cities enclose quarters of buildings, in which the transmission of the viral and bacterial diseases among the neighborhoods is uncomplicated. However, suburban areas are generally more favourable for higher airborne fungal spores. Nearness to large sources of water as rivers and lakes can be a cause of some outbreaks of airborne diseases, after changes in local watershed. Poor sewage systems are usually found in poor countries, especially in the rural areas, and can determine the proliferation of infectious bacteria, that once infecting animal or humans can be transmitted throughout the air.

Working conditions, can also settle infectious airborne diseases. At indoor environments, temperature and relative humidity are mainly affected by HVAC systems (heating, ventilation and air conditioning). Inadequate ventilation is implicated in the airborne transmission of respiratory viruses. Poor maintenance or defects on those systems can foster the conditions for airborne infections. For instance, a poor maintenance of air conditioning systems, can lead to an outbreak of Legionella (mainly Legionella pneumophila), that will spread among the population of the building (workers), before the finding of the focal point. In hospitals, isolation of patients sick of infectious diseases has to be added as a factor, which is noticeable in poor regions, where lack of resources facilitates the spread of infectious diseases.

Prevention

Some ways to prevent airborne diseases include washing hands, using appropriate hand disinfection, getting regular immunizations against diseases believed to be locally present, wearing a respirator and limiting time spent in the presence of any patient likely to be a source of infection. Exposure to a patient or animal with an airborne disease does not guarantee receiving the disease. Because of the changes in host immunity and how much the host was exposed to the particles in the air makes a difference to how the disease affects the body.

Antibiotics are not prescribed for patients to control viral infections. They may however be prescribed to a flu patient for instance, to control or prevent bacterial secondary infections. They also may be used in dealing with air-borne bacterial primary infections, such as pneumonic plague. Additionally the Centers for Disease Control and Prevention (CDC) has told consumers about vaccination and following careful hygiene and sanitation protocols for airborne disease prevention. Consumers also have access to preventive measures like UV Air purification devices that FDA and EPA-certified laboratory test data has verified as effective in inactivating a broad array of airborne infectious diseases. Many public health specialists recommend social distancing to reduce the transmission of airborne infections.

Waterborne diseases

Waterborne diseases are conditions caused by pathogenic micro-organisms that are transmitted in water. Disease can be spread while bathing, washing or drinking water, or by eating food exposed to infected water. Various forms of waterborne diarrheal disease are the most prominent examples, and affect children in developing countries most dramatically.

Infections by type of pathogen

Disease and Transmission	**Microbial agents**	**Sources of Agent in Water Supply**	**General Symptoms**
Protozoan			
Amoebiasis	Entamoeba histolytica	Sewagw, non-treated drinking water, flies in water supply, saliva	Weight loss,diarrhea, bloating, fever
Giardiasis	Giardia lamblia	Untreated water, poor disinfection, pipe-breaks, ground water comtamination	Diarrhea, abdominal discomort, bloating
Bacterial			
Botulism	Clostridium botulinum	Contaminated water sources, gastrointestinal tract	Dr mouth,blurred or blevision,muscle weakness
E.coli Infection	Escherichia coli	Contaminated water	Diarrhea, dehydration, prolonged illness
Dysentery	Shigella and Salmonella	Contaminated water	Requent passage o feces with blood or mucus in some cases vomiting of blood
Viral			
SARS	Coronavirus	Manifests itself in improper treated water	Fever, myalgia, lethargy,gastrointestine symptoms

Hepatitis A	Hepatitis A virus	Water and food	Fatigue, fever, abnormal pain,nausea diarrhea etc.
Agal			
Desmodesmus infection	Desmodesmus armatus	Naturally occurs in water can enter opn wounds	Similar to fingal infecton

Food brone disease:

Foodborne illness (also **foodborne disease** and colloquially referred to as **food poisoning**) is any illness resulting from the food spoilage ofcontaminated food, pathogenic bacteria, viruses, or parasites that contaminate food, as well as toxins such as poisonous mushrooms and various species ofbeans that have not been boiled for at least 10 minutes.

Symptoms vary depending on the cause, and are described below in this article. A few broad generalizations can be made, e.g.: The incubation periodranges from hours to days, depending on the cause and on how much was consumed. The incubation period tends to cause sufferers to not associate the symptoms with the item consumed, and so to cause sufferers to attribute the symptoms to gastroenteritis for example.

Symptoms often include vomiting, fever, and aches, and may include diarrhea. Bouts of vomiting can be repeated with an extended delay in between, because even if infected food was eliminated from the stomach in the first bout, microbes (if applicable) can pass through the stomach into the intestine and begin to multiply. Some types of microbes stay in the intestine, some produce atoxin that is absorbed into the bloodstream, and some can directly invade deeper body tissues.

Foodborne illness usually arises from improper handling, preparation, or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. There is a consensus in the public health community that regular hand-washing is one of the most effective defenses against the spread of foodborne illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as food safety. Foodborne disease can also be caused by a large variety of toxins that affect the environment.

Furthermore, foodborne illness can be caused by pesticides or medicines in food and natural toxic substances such as poisonous mushrooms or reef fish.

Bacteria

Bacteria are a common cause of foodborne illness. In the United Kingdom during 2000, the individual bacteria involved were the following: *Campylobacter jejuni*77.3%, *Salmonella* 20.9%, *Escherichia coli* O157:H7 1.4%, and all others less than 0.56%.[4] In the past, bacterial infections were thought to be more prevalent because few places had the capability to test for norovirus and no active surveillance was being done for this particular agent. Toxins from bacterial infections are delayed because the bacteria need time to multiply. As a result, symptoms associated with intoxication are usually not seen until 12–72 hours or more after eating contaminated food. However, in some cases, such as Staphylococcal food poisoning, the onset of illness can be as soon as 30 minutes after ingesting contaminated food.

Most common bacterial foodborne pathogens are:

- *Campylobacter jejuni* which can lead to secondary Guillain–Barré syndrome and periodontitis.
- *Clostridium perfringens*, *the* "cafeteria germ
- *Salmonella* spp. – its *S. typhimurium* infection is caused by consumption of eggs or poultry that are not adequately cooked or by other interactive human-animal pathogens
  Salmonella
- *Escherichia coli O157:H7* enterohemorrhagic (EHEC) which can cause hemolytic-uremic syndrome

Other common bacterial foodborne pathogens are:

- *Bacillus cereus*
- *Escherichia coli*, *other* virulence properties, *such* as enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC or EAgEC)
- *Listeria monocytogenes*
- *Shigella* spp.
- *Staphylococcus aureus*
- *Staphylococcal enteritis*
- *Streptococcus*
- *Vibrio cholerae*, *including* O1 and non-O1
- *Vibrio parahaemolyticus*
- *Vibrio vulnificus*
- *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*

Less common bacterial agents:

- *Brucella* spp.
- *Corynebacterium ulcerans*
- *Coxiella burnetii* or Q fever
- *Plesiomonas shigelloides*

Enterotoxins

In addition to disease caused by direct bacterial infection, some foodborne illnesses are caused by enterotoxins (exotoxinstargeting the intestines). Enterotoxins can produce illness even when the microbes that produced them have been killed. Symptom appearance varies with the toxin but may be rapid in onset, as in the case of enterotoxins of *Staphylococcus aureus* in which symptoms appear in one to six hours. This causes intense vomiting including or not including diarrhea (resulting in staphylococcal enteritis), and staphylococcal enterotoxins (most commonly staphylococcal enterotoxin A but also including staphylococcal enterotoxin B) are the most commonly reported enterotoxins although cases of poisoning are likely underestimated. It occurs mainly in cooked and processed foods due to competition with other biota in raw foods, and humans are the main cause of contamination as a substantial percentage of humans are persistent carriers of *S. aureus*. The CDC has estimated about 240,000 cases per year in the United States.

- *Clostridium botulinum*
- *Clostridium perfringens*
- *Bacillus cereus*

The rare but potentially deadly disease botulism occurs when the anaerobic bacterium *Clostridium botulinum* grows in improperly canned low-acid foods and produces botulin, a powerful paralytic toxin.

Pseudoalteromonas tetraodonis, certain species of Pseudomonas and Vibrio, *and* some other bacteria, produce the lethaltetrodotoxin, which is present in the tissues of some living animal species rather than being a product of decomposition.

Emerging foodborne pathogens

Many foodborne illnesses remain poorly understood.

- *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*

Preventing bacterial food poisoning

Proper storage and refrigeration offood help in the prevention of food poisoning

Prevention is mainly the role of the state, through the definition of strict rules ofhygiene and a public services of veterinary surveying of animal products in the food chain, from farming to the transformation industry and delivery (shops and restaurants). This regulation includes:

traceability: in a final product, it must be possible to know the origin of the ingredients (originating farm, identification of the harvesting or of the animal) and where and when it was

processed; the origin of the illness can thus be tracked and solved (and possibly penalized), and the final products can be removed from the sale if a problem is detected;

- enforcement of hygiene procedures such as HACCP and the "cold chain";
- power of control and of law enforcement of veterinarians.

In August 2006, the United States Food and Drug Administration approved Phage therapy which involves spraying meat with viruses that infect bacteria, and thus preventing infection. This has raised concerns, because without mandatory labelling consumers would not be aware that meat and poultry products have been treated with the spray.

At home, prevention mainly consists of good food safety practices. Many forms of bacterial poisoning can be prevented by cooking it sufficiently, and either eating it quickly or refrigerating it effectively. Many toxins, however, are not destroyed by heat treatment.

Techniques that help prevent food borne illness in the kitchen are hand washing, rinsing produce, preventing cross-contamination, proper storage, and maintaining cooking temperatures. In general, freezing or refrigerating prevents virtually all bacteria from growing, and heating food sufficiently kills parasites, viruses, and most bacteria. Bacteria grow most rapidly at the range of temperatures between 40 and 140 °F (4 and 60 °C), called the "danger zone". Storing food below or above the "danger zone" can effectively limit the production of toxins. For storing leftovers, the food must be put in shallow containers for quick cooling and must be refrigerated within two hours. When food is reheated, it must reach an internal temperature of 165 °F (74 °C) or until hot or steaming to kill bacteria.

Mycotoxins and alimentary mycotoxicoses

The term alimentary mycotoxicoses refers to the effect of poisoning by Mycotoxins (The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops) through food consumption. Mycotoxinssometimes have important effects on human and animal health. For example, an outbreak which occurred in the UK in 1960 caused the death of 100,000 turkeys which had consumed aflatoxin-contaminated peanut meal. In the USSR in World War II, 5,000 people died due to Alimentary Toxic Aleukia (ALA).[17] The common foodborne Mycotoxins include:

Aflatoxins – originated from Aspergillus parasiticus and Aspergillus flavus. They are frequently found in tree nuts, peanuts, maize, sorghum and other oilseeds, including corn and cottonseeds. The pronounced forms of Aflatoxins are those of B1, B2, G1, and G2, amongst which Aflatoxin B1 predominantly targets the liver, which will result in necrosis, cirrhosis, and carcinoma. In the

US, the acceptable level of total aflatoxins in foods is less than 20 μg/kg, except for Aflatoxin M1 in milk, which should be less than 0.5 μg/kg. The official document can be found at FDA's website.

Altertoxins – are those of Alternariol (AOH), Alternariol methyl ether (AME), Altenuene (ALT), Altertoxin-1 (ATX-1), Tenuazonic acid (TeA) and Radicinin (RAD), originated from Alternaria spp. Some of the toxins can be present in sorghum, ragi, wheat and tomatoes. Some research has shown that the toxins can be easily cross-contaminated between grain commodities, suggesting that manufacturing and storage of grain commodities is a critical practice.

·Citrinin

Citreoviridin

Cyclopiazonic acid

Cytochalasins

Ergot alkaloids / Ergopeptine alkaloids – Ergotamine

Fumonisins – Crop corn can be easily contaminated by the fungi Fusarium moniliforme, and its Fumonisin B1 will cause Leukoencephalomalacia (LEM) in horses, Pulmonary edema syndrome (PES) in pigs, liver cancer in rats andEsophageal cancer in humans. For human and animal health, both the FDA and the EC have regulated the content levels of toxins in food and animal feed.

Fusaric acid

Fusarochromanone

Kojic acid

Lolitrem alkaloids

Moniliformin

3-Nitropropionic acid

Nivalenol

Ochratoxins – In Australia, The Limit of Reporting (LOR) level for Ochratoxin A (OTA) analyses in 20th Australian Total Diet Survey was 1 μg/kg,whereas the EC restricts the content of OTA to 5 μg/kg in cereal commodities, 3 μg/kg in processed products and 10 μg/kg in dried vine fruits.

Oosporeine

Patulin – Currently, this toxin has been advisably regulated on fruit products. The EC and the FDA have limited it to under 50 μg/kg for fruit juice and fruit nectar, while limits of 25 μg/kg for solid-contained fruit products and 10 μg/kg for baby foods were specified by the EC.

Phomopsins

Sporidesmin A

Sterigmatocystin

Tremorgenic mycotoxins – Five of them have been reported to be associated with molds found in fermented meats. These are Fumitremorgen B, Paxilline, Penitrem A, Verrucosidin, and Verruculogen.

Trichothecenes – sourced from Cephalosporium, Fusarium, Myrothecium, Stachybotrys and Trichoderma. The toxins are usually found in molded maize, wheat, corn, peanuts and rice, or animal feed of hay and straw. Four trichothecenes, T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS) and deoxynivalenol (DON) have been most commonly encountered by humans and animals.

Zearalenone

Zearalenols

Viruses

Viral infections make up perhaps one third of cases of food poisoning in developed countries. In the US, more than 50% of cases are viral and noroviruses are the most common foodborne illness, causing 57% of outbreaks in 2004. Foodborne viral infection are usually of intermediate (1–3 days) incubation period, causing illnesses which are self-limited in otherwise healthy individuals; they are similar to the bacterial forms described above.

Enterovirus

Hepatitis A is distinguished from other viral causes by its prolonged (2–6 week) incubation period and its ability to spread beyond the stomach and intestines into the liver. It often results in jaundice, or yellowing of the skin, but rarely leads to chronic liver dysfunction. The virus has been found to cause infection due to the consumption of fresh-cut produce which has fecal contamination.

Hepatitis E

Norovirus

Rotavirus

Parasites

Most foodborne parasites are zoonoses.

Platyhelminthes**:**

Diphyllobothrium sp.

Nanophyetus sp.

Taenia saginata

Taenia solium

Fasciola hepatica

Nematode:

Anisakis sp.

Ascaris lumbricoides

Eustrongylides sp.

Trichinella spiralis

Protozoa:

Acanthamoeba and other free-living amoebae

Cryptosporidium parvum

Cyclospora cayetanensis

Entamoeba histolytica

Giardia amblia

Sarcocystis hominis

Sarcocystis suihominis

Toxoplasma gondii

Natural toxins

Several foods can naturally contain toxins, many of which are not produced by bacteria. Plants in particular may be toxic; animals which are naturally poisonous to eat are rare. In evolutionary terms, animals can escape being eaten by fleeing; plants can use only passive defenses such as poisons and distasteful substances, for example capsaicin in chili peppers and pungent sulfur compounds in garlic and onions. Most animal poisons are not synthesised by the animal, but acquired by eating poisonous plants to which the animal is immune, or by bacterial action.

Alkaloids

Ciguatera poisoning

Grayanotoxin (honey intoxication)

Mushroom toxins

Phytohaemagglutinin (red kidney bean poisoning; destroyed by boiling)

Pyrrolizidine alkaloids

Shellfish toxin, including paralytic shellfish poisoning, diarrhetic shellfish poisoning, neurotoxic shellfish poisoning,amnesic shellfish poisoning and ciguatera fish poisoning

Scombrotoxin

Tetrodotoxin (fugu fish poisoning)

Some plants contain substances which are toxic in large doses, but have therapeutic properties in appropriate dosages.

Foxglove contains cardiac glycosides.

Poisonous hemlock (conium) has medicinal uses.

Other pathogenic agents

Prions, resulting in Creutzfeldt–Jakob disease (CJD) and its variant (vCJD)

13. Industrial production of ethanol

Microbial production of one of the organic feed stocks from plant substances such as molasses is presently used for ethanol production. This alcohol was produced by fermentation in the early days but for many years by chemical means through the catalytic hydration of ethylene.

In modem era, attention has been paid to the production of ethanol for chemical and fuel purposes by microbial fermentation. Ethanol is now-a-days produced by using sugar beet, potatoes, com, cassava, and sugar cane

Both yeasts (*Saccharomyces cerevisiae, S. uvarum S. carlsbergensis, Candida brassicae, C. utilis, Kluyveromyces fragilis, K. lactis*) and bacteria (*Zymomonas mobilis*) have been employed for ethanol production in industries.

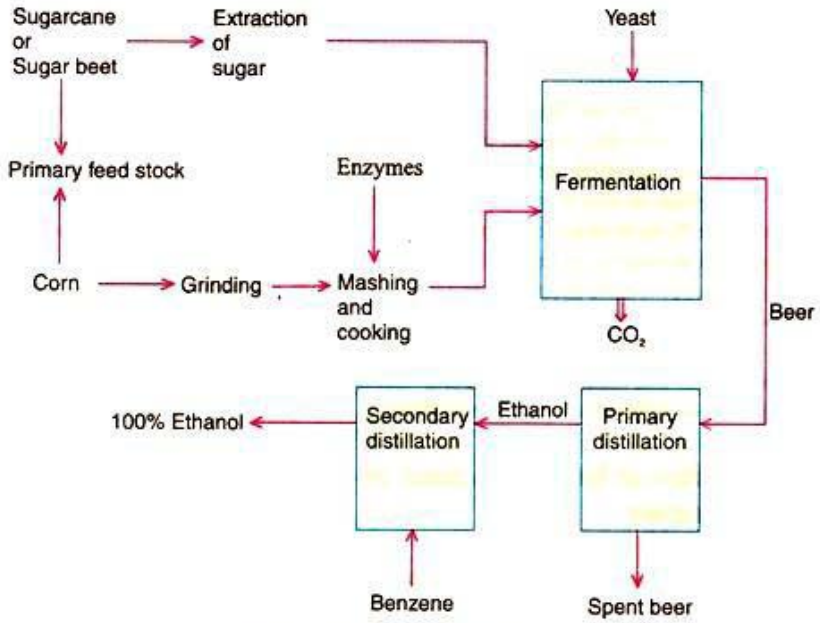


Fig. 20.6: Ethanol production from molasses.

INDUSTRIAL PRODUCTION OF PENICILLIN

In Peoria, Illinois a blue-green mould was found growing on a moldy cantaloupe in aMarket. This mould was identified as *Penicillium chrysogenum* and producedapproximately 200 times as much penicillin than what Florey's team was workingwith (*Penicillium notatum).*Scientists began to try to increase the amount of penicillin produced by *P. chrysogenum* by irradiating it with X-rays and UV rays inorder to induce mutations of this species. They succeeded and developed a mutant thatproduced 1000 times the amount of penicillin than Fleming's original culture. At the same time scientists began to grow the mould in the first deep tank fermenters

INOCULUM DEVELOPMENT-

Inoculum development begins on solid media and subsequently liquid media are used. Inoculate100 ml medium in 500 ml flask with spores of *Penicillium chrysogenum* strains and incubate at 25^{o} C by keeping on a rotator shaker. After 4 days transfer the content of the flask to anotherflask containing the 2 liters medium and incubate for 2 days. The inoculum is prepared usually inthe form of spore suspension, which is transferred into the fermentor by placing it in a metalvessel that is attached to the fermentor.

FERMENTOR-

When a reactor is being designed for a specific purpose there are a number of important parameters that will greatly affect the reactors process performance.

1. *Reactor Size:* How large does the reactor need to be in order to achieveoptimum rates of production? 2. *Reactor Configuration:* How will the reactor be configured? For exampleshould impellers be used to exert mechanical agitation (stirred tank) or will anair-jet system is used for mixing (bubble column) 3. *Mode of operation:* How will substrate be added? Will it be batch fed orcontinuously fed? 4. *Conditions inside the reactor:* What temperature and pH should the reactor bemaintained at? How will contamination be avoided? And how will theseconditions are controlled? Antibiotics are produced in stainless steel fermentor 200-500 m 3 volume. Inoculums of stronglygrowing hyphae are added. Agitation is provided by the impeller batch or fed batch mode. Thefermentor is stem sterilized and loaded with sterilized growth medium but air lift system is alsoused. Oxygen is a vital component for aerobic metabolism. The sparger delivers the oxygen. Oneproblem is the formation of foam and the impellers break the foam. An external cooling jacket isthere to control the temperature. The pH requires adjustment from time to time, to neutralize theammonia produced by the fungi. Temperature is set at first to give the maximum growth rate andthen altered to favour the protein synthesis. Steam is used to keep the reactor free fromcontamination.Aside from what is described above, there are a few specific characteristics of Penicillin that must be considered when attempting fermentation:

- Most penicillin form filamentous broths that are pseudo plastic (non-Newtonian) in nature. This means they can be difficult to mix due to their high(and not constant) viscosity. Also the increasing viscosity of the broth canhinder oxygen transfer. Which leads to the next point?
- Penicillin is an aerobic organism; therefore the rate of oxygen supply is critical to the fermentation. Thus, the reactor must have an efficient oxygen supplysystem.

• The optimum pH for penicillin growth is 6.5. Thus the reactor must maintain pH efficiently (this is frequently done by addition of NaOH

• Strain Stability problems do exist and careful strain maintenance is required.

Biomass doubling is about 6h.

PRODUCTION MEDIUM -

The production medium contains the following compound: Lactose – 3-4% Glucose or Molasses- 10% Corn steep liquor- 4% $CaCO_3$, 1% KH_2 PO4 0.4% Phenyl acetic acid - 0.5–0.8% and Antifoam- 0.25-0.5%

PRODUCTION PROCESS-

Production fermenters are agitated tank 200-250 m3 in volume made of stainless steel.Mechanical agitation is provided at the rate of 100-300 rpm. Temperature is controlled around25-28oC by using cooling coils. Antifoam is added to reduce foam formation. Dissolved oxygenis controlled at >2mg/L and pH at 6.5. Three phases of growth can be differentiated duringcultivation of *Penicillium chrysogenum.*

First phase-

In this phase, growth of the mycelium occurs; yield of antibiotic is quite low.Lactic acid present in corn steep liquor is utilized at the maximum rate by the microorganisms.Lactose is used slowly. Ammonia is liberated into the medium resulting into the rise in pH.

Second phase-

There was intense synthesis of penicillin in this phase, due to rapidconsumption of lactose and ammonia nitrogen.

The mycelia mass increases, the pH remainunchanged.

Third phase-

The concentration of antibiotic decreases in the medium. The autolysis of mycelium starts, liberation of ammonia and slight rise in pH.

RECOVERY-

Once the formation is completed the broth is separated from fungal mycelium and processed byadsorption, precipitation and crystallization to yield the final product. Penicillin is recovered bysolvent extraction at an acidic pH at temperature below 10oC. The solid can be removed by ultrafilteration. Mycelium can be treated, dried and used as soil conditioner. The penicillin

richsolvent can be treated with activated carbon, to remove pigments and other impurities and thepenicillin recovered as the potassium or the sodium salt by adding potassium or sodium acetateto the solvent. Further impurities can be removed by washing the recovered salt with a drysolvent such as isopropanol or n-butanol.

14. Cosmetic microbiology-current trends

Cosmetic products are recognized to be substrates for the survival and development of a large variety of microorganisms, since they possess some of the nutrients that facilitate growth such as: lipids, polysaccharides, alcohol, proteins, amino acids, glucosides, esteroids, peptides, and vitamins. Also, the conditions of readiness (oxygenation, pH, temperature, osmotic degree, superficial activity, perfume, and essential oils) present in the cosmetic products favor microbial multiplication. Routine analyses to determine the microbiological quality of a cosmetic product include the following: Count of mesophilic aerobic microorganisms. Most probable number (MPN) of total coliforms. Count of molds and yeasts. Absence/presence of *Staphylococcus aureu*s probe. Absence/presence of *Pseudomonas aeruginosa* probe.

15. Let’s sum up

About the bacteria section, diversity, classification system, systematic position and molecular identification of bacteria are discussed in some section. The structural organization of cell wall and different growth kinetic parameters also described. Organization and different techniques in replication of genetic material in bacteria are major important section. Microbial ecology with reference to air, water, soil and its different prospect in relation to disease and control are mention. Further more different industrial production of bacterial system and its significance in human welfare are mention.

Suggested Reading

1. Brock biology of microorganism, 10th edition Madigan,Martinko,Bender, Buckley and Stahl. Pearson, New York, San francisco USA.
2. Prescott & Dunn. Industrial Microbology 18. Verma, H.N. Basics of Plant Virology (2003), Oxford and IBH.
3. Dubey, R.C. & Maheswari, D.K. A Text Book of Microbiology, 2005, S.Chand & Company.
4. https://www.wikipedia.org/

Assignments

1. Distinguish between cell wall structure of Gram positive and Gram negative bacteria.
2. Briefly describe the endospore formation of bacteria.
3. With a suitable diagram show the different phases of growth in a bacterial growth curve.
4. Classify bacteria on the basis of nutritional requirements.
5. Describe briefly the process of bacterial transformation.
6. Distinguish food borne infections and food borne intoxication.
7. Describe the structure of different types of immunoglobulins.
8. Define antigen. State the characteristics of antigen.
9. Name the causal organisms, symptoms and control of any food borne disease.
10. Briefly describe the industrial production of penicillin.
11. State the current trends in cosmetic microbiology.

COURSE – BOHCT1.1

Biology and Diversity of Virus, Bacteria and Fungi

Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**

Group – A (Biology and Diversity of Fungi)

Content Structure

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1. Introduction
2. Course Objectives
3. Distinctive features of fungi to form a separate kingdom; modern trends in classification
4. The architecture of fungal cell, cell wall, cell membrane, cell organelles and cytoskeleton, nucleus and its division; biogenesis and protoplast technology; translocation in mycelia
5. Genome organization in fungi; extra chromosomal and transposable genetic elements in fungi
6. Somatic recombination in fungi: heterothallism; heterokaryosis and parasexuality
7. Diversity of somatic, reproductive and fruiting structures in different groups: Myxomycota, Oomycota, Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota, Deuteromycota
8. Fungal spores: types, dispersal, dormancy and germination
9. Let's sum up
10. Suggested Reading
11. Assignment

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1. Introduction

A fungus is any member of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, Fungi, which is separate from the other eukaryotic life kingdoms of plants and animals.

A characteristic that places fungi in a different kingdom from plants, bacteria, and some protists is chitin in their cell walls. Similar to animals, fungi are heterotrophs; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi do not photosynthesise.

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3. Course Objectives

After completion of the course the learners will be able to:

- To know distinctive features of fungi and modern trends in fungi classiiation
- Relate the structure and function of fungal cell
- An understanding of genome organization in fungi
- Be able to distinguish between homothalism and heterothalism
- Define parasextuality
- Describe different group of fungi
- Explain about the fungal spores.

3. Distinctive features of fungi to form a separate kingdom; modern trends in classification

Fungus (pl. fungi) is a Latin word which means mushrooms. Fungi are nucleated, spore bearing, achlorophyllous organisms which generally reproduce sexually and asexually, and whose usually filamentous branched somatic structures are typically surrounded by cell walls containing cellulose or chitin, or both (Alexopoulos, 1952).

In simpler words it may also be defined as "non-green, nucleated thallophytes". The common examples of fungi are the yeasts, molds, mushrooms, polypore's, puff balls, rusts and smuts. The branch of botany that deals with the study of fungi is known as mycology (Gr. mykes = mushroom + logos = discourse) and the person knowing fungi is known as mycologist.

The Italian botanist Pier' Antonio Micheli deserves the honor of being called 'Founder of the science of mycology' because he was the first person to give somatic description of fungi in his book Nova plant-arum Genera published in 1729. Anton De Bary (1831-1888) is called the 'father of modern mycology'. At present about 5100 genera and more than 50,000 species of fungi are known.

Characteristics of Fungi:

1. Fungi are cosmopolitan in distribution i.e., they can grow in any place where life is possible.
2. They are heterotrophic in nature due to the absence of chlorophyll. On the basis of their mode of nutrition, they may be parasite, saprophyte or symbionts.
3. The plant body may be unicellular (Synchytrium, Saccharomyces) or filamentous (Mucor, Aspergillus). The filament is known as hypha (plural, hyphae) and its entangled mass is known as mycelium.
4. The hypha may be aseptate i.e., coenocytic (without septa and containing many nuclei) or septate. The septate mycelium in its cell may contain only one (monokaryotic), two (dikaryotic) or more nuclei.
5. The septa between the cell may have different types of pores: micropore (Geotri- chum), simple pore (most of the Ascomycotina and Deuteromycotina) or dolipore (Basidiomycotina, except rusts and smuts).
6. The cells are surrounded by distinct cell wall (except slime molds), composed of fungal cellulose i.e., chitin; but in some lower fungi (members of Oomycetes), the cell wall is composed of cellulose or glucan.
7. The cells generally contain colourless protoplasm due to absence of chlorophyll, containing nucleus, mitochondria, endoplasmic reticulum, ribosomes, vesicle, microbodies, etc.
8. The cells are haploid, dikaryotic or diploid. The diploid phase is ephemeral (short-lived).
9. In lower fungi like Mastigomycotina, the reproductive cells (zoospores and gametes) may be uni- or biflagellate, having whiplash and/or tinsel type of flagella. But in higher fungi like Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina, motile cells never form at any stage.

10. In response to functional need, the fungal mycelia are modified into different types such as: Plectenchyma, Stroma, Rhizo- morph, Sclerotium, Hyphal trap, Appreso- rium, Haustorium, etc.
11. The unicellular fungi, where entire plant body becomes converted into reproductive unit, are known as holocarpic fungi (e.g., Synchytrium). However, in many others, only a part of the mycelial plant body is converted into reproductive unit, thus they are called eucarpic fungi (e.g., Pythium, Phytophthora).
12. They reproduce by three means: Vegetative, asexual and sexual.
(a) Vegetative reproduction takes place by fragmentation (Mucor, Penicillium, Fusarium), budding (Saccharomyces, Ustilago) and fission (Saccharomyces).
(b) Asexual reproduction takes place by different types of spores. These are zoospores (Synchytrium), conidia (Pythium, Aspergillus), oidia (Rhizo- pus), chlamydospore (Fusarium), etc. The spores may be unicellular (Aspergillus) or multicellular (Alternaria).

Classification of Fungi:

Taxonomy has a dual purpose—first to name an organism according to some internationally accepted system and then to indicate the relationship of the particular organism with other living organisms.

The classification of fungi is still in a state of flux. A stable or ideal scheme is yet to be proposed.

The grouping or categories used in the classification of fungi are as follows:

Kingdom
Division
Class
Order
Family
Genus
Species

The kingdom is the largest of the categories and includes many divisions: each division may include many classes and so on down to the species which is the unit of classification. Each of these categories may be divided into subgroups, subdivisions, subclasses, suborders, if necessary. Species are sometimes broke-down into varieties, biological strains and physiological or cultured races.

In accordance with the recommendations of the committee on International rules of Botanical Nomenclature:

(a) The name of divisions of fungi should end in—mycota.
(b) The name of subdivisions should end in—mycotina.
(c) The name of classes should end in—mycetes.
(d) The name of subclasses should end in—mycetideae.
(e) The name of orders should end in—ales.

(f) The name of families should end in a suffix—aceae.

Genera and species have no standard endings. The name of an organism is binomial. It is composed to parts—the first is noun designating the genus in which the organism has been classified, and the second is often an adjective describing the noun which denotes the species. The first letter of each generic name is always a capital.

Classification of Fungi by Ainsworth G. C. (1966, 71, 73):

Ainsworth G. C. (1966, 71, 73) proposed a more natural system of classification of fungi. This classification is based on morphology, especially of reproductive structure. He includes fungi along with slime molds under the kingdom Mycota.

Based on the presence or absence of Plasmodium and pseudoplasmodium; the kingdom Mycota is further divided into two divisions:

Myxomycota i.e., slime molds and Eumycota or true fungi. Divisions are subsequently divided into subdivision, class, subclass, order, family and then to genus. According to his classification, division ends in mycota, subdivision in mycotina, class in mycetes, subclass in mycetidae order in ales and family in aceae.

A schematic outline of Ainsworth's (1973) classification is given:

Kingdom: Mycota

Important features:

i. Free-living, parasitic or mutualistic symbionts, devoid of chlorophyll.

ii. Cell wall composition is very variable, majority contain chitin and glucan.

iii. Reserve food materials are oil, mannitol and glycogen.

iv. Except some unicellular members, majority are filamentous.

A. Division. Myxomycota:

Wall-less organisms possess either a Plasmodium (a mass of naked multinucleate protoplasm having amoeboid movement) or a pseudoplasmodium (an aggregation of separate amoeboid cells). Both are of slimy consistency, hence slime molds.

1. Class. Acrasiomycetes (cellular slime molds)
2. Class. Hydromyxomycetes (net slime molds)
3. Class. Myxomycetes (true slime molds)
4. Class. Plasmodiophoromycetes (endo- parasitic slime molds).

B. Division Eumycota (True fungi, all with walls):

a. Subdivision Mastigomycotina (motile cells – zoospores present, perfect state spore-oospore).

1. Class. Chitridiomycetes (unicellular, zoospore with single whiplash flagellum).
2. Class. Hyphochytridiomycetes (unicellular, zoospore with single tinsel flagellum).
3. Class. Oomycetes (aseptate mycelium, zoospores with two flagella).

b. Subdivision. Zygomycotina (mycelium aseptate, perfect state spore-zygospore).

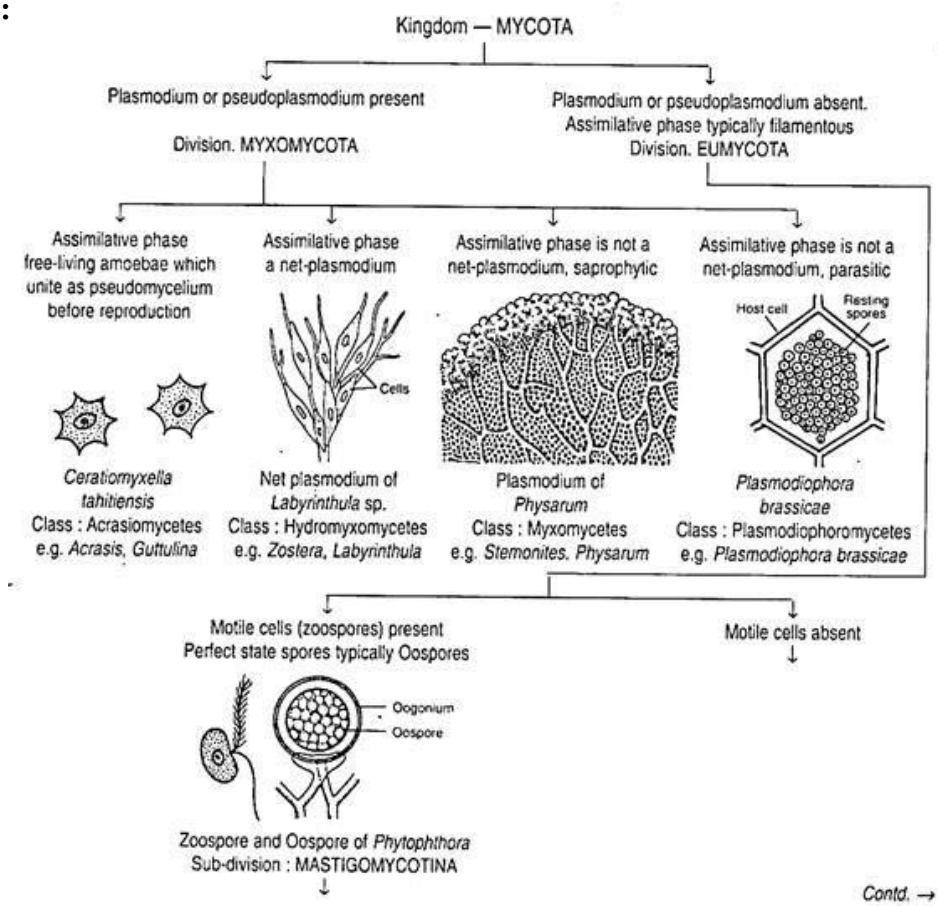
1. Class. Zygomycetes (mycelium immersed in the host tissue).
2. Class. Trichomycetes (mycelium not immersed in the host tissue).

c. Subdivision. Ascomycotina (yeasts or septate mycelium, perfect state spore- ascospores formed in ascus, usually within ascocarp).

1. Class. Hemiascomycetes (no ascocarp, asci naked).

2. Class. Loculoascomycetes (fruit body an ascostroma, asci bitunicate i.e., 2-walled).
3. Class. Plectomycetes (fruit body cleistothecium, asci unitunicate i.e., 1-walled).
4. Class. Laboulbeniomycetes (fruit body perithecium, asci unitunicate, exoparasite of arthopods).
5. Class. Pyrenomycetes (fruit body perithecium, asci unitunicate, not parasitic on arthopods.
6. Class. Discomycetes (fruit body apothecium, asci unitunicate).
d. Subdivision. Basidiomycotina (yeast or septate mycelium, perfect state spore – basidiospore formed on a basidium).
1. Class. Teliomycetes. Basidiocarp lacking, teliospores grouped in sori or scattered within the host tissue, parasitic on vascular plant.
2. Class. Hymenomycetes. Basidio- carp present. Hymenium is completely or partly exposed at maturity. Basidiospore ballistospores.
3. Class. Casteromycetes. Basidiocarp present. Hymenium enclosed in basidiocarp. Basidiospore not ballistospores.
(e) Subdivision. Deuteromycotina or Fungi imperfecti. Yeast or septate mycelium. Perfect state unknown.
1. Class. Blastomycetes. Budding (Yeast or Yeast like) cells with or without pseudomycelium. True mycelium lacking or not well-developed.
2. Class. Hyphomycetes. Mycelia sterile or bearing asexual spore directly or on conidiophore, in various aggregation.
3. Class. Coelomycetes. Mycelial; asexual spore formed in pycnidium or acervulus.

Schematic representation of the outline with figure, the classification of G.C. Ainsworth (1973) is given:



Contd.

Motile cells absent

Sub-division : MASTIGOMYCOTINA

Zoospore uniflagellate (whiplash type)

Synchytrium
Class : Chitridiomycetes
e.g. *Olpidium, Synchytrium*

Zoospore uniflagellate (tinsel type)

Rhizidiomyces
Class : Hyphochytridiomycetes
e.g. *Rhizidiomyces, Rhizidiomycopsis*

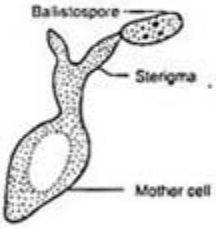
Zoospore biflagellate (one whiplash and other tinsel type)

Phytophthora
Class : Oomycetes
e.g. *Pythium, Phytophthora*

Perfect state absent
Sub-division : DEUTEROMYCOTINA

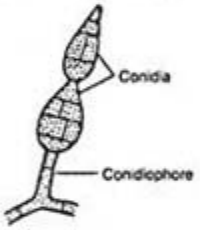
Perfect state present

True mycelium lacking or not well developed



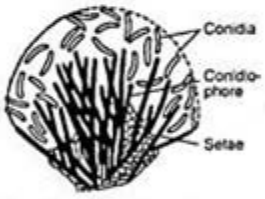
Sporobolomyces roseus
Class : Blastomycetes
e.g. *Sporobolomyces, Bullera*

True mycelium may be sterile or bearing spores directly or on sporophores



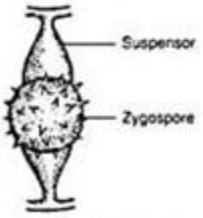
Alternaria
Class : Hyphomycetes
e.g. *Botrytis, Rhizoctonia*

True mycelium aggregated to form pycnidium or acervulus produces spores inside



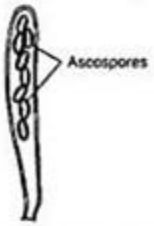
Acervulus of *Colletotrichum*
Class : Coelomycetes
e.g. *Colletotrichum, Pestalotia*

Perfect state spore-Zygospore



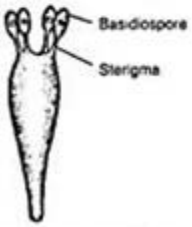
Single zygospore of *Mucor*
Subdivision :
ZYGOMYCOTINA

Perfect state spore-Ascospore



An ascus of *Ascobolus*
Subdivision :
ASCOMYCOTINA

Perfect state spore-Basidiospore



Single basidium of *Agaricus*
Subdivision :
BASIDIOMYCOTINA

Contd. →

Modern trends:

The correct identification of fungi is of great practical importance not only in the clinical setting but also in plant pathology, biodeterioration, biotechnology, and environmental studies. An enormous number of species of fungi are already known, and so taxonomists are being kept very busy with recognizing and describing new species and grouping taxa.

Molecular Techniques

Since the distinguishing morphological characteristics of a fungus are frequently too limited to allow its identification, physiological and biochemical techniques are applied, as has been routinely done for the yeasts. However, for poorly differentiated filamentous fungi, these methods are laborious, time-consuming, and somewhat variable and provide insufficient taxonomic resolution. In contrast, molecular methods are universally applicable.

Two important technical advances have stimulated the use of molecular techniques. Firstly, the advent of PCR has allowed the analysis of small numbers of fungal cells or even single spores, dried herbarium material, or extinct organisms. Second, the selection of universal oligonucleotide primers specific to fungi has provided easy access to nucleotide sequences.

The aim of molecular studies in biodiversity is fourfold:

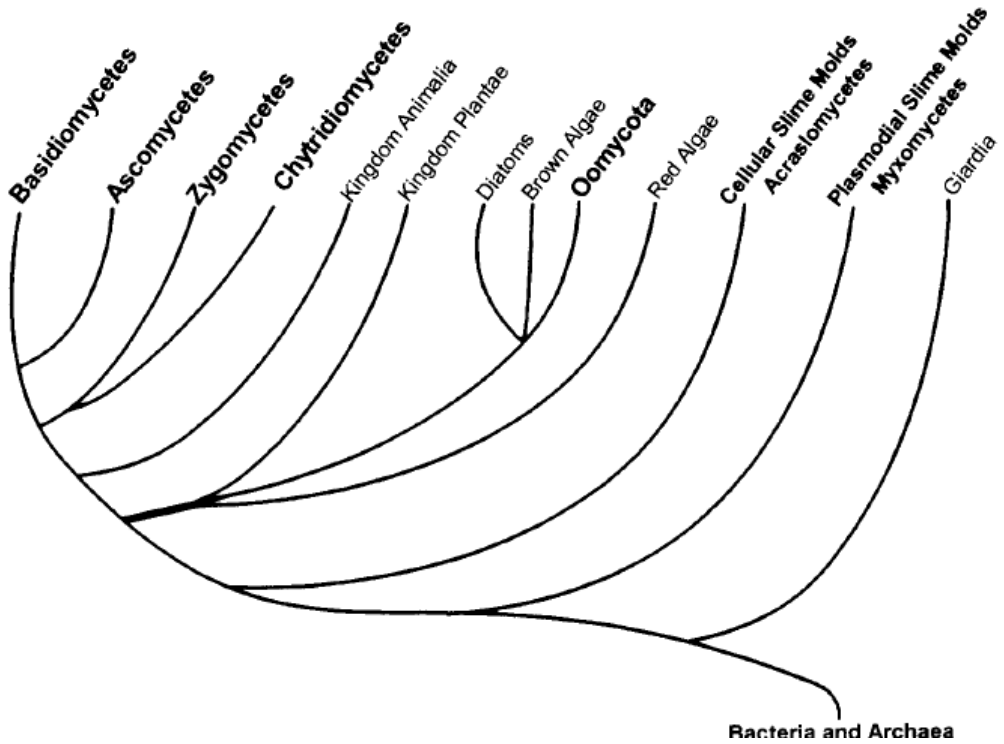
(i) phylogenetic studies, i.e., tracing back the most probable course of evolution and the historic coherence between groups at higher taxonomic ranks;
(ii) taxonomic studies, mostly at the level of genera and species;
(iii) diagnostic applications, i.e., recognition of defined taxonomic entities; and
(iv) epidemiology and population genetics, i.e., monitoring outbreaks of subspecific entities with respect to the analysis of populations and their mode of reproduction. Each of these broad aims and levels of diversity has its own set of optimal techniques. In this review, only phylogenetic and taxonomic studies are discussed.

One of the groups of genes which is most frequently targeted for phylogenetic studies is the one that codes for rRNA. Introns of several protein-encoding genes, such as the β-tubulin, actin, chitin synthase, acetyl coenzyme A synthase, glyceraldehyde-3-phosphate dehydrogenase, lignin peroxidase or orotidine 5′-monophosphate decarboxylase genes, can also be applied and can provide important information. The main reasons for the popularity of rDNA are that it is a multiple-copy, non-protein-coding gene, whose repeated copies in tandem are homogenized by concerted evolution, and it is therefore almost always treated as a single-locus gene. Furthermore, ribosomes are present in all organisms, with a common evolutionary origin. Parts of the molecule are highly conserved and serve as reference points for evolutionary divergence studies. The conserved regions alternate with variable regions or divergent domains. The 5.8S, 18S, and 25S rDNAs are transcribed as a 35S to 40S precursor, along with internal and external transcribed spacers (ITS and ETS). All spacers are spliced out of the transcript. Between each cluster is a nontranscribed or intergenic spacer (NTS or IGS) that serves to separate the repeats from one another on the chromosome. A 5S gene takes a variable position and is transcribed in the opposite direction. The total length of one DNA repeat is between 7.7 and 24 kb.

Comparisons of the 18S (also called the small-subunit [SSU]) rRNA sequences have been performed to assess the relationships of the major groups of living organisms. For phylogeny of filamentous fungi, the 18S sequence is mostly used completely or in subunits of over 600 bp. In the yeasts, the D1 and D2 variable regions of 25S rDNA regions are almost exclusively used. This technique is currently being extended to Heterobasidiomycetes and sometimes also to filamentous ascomycetes. In only a limited number of fungi have both regions been sequenced. Due to this different choice of target regions, comparison of fungi to all possible relatives is hampered. The 25S variable domains are very informative and allow comparisons from high

taxonomic levels down to the species level, although only a limited number of variable positions remain. In the 18S gene, the variable domains mostly provide insufficient information for diagnostic purposes, and thus large parts of the molecule must be sequenced to obtain the resolution required. The ITS regions are much more variable, but sequences can be aligned with confidence only between closely related taxa. These regions are generally used for species differentiation but may also demonstrate patterns of microevolution. In contrast, 5.8S rDNA is too small and has the least variability. 5S has been used mainly to infer relationships at the ordinal level, where differences could be traced back to the secondary structure of the molecule.

The phylogenetic relationships among higher fungal taxa remain uncertain, mainly because of a lack of sound fossil. evidence, and remains a source of much controversy. The proposed phylogenetic relationships among the Animalia, Plantae, and Fungi kingdoms depend on the molecular regions and methods used by different investigators. Phylogenetic analysis has shown that the fungal kingdom is part of the terminal radiation of great eukaryotic groups



Phylogenetic tree based on the nucleotide sequence of 18S rRNA.

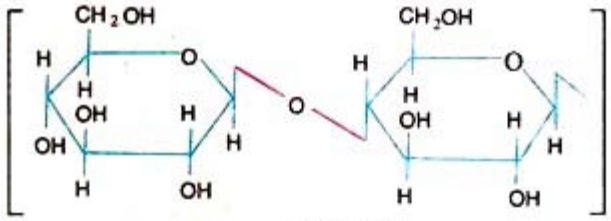
4. The architecture of fungal cell, cell wall, cell membrane, cell organelles and cytoskeleton, nucleus and its division; biogenesis and protoplast technology; translocation in mycelia

a) The Cell Wall of the Fungal Cell:

Except slime molds (Myxomycetes), the fungal cell consists of a rigid cell wall and cell organelles. However, composition of cell wall of different fungal groups differs. Chemical

analysis of cell wall reveals that it contains 80-90% polysaccharides, and remaining proteins and lipids.

Chitin (a polymer of N-acetyl glucosamine), cellulose (a polymer of D-glucose) or other glucans are present in cell walls in the form of fibrils forming layers. In most of the fungi the cell wall lacks cellulose (except Oomycetes) usually chitin and cellulose are found together e.g. Ceratocystis and Rhizidiomyces contain a form of chitin called fungus cellulose. It is similar to the chitin of insects. Structural formulae of repeating units of cellulose and chitin are given in



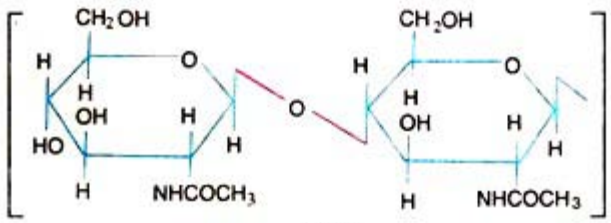


Fig. 4.36 : Structural formula representing the units of cellulose and chitin.

The micro-fibril layers run parallel to the surface. Several non-fibril materials are also associated with micro-fibrils. Though chitin is the most usual component yet cellulose is present in cell walls of Oomycetes along with glucans. An amino acid and hydroxy-protein are present in the cell wall of Oomycetes along with cellulose.

Several other substances have also been found to be associated together in cell walls and cell wall components such as proteins enzymes, etc. In Peronospora and Saprolegnia true cellulose is present, but in Phytophthora and Pythium cellulose is totally absent and glucans predominates in their walls. In the cell wall of some fungi the presence of chitin has been reported.

The basic constituents of cell walls of Zygomycetes, Ascomycetes and Basidiomycetes are chitin. But in yeasts and some Hemiascomycetidae chitin is absent. Micro-fibrils of mannans and β-glucan constitute their cell wall. Various chemical substances found in cell walls seem to be correlated with fungal taxonomy.

Table 4.5 : Taxonomy of fungal cell walls.

Categories	*Taxonomic groups*	*Features*
Cellulose-glycogen	Acrasiales	Pseudoplasmodia
Cellulose-glucan	Oomycetes	Biflagellate zoospores
Cellulose-chitin	Hypochytridiomycetes	Anteriorly uniflagellate zoospores
Chitosan-chitin	Zygomycetes	Zygospores
Chitin-glucan	Chytridiomycetes	Posteriorly unitlagallate zoospore
	Ascomycetes	Septate hyphae, ascospores
	Basidiomycetes	Septate hyphae, basidiospores
	Deuteromycetes	Septate hyphae
Mannan-glucan	Saccharomycetaceae	Yeast cells, ascospore
	Cryptococcaceae	Yeast cells
Mannan- chitin	Sporobolomycetaceae	Yeast cells, ballistospores
	Rhodotorulaceae	Yeast (carotenoid pigment)
Polygalacturosamine-galactan	Trichomycetes	Heterogenous group

(b) The Protoplast in the Fungal Cell:

The living substance of the cell within the cell wall is the protoplast. It lacks the chloroplasts but is differentiated into the other usual cell parts such as plasma or cell membrane, vacuolated cytoplasm, cell organelles and one or more nuclei.

Cell Membrane:

It is a delicate, extremely thin, living membrane which closely invests the protoplast. The cell or plasma membrane is pressed against the cell or hyphal wall except for occasional invaginations in some regions. The Invagination is either in the form of an infolded convoluted pocket or a pouch enclosing granular or vesicular material.

Moore and Mc Lear (1961) named it lomasome. Actually the plasma membrane is the surface layer of the protoplast altered to perform special functions. It is differentially permeable and shows a typical tripartite structure under the electron microscope. There is an electron dense layer on either side of the less dense central region.

Cytoplasm:

Within the plasma membrane is the colorless cytoplasm in which sap-filled vacuoles may occur. In young hyphae and hyphal tips, the cytoplasm appears rather uniform and homogeneous. Immersed in the cytoplasm are structures known as the organelles and inclusions.

The organelles are living structures, each with a specific function. The inclusions are dead, have no specific function and thus are not essential to cell survival.

Amongst the cell organelles are included the endoplasmic reticulum, mitochondria, ribosomes, Golgi apparatus and vacuoles. Lomasomes which are membranous structures lying between the cell wall and plasma membrane are common. Examples of inclusions are the stored foods (glycogen, and oil drops) pigments and secretory granules.

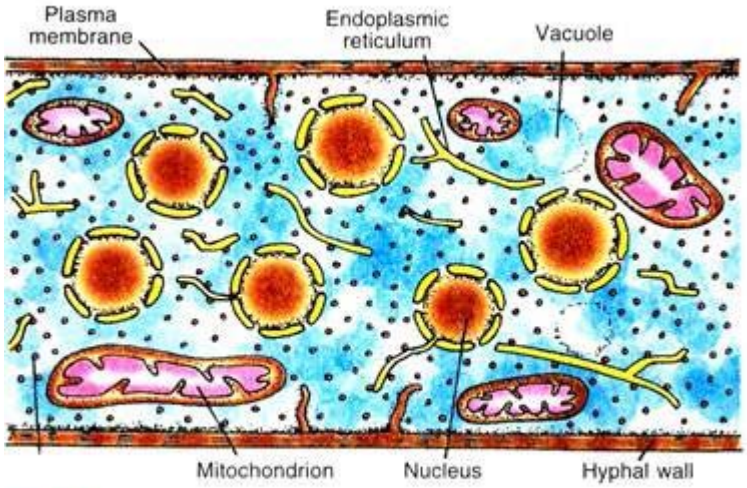


Fig. 1.9. *Fungi*. Fine structure of a hypha near the growing tip of *Mucor* based on an electron micrograph.

(i) Endoplasmic Reticulum:

The presence of endoplasmic reticulum in the fungal cytoplasm has been demonstrated by the use of electron micro-scope. It is composed of a system of membranes or microtubular structures usually beset with small granules which by some scientists are likened to the ribosomes. In many fungi, the endoplasmic reticulum is highly vesicular. Usually it is loose and more irregular than in the cells of green plants.

(ii) Mitochondria: The cytoplasm contains small, usually spherical bodies known as the mitochondria. Each mitochondrion is enveloped by a double membrane. The inner membrane is infolded to form the cristae which are in the form of parallel flat plates or irregular tubules.

The cristae contain the same fluid that fills the space between the two membranes. The mitochondria function as the power house of the cell. There is no fundamental difference between the mitochondria of fungi and those of green plants. However, Hawker (1965) holds that the cristae of fungal mitochondria are fewer, flatter and more irregular than those of the green plants.

(iii) Golgi Apparatus (Dictyosomes):

With the exception of Oomycetes there is less certainty of the occurrence of structures similar to those of the golgi apparatus (dictyosomes) m fungi. Moore and Muhlethaler (1963) reported a golgi apparatus consisting of three flattened sacs surrounded by many bubble-like structures in Saccharomyces cells.

(iv) Vacuole:

The cytoplasm of young hyphae or fungal cells and hyphal tips lacks vacuoles. They appear further back or in the old cells. With age, they enlarge and show a tendency to coalesce and ultimately reduce the cytoplasm to thin lining layer immediately within the cell wall.

(v) Inclusions:

The cytoplasm contains various kinds of inclusions. Examples of stored foods are lipid globules, granules of glycogen, oils and the carbohydrate trehalose, proteinaceous material and volutin. The glycogen may occur in vacuoles.

There are no starch grains. Of the pigments, the fungi lack chlorophyll. Carotenoids are often conspicuous by their presence and may occur throughout the cytoplasm or concentrated in the lipid granules or distributed in the cell wall. The cytoplasm, in addition, secretes several kinds .of ferments, enzymes and organic acids.

Nucleus:

The cytoplasm in the individual cells contains one, two or more globose or ellipsoid nuclei which in the somatic portion are small and usually range from 1-2 or 3μ in diameter. They cannot be seen without special techniques.

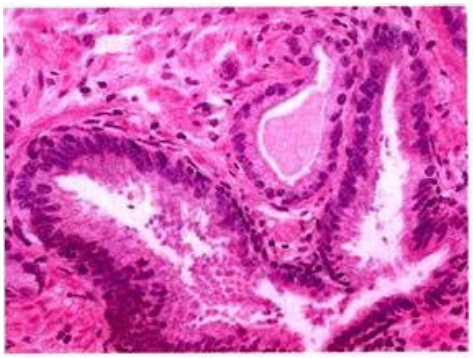
Structurally the nucleus consists of:

(i) A central, dense body with a clear area around it.

(ii) Chromatin strands, and

(iii) The whole structure surrounded by a definite nuclear, membrane.

The central body takes heavy iron haematoxylin stain and is usually Feulgen-negative. In electron micrographs, it appears as an amorphous or granular mass. Mycologists usually designate it as the nucleolus. Bakerspigel (1960) stated that it contains RNA. During nuclear division, the chromatin strands become organised into chromosomes which are extremely small and difficult to count.



Nucleolus.

Under the electron microscope, the nuclear membrane is seen to consist of inner and outer layers of electron dense material and the middle one of electron transparent substance. The nuclear membrane has pores. At certain points, the nuclear membrane is continuous with the endoplasmic reticulum.

Protoplast fusion may be used to produce interspecific or even intergeneric hybrids.Protoplast fusion becomes an important tool of gene manipulation because it breakdown the barriers to genetic exchange imposed by conventional mating systems.Protoplast fusion technique has a great potential for genetic analysis and for strain improvement.It is particularly useful for industrially useful microorganisms

Mechanism of protoplast fusion:

When the protoplasts are brought into close proximity ,this is followed an induction phase thereby changes induced in electrostatic potential of the membrane results in fusion .After the fusion,The membranes stabilizes and the surface potential returns to their former state.Other literature showed when the protoplasts are closely adhered ,the external fusogens cause disturbance in the intramembranous proteins and glycoproteines.This increases membrane fluidity and creates a region where lipid molecule intermix,allowing coalescence of adjacent membranes.The negative charge carried by protoplast is mainly due to intramembranous phosphate groups .The addition of Ca ++ ions causes reduction in the zeta potential of plasma membrane and under this situation protoplasts are fused(Peberdy J.F 1980).The high molecular weight polymer (1000-6000) of PEG acts as a molecular bridges connecting the protoplasts. calcium ions linked the negatively charged PEG and membrane surface .On elution of the PEG ,the surface potential are disturbed ,leading to intramembrane contact and subsequent fusion, Besides this ,the strong affinity of PEG for water may cause local dehydration of the membrane and increase fluidity,thus inducing fusion.Protoplast fusion takes place when the molecular distance between the protoplasts is 10A or less .This indicates that protoplast fusion is highly a traumatic events.(Jogdand S.N,2001)(Narayanswamy S 1994)

Protoplast fusion in fungi:

Production and regeneration of protoplasts is a useful technique for fungal transformations.Commercial preparation of enzymes which contain mixture of products to digest fungal cellwall used .Novozyme 234 includes (glucanase and chitinase) enzyme mixture is added to rapidly growing fungal tissue suspensed in an osmotic buffer (e.g.0.6 mol-1,KCl,1.2 mol-1,Sorbitol or 1.2 mol-1MgSO4).The protoplasts and DNA are mixed in presence of 15%(w/V)PEG 6000 and pH buffer (TRIS HCl).10 mml-1.PEG causes clump formation in protoplasts. At 370 C ,grow mycelium on cellophone placed on agar overnight.Incubte with enzyme at 300 C for 1.5 hours in empty petridish having KCl,than filter protoplasts,wash protoplast in KCl (Centrifuge and resuspended the pellets).Protoplast fusion frequency in fungi is 0.2-2%.

Applications of protoplast fusion technology:

Protoplasts contained all the intracellular organelles of cells and form a vital link in transfer of micromolecules between cyto organelles,currently most of the laboratories engaging in fungal genetics are using gene manipulation procedures based on protoplasts.Therefore to further improve the genetic properties of these strains using protoplast fusion are attempt to develop methods for preparation and regeneration of protoplasts.The process involves protoplast mutagenesis,transformation and protoplast fusion (Evans D.A.1983). The direct bioconversion of cellulosic materials to ethanol by the intergeneric fusants between *T.reesei* and *Saccharomyces cerevesie* appears to be are of the best technique for an alternative approaches for ethanolproduction.

Not unexpectedly, the developments of a fusion technology have attracted interest for application in several genetic fields, and significant advances have been made at intra- and inter-specific and intergeneric levels. Thus protoplast fusion involving like mating types has been obtained in

Saccharomyces cerevisiae and *Schizosaccharomyces pombe*, yielding diploid progeny in both cases. Similar reports have been made for *Rhodosporidium* and *Saccharomycopsis lipolytica*.
In the filamentous fungi protoplast fusion was used successfully to produce heterokaryons between strains of *A. nidulans* that are incompatible by using conventional methods and has proved particularly valuable for basic genetic studies in *Cephalosporium acremonium* and *Candida tropicalis*, two organisms in which the conventional manipulations have proved difficult. The implications of this technique in the breeding of industrially important fungi were demonstrated in the isolation of a segregant that had higher *cephalosporin* C titer and other superior properties to the best parental strain. Protoplast fusion techniques have also proved useful in conventional crosses in yeasts, and recently in providing the possibility for genetic studies in Mucor.
Of broader significance is the use of fusion techniques in the production of interspecific and intergeneric hybrids. The earliest studies suggested that hybrid formation with subsequent segregation of nonparental types could be achieved only if closely related parental species were used, but more recent experiments suggest this might not be the case (3). Crosses between *A. nidulans* and *Aspergillus rugulosus*. both of the same species group, first gave heterokaryons following the reversion of protoplasts on minimal medium. Arising from the heterokaryons were sectors of more vigorous growth, which on the basis of DNA determinations and segregation properties were deemed to be interspecific hybrids whose origin rested in fusion of the two parental nuclei. Crosses with the closely *related Penicillium chrysogenum* and *Penicillium cyaneo-julvum* yielded colony types that behaved similarly.

translocation in mycelia

Most fungi are built up from hyphae, and often a continuous hyphal network, i.e. the mycelium, of a fungal individual may extend over considerable distances (Thompson & Rayner, 1983; Olsson, 1999). Many, if not most, fungi may transport substances between their cells, and some, particularly basidiomycetes, translocate resources such as carbohydrates, nutrients and water freely throughout their whole mycelium (reviewed by: Jennings, 1987; Cairney, 1992; Boddy, 1999; Olsson, 1999). The ability to translocate resources implies that a mycelium is much more than a colony of physically connected, but otherwise independent, hyphae. The whole mycelium may be integrated into a single entity, where the local environment of one part of the mycelium may affect distant parts. Furthermore, the performance of a single hyphal tip may depend on the nutritional status of the whole mycelium. As resources can be removed from areas with a surplus to enable activity at other sites where resources are lacking, translocation makes fungi well adapted to colonise solid substrates with a high degree of spatial heterogeneity. Many studies of mycelial translocation have used radioactive tracer isotopes. For example, Lindahl et al. (2001a) demonstrated transport of radioactive phosphorus between two wood blocks placed ~10 cm apart in soil and connected by mycelium of the wooddegrading fungus Hypholoma fasciculare. When the isotope 32P was added to one of the wood blocks and 33P to the other, phosphorus was found to be transported in both directions simultaneously. Similarly, Tlalka et al., (2002) showed bidirectional translocation of the 14C-labelled amino acid analogue aminoisobutyric acid in mycelia of the wood-degrading fungus Phanerochaete velutina. These microcosm studies confirm the suggestion by Olsson & Gray (1998), that many fungi circulate substances throughout their mycelia. Circulation of resources would facilitate net translocation of resources from sites with high cytoplasmic availability to sites with low cytoplasmic availability, i.e. from sources to sinks.
Many fungi translocate resources, such as carbohydrates, amino acids and phosphate, throughout their mycelia. Translocation enables growth and activity in substrates where available resources

are scarce or absent. Fungi may use translocated resources to build up 'mycelial impact' in a substrate, thereby conditioning the substrate to increase resource availability. A high mycelial impact also enables fungi to interact with other microorganisms; to interfere with other fungi in order to monopolise the substrate, and to modify the composition of the bacterial community. The capacity to translocate makes fungi well adapted to colonise solid substrates where diffusion and mixing is limited. The benefit of translocation is likely to be highest in environments with a high degree of spatial heterogeneity, such as forest soils. A wider knowledge and recognition of how, when and where fungal translocation takes place may radically change established models of processes where fungi are involved, such as decomposition, plant nutrition and plant disease (Lindahl et al., 2002), and may eventually lead to a better understanding of fungus-dominated ecosystems.

5. **Genome organization in fungi; extra chromosomal and transposable genetic elements in fungi**

Fungal genome

Recently, the genome sequencing technology has emerged as one of the most efficient tools that can provide whole information of a genome in a small period of time. Since the completion of genome sequencing of the model fungus *S. cerevisae* in 1996, sequencing of large numbers of fungal genomes are now completed. Sequencing of large numbers of fungal genomes will allow us to understand the diversity of genes encoding enzymes, and pathways that produces several novel compounds. Although the fungi are very diverse in nature, their basic cellular physiology and genetics shares some common components with plants and animal cells. These include multi-cellularity, cytoskeletal structures, cell cycle, circadian rhythm, intercellular signaling, sexual reproduction, development and differentiation

The genome size of model fungi *S. cerevisae* is bit more than 12 Mb. From the studied 172 fungal species, only seven species have genome sizes larger than 100 Mb. So, the probability of occurrence of larger genomes in fungi is very small. The genome size of *Cenococcum geophilum* (177.57 Mb) is the largest and the genome size of *Hansenula polymorpha* (8.97 Mb) is the smallest from the studied species. Both species belong to Ascomycota. In the group of Basidiomycota species, the genome size of *Wallemia sebi* (9.82 Mb) is the smallest one and genome of *Dendrothele bispora* (130.65) is the largest one. No single species from Chytridiomycota, Glomeromycota, Oomycota, Stramenopiles, Mucoromycotina have genome size larger than 100 Mb. Although there is large variation in genome size in fungi, the average genome size of fungal species taken during this study is 42. 30 Mb. The average genome size of Oomycota group of fungi is 74.85 Mb which is the highest among all groups. If we consider about the coding gene sequence in fungi, in average the Acomycota, Basidiomycota, Oomycota and Mucoromycotina groups encodes for 11129.45, 15431.51, 24173.33, 13306 no. of genes respectively in their genomes.

Genomes are aggregates of genes and this concept nicely fits with the prokaryotic organisms and viruses. This concept is very inappropriate for eukaryotic organisms as most of the eukaryotic genomes are studded with nongenic and unconstrained repetitive DNA. This can lead to approximately 200,000 fold variation in genome size. The genome size of an organism depends on the particular developmental and ecological need of the organism. The genes are made up of DNA and it is a general assumption that more complex organisms requires more genes and thus

contain more DNA in its genomes. The simple organisms probably contain fewer essential genes compared to more complex organisms and thus contain less DNA in its genomes.
The smallest eukaryotic genomes, of some yeasts, are in the region of 10 Mb (Mb is the usual abbreviation for a million base pairs or a '***megabase***'), and the largest (in vertebrates and plants) are over 100,000 Mb, so we can observe some surprising structural differences when we compare different eukaryotes. Generally speaking (but remember there are exceptions to all generalisations), it appears that space is saved in the genomes of less complex organisms by having the genes more closely packed together and by having much less repetition (Fig. 14, above).
The genome of *Saccharomyces cerevisiae* contains more genes per unit length of DNA than occur in human or maize DNA. On the other hand, up to 40% of the gene sequences of *Saccharomyces cerevisiae* are ***duplicated***. In most cases the duplicated sequences are so similar that their protein products are identical and, presumably, either functionally redundant, or (more likely) under very different ***regulatory control***. Nevertheless, the small size of the yeast genome is one reason why yeast geneticists and molecular biologists pioneered eukaryote genome analysis.
Although some of the more unusual aspects of genome structure observed in higher animals and plants might not be represented in fungi, the genomes of yeast and other fungi remain good models of eukaryotic genetic architecture and their smaller size means that the information they contain is technically more accessible. In terms of genetic information content, the organisation of the fungal genome is, generally, much more economical than that of animals and plants. Although fungal genes may be generally more compact with fewer introns and shorter spaces between genes, a major difference is that fungi contain much less of the so-called redundant DNA, which is devoted to repetitive noncoding sequences in so many animals and plants. But then, fungi have made specialist use of heterokaryosis, which is uncommon in the other kingdoms. What better way is there to increase genome size than being a syncytium?
So, despite the differences that undoubtedly exist, ***fungi are typical eukaryotes***, featuring all the basic cell biology expected of this grade of organisation. Even though the yeast genome is only in the same size range as some of the more advanced prokaryotes, the genetic structure and functioning of genes of filamentous fungi are representative of all eukaryotes and we can use their sequences to learn about genomics

Transposable elements in fungi:
Unlike most bacterial transposons, which carry genes conferring a selective advantage to the host, for example antibiotic resistance, eukaryotic transposable elements, including those from fungi, carry no selectable genes. Fungal transposons, like other eukaryotic transposons, can be divided into two classes. Class I elements transpose via an RNA intermediate employing a reverse transcriptase. By contrast, most class II elements transpose at the DNA level by excision from a donor site and reintegration elsewhere in the genome. Approximately one-half of all identified fungal transposons belong to class I . So far three types of class I elements are known in filamentous fungi:
1. Retrotransposons: These elements carry long terminal repeats (LTR) and encode gag (viral coat proteins) and pol (reverse transcriptase, RNase H, integrase, and protease) genes. Retrotransposons have been identified in a number of plant pathogens, e.g., Cft-1 in *Cladosporium fulvum* and Skippy from *Fusarium oxysporum*. Although there is no apparent correlation between the genomics location of these elements and the pathogenity of their host, retrotransposons represent useful genetic markers for strain identification. Remarkably, all but

two are members of the gypsy retrotransposon subfamily, with only two (MARS2 and MARS3 being a copia like retrotransposon. The two subfamilies differ in the order of their pol genes.

2. Retroposons, or LINE-like retroelements: These elements usually possess poly-A-tails but no LTRs. LINE elements were originally identified in mammals; intact retroposons of this type also carry gag and pol genes. Fungal members of this group include MARS1 from *Ascobolus immersus* and the Tad element from *N. crassa.* Tad has some interesting features, as it occurs in a fungus that usually inactivates repeated DNAs by a mechanism known as ‘‘RIP’’.

3. SINE-like elements: These elements are derived from RNA polymerase III transcripts. They do not possess special structural features or gag or pol genes. Instead it is believed that they are trans-activated by reverse transcriptases provided by LINE-like elements or retrotransposons. Class II elements in fungi are mostly Fot1/Pogo-like or belong to the Tc1/mariner superfamily. Typical examples for these transposon families are the Fot1 and Impala elements from *F. oxysporum*. It is unknown why this class II transposon family is predominant in fungi. However, since transposon traps were used to identify most of these transposons, the Fot1/Pogo and Tc1/mariner-like elements may simply represent a very active type of transposon that may have been more successful than others in spreading by horizontal gene transfer. Indeed there is evidence for horizontal gene transfer of Fot1 elements in different *Fusarium* species. Members of other families have also been detected in fungi.

6. Somatic recombination in fungi: heterothallism; heterokaryosis and parasexuality

Heterothallism

The term Heterothallism was first used by an American geneticist A.F. Blakeslee in 1904 when he observed that zygospores could develop in some spp. only when two mycelia of different strains were allowed to come in contact with each other.

Blakeslee made these observations as a result of his studies on zygospore formation in Mucorales. He found that in some species of Rhizopus, zygospores were formed freely while in others like R. nigricans zygospores were formed rarely.

In Mucor hiemalis, too zygospores were formed rarely. On the basis of his studies, he divided the various species of Mucorales into two groups: Heterothallic and Homothallic.

Thus heterothallic species are those which require mycelia of two different strains to interact to enable the zygospores to be formed while the homothallic species are those which require myceha of only one strain to interact for the formation of Zygospores.

According to Blakeslee (1904) Heterothallic condition is **“essentially similar to that in dioecious plants and animals and although in this case the two complimentary individuals which are needed for sexual reproduction are in general not so conspicuously differentiated morphologically as in higher forms, such a morphological difference is often distinctly visible.”**

He concluded that the Zygospore formation is a sexual process. In homothallic species, the mycelium is bisexual while the mycelium in heterothallic species is unisexual, (+) and (-) strains represent the two different sexes.

Heterothallism may therefore be defined as the condition in which Zygospore formation takes place only when mycelia arising from asexual spores of two genetically different mating types (+) and (-), are allowed to interact.

On the other hand the condition, in which one individual originating from a single asexual spore is capable of forming zygospores independently, is known as Homothallism.

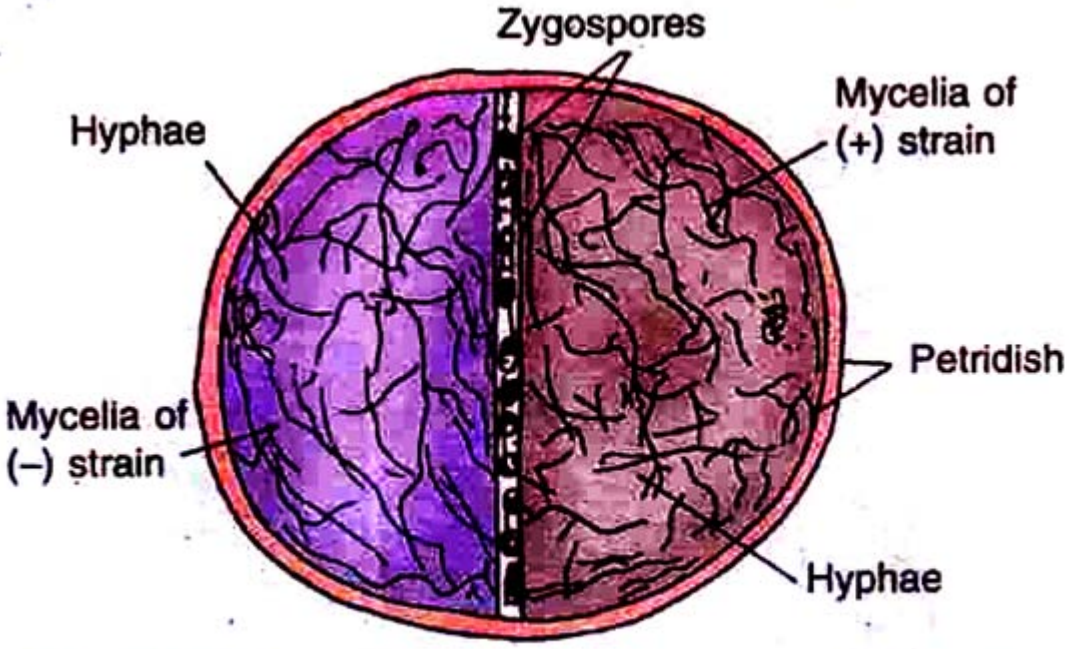


Fig. 17.2 *Mucor hiemalis*. Zygospore formation indicating Heterothallism.

Blakeslee proved the phenomenon of heterothallism on the basis of the experiments he conducted using several species of Mucorales. He inoculated spores of two different strains of Mucor hiemalis on a petridish containing synthetic agar medium.

After a few days it was observed that the zygospores were formed along the zone of contact of two myceha. In another experiment he inoculated spores of only one strain on a petridish containing synthetic agar medium and after some time it was observed that absolutely no zygospores were produced in this experiment.

From these experiments, he concluded that zygospores could be formed only when mycelia of two different strains were allowed to come in contact.

Blakeslee and his coworkers (1928) examined different genera of Mucorales to test whether these were homothallic or heterothallic. Most of the genera tested were homothallic while only a few genera were found to be heterothallic.

In heterothallic species of Mucor mucedo, the zygospores upon germination produced germsporangia which contain spores of only one strain (either + or -).

In this case, zygospores could be formed only when mycelia formed from spores of (-) strain formed in germsporangia produced on germination of zygospores are allowed to come in contact with the mycelia produced from the spores of (+) strain.

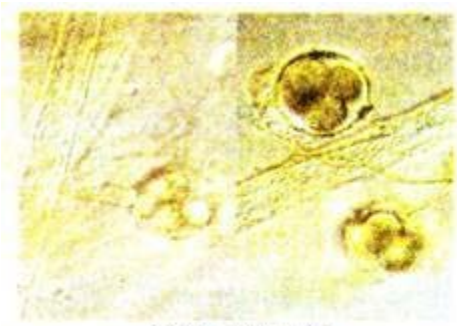
But in the heterothallic species of Phycomyces nitens Spores of (+) and (-) strains are produced in the same germsporangium. Blakeslee thus regarded the (+) and (-) strains of heterothallic species as differing in sex and the term heterothallism may therefore be treated as equivalent to dioecism in haploid organisms.

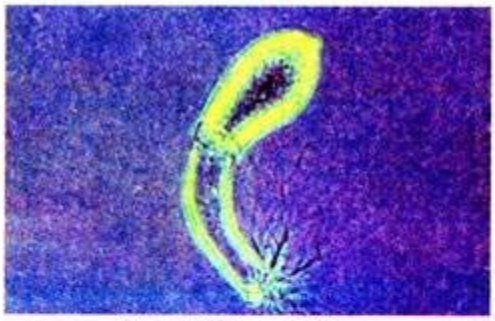
Since the discovery of heterothallism in Mucorales by Blakeslee, the phenomenon has been reported in several groups of fungi. Though variations may occur, but all heterothallic species share one common feature of intermycelial contact.

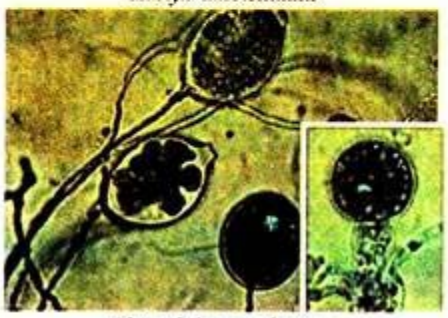
While in Dictyuchus monosporous it is dioecism, in Ascobolus magnificus, it is expressed as self- sterility or self-incompatibility. Both the fungi resemble the heterothallic Mucorales in that sex-organs or gametangia are formed only when opposite strains come in contact.
In Dictyuchus monosporous the two sex-organs are formed by different strains but in Ascobolus magnificus, each strain produces sexorgans but these are self-sterile.

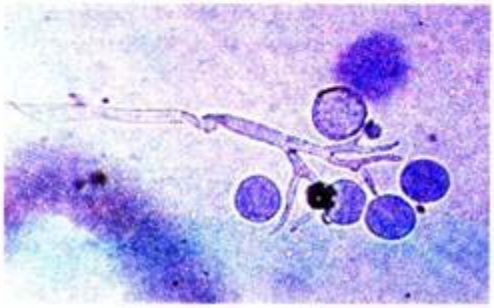
On the basis of these observations, the heterothallism may be of two types: Morphological heterothallism and Physiological heterothallism.

Morphological heterothallism:
Morphological heterothallism may be defined as the condition when morphologically different male and female sex organs are produced in two closely associated mycelia.
The two sex organs or gametes are so morphologically different that it is easier to term one of them as male and the other as female-examples of such type of morphological heterothallic fungi are: Achlya ambisexualis, A. bisexualis, Blastocladiella variabilis, Dictyuchus monosporus, Phytophthora palmivora and Peronospora parasitica (photographs given below).
However, in Blastocladiella variabilis the male and female gametangia are morphologically distinct, the male being smaller than the female.
Whitehouse (1949) also used the term haplodioecious for morphologically heterothallic species of fungi.


Achlya ambisexualis


Blastocladiella variabilis


Phytophthora palmivora


Phytophthora parasitica

Physiological Heterothallism:
In physiological heterothallism, the interacting thalli differ in mating type or incompatibility, irrespective of the presence or absence of the sex organs or gametes. This means that sexual reproduction takes place by two morphologically similar but physiologically different hyphae in physiological heterothallism.
The gametangia as well as gametes do not show morphological differentiation but physiologically they behave differently.
Physiological heterothallism may be of two types:

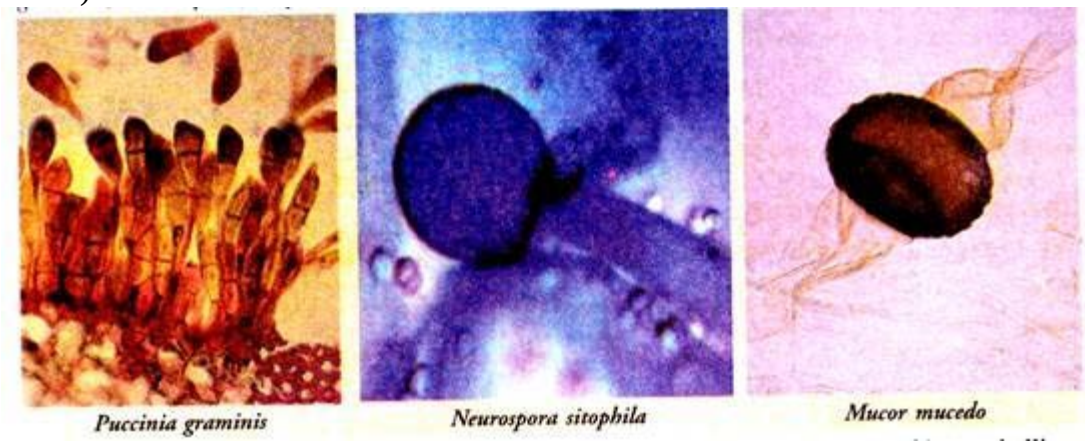
(i) Two Allelomorphs or Two-Allele Heterothallism:

When nuclei of both the mating types are different in genetic characters, this type of Heterothallism is known as Two-Allele heterothallism. In these types compatibility is governed by a pair of Alleles represented by A and a located at single same locus of the chromosome.

Due to the dominance of A over a, A is represented by (+) and a by (-). At the time of meiosis, separation of the chromatids take place. Half of the haploid spores thus have (+) and the other half (-) allele.

The spores bearing (+) allele will produce (+) mycelia and the spores with (-) allele will give rise to (-) mycelia. The mycelia of (+) and (+) and (-) and (-) are self-sterile or self-incompatible. Thus, two complimentary mating types (+) and (-) are essential for sexual reproduction.

Two-Allele heterothallism has been reported in several fungi of like Ascobolus magnificus, Puccinia graminis, Neurospora sitophila Mu mucedo, Ustilago kolleri etc. (photographs given below)



(ii) Multiple Allelomorph or Multiple Allele Heterothallism:

In this type of heterothallism, more than two (multiple) alleles determine the sexual compatibility. These may be located at one (bipolar) or two (tetrapolar) loci.

Because of the larger number of alleles involved in this type of heterothallism, chances of mating of compatible strains increase.

As stated above, the multiple allele heterothallism may be of two types:

(a) Bipolar Multiple-allele heterothallism

(b) Tetrapolar multiple-allele heterothallism.

(a) Bipolar Multiple-Allele Heterothallism:

This type of heterothallism is controlled by multiple alleles at a single locus, instead of a pair of Alleles. For example, if the locus is named as L, the multiples alleles will be designated as L_1, L_2, L_3, L_4—Ln and these are present on the single locus L.

The meiotic division will give rise .to thalli which may be of several mating types, generally equal to the number of alleles. The thallus containing the allele L_1 can mate with a thallus of any mating type except L_1.

Similarly L_2 can mate with any thallus except that containing L_2 allele and so on. In this type of heterothallism, incompatibility factors are more commonly involved. Bipolar multiple allele heterothallism is characteristic of Basidiomycetes except rusts and smuts

(b) Tetrapolar Multiple Allele heterothallism:

	L_1	L_2	L_3	L_4
L_1	N	C	C	C
L_2	C	N	C	C
L_3	C	C	N	C
L_4	C	C	C	N

Fig. 17.3. Bipolar multiple allele heterothallism. C-compatible, N-non compatible

This type of heterothallism is characteristic of Basidiomycetes except rusts. In this type of heterothallism, which is very similar to bipolar multiple allele heterothallism, compatibility is determined by two loci.

Multiple allele—the compatible factor is present on two loci L_1 and $L_2$of two Chromatids of a chromosome. At the time of meiotic division, both the loci are separated with chromatids.

It is estimated that at least 100 alleles are present on each locus. In Schizophyllum commune, 122, alleles of factor A and 61 of B have been identified in the laboratory.

According to rough estimates, the number of alleles may be even more, about 350-450 of L_1, and 65 of L_2. Any two mating types, which differ in allele present on L_1 and L_2 are compatible.

If the allele composition of mating type is A_1 B_1, it would be compatible with any other type of allele composition except A_1 B_1. But the mating type with allele composition is not fully compatible with allele composition A_2 B_2 or A_2 B_2.

Figure 17.4 fully explains type of heterothallism which has been reported in Ustilago maydis and Comprinus firmaterius.

	A_1B_1	A_1B_2	A_2B_1	A_2B_2
A_1B_1	N	NFC	NFC	C
A_1B_2	NFC	N	C	NFC
A_2B_2	NFC	C	N	NFC
A_2B_2	C	NFC	NFC	N

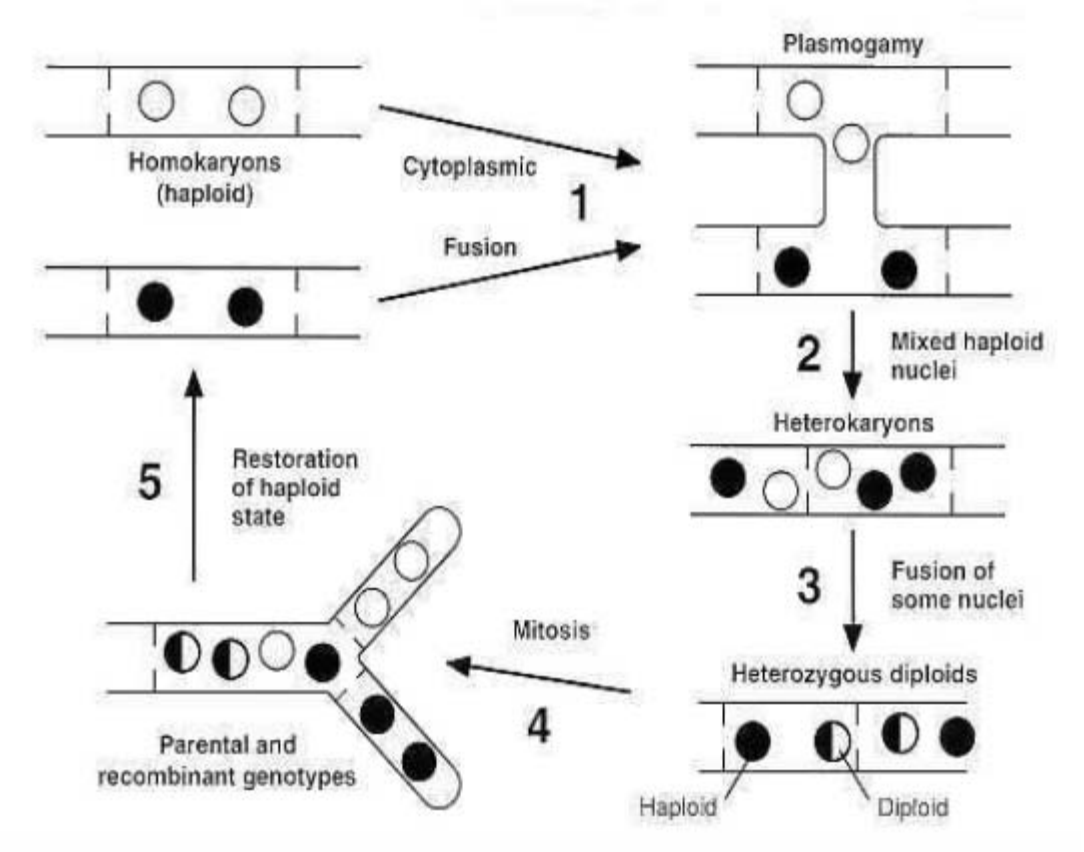
Fig. 17.4. Tetrapolar multiple allele heterothallism. N-non compatible, NFC—Not fully Compatible, C-Compatible.

This type of heterothallism encourages out-breeding. Whereas in bipolar multiple Allele heterothallism, the out-breeding is 25%, in tetrapolar, it is 100%. This may be due to enormous increase in the number of possible mating types of thalli.

According to Garrett (1963), **"heterothallism promotes the out-breeding and therefore subserves the same end as the sexual process, which it renders most efficient. Hetrothallism is not the same as sex, it is refinement super imposed upon it."**

Heterokaryosis

The presence in the same cell of two or more genetically different nuclei. Heterokaryosis occurs naturally in certain fungi, in which it results from the fusion of the cytoplasm of cells from different strains without the fusion of their nuclei. The cell, and the hypha or mycelium containing it, is known as a *heterokaryon*; the most common type of heterokaryon is a dikaryon. Heterokaryosis can also be induced *in vitro*, to study the interaction between the cellular components from different species



Heterokaryons are found in the life cycle of yeasts, for example *Saccharomyces cerevisiae*, a genetic model organism. The heterokaryon stage is produced from the fusion of two haploid cells. This *transient* heterokaryon can produce further haploid buds, or cell nuclei can fuse and produce a diploid cell, which can then undergo mitosis.

Parasexual Cycle:

In some fungi, true sexual cycle comprising of nuclear fusion and meiosis is absent. These fungi derive the benefits of sexuality through a cycle known as Parasexual Cycle.

The Parasexual Cycle is defined as a cycle in which plasmogamy, karyogamy and meiosis (haploidisation) take place but not at a specified time or at specified points in the life-cycle of an organism.

Generally parasexual cycle occurs in those fungi in which true sexual cycle does not take place. The members of class Deuteromycetes (Deuteromycotina) in which sexual cycle does not occur, exhibit parasexual cycle generally.

Parasexual cycle was first discovered by Pontecarvo and Roper of University of Glasgow in 1952 in Aspergillus nidulans, the imperfect stage of Emericella nidulans.

Since then parasexual cycle has been discovered not only in several members of Deuteromycetes but also in fungi belonging to Ascomycetes and Basidiomycetes.

Steps Involved in Parasexual Cycle:

According to Pontecarvo (1958), parasexual cycle in A. nidulans involves the following steps:

(i) Formation of heterokaryotic mycelium

(ii) Fusion between two nuclei (Karyogamy)

(a) Fusion between like nuclei

(b) Fusion between unlike nuclei

(iii) Multiplication of diploid nuclei
(iv) Occasional Mitotic crossing over.
(v) Sorting out of diploid nuclei
(v) Occasional haploidisation of diploid nuclei, and
(vii) Sorting of new haploid strains.

A brief account of these steps are being presented below:

(i) Formation of heterokaryotic mycelium:

Heterokaryotic mycelium is formed in several ways. The most common is by the anastomosis of somatic hyphae of different genetic combinations.

The foreign nucleus or nuclei introduced into a mycelium multiplies and its progeny spreads through the mycelium rendering it heterokaryotic. Mutation in one or more nuclei of a homokaryotic mycelium also makes it heterokaryotic.

It happens in some of the fungi belonging to Ascomycetes. Still a third way is by the fusion of some of the nuclei and their subsequent multiplication and spread among the haploid nuclei. In this type of heterokaryotic mycelium a fixture of haploid and diploid nuclei occur.

(ii) Fusion between two nuclei (Karyogamy):

The fusion of nuclei in the mycelium has been demonstrated. The nucelar fusion may be of two types: (a) fusion between like nuclei and (b) fusion between unlike nuclei. The nuclear fusion results in the formation of homozygous or heterozygous diploid nucleus respectively.

If the genotype of unlike nuclei present in the heterokaryotic mycelium is A and B, then five types of nuclei can be formed by their fusion: two types of haploid nuclei (A and B), two types of homozygous diploid nuclei (AA and BB) and one type of heterozygous diploid nucleus (AB).

(iii) Multiplication of diploid nuclei:

The above mentioned five types of nuclei multiply at about the same rate but the diploid nuclei are present in much smaller number than the haploid nuclei. Portecarvo (1958) estimates a proportion of one diploid heterozygous nucleus to 1000 haploid nuclei.

(iv) Occasional mitotic crossing over:

During multiplication of diploid nuclei, mitotic crossing over may take place. This results in the formation of new gene combinations. These recombinations, which are dependent on the existence of heterokaryosis, give the fungus some of the advantages of sexuality within the parasexual cycle.

According to Pontecarvo's (1958) estimates, the amount of recombinations which may be expected to occur in an ascomycete through its parasexual cycle is 500 times smaller than through its sexual cycle.

However, in Penicillium chrysogenum and Aspergillus niger, diploidisation and mitotic crossing over occur more frequently indicating the importance of parasexual cycle in evolution of new strains.

(v) Sorting out of Diploid nuclei:

In those fungi which produce uninucleate conidia, sorting out of the diploid nucleus occurs by their incorporation into conidia which germinate to produce diploid mycelia. Diploid strains of several important imperfect fungi have been isolated.

Roper (1952) first synthesized and isolated diploid strains of Aspergillus nidulans. The conidia of diploid strains are somewhat larger than those of haploid strains.

(vi) Occasional haploidisation of the diploid nuclei:

Occasionally, some hyphae of diploid mycelium form haploid conidia which form haploid mycelia on germination. The formation of haploid conidia by diploid mycelium indicates that haploidisation occurs in some diploid nuclei.

(vii) Sorting of new haploid strains:

Some diploid nuclei undergo haploidisation in the mycelium and are sorted out by incorporation of haploid nuclei in the uninucleate conidia. Some of these haploid strains are genotypically different from their parents because of their mitotic recombinations.

Thus, after the parasexual cycle has operated for some time, the mycelium may contain the following types of nuclei:

(a) Haploid nuclei like those of both the parents,
(b) Haploid nuclei with various new genetic recombinations,
(c) Several types of diploid homozygous nuclei,and
(d) Several types of diploid heterozygous nuclei.

Significance of Parasexual Cycle:

Parasexual cycle is of importance in industrial processes. Several fungi which are used in various industrial processes belong to fungi imperfecti or Deuteromycetes and in these fungi only parasexual cycle operates.

New and better strains of these fungi are obtained by mutation through parasexual cycle. The strains of desirable characters can be developed through mitotic recombinations.

Parasexuality can also be applied in the analysis of genetic and physiological processes of perfect and imperfect fungi. Parasexual cycle has also been successfully employed in genetic control of pathogenicity and host-range in several species of Fusarium.

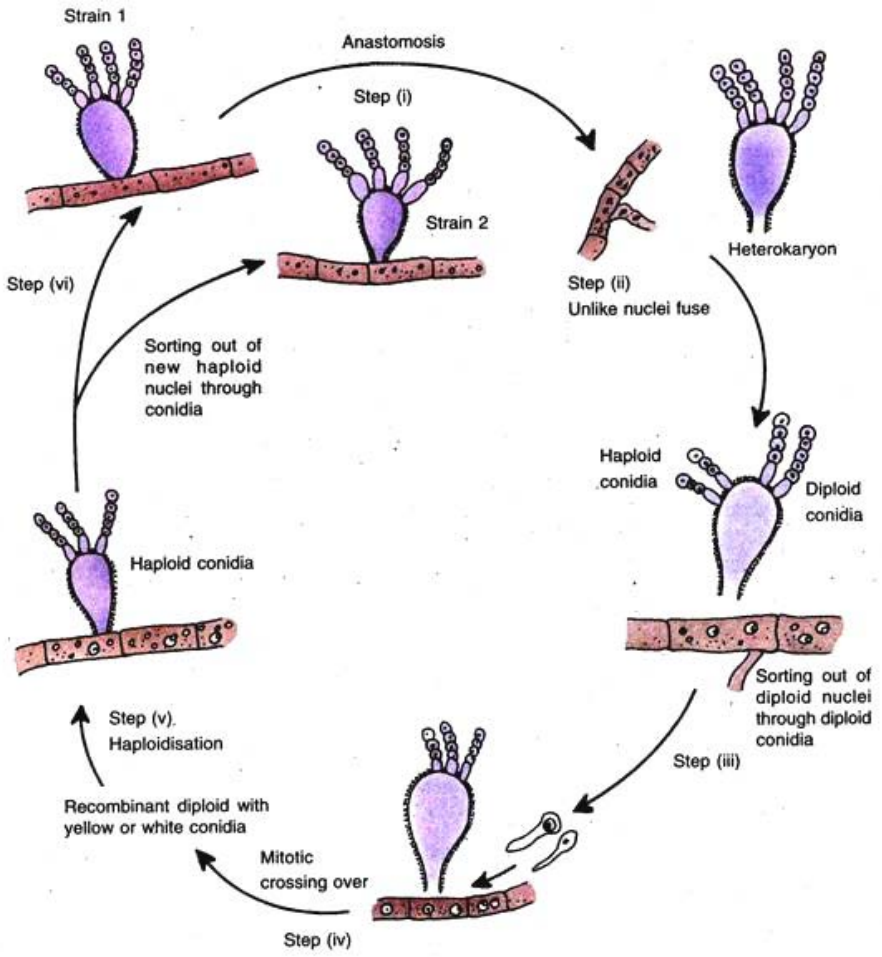


Fig. 17.10. Pontecarvo's (1958) idea of Parasexual cycle

7. Diversity of somatic, reproductive and fruiting structures in different groups
8. Fungal spores: types, dispersal, dormancy and germination

Myxomycota
Salient features

1. (Gr. myxa = slime); Members are called the **slime moulds**. Presently classified in the Kingdom **Protista (=Protozoa)**, they were once thought to be fungi because they produce **spores** that are borne in **sporangia**, a characteristic common to some fungi. But the spore contains cellulosic wall instead of chitin.

2. Slime moulds differ from the Eumycota not only in phylogeny, but also in their mode of nutrition, physiology and ecology.

3. Their vegetative state consists of individual amoebae in the cellular slime moulds, or multinuclear (coenocytic) plasmodium in the plasmodial slime moulds.

4. Motile stages with two anterior whiplash-type flagella is present in the plasmodial slime moulds and Plasmodiophoromycota.

5. Amoebae or plasmodia feed by the ingestion (phagocytosis) of bacteria, yeast cells or other amoebae, followed by intracellular digestion in vacuoles. Thus the mode of nutrition in slime moulds is fundamentally different from extracellular degradation and absorption as seen in the Eumycota.

Classification of slime moulds (Ainsworth, 1973)
Division – Myxomycota - (4 Classes)

1a. Assimilative phase free-living amoeba which unites as pseudo-plasmodium before reproduction - Acrasiomycetes
1b. Assimilative phase a plasmodium
  2a. Plasmodium forming network - Hydromyxomycetes
  2b. Plasmodium not forming network
    3a. Plasmodium saprobic, free-living - Myxomycetes
    3b. Plasmodium parasitic within cells of host plant – Plasmodiophoromycetes

Classification of slime moulds
(Alexopoulos, Mims & Blackwell, 1996)
Kingdom: Protista
Fungus -like organisms of uncertain affinity Phylum **Acrasiomycota** (acrasid slime moulds)
Phylum **Dictyosteliomycota** (dictyostelid slime moulds)
Phylum **Myxomycota** (plasmodial slime moulds)
Phylum **Plasmodiophoromycota** (plasmodiophorids)
Kingdom: Stramenopila
Probably derived from the protist group containing golden-brown algae, diatoms, etc. Phylum **Oomycota**
Phylum **Hyphochytridiomycota**
Phylum **Labyrinthulomycota**

Classification of Slime mould (Webster & Weber, 2007)

KINGDOM PROTOZOA

Phylum Myxomycota

Class Myxomycetes (plasmodial slime moulds)

Class Dictyosteliomycetes (cellular slime moulds)

Class Protosteliomycetes (cellular slime moulds)

Class Acrasiomycetes (cellular slime moulds)

Phylum Plasmodiophoromycota (plasmodial slime moulds)

KINGDOM STRAMINIPILA

Phylum Hyphochytriomycota

Phylum Labyrinthulomycota (net slime moulds)

Phylum Oomycota

Class Myxomycetes

True or plasmodial slime molds; found in moist dark places on decaying organic matter, dead twigs, bark, leaf litter; some occur in soil and dung; species of *Lepidoderma* associated with snow banks occur during late spring and early summer in alpine regions of temperate zone.

These organisms are characterized by:

- A phagotrophic nutrition
- A multinucleate somatic phase known as a **plasmodium** that moves and exhibits a reversible shuttle streaming of its protoplasm,
- A resistant stage consisting of a sclerotium,
- A reproductive phase that ends in the production of stationary **sporophores** containing walled spores.
-

Fig. *Lepidoderma*, Source: https://www.discoverlife.org/mp/20q?search=Lepidoderma

Range of plasmodium

he vegetative phase of the members of Myxomycetes is a free-living **plasmodium**, i.e. a multinucleate wall-less mass of protoplasm. This may or may not be covered by a slime sheath. **Plasmodia** vary in size and can be loosely grouped into **three** categories.

1. **Protoplasmodia** are inconspicuous microscopic structures protoplasm granular with slime sheath; usually giving rise only to a single sporangium when it fruits, e.g. *Dictydium*

Fig. *Dictydium*, Source: https://de.wikipedia.org/wiki/Dictydium

2. **Aphanoplasmodia** (Gr. aphanes = invisible)

Fig. *Stemonitis*, Source: https://en.wikipedia.org/wiki/Stemonitis

resembles a protoplasmodium in its initial stages, but soon elongates, branches, and becomes a network of very fine, transparent strands, with individual strands only 5-10 μm wide and the entire plasmodium about

100-200 µm in diameter, non-granular, cytoplasmic streaming rapid and reversible, e.g. *Stemonitis*

3. **Phaneroplasmodium** (Gr. phaneros = visible) also resembles a protoplasmodium at first; later it grows larger, becomes colourful, massive and visible to the naked eye. Its protoplasm is very granular and shows rhythmic and reversible streaming, each pulse lasting about 60-90 s. This striking phenomenon is readily observed with a dissection microscope and is probably due to interactions of Ca^{2+} ions with cytoskeletal elements lining the veins. At maturity, it exhibits polarity and directional movement, terminating anteriorly in an advancing fan-shaped feeding edge and posteriorly in a trailing network of veins. e.g. *Physarum.*

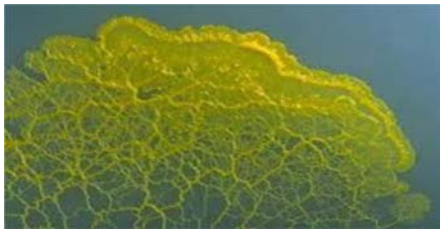


Fig. *Physarum*, Source: http://www.sharnoffphotos.com/nature/myxomycetes/myxo_index3.html

Sclerotia

Under conditions of low nutrients (starvation), plasmodia can be converted either into sporophore in presence of light or sclerotia in darkness. Low temperature, decreased moisture and aging are also responsible for sclerotia formation.

Sclerotia are diploid and composed of small multinucleated cells called macrocysts or spherules varying in size from 10 -25 µm covered by a horny covering. Upon wetting, sclerotia become highly vacuolated and pseudoplasmodia penetrate through the fragmenting spherule walls to fuse with those of other spherules to reconstitute the plasmodium. This is followed by clumping-up and forming of anyone of fruiting body.

Range of mature fructifications in Myxomycetes

Under condition of high moisture, nutrient, light, Ca2+ and malate, plasmodium converted irreversibly into sporophore that contains walled spores. Mature fruiting bodies are of following types:

1. In *Ceratiomyxa* plasmodium forms simple erect or brached pillars. Surface of the pillars consist of unicellular globose spores on short stalks.

2. **Sporangia**

- Small fruiting bodies, each with its own peridium or covering.
- One to several thousand develop from a single plasmodium.
- May be stalked (*Stemonitis*) or sessile (*Diderma*), seated on a membranous persistent layer called **hypothallus**.

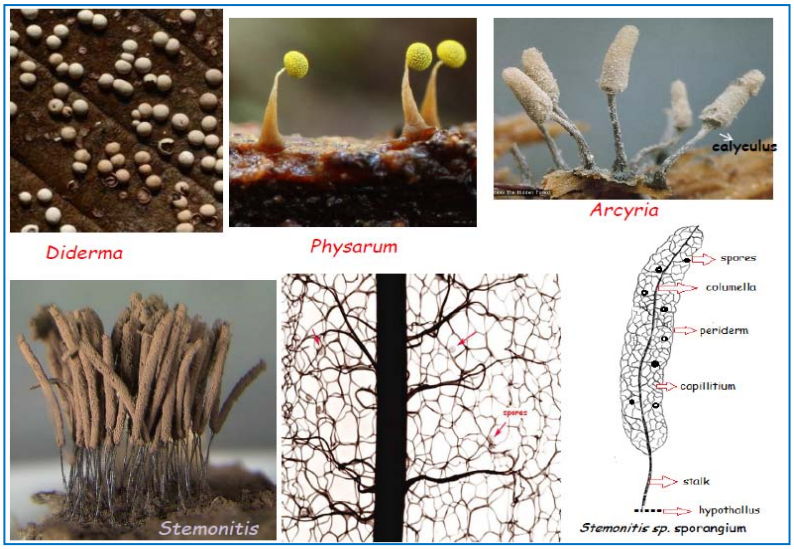


Fig. mature fructifications in Myxomycetes

- When stalked – the stalk extends into the body of the sporangium as **columella**. Branched capillitial threads traverse the cavity of sporangia, separating and supporting the spores. Made of Ca salt and is non-living.

- In some species (*Arcyria*), lower portion persists as

- a cup or saucer-like base known as **calyculus**.

3. Plasmodiocarps sessile, elongated, worm-like, branched, forms a network over the substratum following the veins of the plasmodium from which it develops. Peridium is laid down around the outside. e.g., *Hemitrichia*

4. Aethallium (Gr. aethes =irregular, curious, unusual)

- Large, sessile, cushion shaped fruiting body, formed from one plasmodium that does not differentiate into sporangium

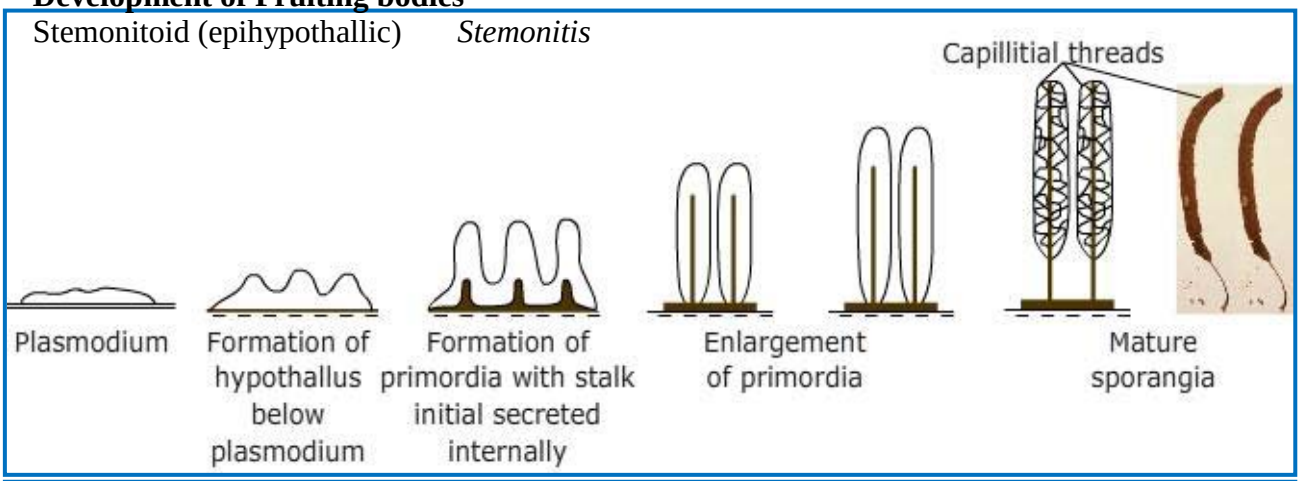
- covered by a silvery periderm

- *Fuligo septica* – largest fruiting body (as much as 20 cm diameter)

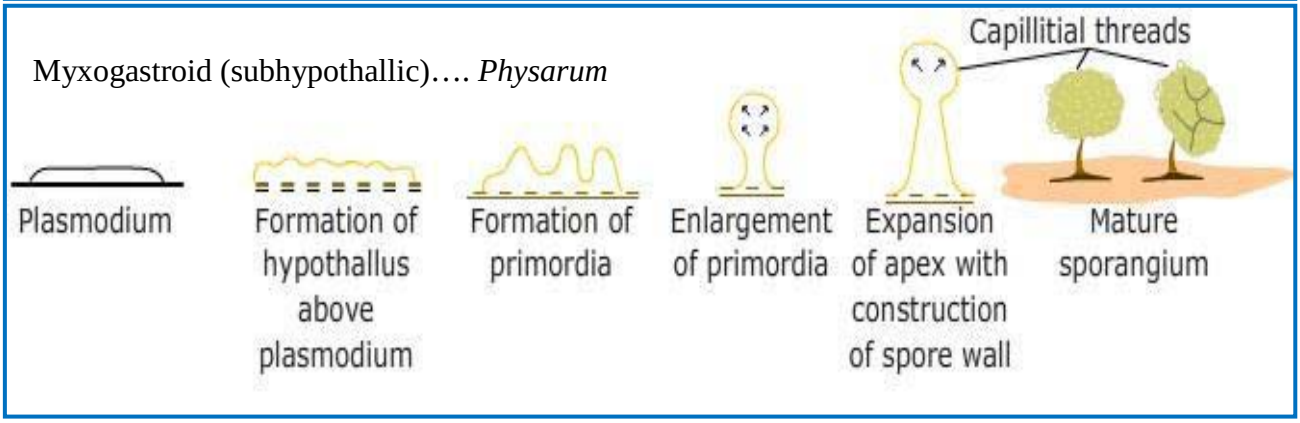
5. Pseudoaethalium many sporangia are packed so closely together (*Dictydiaethalium*) or only partially fused (*Tubifera*). Individual sporangia are clearly distinguishable at maturity, formed on a common hypothallus

Development of Fruiting bodies

Stemonitoid (epihypothallic) *Stemonitis*



Myxogastroid (subhypothallic)…. *Physarum*

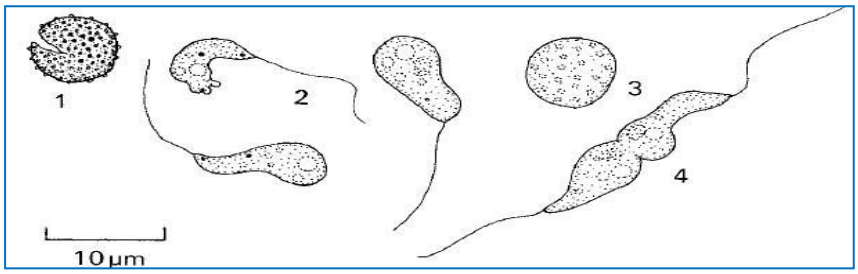


Spores: formation, structure & dispersal

- Within the sporangium, spores are formed due to cleavage of protoplasm. Meiosis occurs in young spore after about 18-30 h of cleavage. Out of 4 haploid nuclei 3 degenerate.
- Spore generally globose in shape, free or aggregated into loose or tight clusters or spore balls of 2-40.
- Wall of spores may be smooth, spiny, warty, reticulate.TEM shows spore wall is bi-layered- an inner electron transparent layer and outer electron dense layer that bears ornamentations.
- Spore wall of Physarum polycephalum contains 81% galactosamine polymer and 15.4 % melanin. This property suggests that myxomycetes are not closely related to fungi (that contains glucosamine polymer, chitin).
- Some myxomycetes spores are exceptionally resistant to unfavourable conditions like prolonged dessication and some remain viable upto 75 years.
- Capillitium or capillitial threads help spores to be discharged by air currents.
- Spores are dispersed by animals like arthropods, rain, wind.

Germination of spore

During germination spores imbibe water, their protoplsm swell, and they open up by a V-shaped opening which is formed by splitting of spore wall and releasing one or more myxoamoebae or flagellated cells (swarmers) that contain 1-4 anterior flagella.

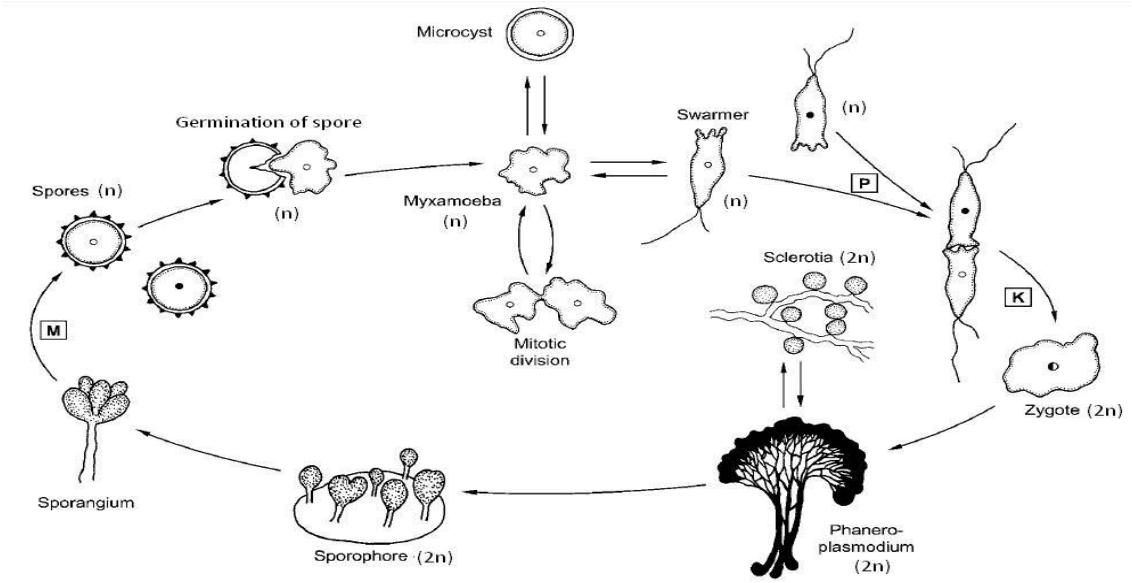


1) spore showing crackedwall;
2) swarmers, one with pseudopodia;
3) encystment stage;
4) fusion between two swarmers.

Mitotic division

- Centric (centrioles are present) and open (nuclear envelope is non-persistent i.e., it breaks down in prophase) and observed in myxoamoebae
- Acentric (centrioles are absent) and closed (nuclear envelope remains intact till late anaphase or early telophase) and is observed in plasmodium.

Life cycle of the myxomycete
Physarum polycephalum



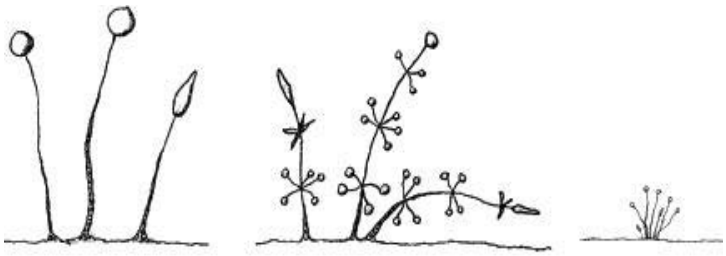
Class Dictyosteliomycetes
(cellular slime moulds)

●Members occur in soils, especially on the surface soil and leaf litter of deciduous forests.

●Vegetative phase- uninucleate, haploid, naked amoebae with filose (acutely pointed) pseudopodia that can creep out of soil andconsume bacteria.

●At the end of feeing phase, sorocarp develop which are stalked fruiting bodies with aggregation of amoebae. Stalk may be acellular (*Acytostelium*), cellular and unbranched to sparcely branched (*Dictyostelium*) or cellular with whorls of branches (*Polysphondylium*).

●*Dictyostelium discoideum* has been studied intensively by biologists and biochemists for cellular interactions and developmental processes.



A. *Dictyostelium mucoroides*

B. *Polysphondylium violaceum*

C. *Acytostelium leptosomum.*

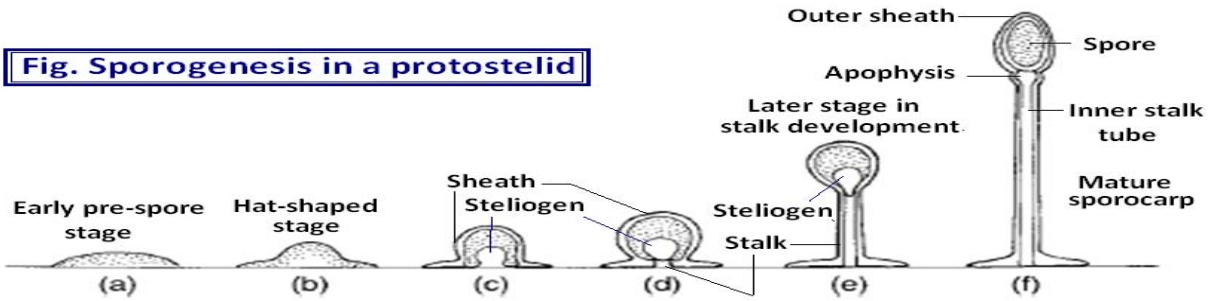
●Important model organism for study of cytokinesis, signaling, chemotaxis, phagocytosis, motility, cell sorting, cell-type determination.

Class: Protosteliomycetes

- Protostelids are ubiquitous on decaying plant parts in soil and humus,dung or in freshwater.
- They occur in all climatic zones from the tundra to tropical rainforests.
- Protostelids produce amoebae with **filose pseudopodia** feeding phagocytotically on bacteria, yeast cells or spores of fungi.

- **Sporulation** occurs by the conversion of a feeding amoeba or plasmodium into a round **prespore cell** which then rises at the tip of a delicate acellular stalk, ultimately forming one or several spores in a single sporangium.

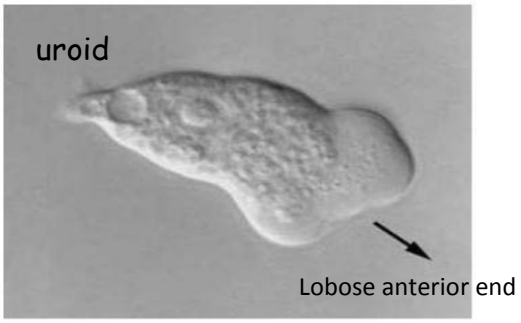
Fig. Sporogenesis in a protostelid



- When feeding stops, the amoeba rounds off forming pre-spore cell that heaps its protoplasm in the centre to form the 'hat-shaped' stage.
- A membranous, pliable, impermeable sheath develops on the surface of the cell.
- The protoplast contracts into the central hump, the sheath collapses at the margins, forming the disc-like base to the stalk of the sporocarp. Within the protoplast, a granular basal core - steliogen, differentiates and begins to form a hollow tube.
- As the tube extends at its tip, the protoplast migrates upwards, sits on top of the growing tip. The entire structure remains covered by the sheath.
- The steliogen is left behind at the tip of the stalk and forms the apophysis, and the protoplast secretes a cell wall and becomes the spore.

Class Acrasiomycetes

- The trophic stage consists of amoebae morphologically distinct from those of the dictyostelid cellular slime moulds in having a cylindrical, rather than flattened, body bearing a single large-lobed (lobose) anterior pseudopodium.
- The posterior end is knob-shaped and is called the uroid, containing a contractile vacuole and may produce small filose pseudopodium.
- The cellular contents are granular.
- Acrasid slime moulds are common on decaying plant matter, in soil, on dung and on rotting mushrooms.



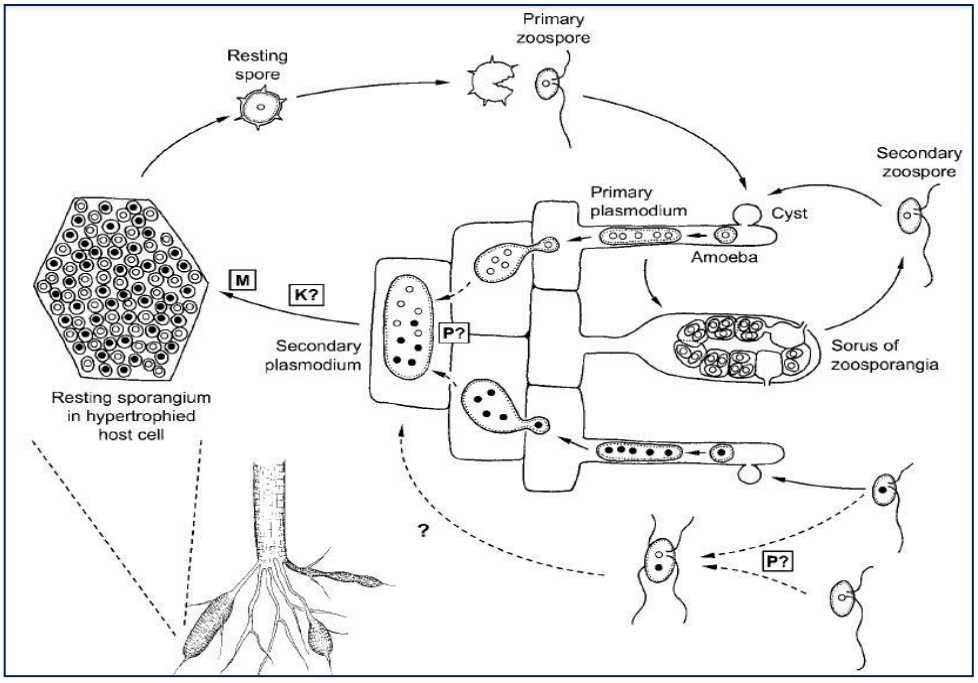
- The uninucleate amoebae feed on yeast cells, bacteria or fungal spores and encyst under unfavourable conditions, to form **microcysts**.
- Each microcyst germinates again to release a single amoeba.

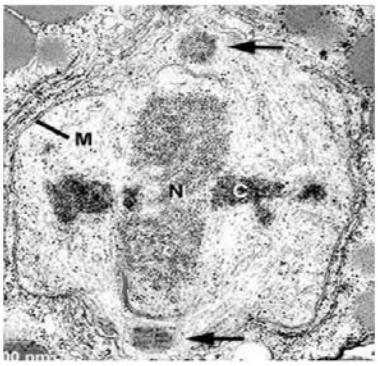
- Amoebae aggregate to form a pseudoplasmodium, in which the individual amoebae retain their identity but are surrounded by a common sheath.
- The chemical signal for aggregation is unknown but it is not cyclic AMP (cAMP) as in the dictyostelid slime moulds.
- No migration of pseudoplasmodium.
- The pseudoplasmodium develops into a branched fruiting body called **sorocarp**- in which the amoebae align themselves in single rows and then round off, each forming a walled spore.
- All cells of sorocarp are able to germinate.
- Each spore germinates to release a single amoeba.
- The cells making up the stalk of the sorocarp also encyst and are capable of germination.
- Sexual reproduction in the acrasid slime moulds is unknown.

Phylum Plasmodiophoromycota

- Endoparasitic slime molds - Trophic stage formed inside host cells
- Obligate parasites of aquatic and terrestrial plants, algae and fungi.
- Cause abnormal enlargement of host cells. (**hypertrophy**) or abnormal multiplication of cells (**hyperplasia**); may also cause stunting. e.g. club root of crucifers by *Plasmodiophore brassicae*
- Polymyxa, Spongospora act as vectors of important plant viruses.
- Plasmodial phases are of two types: (i) **Primary (Zoosporangial)** (ii) **Secondary (Sporangiogenous/cystogenous)**;
- Resting spore (n) germinates to form biflagellate primary zoospore (n). The flagella are inserted laterally and are of unequal length, the anterior one being shorter- **anisokont.** Both flagella are of the whiplash type. The zoospore encysts on a suitable surface by secreting a cell wall. After a while, an amoeba is injected from the cyst into a host root hair cell where it enlarges to form a primary plasmodium (n). The plasmidium is then differentiated into zoosporangium that releases secondary zoospores (n). Once released, secondary zoospores may re-infect the host to give rise to further primary plasmodia and zoosporangia. Secondary plasmodium (2n) is formed in the cortical cells after fusion of primary plasmodia and/or zoospores of two different mating types. Secondary plasmodium undergoes meiotic nuclear divisions and produces thick-walled resting spores (n). It is not clear where plasmogamy and karyogamy occur in the life cycle of the Plasmodiophorales.

Life cycle of *Plasmodiophora brassicae*





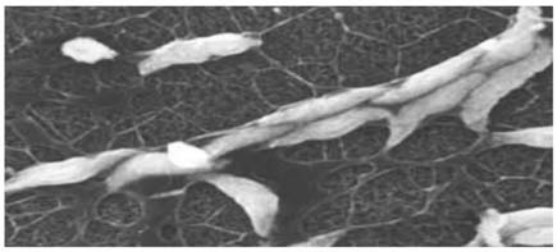
- An unique feature of the group is that nuclear divisions are cruciform. An intranuclear spindle is formed on which chromosomes are arranged in a circle around the nucleolus. Nucleolus persists, elongates, and becomes dumb-bell shaped.
- Thus the chromosomes and the nucleolus appear like 'cross' at metaphase, hence the name cruciform (L. crux = cross) is given to the division.
- Cruciform nuclear divisions are observed during mitotic division of primary and secondary plasmodium within host cell, but not during transition of primary plasmodium to zoosporangium and secondary plasmodium to resting spores.
- Genera based on arrangement of cysts inside host cells.

Characteristics	Plasmodiophoromycota	Myxomycota
Mode of nutrition	parasitic	saprobic
Plasmodium	formed inside host cells; lacks phagotrophy; formed inside host cells absent; differentiated into primary and secondary plasmodia	formed outside on the substratum; phagotrophic; shows translocation movements; not differentiated
Zoospores	Presents, produced twice in the life cycle	Absent; swarmers occur once in life cycle
Resting spore	Cyst, one type, wall chitinous	Some produce micro- and macro cysts, sclerotia, spore wall has galactosamine polymer

Phylum Labyrinthulomycota

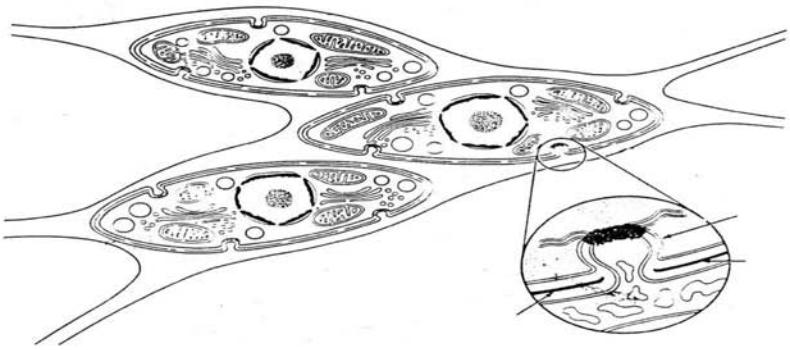
- Members are commonly called **net slime molds**, occur in freshwater and marine environments where they are attached to solid substrata by means of networks of slime within which individual vegetative cells are present.

- The plant body consist of an ectoplasmic network of branched, anastomosing, wall-less filaments produced by spindle shaped or spherical cells that move by gliding within the network.

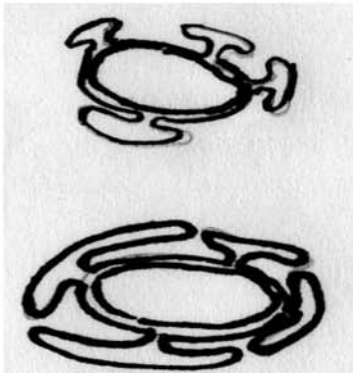


- The vegetative cells possess a wall which, uniquely, is produced from Golgi-derived scales of a polymer of L-galactose. These scales are located between the plasma membrane and the inner membrane.

- The branched, anastomosing wall-less filaments are produced by cells with a specialized cell surface organelle, called a **bothrosome** or **sagenogen** or **sagenogenetosome**.

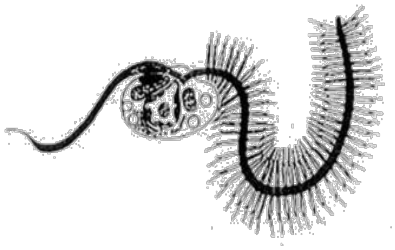


bothrosome



Cell plasma membrane

Membranes balloon out at the plasma membrane of each cell from the bothrosomes and at maturity an outer plasma membrane forms the boundary of the filaments with the outer environments.
A closely appressed inner plasma membrane isolates the uninucleate cells in pockets within the membrane formed filaments.
The inner membrane of the filaments is continuous with the plasma membrane of the parent cell it encloses at the



zoospore

bothrosomes.
Members feed by absorption (osmotrophy) of nutrients. The nets contain degradative enzymes which can lyse plant material or microbial cells.
Some members produce zoospores each provided with two flagella (**heterokont**), the longer directed anteriorly provided with mastigonemes (i.e. tinsel flagellum) and the shorter is whiplash directed posteriorly.
During sexual reproduction the somatic cells aggregate within the ectoplasmic net to form **reticulate sori**.
The spindle shaped cells assume spherical shape and enlarge in size and develop into sporangia.

OOMYCOTA

Straminipila

Presence of straminipilous (L. stramen = straw; pilus = hair) flagella, chlorophyll c instead of ch b, chloroplast enclosed in sheets of endoplasmic reticulum
Two types: photosynthetic (brown algae, golden algae, diatoms, cryptomonads), Non-photosynthetic (**oomycota**, hyphochytriomycota & labyrinthulomycota)

Difference between oomycota & eumycota (true fungi)

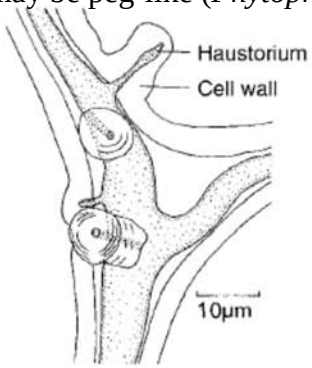
Characters	**Oomycota**	**True fungi**
Motile stage	Zoospore biflagellated: heterokont, tinsel (anterior) & whiplash (posterior) type in secondary zoospore, and both anterior in primary zoospore of some spp.	Only in chtrids, zoospore & gametes uniflagellate, posterior whiplash flagella; zoospore multiflagellate in *Neocallimastix*
Cell wall	Composed of β-glucan, hydroxyproline, cellulose	Composed of chitin, proline, no cellulose
Lysine biosynthesis via Sterol in cell membrane	α-diaminopimelic acid (DAP) pathway as in plants & bacteria Fucosterol like plants	α-aminoadipic acid (AAA) pathway Ergosterol like animal
Reserve compounds	Mycolaminarin	Glycogen, lipids, sugar alcohol, trehalose
Inner mitochondrial membrane	Consists of tubular cristae as in plants	Consists of lamellate cristae
Golgi bodies	With multiple flattened cisternae as in protozoa & plants	With simple & single cisterna
Sexual reproduction	Oogamous type only	Iso-, aniso-, oogamy and other special types
Meiosis	Gametangial, main thallus 2n	Zygotic, main thallus either n or n+n

Habit:
Plant parasites:
Phytophthora infestans – late bight of potato – saprotroph or necrotroph
Pythium ultimum –Damping off of seedlings- saprotroph or necrotroph
Plasmopara viticola – downy mildew of grapes - biotroph
Albugo candida – white rust (blister) of crucifers – biotroph
Animal parasites:

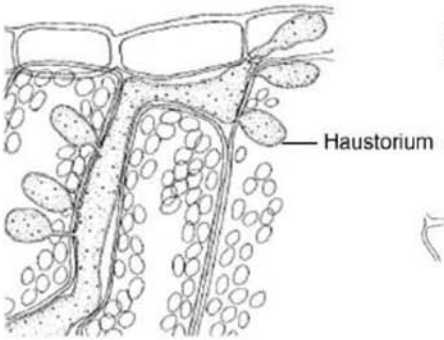
Saprolegnia diclina, *S. parasitica* on cat fish & salmond fish– used as BCA *Lagenidium giganteum* on nematode & mosquito larvae
Pythium insidiosum – Pythiosis, an animal disease Mycoparasites: *Pythium oligandrum* on *Pythium ultimum* – used as BCA
Aqualinderella fermentans obligately anaerobic and lacks mitochondria, can grow in stagnant &/or polluted water
Lagena radicola occurs as symptomless parasite on plant roots

Thallus structure

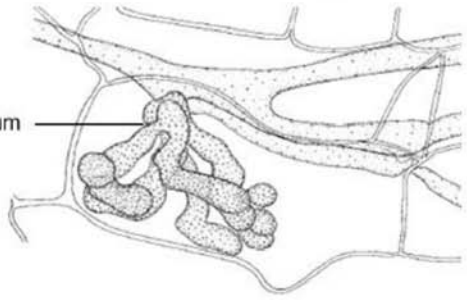
Unicellular holocarpic (Lagenidium) to eucarpic filamentous form that is composed of profusely branched coenocytic hyphae with diploid nuclei; nuclei and other organelles are pushed towards the periphery of the hypha by a large central vacuole.
Simple septa are formed to isolate reproductive structures or older hyphae.
Obligate parasitic forms grow intercellularly and absorb nutrients by producing haustoria which may be peg-like (*Phytophthora*), spherical (*Bremia*), lobed (*Peronospora*).



Finger-like haustoria
Phytophthora

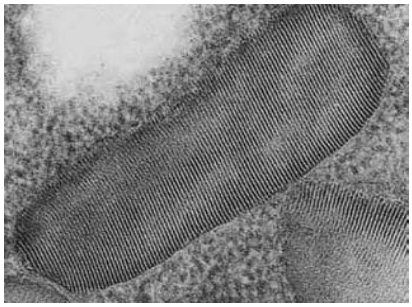


Knob-shaped haustoria
Bremia



Lobed haustoria
Peronospora

- Hyphal apex consists of a large number of apical vesicles (instead of Spitzenkorper), helps in growth of hyphal tip.
- The vacuolar system consists of dense-body vesicles or '**fingerprint vacuoles**' which consist of deposits of a phosphorylated β-(1,3)-glucan polymer, mycolaminarin. It may serve as a storage compound for carbohydrates as well as phosphate.
- Vacuoles show membranous continuity linking adjacent vacuoles and provide a means of transport by peristalsis (as in true fungi).



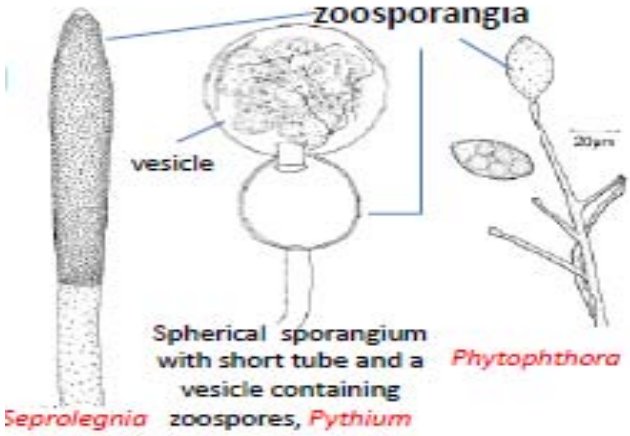
Fingerprint vacuoles

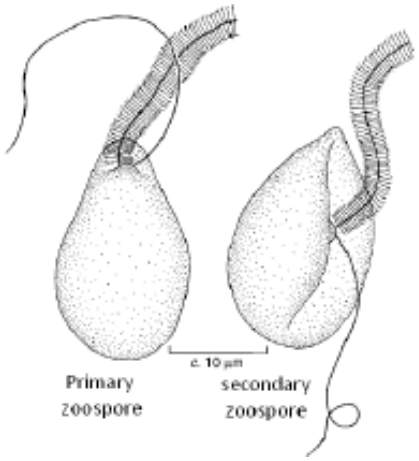
- Both mitotic and meiotic divisions are intranuclear and centric as in true fungi.

Asexual reproduction

By formation of heterokontic zoospores produced in a **zoosporangium**, that is separated from the hyphae by a complete septum, or sometime into an evanescent vesicle protruding from the sporangium. They are swollen, lobed portion of hyphae (*Seprolegnia*), spherical (*Pythium*), oval or lemon-shaped (*Phytophthora*). The sporangial contents are cleaved resulting diploid zoospore.

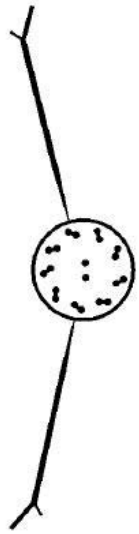
In some species, two morphologically distinct zoospores are produced: **primary (auxiliary) zoospore** – pear-shaped with both flagella inserted apically. Soon after its formation It encysts and withdraw flagella. The cyst germinates to give rise to the **secondary (principal) zoospore** which is kidney-shaped, with the flagella inserted laterally in a kinetosome boss which in turn is located within the lateral groove.





Ultra structure of tinsel flagella:

The flagellum is decorated with hair-like structures 1-2 µm long. The hairs are called mastigonemes or tripartite tubular hairs (TTHs) because they are divided into three parts. Each TTH is attached to the flagellum by a conical base pointed towards the axoneme. The main part of the TTH is a long tubular shaft thought to consist of two fibres of different thickness coiled around each other. At the tip of the TTH, the two fibres separate from each other to form loose ends. In the TTHs of some straminipilous organisms, only one loose end is visible. TTHs are assembled in antiparallel arrays in Golgi-derived vesicles of the maturing zoospore, and are released by fusion of the vesicles with the plasma membrane. TTHs are arranged in two rows along the axoneme. The cones of each row are adjacent to an outer microtubule doublet, and because there are nine such doublets, the two rows of TTHs are at an angle of about 160° rather than 180° to each other. In zoospores of straminipilous fungi, the straminipilous flagellum always seems to point towards the direction of movement. An anterior straminipilous flagellum pulls the spore through the water, whereas a backwardly directed whiplash flagellum pushes the spore.



T.S. of the straminipilous flagellum

Terminology based on motility

●**dimorphic** (*Seprolegnia*)- species produce both primary and secondary zoospores

●**monomorphic** (*Dictyuchus*) those producing only one type of zoospore

The zoospores have a period of swarming. Based on swarming period, they are

●**monoplanetic** (1 swarming period by one type of zoospore *Aphnomyces euteiches*),

●**diplanetic** (2 swarming periods by two types of zoospore *Saprolegnia*),

●**polyplanetic** (several swarming periods by one type of zoospore, *Dictyuchus*),

●**aplanetic** (do not produce zoospore, *Geolegnia*)

Germination of zoospore:

Swarming periods of zoospores are separated by periods of encystment, when zoospores withdraw flagella, adhesive and cell wall material is secreted by the synchronized fusion of pre-

formed storage vesicles with the zoospore plasma membrane. Depending on species & environmental conditions, a cyst germinates **directly** (by formation of germ tube) or **indirectly** (through the formation of another zoospore).

Sexual reproduction

Oogamous type, involves production of a male gametangium (antheridium) and a female gametangium (oogonium). Both the structure may develop from the same thallus (**homothallic**, *Pythium* spp., *Achlya colorata*) or from different thalli (**heterothallic**, *Achlya bisexualis*).

Oogonia are globose to oblong in shape containing one to many (*Pythium multisporum*) non-motile eggs or **oospheres**. The mature oospore wall is bi-layered: **epispore** & **endospore**. Oosphere is differentiated into peripheral oogonial cytoplasm (**periplasm**) containing a prominent storage vacuole termed as **ooplast**. It arises by the fusion of dense-body vesicle and contains mycolaminarin and phosphate. Lipid droplets present in the oospore provide energy during germination.

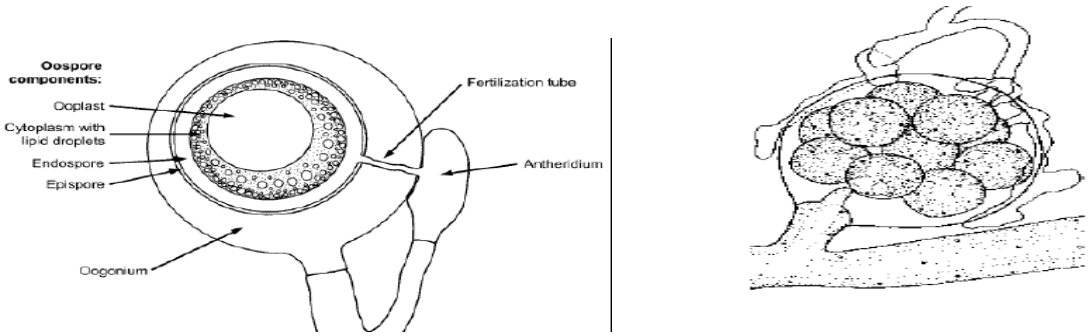


Fig. Sexual reproduction

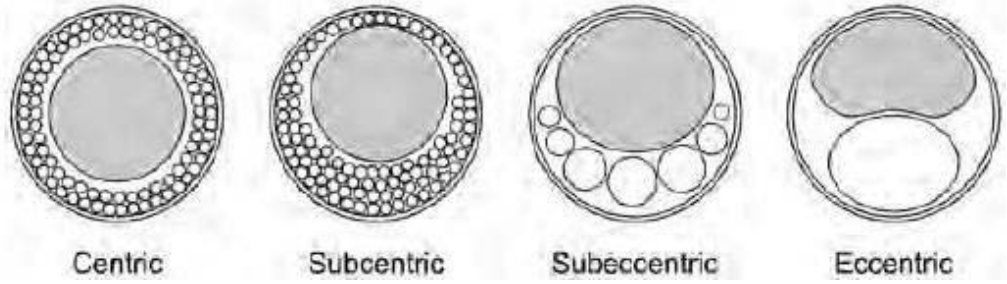
The **position of the ooplast in the oospore** is used for species identification, and four types of oospore have been distinguished:

Centric oospores have a central ooplast surrounded by one or two peripheral layers of small lipid droplets (e.g. *Saprolegnia hypogyna, S. ferax*).

Subcentric oospores have several layers of small lipid droplets on one side of the ooplast and only one layer or none at all on the other (e.g. *S. unispora, S. terrestris*).

In subeccentric oospores, the small lipid droplets have fused into several large ones all grouped to one side, with the ooplast contacting the plasma membrane on the opposite side (e.g. *S. eccentrica*).

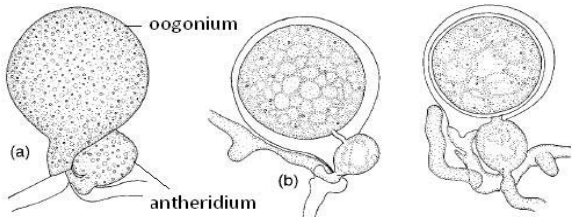
The **eccentric type** (*S. anisospora*) is similar to the subeccentric type except that there is only one very large lipid drop.



Arrangements of the ooplast (shaded organelle) and lipid droplets (empty circles or ellipses) in oospores of *Saprolegnia*

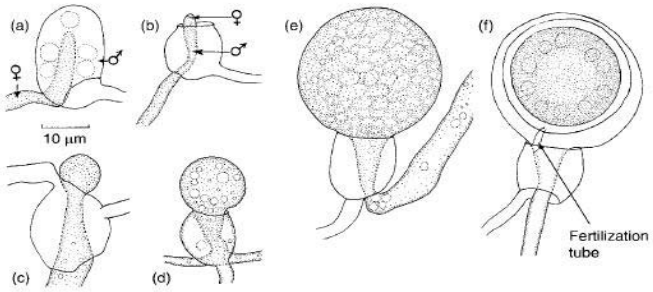
Antheridia are multinucleate, elongated bodies borne terminally on slender branches and are much smaller. Two distinct types of **antheridial arrangement** are found. In *Phytophthora fragariae, P. megasperma*, antheridia are attached laterally to the oogonium and are described as **paragynous** meaning 'beside the female'.

Paragynous arrangement



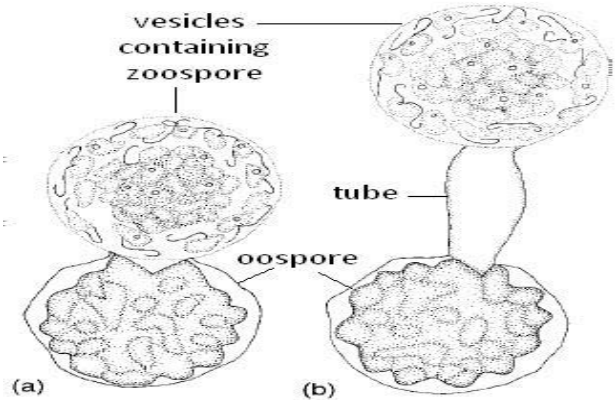
In *Phytophthora infestans, P. cinnamomi and P. erythroseptica*, the oogonial hypha penetrates and grows through the antheridium, emerges above the antheridium and then swells to form the oogonium, with the antheridium persisting as a collar around its base. This arrangement of the antheridium is termed **amphigynous** ('around the female').

Amphigynous arrangement



Under conditions favourable for sexual reproduction, hyphae producing antheridia are attracted to oogonia by hormones and produce fertilization tubes. One or more antheridia become attached to each oogonium. Mating of Achlya is controlled by **antheridiol and oogoniol**.

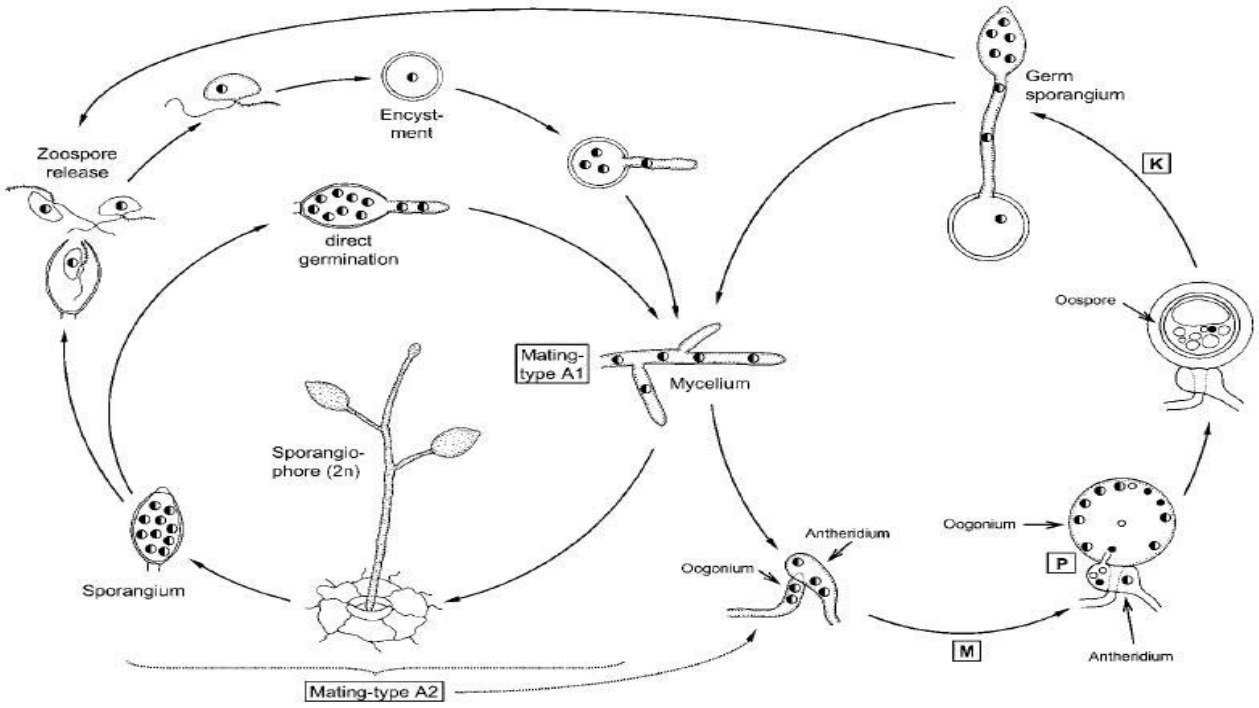
Meiosis occurs within these sex organs producing haploid oosphere in oogonia and haploid male nuclei in antheridia. **Fertilization tubes** originating from the antheridium penetrate the oogonial wall and reach the oospheres. On entering the oogonium, a fertilization tube may branch out and send one branch to each oosphere. Antheridial nuclei migrate from antheridium to the oosphere through fertilization tube; one nucleus enters each oosphere, approaches its nucleus, fuses with it to form a **diploid zygote** nucleus.



Two methods of oospore germination in *Albugo candida*

Oospore germination: After a long period of rest, outer wall of the oospore bursts and the endospore is extruded as a thin, spherical vesicle, which may be sessile or formed at the end of a wide cylindrical tube. Within the thin vesicle 40-60 zoospores are differentiated and are released on its breakdown.

Life cycle of *Phytophthora infestans* (heterothallic)



Classification of Oomycota:

Members range from unicellular, holocarpic endobiotic parasites to well-developed mycelial forms.

A : Spores formed within sporangium, monomorphic or dimorphic, rarely aplanetic

Holocarpic or eucarpic; hyphae when present lacks constrictions -------- **Saprolegniales**

Eucarpic, hyphae with constriction -------

Leptomitales

- Spores formed within sporangium or an evanescent sporangial vesicle, monomorphic, reniform.

C: Holocarpic---------- **Lagenidiales**

CC: Eucarpic---------- **Perenosporales**

Order Saprolegniales

Coenocytic mycelium, septation to separate sporangial / sexual structures

Saprolegnia --- Sporangial proliferation

Achlya- some species show heterothallism sex hormones play an important role in sexual fusion – produces **antheridiol & oogoniol**

Order Lagenidiales

Consists of microscopic, holocarpic, endobiotic forms – mostly freshwater, few marine – mostly parasitic on algae, other water molds, mosquito larvae, nematodes, insects.

Ex. *Olpidiopsis, Lagenadium*

Order Leptomitales

Saprobic in freshwater/polluted water. Characterized by constriction of aseptate hyphae at intervals, plugged with cellulin.

Ex. *Leptomitis, Sapromyces*

Order Perenosporales

Highly advanced order consisting of either saprophytes or highly specialized parasites of plants.
Sexual reproduction – oogamous type in all families of Perenosporales

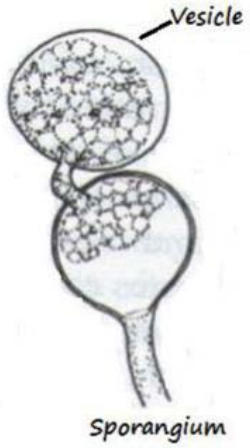
Family Pythiaceae :

Saprophytes or facultative parasites. Sporangiophore with determinate growth. Sporangiophore cannot be differentiated from vegetative hyphae.

Sporangia may or may not produce vesicle, where zoospore differentiation takes place.

Ex. *Pythium* – vesicle present *Phytophthora* – vesicle absent.

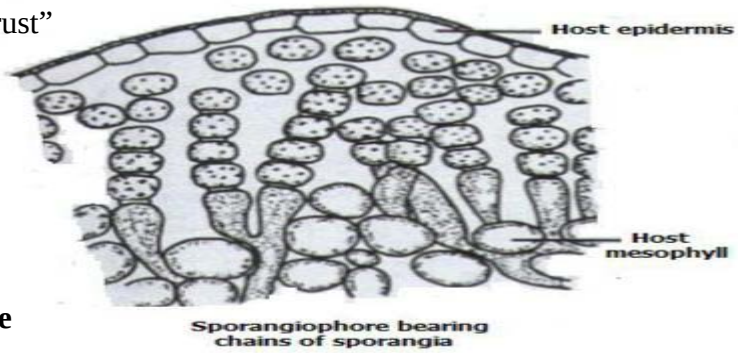
Causes damping off diseases (*Pythium*) or leaf blight (*Phytphthora*)



Family Albuginaceae

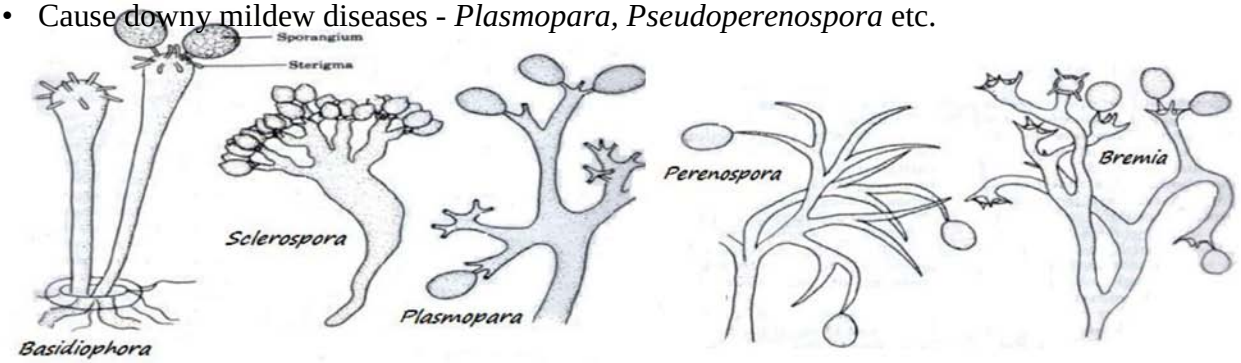
- Obligate parasites
- Mycelium produces short, club shaped sporangiophores – which produce a chain of sporangia in basipetal succession.
- Sporagiophore is of indeterminate growth.

Ex. *Albugo* sp. – "white rust"



Family Perenosporaceae

- Obligate parasites
- Sporangiophore distinct from mycelium and much branched.
- Sporangiophore reach maturity, stop growing and then produce a crop of sporangia on sterigmata at the apices of its branches.
- Cause downy mildew diseases - *Plasmopara, Pseudoperenospora* etc.



CHYTRIDIOMYCOTA

Salient features

- Gr. chytridion = little pot; common name chytrids, due to presence of small, simple structure containing unreleased spores, known as chytridium. Simplest and smallest among true fungi,

Presence of motile stage in their life cycle; both zoospores and gametes contain one posterior whiplash (smooth) flagella; multi-flagellate zoospore in *Neocallimastix*

- Thallus unicellular, filamentous without septa
- Cell wall made up of chitin and glucan
- Nuclear division intranuclear, centric
- Asexual reproduction by uninucleated, uniflagellate (posterior whiplash type) zoospores formed by cytoplasmic cleavage in a zoosporangium
- Sexual reproduction isogamy, anisogamy, oogamy
- Some are obligate anaerobes; Neocallimastigales (rumen fungi) e.g., *Anaeromyces* can flourish in the rumen of herbivorous mammals (which regurgitate and masticate previously ingested food like cows, sheep and deer) because oxygen is depleted there by the intense respiratory activity of a dense population of protozoa and bacteria. They have a **hydrogenosome**, equivalent to a mitochondria, for generating energy.

Habitat:

Mostly aquatic saprophytes, few parasitic on algae, aquatic plants and animals; *Olpidium* spp. biotrophic parasites of *Spirogyra, Vaucheria, Ceratium Rhizophydium planktonicum* parasite on phytoplankton.
Synchytrium endobioticum – black wart disease of potato
Catenaria on nematodes, *Coelomomyces* on mosquito larvae,
Rozella allomycis biotrophic parasite on *Allomyces* sp. (another chytrid)
Caulochytrium mycoparasite on mycelia & conidia of terrestrial fungi
Zoospores of *Olpidium brassicae* vectors for tobacco necrosis virus (TNV)

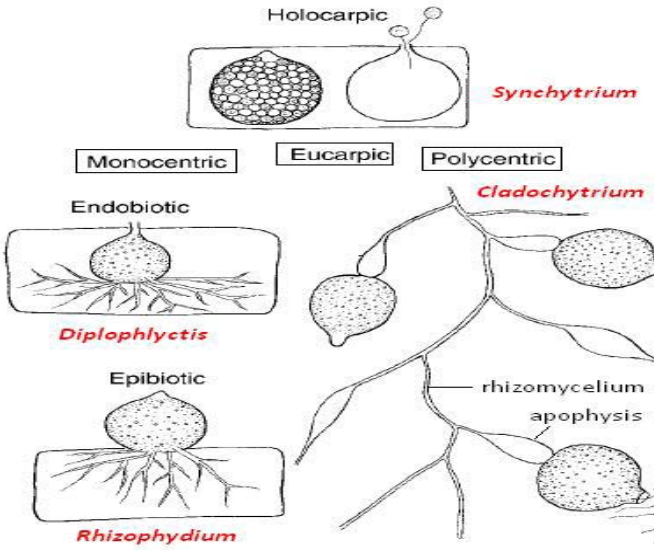


Rozella allomycis on Allomyces wart disease Coelomomyces on mosquito larvae of potato

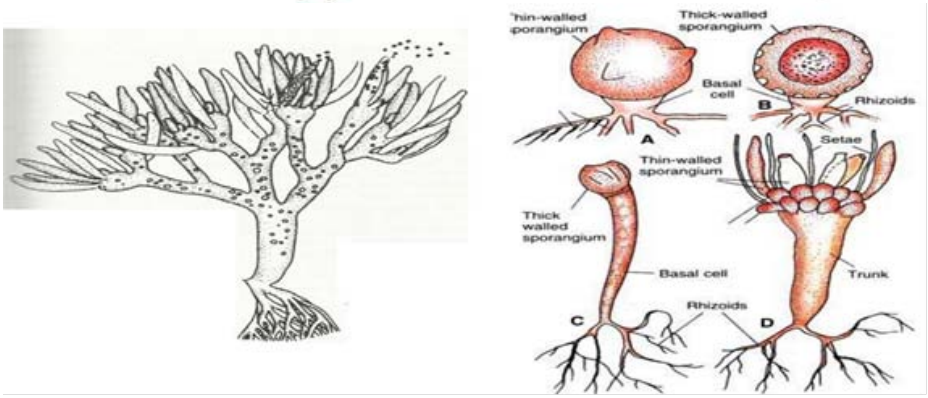
Range of thallus structure

Holocarpic: Primitive, endobiotic, unicellular globose thallus which except wall surrounding it becomes converted at maturity into one or more reproductive structures. *Olpidium, Synchytrium*
Eucarpic: consists of vegetative part with rhizoids and reproductive parts arising from certain portions of the thallus.

Rhizoids are short, delicate filaments that contain cytoplasm but no nuclei and eventually separated from rest of the thallus by septa. These may be limited or inconspicuous or well developed, much branched (**Rhizomycelium**) depending upon the species, their function is to attach the thallus to its substratum and nourish it by digesting and absorbing food.



Rhizoidal system with a single reproductive structure – **monocentric**; several reproductive structures are interconnected by rhizomycelium – **polycentric**; e.g. *Cladochytrium*; the vegetative system may bear intercalary swellings and septate turbinate cells (**spindle organs**); *Rhizophlyctis rosea* shows both moncentric & polycentric forms. Septa may be formed at the base of the reproductive structures or older portion of the thallus – **pseudosepta** – chemical composition is different from the wall of the hyphae. Rhizoids extend directly from the reproductive body or from a broad globose part of the thallus – **apophysis** – under the reproductive body.



Endobiotic – thallus & reproductive body is entirely within the host cells.

Epibiotic – rhizoids penetrate the host / substratum & reproductive body is not in Contact with the host/ substratum.

Physoderma shows both epibiotic and endobiotic sporangia.

The more advanced members – *Blastocladiales* and *Monoblepharis.*

Thallus growth & Reproductive development:

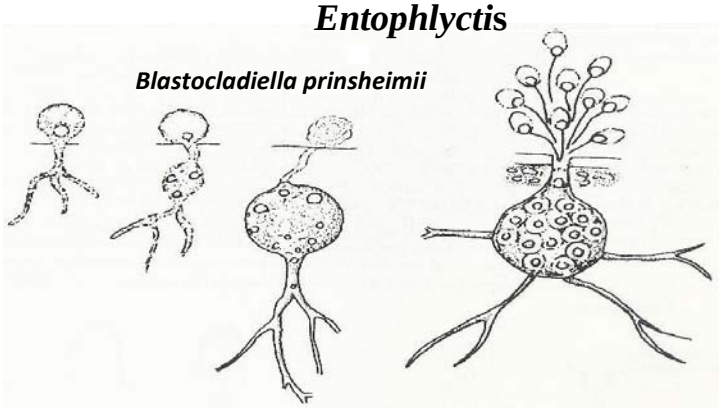
Occur following encystment of an uninucleate zoospore.

Two principal modes of zoosporangium development occurs:

Endogenous – zoosporangium endobiotic; the nucleus remains in the cyst, which enlarges to form one (moncentric) or more (polycentric) sporangia.

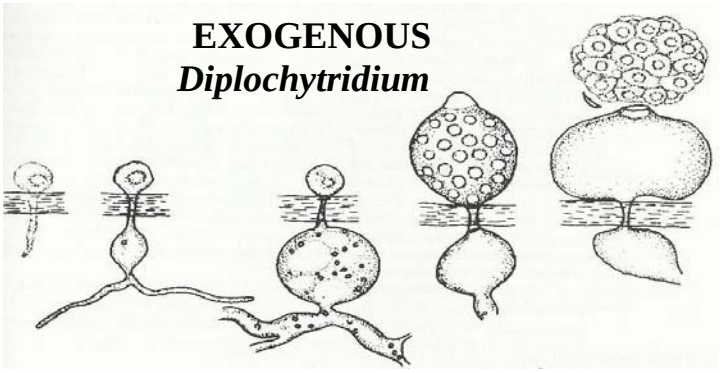
ENDOGENOUS

Entophlyctis



Exogenous – single nucleus migrates from cyst into a germ tube where it divides. Apophyses may be formed below cyst. The cyst enlarges to form one (moncentric) or more (polycentric) epibiotic zoosporangia.

Exogenous-polycentric forms may be either colonial or filamentous where rhizomycelia causes unlimited growth under favourable condition.



Asexual reproduction

By of uninucleated, uniflagellate (posterior whiplash type) zoospores formed by cytoplasmic cleavage in a zoosporangium which is spherical or pear-shaped structure. In holocarpic spp. (*Rhizophlyctis*), unicellular somatic cell wholly converted into 1-2 zoospores; in eucarpic (*Allomyces*), sporangia formed on part of the thallus contain numerous zoospores

Release of zoospores from zoosporangium:

In *Rhizophydium* entire sporangial wall deliquesce or split to release zoospore. In others, zoosporangium may be operculate or inperculate.

The sporangium of **inoperculate** chytrids (*Olpidium*, *Synchytrium*) zoospores exit through a **discharge papilla**, each a lens- or nipple-shaped protrusion. Discharge papilla occur directly on the surface of sporangium or at the end of an elongated **exit tube**.

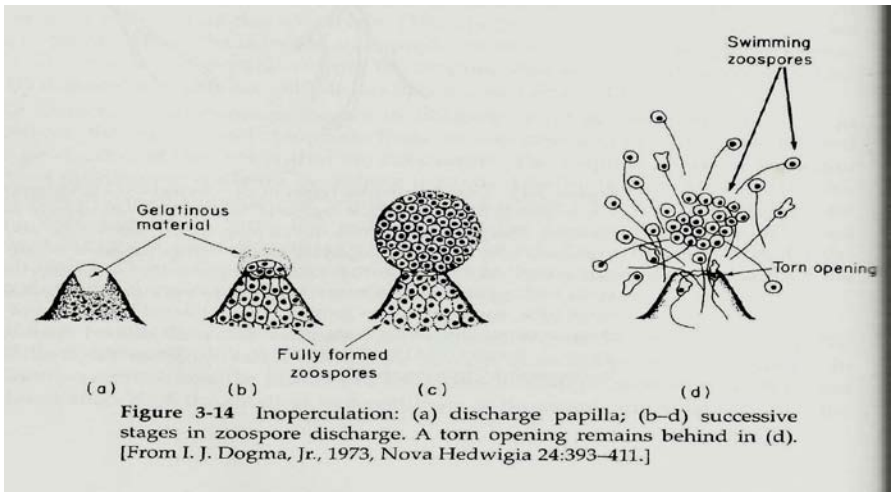
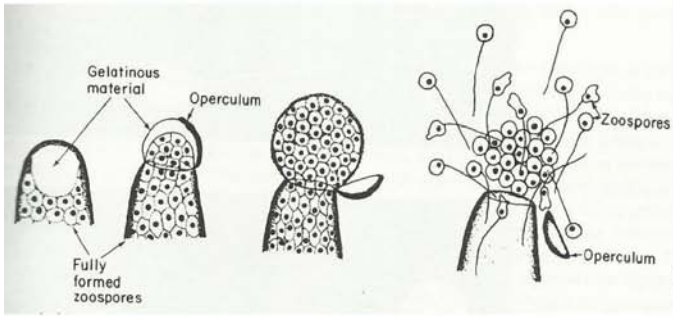


Figure 3-14 Inoperculation: (a) discharge papilla; (b–d) successive stages in zoospore discharge. A torn opening remains behind in (d). [From I. J. Dogma, Jr., 1973, Nova Hedwigia 24:393–411.]

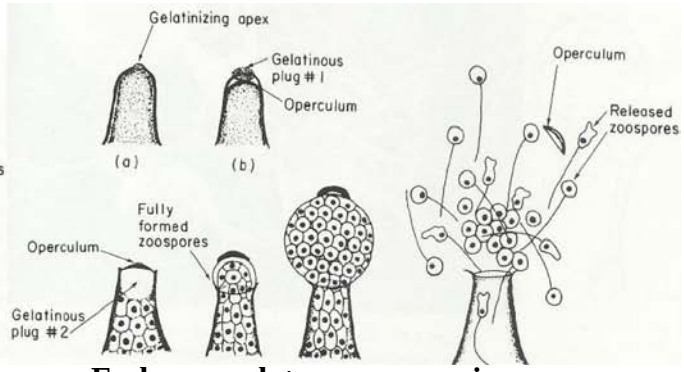
A hyaline refractive gelatinous mass occurs inside the apical wall of the papilla. The wall of the tip of discharge papilla become thin and ruptures to form a pore. The gelatinous mass is expelled, expands and ejects the zoospores passively.

In operculate chytrids the tip of the sporangium forms a lid-like flap, operculum, which may be of exo- and endo- types.

- **Exooperculate (*Chytridium*)**: the wall at the tip of discharge papilla develops a line of weakness, delimiting a circular cap. When it dehisces, the gelatinous mass is extruded



Exooperculate sporangium



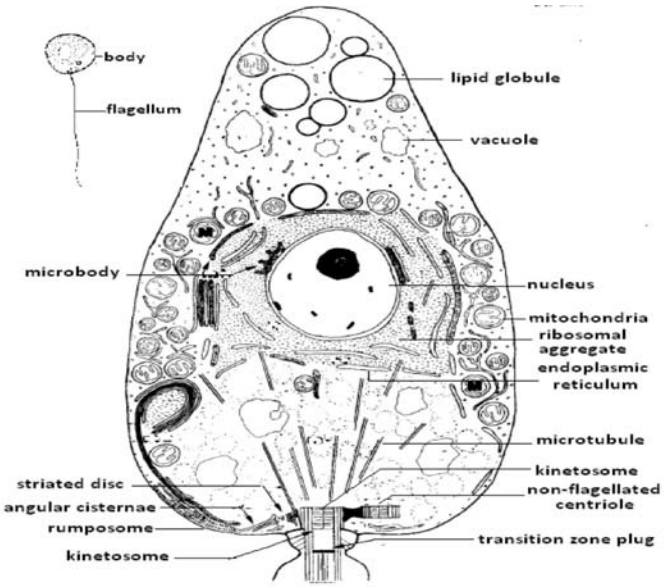
Endooperculate zoosporangium

outwards, forming an envelope around the zoospores which are released passively.

- **Endooperculate (*Nowakowskiella*)**: a cap is derived from a modified membrane that forms over the surface of the protoplasm and in older zoosporangium may become thickened and rigid. Endooperculum is cast out as the gelatinous plug(s) releasing zoospores.

Zoospore ultrastructure of *Monoblepharis polymorpha*

Zoospore is spherical or ellipsoidal body with a long-trailing flagellum. They show characteristics jerky or hopping movement. The body of the zoospore is differentiated into three regions: an anterior region which is often devoid of organelles apart from lipid globules, a few vacuoles and tubular cisternae; a central region which contains the nucleus, surrounded by ribosomal aggregations (sometimes termed the nuclear cap), microbodies and spherical mitochondria with flattened cristae; and a posterior 'foamy' region at the base of which are the functional kinetosome, a non-functional kinetosome and a rumposomal complex. The functional kinetosome is surrounded by a striated disc, apparently anchored to annular cisternae. From an electron-dense region of the striated disc, about 31-34 microtubules extend outwards into the body of the zoospore.



The flagellar apparatus: The whiplash flagellum resembles that of other eukaryotes, with a smooth membrane enclosing a cylindrical shaft, the **axoneme**, made up internally of nine doublet pairs of microtubules surrounding two central microtubules. The **base of the axoneme** comprises three regions, the **flagellum proper**, the **transitional zone** and the **kinetosome**. The function of the kinetosome is to generate the flagellum. An interesting feature found in several species is a **second kinetosome** or the remainder of one, the **dormant kinetosome**. Its presence has led to the suggestion that the ancestors of the Chytridiomycota may have had biflagellate zoospores, the second flagellum having been lost in the course of evolution.

In transverse section, the **kinetosome** resembles a **cartwheel**, because to each of the nine outer microtubule doublets seen in the flagellum proper, a third microtubule is attached. This is called the **C-tubule**; in the doublets, that tubule with extended dynein arms is the **A-tubule**, and its partner is labelled **B**. These flagellar microtubules radiate as kinetosome props into the zoospore, perhaps providing structural support and anchorage of the flagellum. Microtubules may also be attached laterally to the kinetosome, contributing to the flagellar root system. In the innermost (proximal) part of the transitional zone, the nine microtubule triplets of the kinetosome are converted into the doublets of the flagellum proper; concentric fibres, possibly arranged helically, surround the nine doublet pairs. Also within the transitional zone the two central microtubules arise near a terminal plate.

The microbody-lipid complex: The MLC is made up of a microbody which is often closely appressed to a large lipid globule and to simple membrane cisternae or a tubular membrane system, the **rumposome**. This is defined as a cisterna in which there is an area with hexagonally

arranged, honeycomb-like pores called **fenestrae**. The rumposome may be involved in signal transduction from the plasma membrane to the flagellum because it is known that this organelle sequesters calcium. Regulation of external calcium concentrations has an effect on the symmetry of flagellar beat and hence on the direction of zoospore movement.

Germination of Zoospore:

Zoospores have ability to undergo prolonged amoeboid crawling by pseudopodium-like extension of cell. After a period of swimming (a few min to several hours) zoospore comes to rest and encyst; its flagellum may be shed, may contract or may be completely withdrawn.

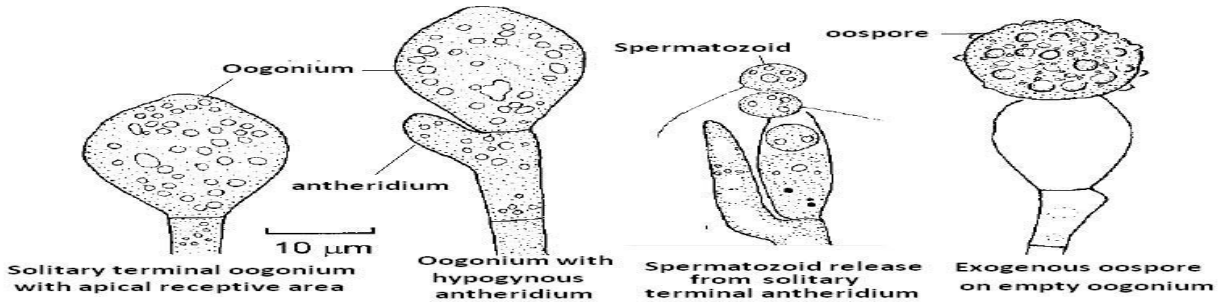
In holocarpic parasites, the zoospore encysts on the host surface and injects the cytoplasmic contents into the host cells.

In monocentric chytrids, rhizoids develop from one point on the cyst and it develops into a zoosporangium. In polycentric chytrids, the zoospore may form a limited rhizomycelium on which swollen cells arises. Monopolar (Chytridiales) or bipolar (Blastocladiales) germination occur.

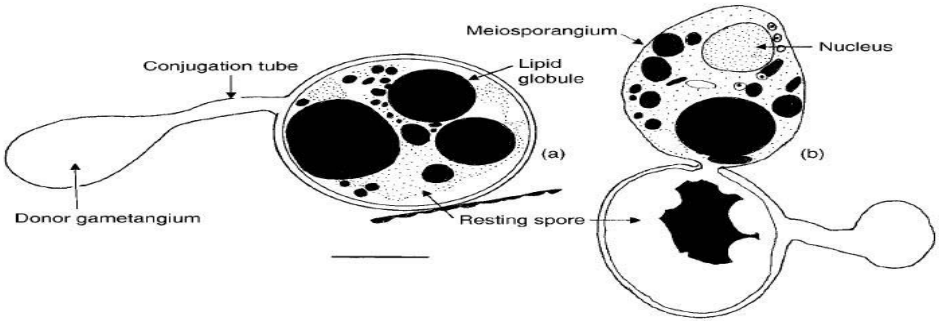
Sexual reproduction

Rare in Chytridiales and not known in Blastocladiales

Isogamy: *Synchytrium* fusion between two morphologically identical gametes **Anisogamy:** *Allomyces* Gametophytic thallus bears two types of **gametangia** at the tips of branches – **antheridia** (orange in colour & globular) and the female gametangia (colourless, cylindric or ovoid), with varying position i.e. below & above or *vice versa.* Larger, sluggish female gamete releases a pheromone, **sirenin** to attract male gametes that are smaller, more actively motile and any one of the male gamete fuses with the female gamete. Sporophytic thallus roduces zoosporangia - alternation of generation **Oogamy:** *Monoblepharis* anthredium releases motile spermatozoids any one of which fuses with a much large, non-flagellate immobile globose egg, present within Oogonium. Fertilization results zygote that forms a thick-walled Oospore.

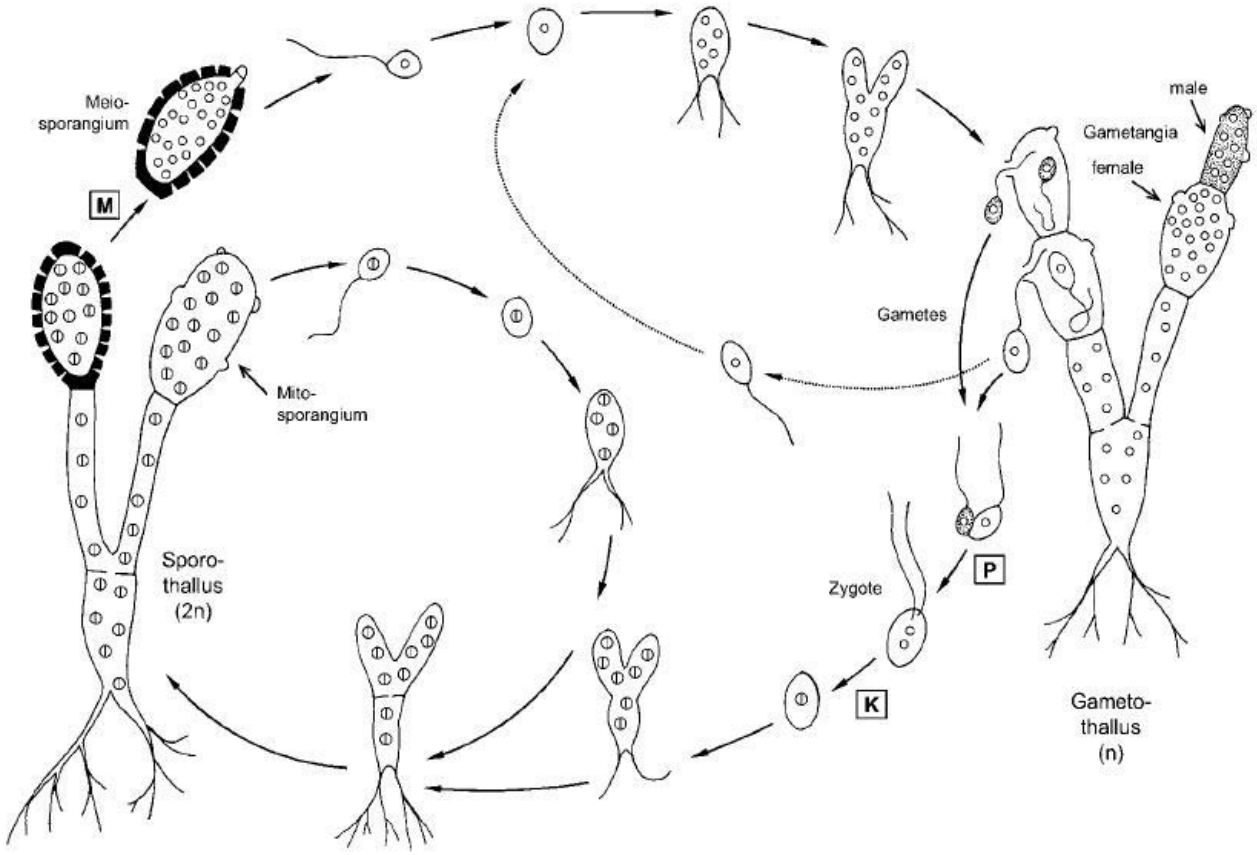


Somatogamy: fusion of undifferentiated hyphae or rhizoid, cultures of *Chytriomyces hyalinus* **Gametangio-gametangiogamy:** *Zygorhizidium planktonicum* fusion of gametangia; zoospores released from the zoosporangia germinate and form new zoosporangial thalli or gametangial thalli of two sizes with globose uninucleate gametangia. Conjugation occurs when a conjugation tube grows from the smaller donor to the larger recipient gametangium. The larger gametangium develops a thick wall after fusion and functions as a diploid resting spore. After a period of maturation, the resting spore acts as a prosporangium and gives rise to the thin-walled meiosporangium where meiosis takes place. Zoospores are formed in the meiosporangium



Sexual reproduction in *Zygorhizidium planktonicum*

Life cycle of *Allomyces macrogynus*



ZYGOMYCOTA: Salient features
Gr. Zygos = yoke + spora = spore
Mycelium filamentous coenocytic;
Asexual reproduction is by production of sporangiospores;
Production primary saprophytic of thick-walled sugar resting fungi- utilize spore simple–zygosposuga**r**e;;

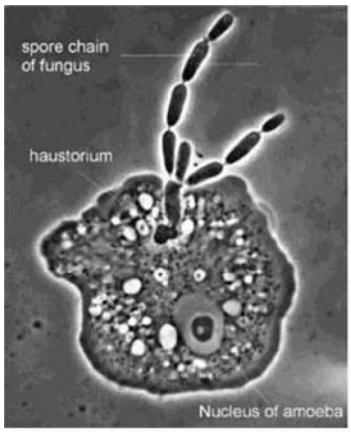
Consists of two classes - **Zygomycetes** and **Trichomycetes**

- **Zygomycetes** consists of orders such as Mucorales, Endogonales, Glomales, Entomophthorales, Zoopagales
- **Trichomycetes** – „**hair fungi**" – obligately associated with insects, millipedes, crustaceans etc.

•Thermophilic (60°C): *Thermomucor*
•Psychrophilic (0°C): *Thamnidium*

Habitat

Saprophytes on substrates such as fruit, soil, and dung (Mucorales),
Facultative or weak parasites (pathogens) of insect (Entomophthorales), animals, plants, amoebae and other fungi



Aquamortierella elegans aquatic with appendaged sporangiospore

Amoebophilus simplex (Zoopagales)

produces a haustorium that enters its amoeba host

Sporangia of ***Spinellus fusiger*** (Mucorales)

parasitic on fruitbodies of the mushroom *Mycena pura*

Symbiotic: Plant mutualists forming ectomycorrhizae (Endogonales), endomycorrhizae or VAM (Glomales)

- Harmless inhabitants (commemsalists) of arthropod guts: Trichomycetes-Harpellales *Genistellospora homothallica* feed on nutrients that are not utilized by the arthropod

Beneficial effects

Fungi	Products/uses
Rhizopus stolonifer	Cortisone (steroid)-used in various skin problem
***Blakeslea* sp.**	β-carotene- colourant, foodadditive
Mucor ramannianus	Raymycin- antibiotic against Gram+bacteria
Mucor miehei	Lipase

Mucor rouxii	Amylase
***Thamnidium* sp.**	Protease

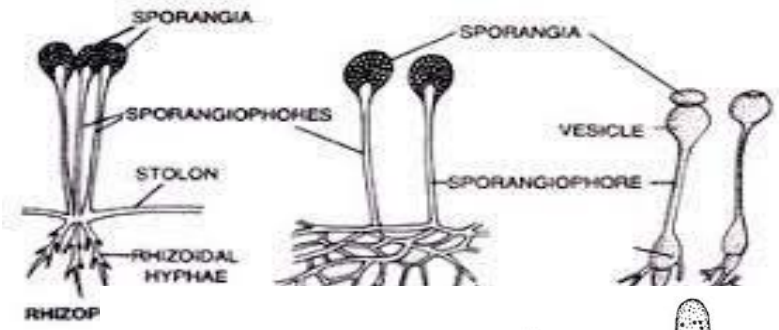
Harmful effects

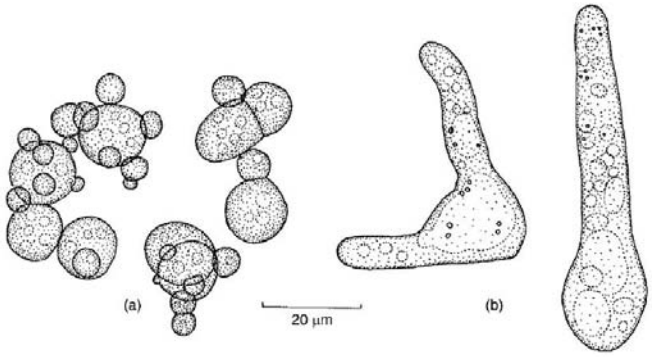
Post harvest disease, soft rot of fruits & vegetables by ***Rhizopus stolonifer,*** ***Mucor racemosus***

Human disease: zygomycosis/ mucoromycosis by ***Mucor hiemalis***, ***Cunninghamella ertholletiae***

Animal disease by ***Basidiobolus ranarum*****fect**

Range of Thallus Structure

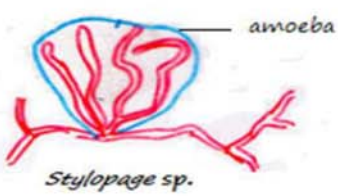


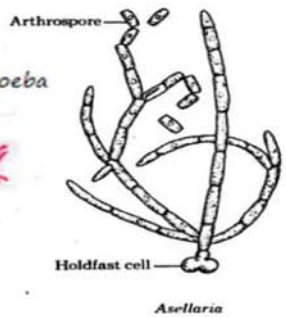


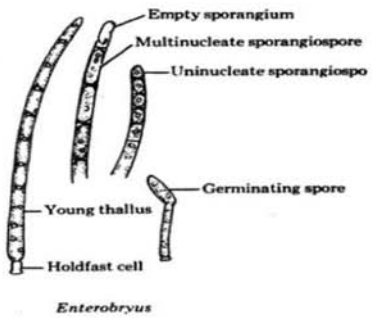
In Mucorales, thallus consists of extensive, stout, much branched, non-septate (coenocytic) hyphae. In *Rhizopus*, thallus is differentiated into **stolons**, **rhizoids** and **sporangiophores**. [stolon & rhizoids are absent in *Mucor*, absorbed food by Inentire*Pilobolus*mycelium, sporangiophore] with basal inflated pigmented trophocyst and a subsporangial veIn**s**icleome,. septa are formed that divide the hyphae into **pleurinucleate coenocytic** segments which are not truly cellular. Septa are also formed during delimitation of sporangia & gametangia. Septa are solid without pore (*Mucor*) or perforated (*Coemansia*). A few are **dimorphic**; in anaerobic liquid culture, especially in the presence of CO_2, several species of *Mucor* (e.g. *M. rouxii*) grow in a yeast-like instead of a filamentous form but revert to filamentous growth in the renewed presence of O_2. [*Rhizopus oryzae* can grow under anaerobic condition but still remain mycelial, *Phycomyces*

In Entomophthorales – vegetative mycelium is reduced. The original coenocytic mycelium later becomes septate and breaks up into small fragments – **hyphal bodies** – which act as propagative units.

In Zoopagales – thallus is of two types (a) minute and coiled inside the host cell (animal) – *Cochlonema* (b) mycelium very delicate, non-septate or sparingly septate (ectoparasites) – *Stylopage*.



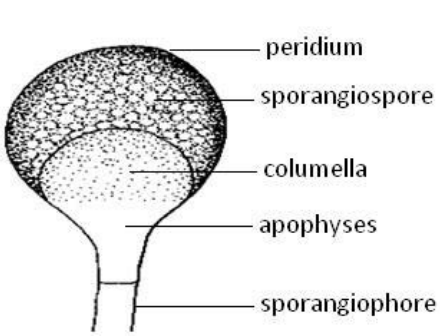


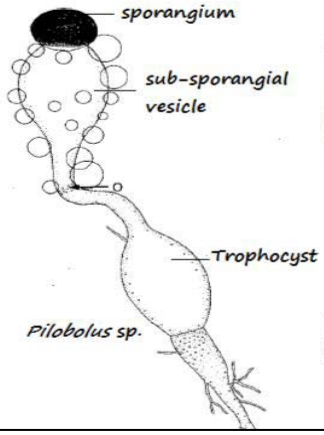


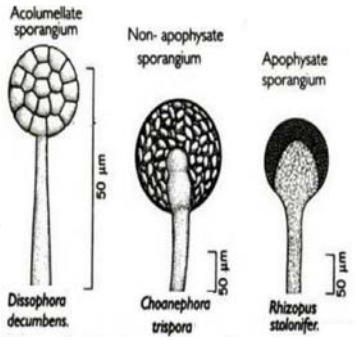
In Trichomycetes – thalli are unbranched (Enterobryus) or branched (*Asellaria*), septate; perporated with flared septal wall around an electron-dense non-membranous biumbonate plug; anchored to the hind gut of arthopods by holdfast.

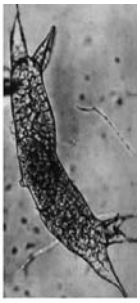
Asexual reproduction

By formation of non-motile **sporangiospores** – produced within **sporangia** borne on simple or branched **sporangiophore.** Shape of sporangia varies (as taxonomic marker), sporangia – flask-shaped (*Saksenaea*), dumbbell-shaped (*Halteromyces*), bilobed, cylindrical with horn- like projection (*Echinosporangium*), obovoid, obpyriform due to presence of **apophyses** (*Rhizopus*). In Mucorales – sporangia are large, globose with central columella and spores are formed outside the **columella**.





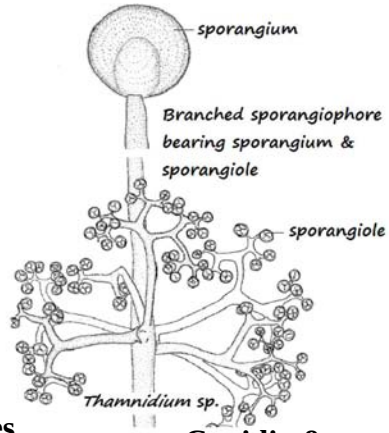




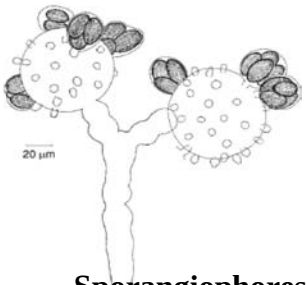
Echinosporangium In *Pilobolus*, sporangiophore unbranched with basal inflated pigmented trophocyst and a subsporangial vesicle.

Some produce smaller sporangia, with or without columella. Smaller sporangia are called **sporangioles**. Both sporangia & sporangioles may be formed by the same species (*Thamnidium*). Sporangioles may contain a few (*Blakeslea* sp.) to single spore (**stylospores** as in *Mortiella zonota*). In single spore sporangiole, spore wall indehiscent and fused with sporangiole wall – resembling conidia (*Cunnighamella*).

Stylospores
Mortiella zonota

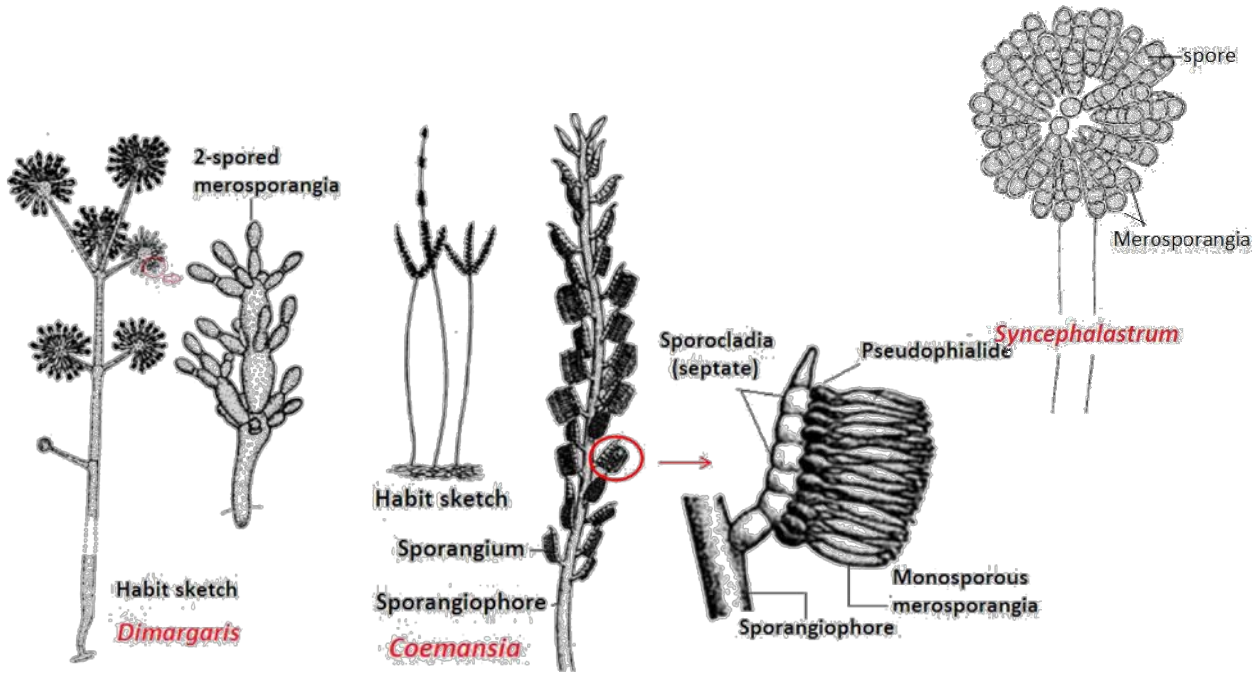


Conidia & sporangiole
Cunnighamella



Sporangiophores with globose terminal vesicles bearing sporangiola containing 3-4 spores
Blakeslea

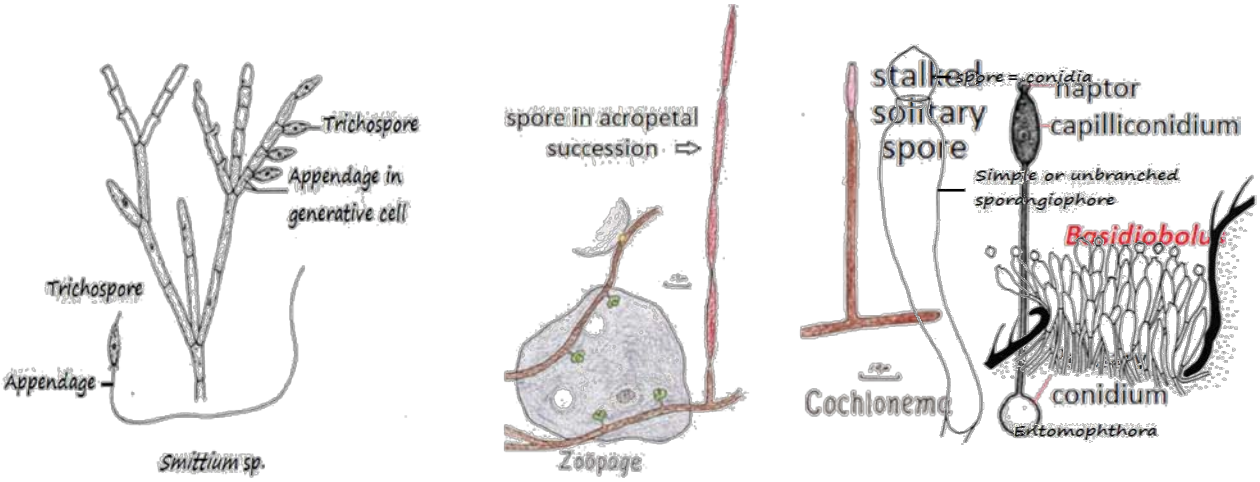
In some members, sporangia are cylindrical containing 10-14 spores in a row (uniseriate) – **merosporangia**. They radiate from the inflated head of the sporangiophore (*Syncephalastrum*). In *Dimargaris* – 2 spores, *Coemansia* – 1 spore per merosporangium.



In Entomophthorales, conidia borne on simple or branched conidiophore; conidia are forcibly discharged – germinate by germ tube; in absence of appropriate substrate, smaller secondary conidia or **capilliconidia** (with haptor, an attachment organ) develop.

In Zoopagales – slender aerial mycelium produce spindle shaped conidia.

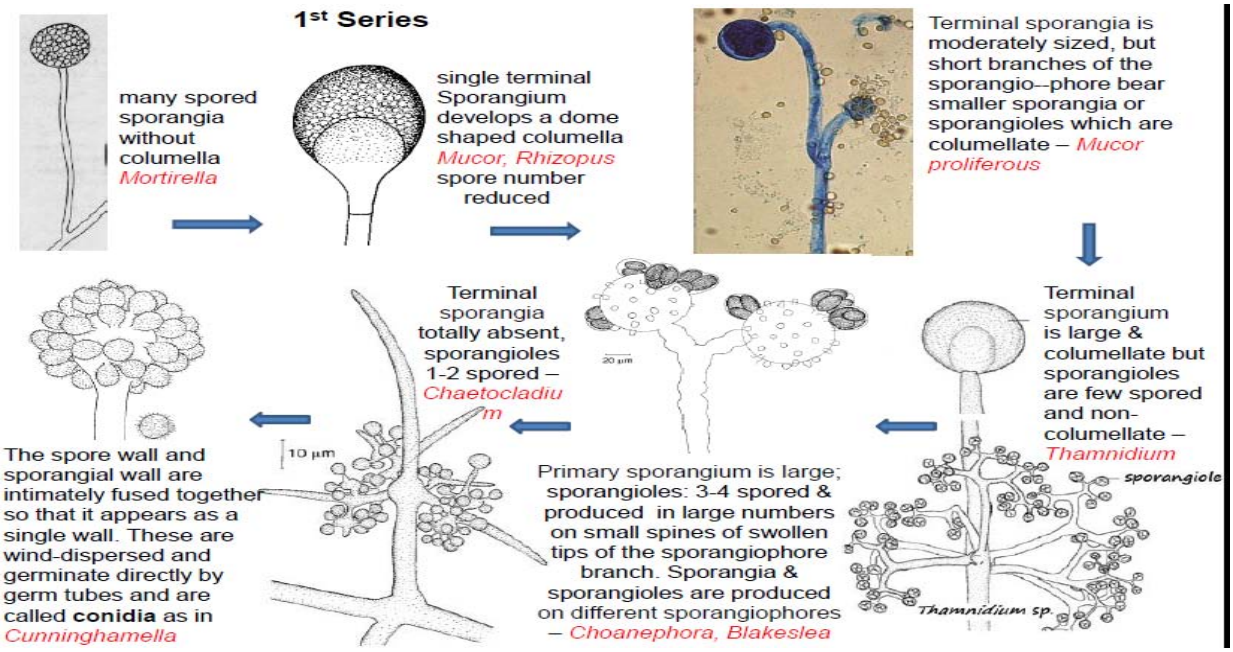
In Trichomycetes – coenocytic thallus cleaves to form sporangia beginning at the apex downwards - forming endogenous sporangiospores or exogenously branched **trichspores**.



Evolution of conidia from sporangia in mucorales

Mucorales show a wide range of structures of asexual reproduction.

There is a gradual tendency among the species of this order towards reduction in size of sporangium and number of spores, until they become 1-2 spored. Finally, the spore wall and sporangium wall fuse and the whole structure becomes deciduous, wind dispersed and germinate directly. At this stage , the sporangium is called **conidium**. In Mucorales- 2 series of evolution of conidia has occurred.



1st Series

1. Sporangia are many spored without columella - ***Mortierella.***
2. In the next stage of evolution – single terminal sporangium develops a dome shaped columella – ***Mucor, Rhizopus*** – spore number is reduced
3. Terminal sporangia is moderately sized, but short branches of the sporangiophore bear smaller sporangia or sporangioles which are columellate – ***Mucor proliferous.***
4. Terminal sporangium is large & columellate but sporangioles are few spored and non-columellate – ***Thamnidium***.
5. Primary large sporangium is produced but sporangioles are only 3-spored & produced in large numbers on small spines of swollen tips of the sporangiophore branch. Sporangia & sporangioles are produced on different sporangiophores - ***Choanephora***.
6. Terminal sporangia often absent, sporangioles -1-2spored - ***Dicranophora***.
7. Terminal sporangia totally absent, sporangioles 1-2 spored – ***Chaetocladium***.
8. The spore wall and sporangial wall are intimately fused together so that it appears as a single wall. These are wind-dispersed and germinate directly by germ tubes and are called conidia as in ***Cunninghamella***.

2nd Series

1. In ***Syncephalastrum*** – the Mucor type of sporangium is completely absent. Sporangiophores are branched and their tips are swollen. On these tips, bunch of elongated, cylindrical sporangia (merosporangia) with one row of spores are produced. Further reduction in the number of spores is seen in Dimargaris, where 2-spored merosporangia are found.
2. In ***Syncephalis, Piptocephalis*** – merosporangia are divided into many locules as there are spores formed by septation, so that each locule contains one spore. Ultimately, sporangium wall disintegrates and the spores stick end to end in chains to form Aspergillus-like condition. These monosporous segments may be considered as conidia and compared to chains of conidia found in Aspergillus.
3. In ***Coemansia***, monosporous merosporangia resembling conidia are present.

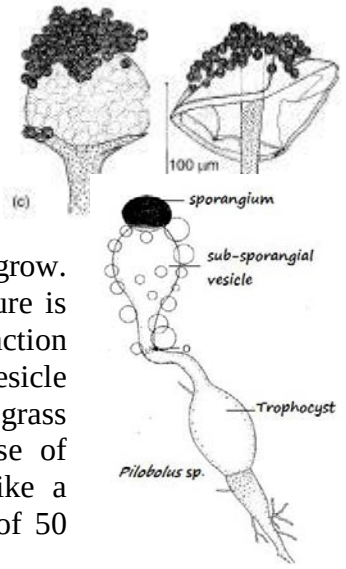
Dispersal of spore

In some Mucor spp. (*M. hiemalis*) sporangium wall dissolves at maturity leaving a stalked spore drop at the tip of the sporangiophore. The spores absorb water and adhere to the columella with the ramnants of sporangial wall.

The sporangial walls of Phycomyces & *M. mucedo* are thin, readily rupture at maturity to expose spores in a mucilaginous matrix that are dispered by rain splash, insects or after drying.

In Mortierella, dry spore mass is dispersed by air currents.

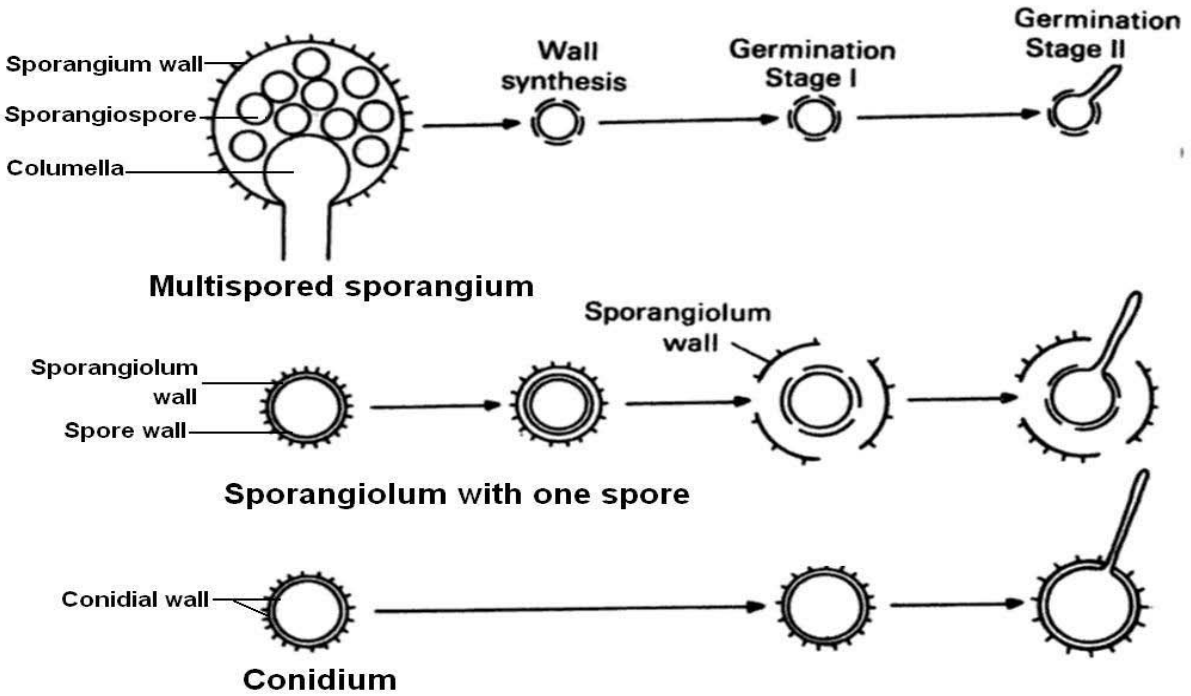
In *Rhizopus stolonifer*, when sporangia dry, columella collapses and found as an inverted pudding bowl at the tip of the sporangiophore. This leads to the fragmentation in sporangial wall releasing dry spores in wind currents.



Shot-gun mechanism: In *Pilobolus*, light signal is perceived by orange carotenoid at the base of subsporangial vesicle and the sporangiophores bend towards bright light where grass is likely to grow. The vesicle has high sugar content at maturity i.e., its turgor pressure is high and water is drawn by osmosis. Meanwhile enzymatic reaction weakens wall of the vesicle and the sporangium. Suddenly, the vesicle bursts like an over inflated balloon and blows its spore towards the grass where it sticks to its surface by the mucilage released from base of sporangium. Thus the entire sporangium is forcibly discharged like a callon-ball and travels to a distance of 2 m or more with a speed of 50 km/hr.

Mucor racemosus produces chlamydospores both in vegetative hyphae and within sporangiophores and remains at the site of production until conditions favourable for growth recur. *M. rouxii* produces arthrospore under certain cultural conditions.

Morphological changes during germination of sporangiospore

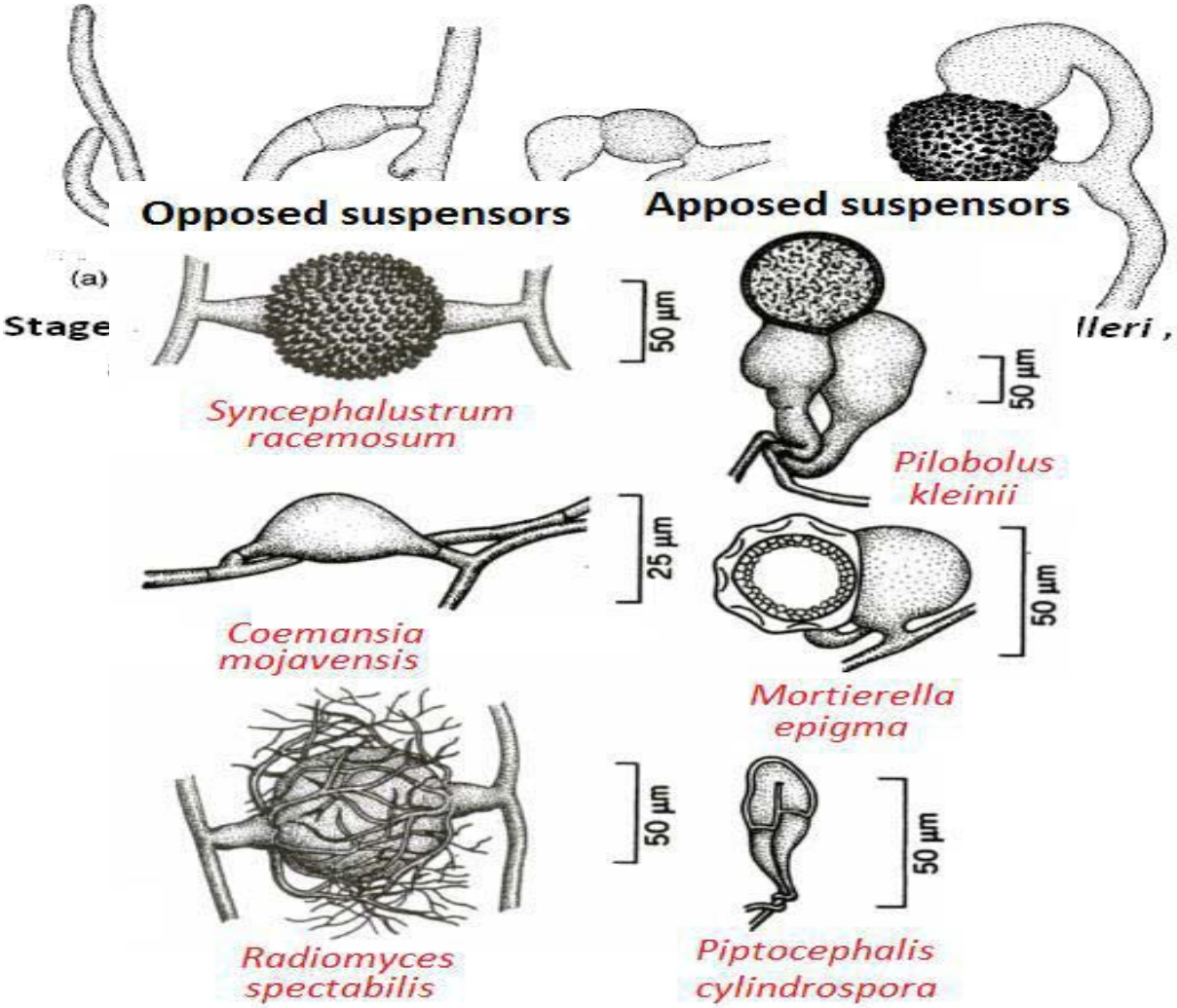


Sexual Reproduction

- Gametangial copulation i.e. fusion of two multinucleate gametangia resulting formation of zygospore.

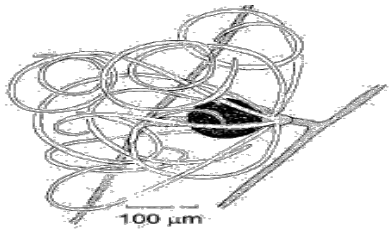
• Some are homothallic (self-sterile) – *Rhizopus sexualis*, *Mucor genevensis*, *Syzygites megalocarpus, Zygorhynchus moelleri*
• Some are heterothallic (self-sterile) - *Rhizopus stolonifer, Mucor hiemalis, Phycomyces blakesleeanu*s, and formation of gametes and sexual reproduction is controlled by sex hormones (**trisporic acid**).
• The shape and size of gametangia may differ, e.g., *Mortierella umbellate*

• In response to volatile stimulus, two zygophores approach each other, come into contact, swell to form progametangia, delimited by septa to form terminal gametangia and adjacent suspensor. Cross wall breaks forming a zygote with numerous nuclei in a common cytoplasm . The zygote enlarges, develops thick multi-layered wall of zygosporangium enclosing a single zygote. Ornamentation of zygospore wall is of taxonomic importance.



Zygosporangia with different suspensors

- In some, union of hyphal segments (hyphal bodies) forms zygospore.
- Zygospore formation is affected by environmental conditions – darkness, low temperature favoured zygospore formation.
- The suspensors may bear **appendages** which arch over the zygospore; possibly to assist in attaching zygospores to the passing animals.

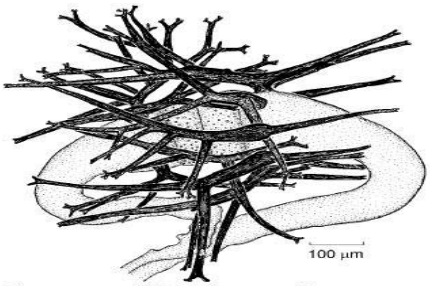


Absidia glauca Zygospore with arching suspensor appendages

Zygospore germination

In *M. mucedo* and *P. blakesleeanus*, the wall of the zygospore is rich in sporopollenin making it extremely resistant to degradation and enables to remain dormant but undamaged in the soil for long periods.

After a period of resting stage (6-7 months), under favourable condition zygospore germinates by producing a vegetative mycelium, a germ sporangium or germ sporangiolum depending on species.



Phycomyces blakesleeanus Young zygospore overarched by dichotomous suspensor appendages

Cytology of zygospore formation

(1) In *Mucor hiemalis*, *Absidia spinosa* and some other species, all the nuclei fuse in pairs within a few days, then quickly undergo meiosis so that the mature zygospore contains only haploid nuclei.

(2) In *Rhizopus stolonifer* and *Absidia glauca*, some of the nuclei entering the zygospore do not pair, but degenerate. The remainder fuse in pairs, but meiosis is delayed until germination of the zygospore.

(3) In *Phycomyces blakesleeanus*, the haploid nuclei continue to divide mitotically in the young zygospore and then become associated in groups, with occasional single nuclei also present. Before germination some of the nuclei pair up, and in the germ sporangium diploid nuclei and also haploid nuclei are found; some of these may be products of meiosis, others may represent the scattered solitary nuclei which failed to pair up.

(4) In *Syzygites megalocarpus* mitotic nuclear divisions continue in the young zygospore, but nuclear fusion and meiosis apparently do not occur. This fungus can therefore be described as **amictic** (Burnett, 1965).

Mating-types represented in germ sporangia

1. **Homothallic species showing pure germinations** in which all the spores produce homothallic mycelia, e.g. in *Mucor genevensis, Zygorhynchus dangeardi* and *Syzygites megalocarpus*.

2. **Heterothallic species showing pure germinations** in which all sporangiospores are any one of mating type, i.e. (+) or (-). *Mucor mucedo, M. hiemalis* and *Phycomyces blakesleeanus*. Here, only one diploid nucleus survives in zygospore that undergoes meiosis and one or more of the resultant nuclei divide mitotically to provide nuclei for the germ sporangium. In some, germ

sporangia contains heterokaryotic spores, the mycelium which develops from them may be abnormal and „neuter", i.e. it is unable to mate with (+) as well as (-) strains.

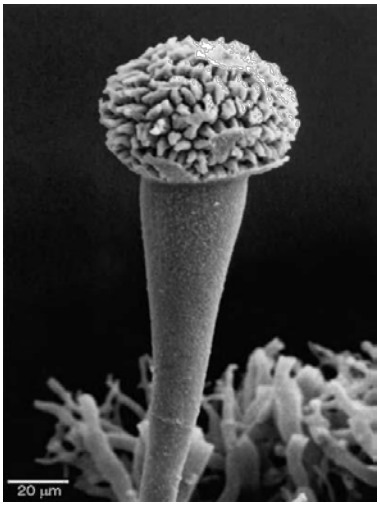
3. **Heterothallic species showing mixed germinations**. In *Phycomyces nitens*, the same germ sporangium sometimes contains (+), (-) and homothallic (i.e. self-fertile) spores from the diploid nuclei that enter the germ sporangia. The survival of more than one meiotic products results this type of spore. Also some 2n nuclei do not undergo meiosis resulting homothallic spore. This is also known as **secondarily homothallic**.

4.

Azygospores

In some Mucorales, if gametangial copulation fails to take place normally, one or both gametangia may give rise **parthenogenetically** to a structure morphologically similar to the zygospore, termed an azygospore (azygosporangium).

Azygospores therefore usually appear as warty spherical structures borne on a single suspensor-like cell, or occasionally on a sporangiophore. They are formed regularly in cultures of *Mucor bainieri* and *M. azygospora*



Azygospores

BASIDIOMYCOTA

SALIENT FEATURES

Large, heterogeneous group; **genera 1353, species > 30,000 (37%** of known true fungi**)**.Gk. *basidion* = small base; presence of basidia & basidiospores. Mycelia septate, with dolipore septa; Presence of clamp connection. Absence of motile cells in life cycle. Complete degeneracy of sexual reproductive organs.

Habitat:

TERRESTRIAL APROPHYTIC: *Agaricus, Ganoderma, Coprinus, Mycena*

SYMBIOTIC: *Cora pavonia* (lichen); *Amanita* (mycorrhiza)

PARASITIC: Plant parasites: *Puccinia graminis* (rust fungus)

Mycoparasites: *Agaricus tuberosus, A. racemosus, A. loveianus* on another spp. of *Agaricus* (eg., *A. nebularis*)

AQUATIC: *Nia vibrossa* (marine); *Bulbillomyces furinosus* (fresh water)

BENEFICIAL ROLES

Edible mushrooms	**Common name**
Agaricus bisporus, A. campestris	White button mushroom
Volvariella volvacea	Paddy-straw mushroom
Pleurotus ostreatus	Oyster mushroom

Lentinula edodes	Shiitake mushroom
Flammulina velutipes	Enokitake or enoki mushroom (Japan)

HARMFUL ROLES

PLANT PATHOGEN-rust & smut fungi

HUMAN PATHOGEN-*Filobasidiella neoformans* cryptococcosis, a fatal disease of brain in HIV '+' patients

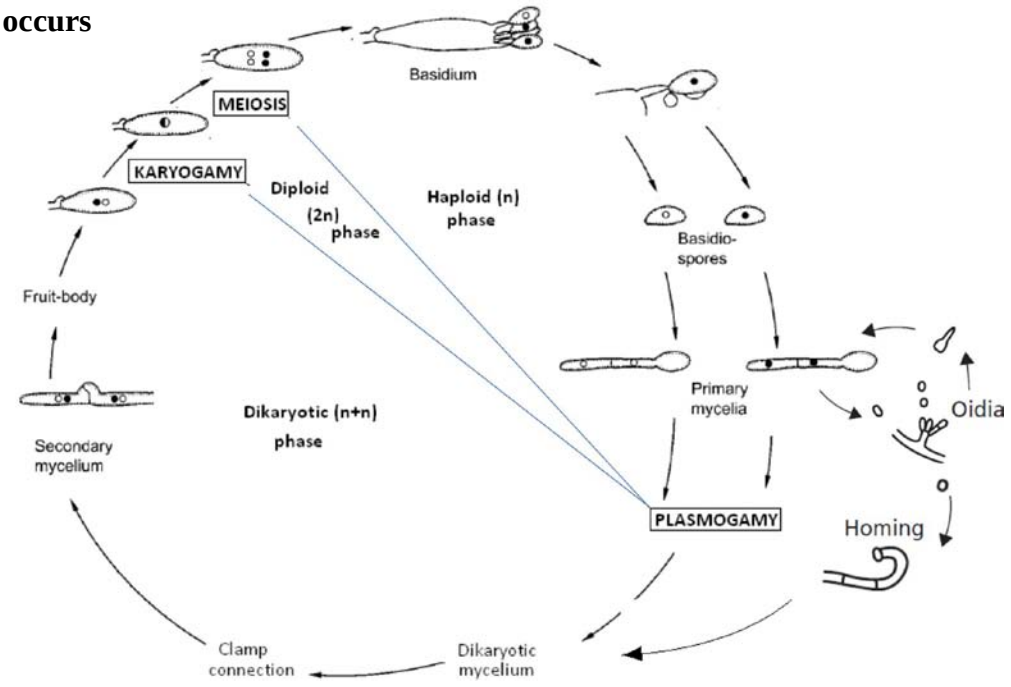
(unicellular form) POISONOUS-*Amanita phalloides*

Amanita phalloides

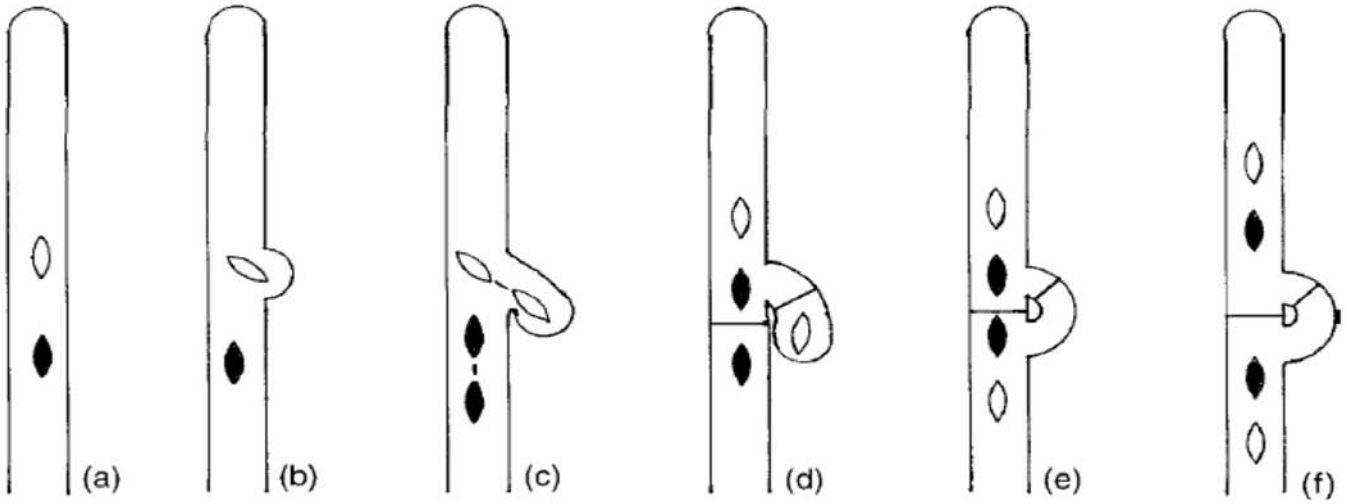
LARGEST ORGANISM OF THE PLANET

The largest and longest-living wood fungi are ***Armillaria*** species. From a single spore of ***A. gallica*** the mycelia grew below ground through the soil and produced mushrooms through an area of about 15 ha in a Michigan forest and was estimated at an age of about 1,500 years and a total biomass of 1,000 t (Smith et al. 1992). A clone of ***A ostoyae,*** known as the honey mushroom estimated at 400-1,000 years covered an area of 6 km2 in the Rocky Mountains (Anonymous 1992). In August 2000, another ***A. ostoyae*** fungus was found in the forest east of Prairie City, Oregon. The mushrooms stretched over an area of about 9 km2, an area as big as **1,600 football fields**. The fungus was found to a depth of about 100 centimeters into the ground. Nobody has estimated the weight of this fungus yet, but it has been estimated that it took the fungus filaments about **2,400 years to grow** from the single spore to the giant fungus size it has reached to date. That all of these mushrooms near the periphery of the fungus growth were derived from one organism was proven by the identity of the DNA in dozens of samples of mycelium and growing mushrooms within the area in question.

Generalised life cycle of a typical member of the basidiomycotina in which basidiocarp formation occurs



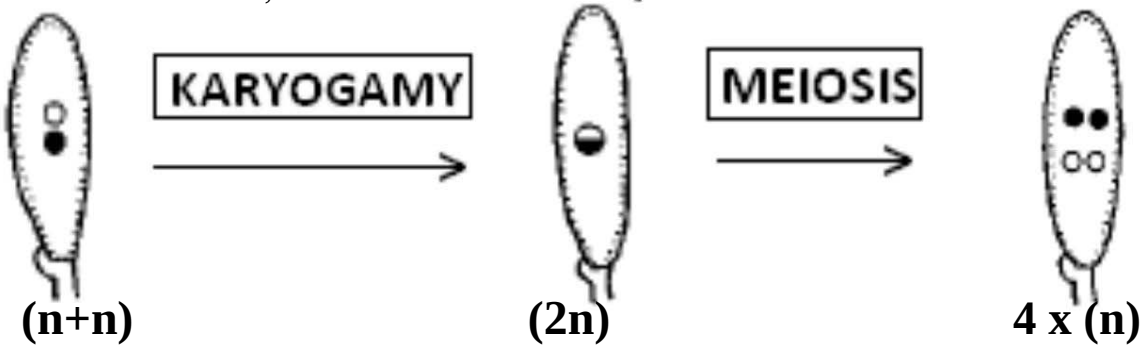
Diagrammatic representation of the sequence of events associated with the formation of a clamp connection in the dikaryotic hypha of a basidiomycete

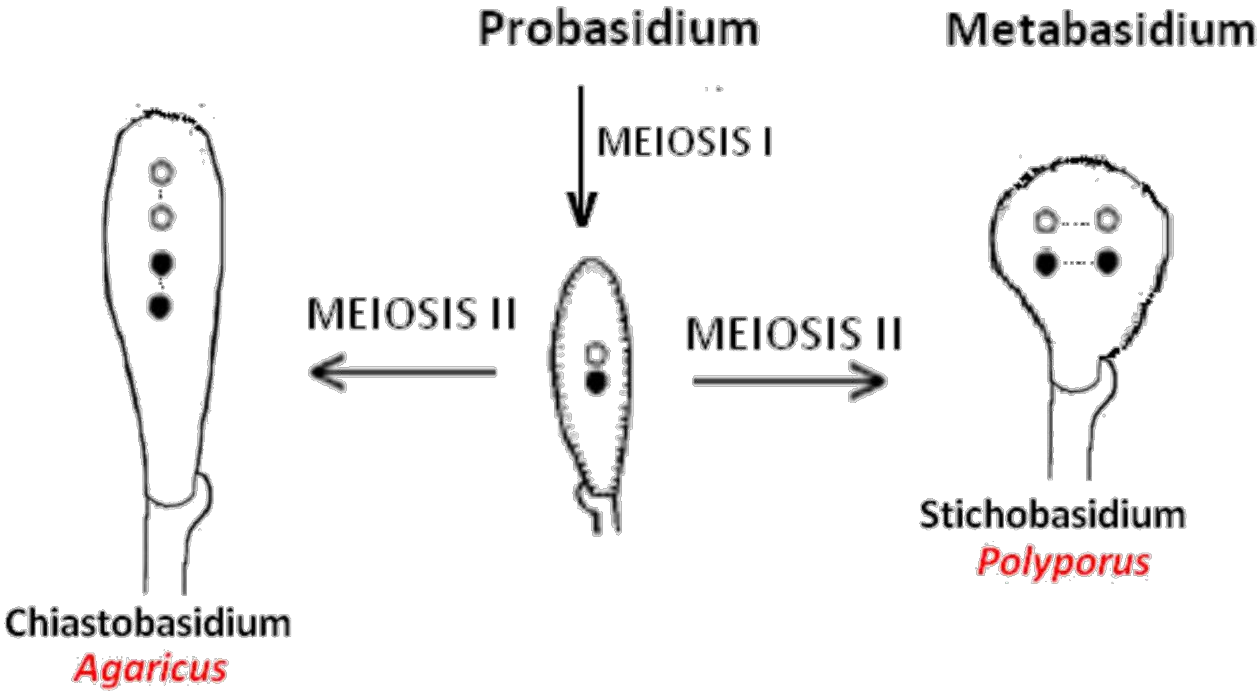


Terminal segment of a hypha with two genetically dissimilar nuclei	A lateral bulge in the hypha appears near the paired undergo the leading nucleus move into the the bulge	The two nuclei in the hypha conjugate (i.e simultaneous) nuclei. The nuclear division. Mitosis of the leading nucleus occurs within the bulge	The lateral bulge has developed into a backwardly directed branch or hook with one daughter of the leading nucleus at its tip. One of the daughter subterminal nuclei moves forward. Transverse septa have developed main hypha and at the base of the hook	The tip of the hook has fused with the wall of the main hypha. Its nucleus has moved into the main hypha and taken up position behind the daughter of the subterminal nucleus	The pairs of nuclei move away from the transverse septum in the main the terminal pair moving nearer the hyphal tip and the subterminal pair distally note that both segments contain dissimilar nuclei but that their arrangement

Terminology regarding nuclear events of basidium development

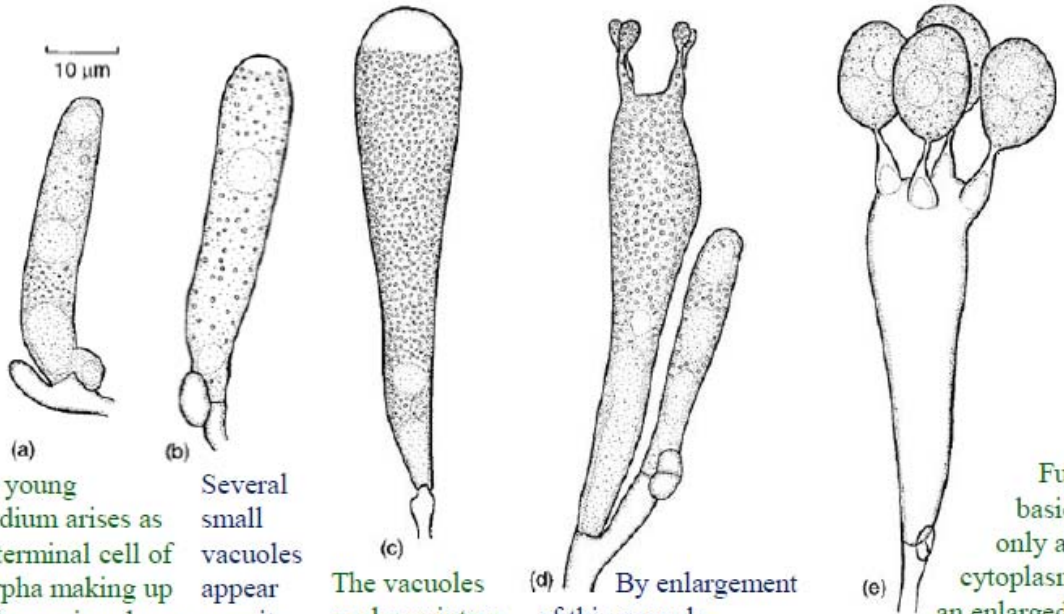
Division is intranuclear; the nuclear membrane remains intact





DEVELOPMENT OF BASIDIUM

Cytological aspects (holobasidium in *Oudemansiella radicata*)



(a) The young basidium arises as the terminal cell of a hypha making up the hymenium layer of the basidiocarp. The cell is filled with dense cytoplasm

(b) Several small vacuoles appear near its base

(c) The vacuoles coalesce into a single large vacuole at its base, and a clear cap is differen--tiated at the tip

(d) By enlargement of this vacuole, cytoplasm is pushed towards the apex of the basidium and from the apical cap region sterigmata develop

(e) Fully developed basidium contains only a thin lining of cytoplasm surrounding an enlarged vacuole. The vacuole, which is filled with liquid, keeps the basidium turgid until spore discharge has occurred

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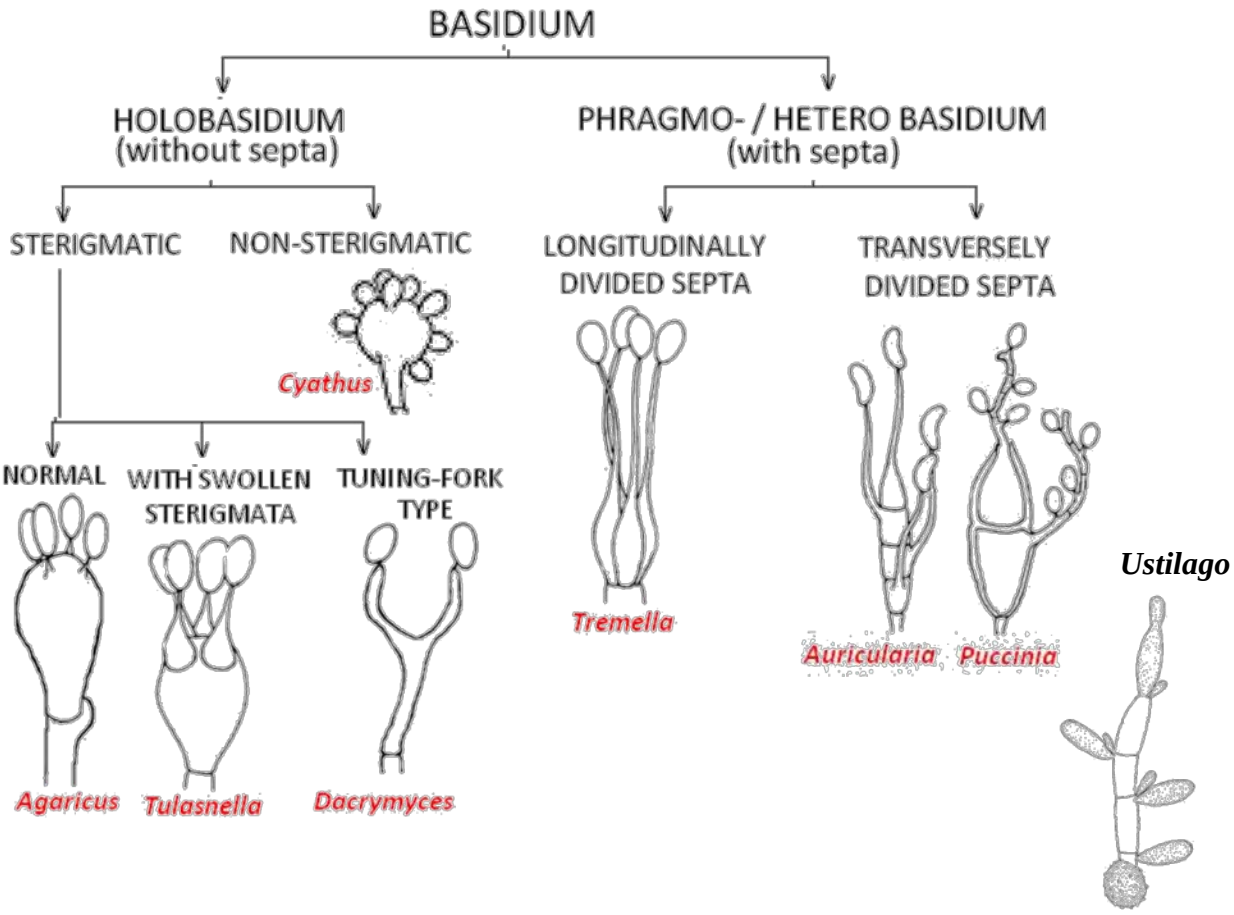


Basidiospore

Basidiospores are unicellular non-motile meiospore produced exogenously on a basidium. Each spore receives single haploid nucleus from basidium and is generally uninucleate, but sometimes becomes binucleate due to mitosis of the nucleus.

Number of basidiospore / basidium

4 (*Agaricus campestris, Polyporus* spp.)

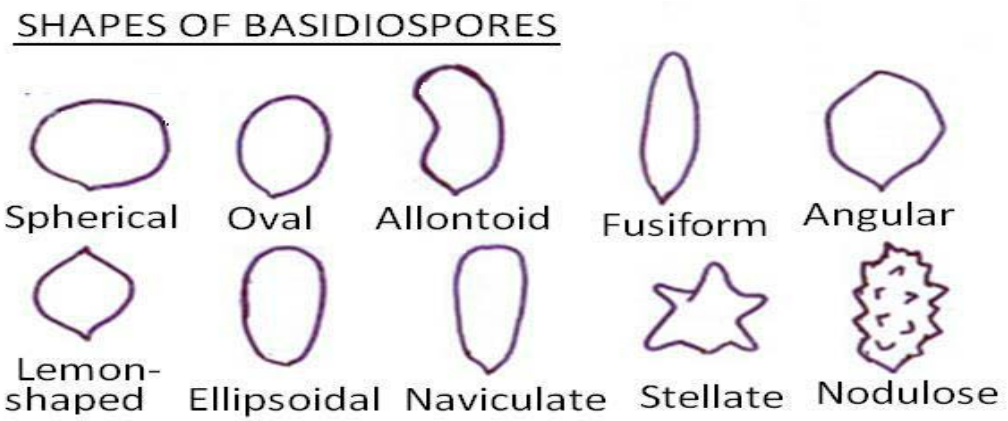
1 (*Itersonilia perplexans*)

2 (*Agaricus bisporus, Dacrymyces* spp.)

9 (*Cyathus* spp., *Phallus impudicus*)

Basidiospore as taxonomic marker

Basidiospore varies in size, shape, colour, & ornamentation which are important taxonomic marker.



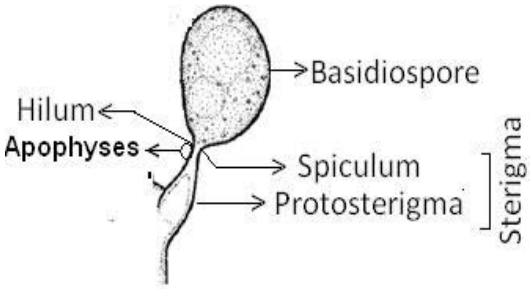
PARTS OF STERIGMA (Gk. sterigma = support)

Protosterigma- the basal filament or inflated part of sterigma,

Spiculum- the apical spore bearing point of sterigma

Hilum- the narrow point of attachment of the spore at the tip of the sterigma from where spore separates from basidium

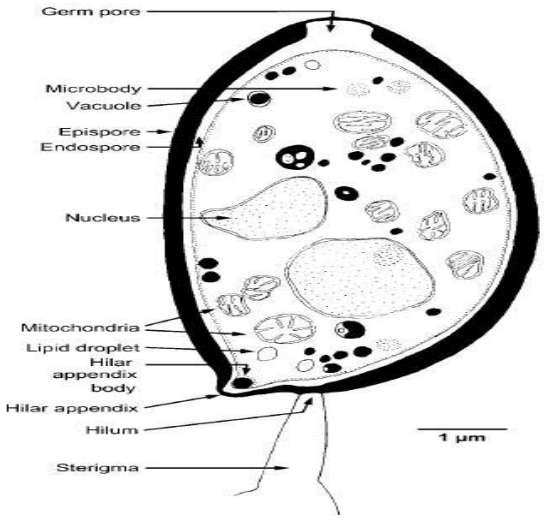
Apophysis (Gr. apo- = away from, separate; physis = growth)- a small spherical knob formed by expansion of the tip of the sterigma



Basidiospore- ultrastructure

The wall of a basidiospore consists of **endosporium (innermost), episporium & exosporiu**m. Some contains more layers: perisporium & ectosporium. Hilar appendix- a small projection present at the base of spore close to the hilum

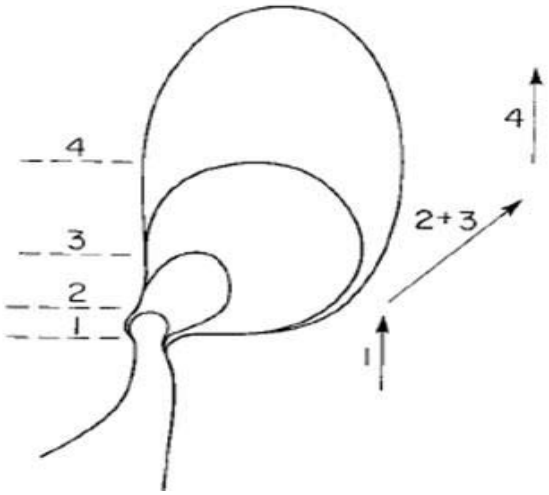
Hilar appendix body (HAB)- an electron-dense hemispherical or conical cytoplasmic region lying immediately within the plasma membrane of the apophysis and closely appressed to the wall of the hilar appendix. **Suprahilar plage**- the smooth adaxial face of basidiospore immediately above the hilar appendix found in *Lactarius* and *Russula*. It plays an important part in the spore discharge



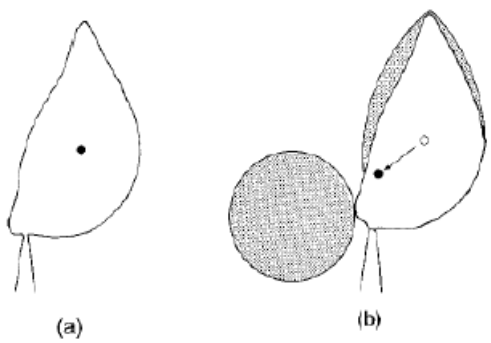
Basidiospore development

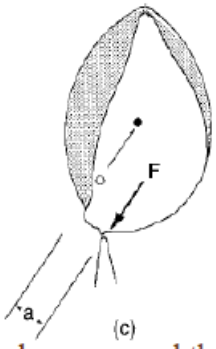
In spherical basidiospore (*Amanita vaginata*), wall expansion is homogenous, but in *Coprinus cinereus*, McLaughlin (1977) has distinguished **four** successive stages of basidiospore expansion: Stage 1, **inception**. Spherical enlargement of the sterigma apex leads to formation of basidiospore primordium 0.6-0.8 µm in diameter. The thin basidiospore wall is three-layered at first. Microtubules are occasionally present in the sterigma, being orientated parallel to its long axis. Stage 2, **asymmetric growth**. The basidiospore initial grows asymmetrically on its abaxial side, and the hilar appendix develops. The basidiospore wall thickens, being thickest at the apex of the spore, and is six-layered.

Stage 3, **equal enlargement**. Basidiospore grows at an angle of about 45° to the sterigma apex and becomes spherical. Stage 4, **elongation**. Basidiospores grow in length and a pore cap is formed at the upper end of the spore. The spore wall becomes darkly pigmented, starting at the upper end.



Ballistospore discharge- Buller's drop phenomenon





Ballistospore attached to its sterigma before drop formation. The closed circle within the spore indicates the centre of mass of the spore.

Buller's drop appears at the hilar appendix. The adaxial drop emerges on the spore wall above it and extends downwards as it increases in size. The centre of mass of spore plus drop moves to a position nearer the hilar appendix.

Contact between the two drops is followed by immediate coalescence and the combined mass of liquid moves rapidly up the adaxial face of the spore away from the hilar appendix. The centre of mass moves very rapidly in the direction of the thin arrow and the spore-drop system gains kinetic energy and momentum in the same direction, simultaneously exerting an opposite force F on the sterigma at the hilum (thick arrow). Some angular momentum is also exerted on the spore, related to the distance a between the hilum and the hilar appendix

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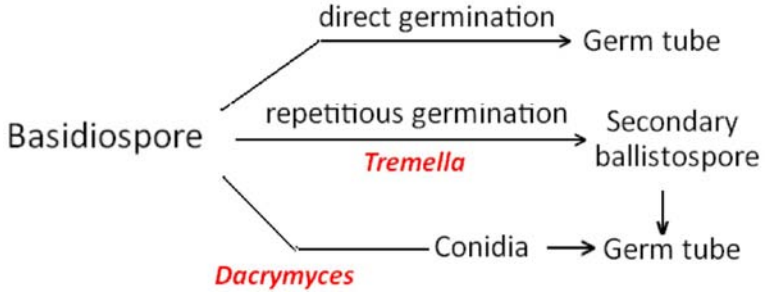
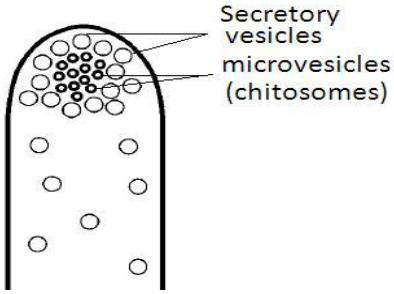
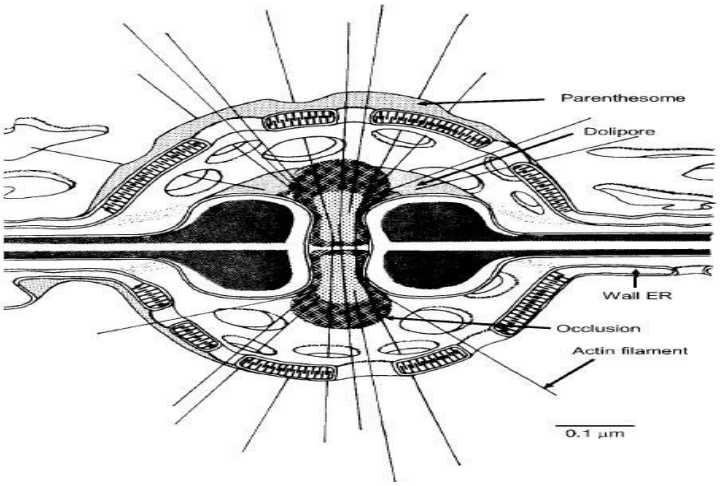
Germination of basidiospore



Spitzenkor



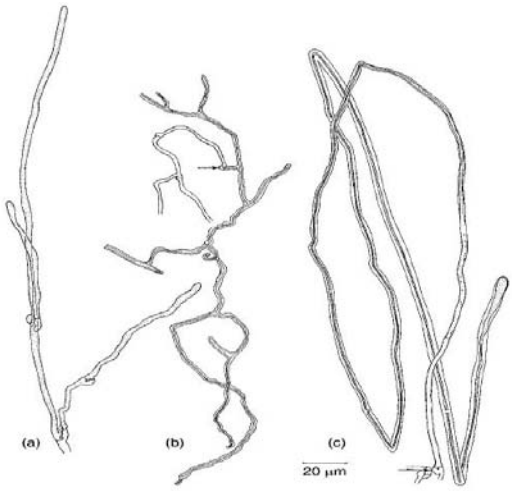
Dolipore/parenthesome septum



Perforated (homobasidiomycetes)
Imperforated (heterobasidiomycetes)
FUNCTIONS:
1.To secure the integrity of hyphal cell;
2.To maintain intercellular communication and transfer of cell organelles, except nuclei; 3.Repair of hyphal damage - pore rapidly plugged by electron dense material in the compartment of a hypha adjacent to the damaged segment.

Hyphal analysis

There are three main hyphal types:



(a) Generative hyphae (GH)
Thin-walled, branched, with clamp connections, produce basidia BH & SH are developed from GH, are thick-walled, sterile, do not have clamp connection

(b) Binding hyphae (BH) or ligative hyphae
Much-branched, narrow, thick-walled of limited growth, weave themselves between the other hyphae and bind them together.

(c) Skeletal hyphae (SH)
Unbranched, form a rigid frame.

Hyphal analysis

INTERMEDIATE TYPES

Sarco-hyphae- are composed of long (10-30 µm), unbranched skeletal, inflated generative hyphae.

Skeleto-ligative hyphae- skeletal hyphae but function as binding hyphae. eg. ***Amauroderma rugosum***

In ***Ganoderma***, SH are of two types:

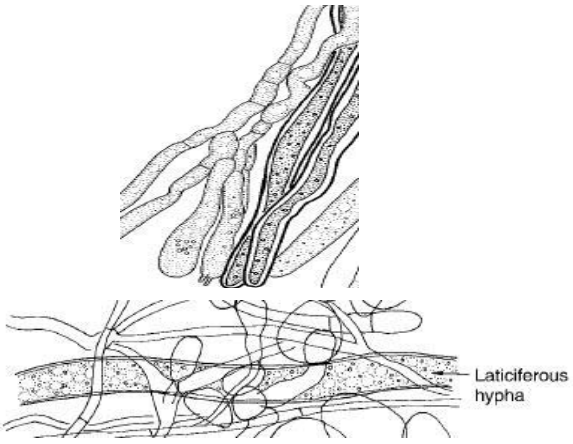
Arboriform- unbranched basal part with a branched tapering end;

Acicular- unbranched & usually with a sharped tip

Gloeoplerous hyphae have dense oily contents.

Lactifers- latex containing hyphae, ***Lactarius rufus***

Sanguinolentous hyphae- modified skeletal exude a red fluid when damaged



Sanguinolentous hyphae
Stereum ruaosum

MITIC SYSTEM

(Gr. *mitos* = a thread of the warp)

Diverse hyphal types are present in basidiocarps in different combinations

Monomitic- basidiocarps are made up of generative hyphae

only.

Polyporus adustus

Sarcomitic- basidiocarps are made up of inflated sarco-hyphae which function as skeletal elements; ***Meripilus*** sp.

Dimitic- basidiocarps are made up of two types of hyphae; 3 types:

(i) generative + binding hyphae; ***Laetiporus sulphureus***

(ii) generative + skeletal hyphae; ***Heterobasidion annosum***

(iii) generative + skeleto-ligative; ***Lentinus, Ganoderma***

Trimitic- fruit bodies contain all three kinds of hyphae, generative, binding, & skeletal hyphae; ***Trametes versicolor***

Sarcotrimitic- sarco-hyphae + binding + skeletal hyphae; ***Trogia*** sp.

Hyphal aggregates

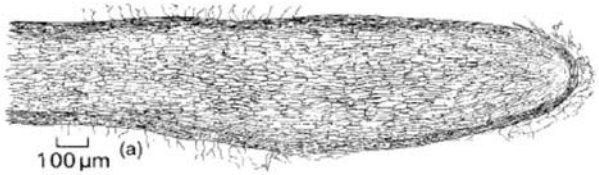
Mycelial strands- aggregates of parallel, relatively undifferentiated hyphae arise from a well developed mycelium extending as underground mycelium from an exhausted food base into nutrient-poor surroundings. e.g. ***Agaricus bisporus***.

Mycelial cords –aggregates of parallel, longitudinally aligned hyphae the tip of which differentiates into an egg-like basidiocarp upon reaching the soil surface. e.g. ***Phallus impudicus***.

Mantle of ectomycorrhiza-continuous sheet of fungal hyphae, several layers thick covering the root tip of many conifers and deciduous tree with ectomycorrhizal associations. The mycelium extends outwards into the litter layer of the soil and inwards as single hyphae growing intercellularly in the cortical cells of the root to form **Hartig net**.

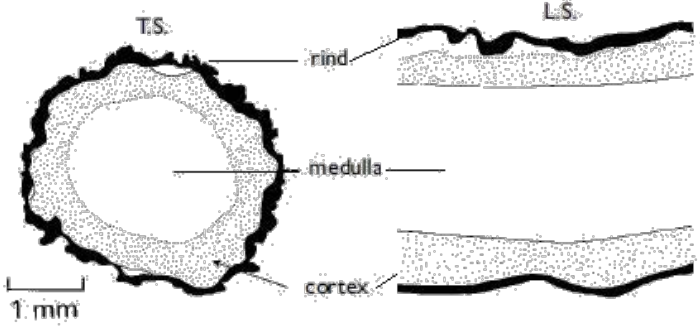
e.g. ***Amanita muscaria***.

Rhizomorph- root-like form of mycelial aggregates spread underground from one tree root system to another. In nature, two kinds are found – a dark **cylindrical** type and a paler, **flatter** type.



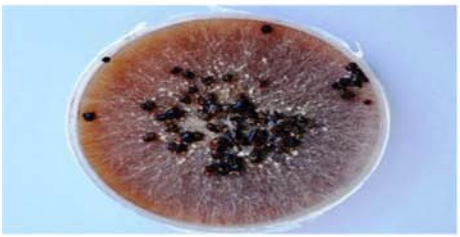
The centre of the rhizomorph may be hollow or solid. Surrounding the central lumen or making up the central **medulla** is a zone of enlarged hyphae 4-5 times wider than vegetative hyphae, called **vessel hyphae** serve in translocation. Towards the periphery of the rhizomorphs the cells become smaller, darker and thicker walled.

e.g. ***Armillaria mellea*.**



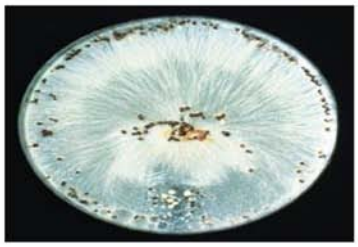
Sclerotia- pseudoparenchymatous aggregations of hyphae, adapted for prolonged survival and propagation. They vary in size from 50 µm to several cm and in weight from 10 µg to several kg.

Several kinds of development in sclerotia are:



Loose type

Rhizoctonia solani



Strand type

Sclerotium rolfsii

Massive type

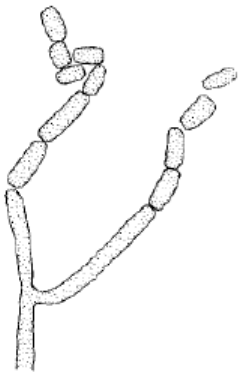
complex type (upto 30 cm in diameter)

Polyporus mylittae

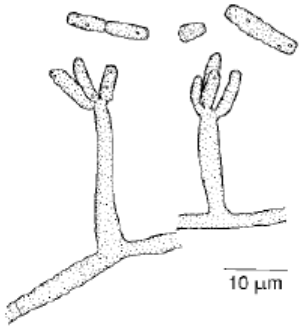
Pseudosclerotia- with a similar function to sclerotia, but consisting of a compacted mass of intermixed substratum, soil, stones, etc., support the fruiting of certain polypores such as ***Polyporus tuberaster***.

Asexual reproduction

Oidia (arthroconidia)

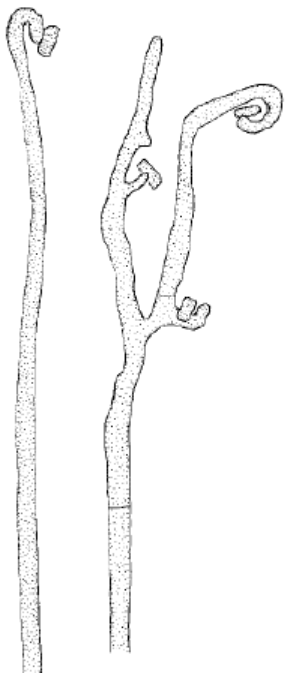


Flammulina velutipes



Coprinus cinereus

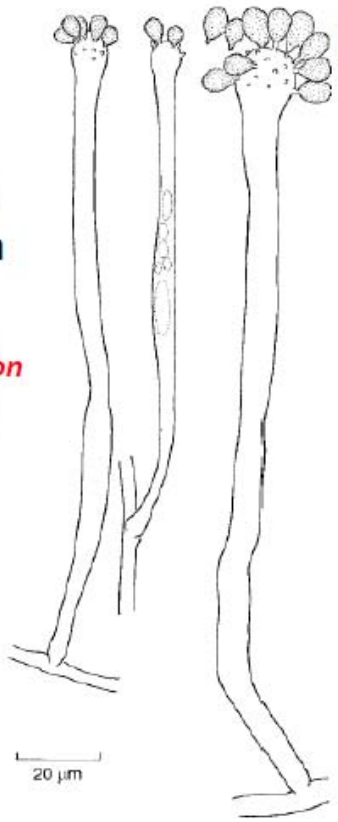
'Homing reaction'



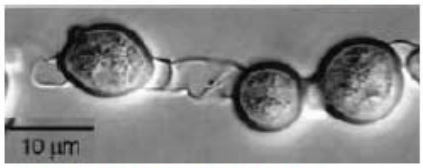
If an oidium is placed a little distance ahead of a monokaryotic hypha, the growing hypha changes direction, being attracted chemotropically towards the oidium. Oidia function as spermatizing agents.

Blastic conidia

Heterobasidion annosum

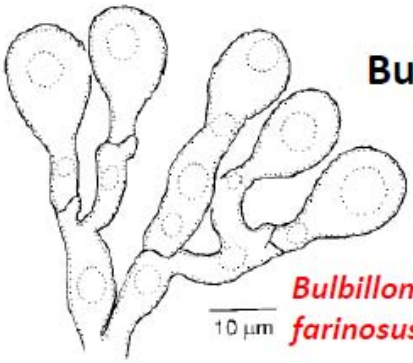


Intercalary chlamydospores



Laetiporus sulphureus

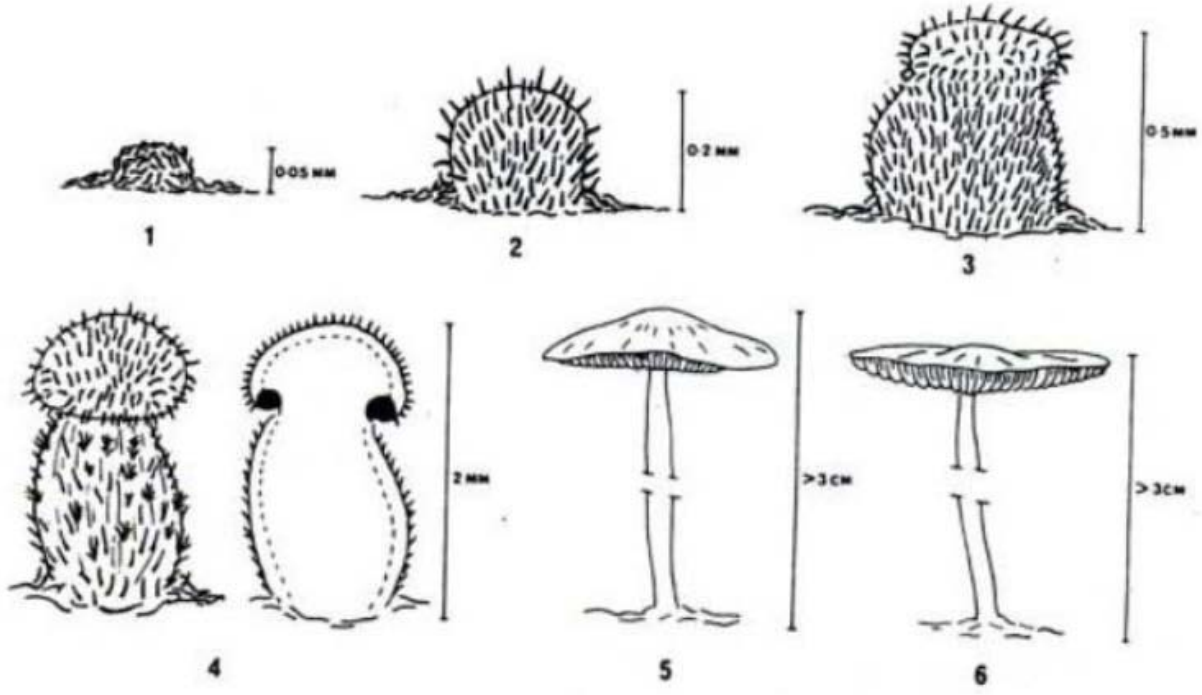
Bulbils



Bulbillomyces farinosus

Basidiocarp development

Five developmental stages of basidiocarp- **bulb, button, egg, elongation** and **mature**.

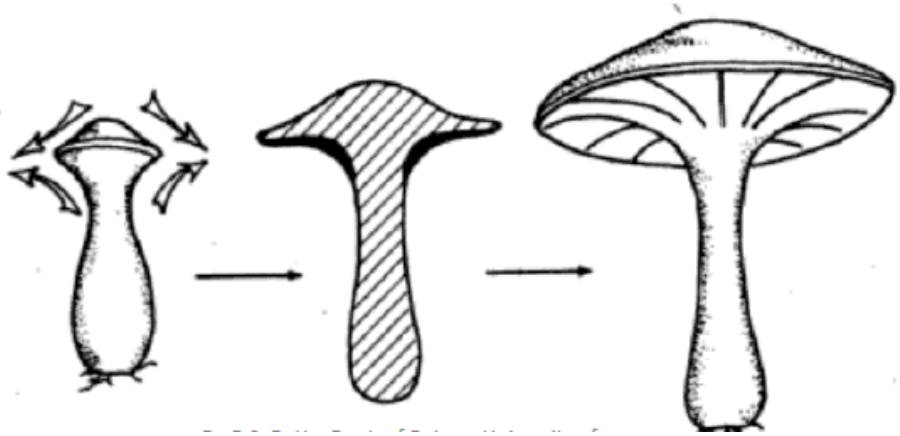


Types of basidiocarp development

Based on development, basidiocarps are of three types:

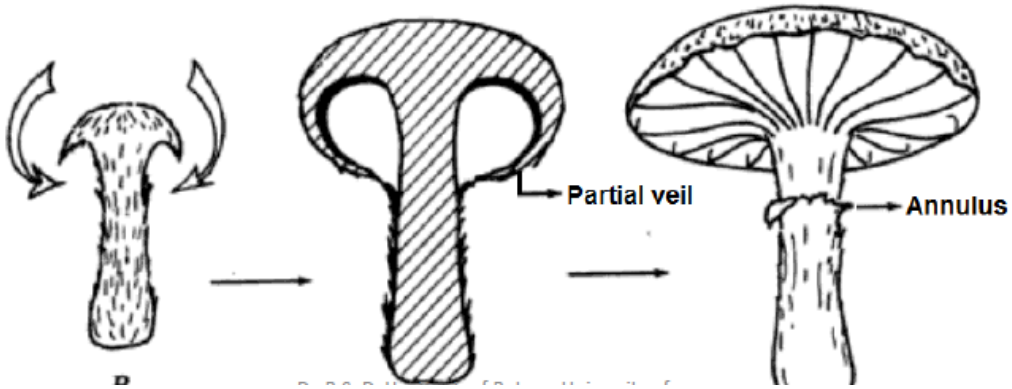
1. Gymnocarpous development

The hymenium remains naked from the time of its first appearance and is never enclosed by tissue. The pileus developed at the tip of the stipe and hymenophore differentiates on the lower side of pileus. ***Russula emetica***



2. Pseudoangiocarpic/ Secondary angiocarpic development

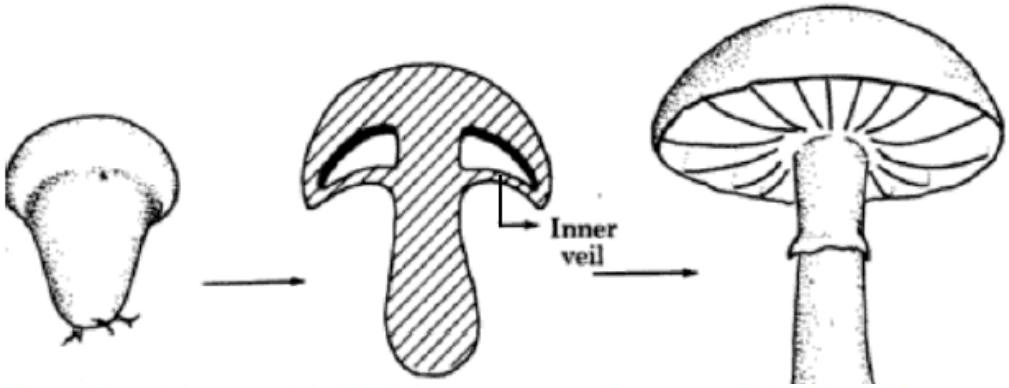
The hymenium, at first remains naked, but later becomes enclosed by incurving margin of the pileus as well as sometimes by an outgrowth of the stipe. The incurving edges of the young pileus become joined to the stipe by **partial veil**. Thus a small cavity is formed within which gills are differentiated. The hymenium remains enclosed until the mushroom matures and the pileus expands, once again revealing the hymenium. The broken partial veil is left as a collar-like **annulus** around the stipe or remnant may remain on the edges of pileus as a **marginal veil** (e.g. ***Agaricus***). Sometime the remaining veil is thin and cobweb-like and is called **cortina** (e.g. ***Cortinarius***).



3. Hemiangiocarpic/ Primary angiocarpic development

(3a. with inner veil only) ***Lentinus tigrinus***

The hymenium is formed endogenously within the gill cavity. The margin of the pileus is connected to the stipe by membranous tissue called **inner veil**. The hymenium is not exposed until the cap expands, tearing the inner veil. The veil often becomes severed from the margin of the pileus and remains attached to the stipe in the form of a ring or annulus.



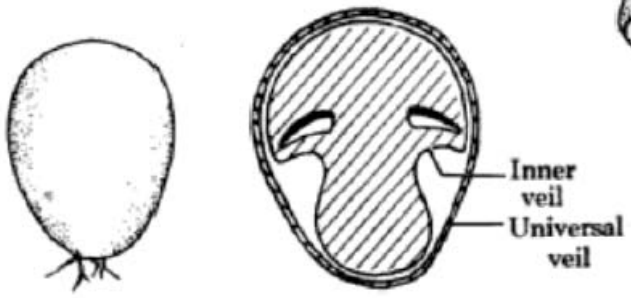
3. Hemiangiocarpic/ Primary angiocarpic development

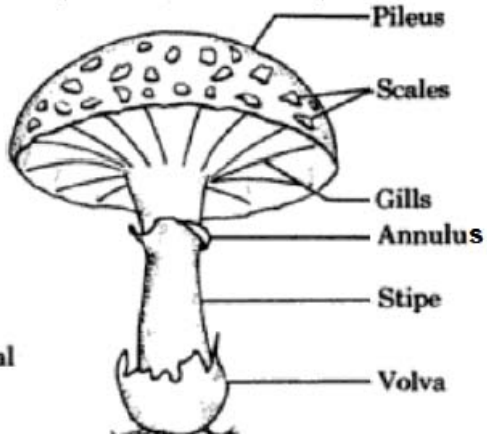
(3b. with universal veil only) ***Volvariella***

(3c. with both universal veil and inner veil) ***Amanita***

The entire primordium is covered by a membrane called **universal veil**. When the basidiocarp enlarges and the pileus finally expands, the universal veil tears and leaves a cup-shaped body, the **volva** around the base of the stipe. The remnants of the part of the universal veil that covered the pileus often left scattered as scales on the cap. e.g. ***Volvariella*** contains only volva, no annulus.

In ***Amanita*** both universal veil & partial veil participate in basidiocarp development, hence its basidiocarp contains both annulus & volva.





Parts of basidiocarp

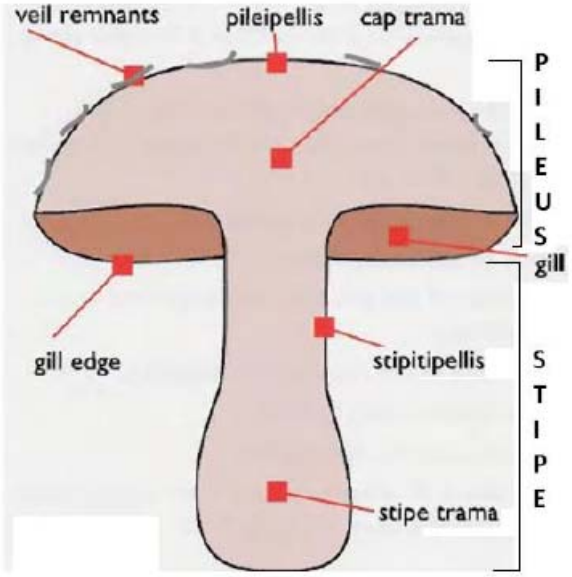
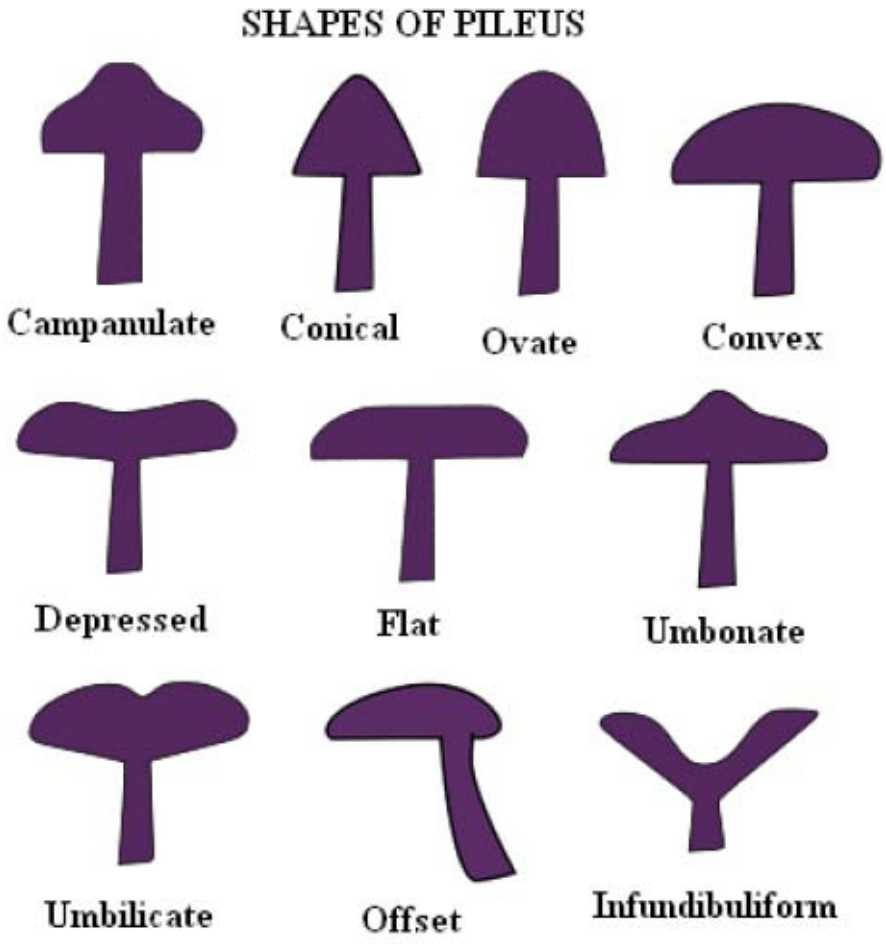
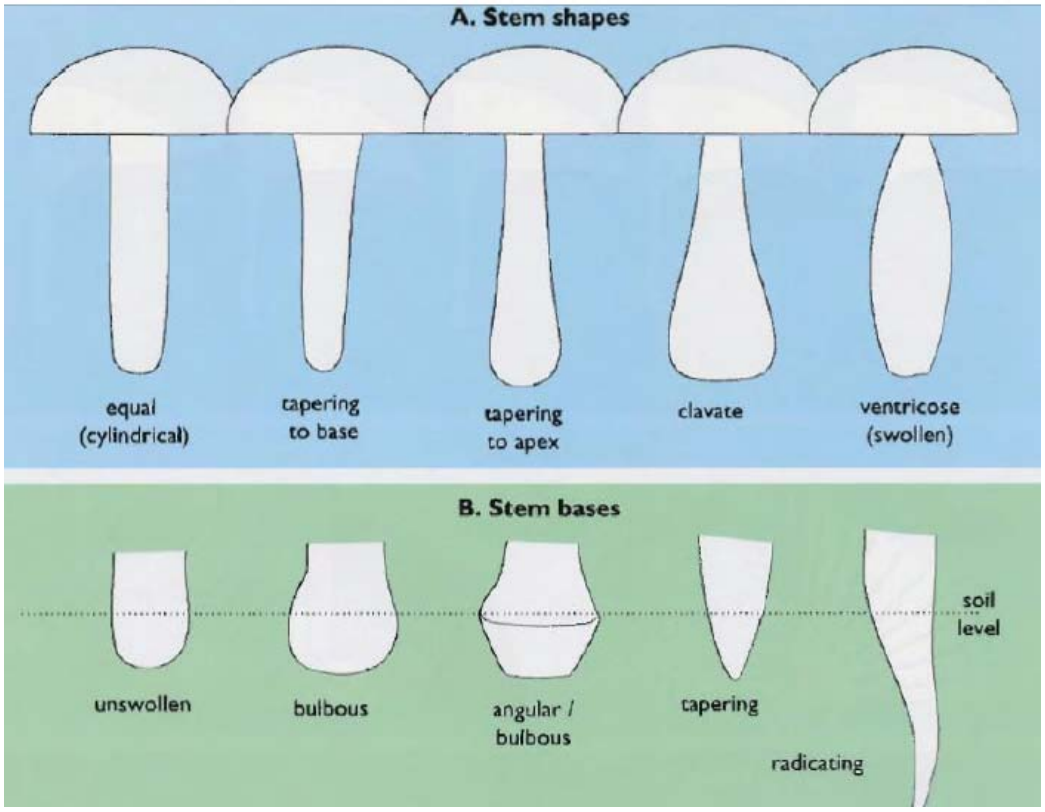
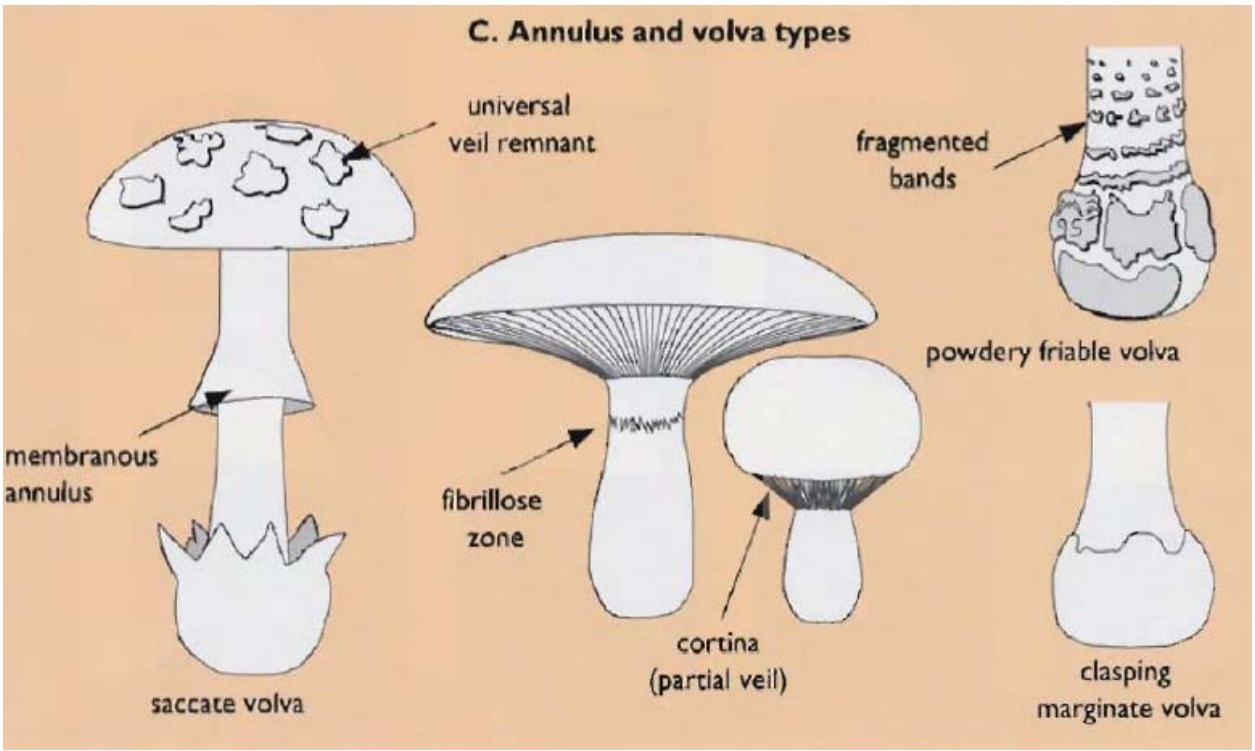


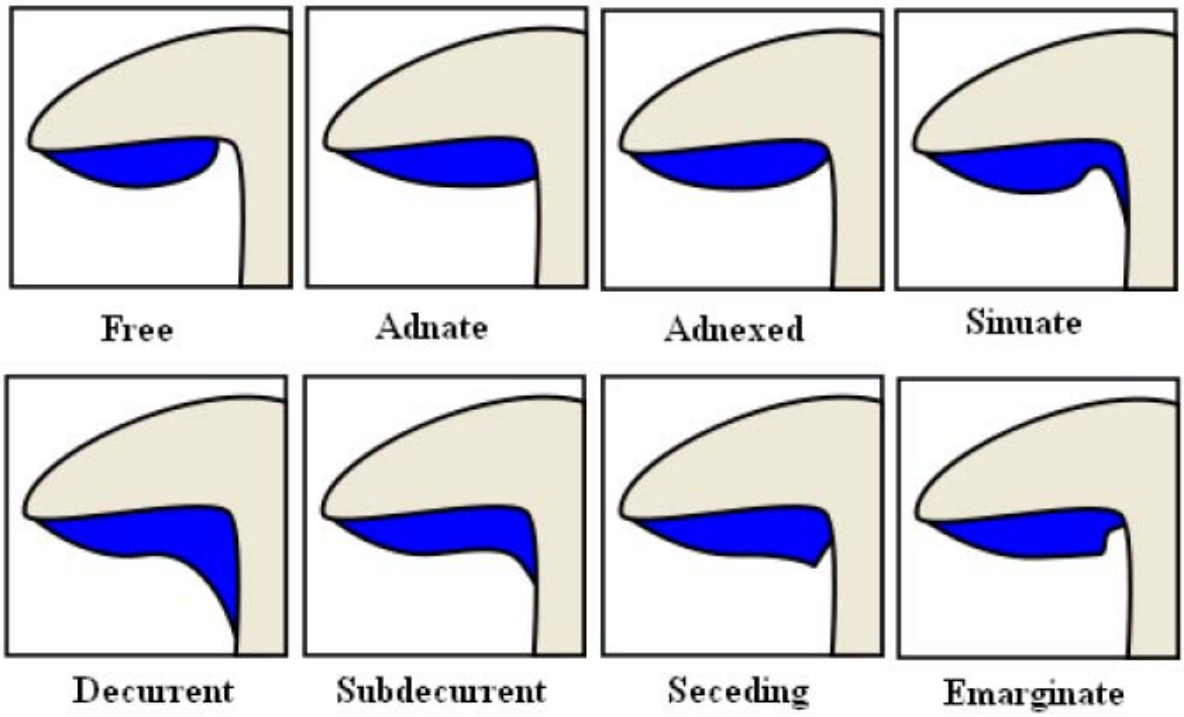
FIG. **Types of pillis (cuticle) & trama (central portion) based on their position**




A. Stem shapes
equal (cylindrical)
tapering to base
tapering to apex
clavate
ventricose (swollen)
B. Stem bases
soil level
unswollen
bulbous
angular / bulbous
tapering
radicating


C. Annulus and volva types
universal veil remnant
fragmented bands
powdery friable volva
membranous annulus
fibrillose zone
cortina (partial veil)
saccate volva
clasping marginate volva

ATTACHMENT OF GILL

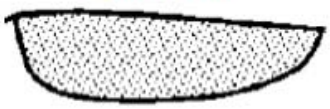


LAMELLAE / GILLS

Aequi-hymenial

wedge-shaped in L.S., hymenium develops in an equal manner all over the surface

Agaricus

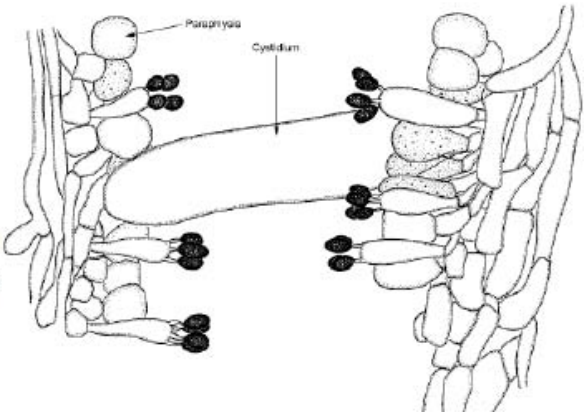


Inaequi-hymenial

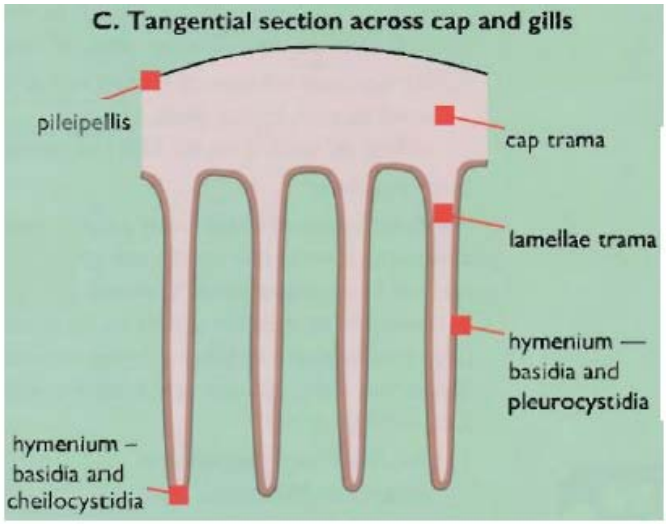
parallel-sided, often held apart by cystidia the hymenium develops in an unequal manne with basidia ripening in zones

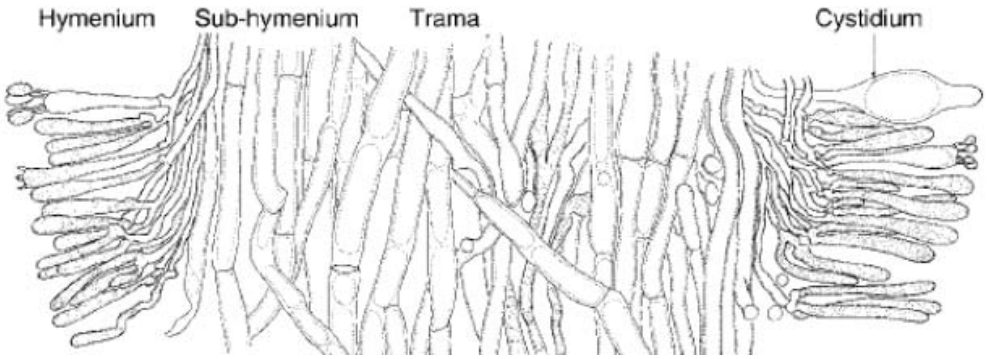
Coprinus

Vertical section of the parallel-sided gills of *Coprinus atramentarius* showing basidia, interspersed by globose paraphyses and a cystidium extending across the space between adjacent gills to make contact with the surface of the opposing gill.



Parts of the Gill





HYMENOPHORAL TRAMA

HOMOIOMEROUS

HETEROMEROUS

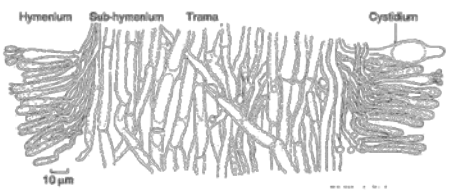
Interwoven

Parallel

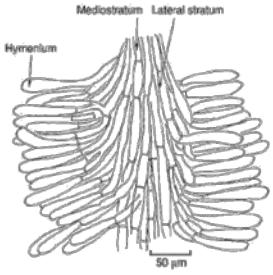
Inverted

Divergent

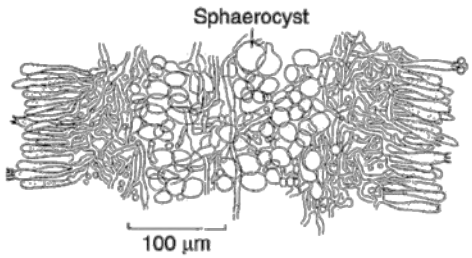
Bilateral



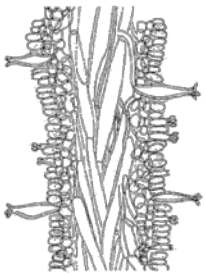
Agaricus



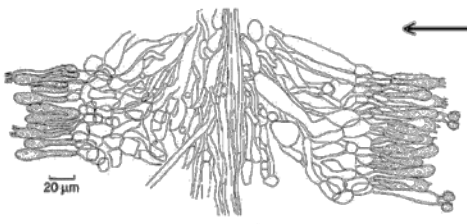
Boletus



Russula

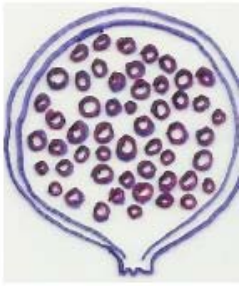


Pleurotus

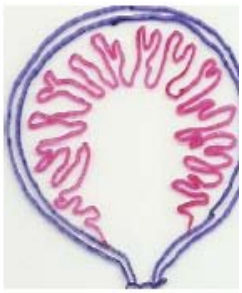


Amanita

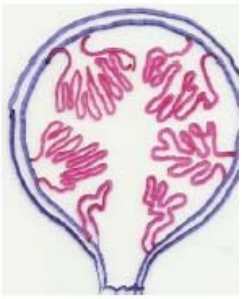
Various types of gleba



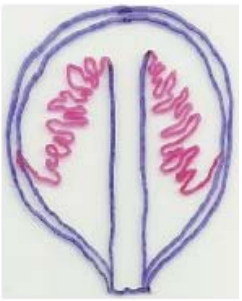
LACUNAR- a single cavity (*Sphaerobolus*) or more cavities (*Cyathus*) lined with hymenium arise within basidiocarp, Nidulariaceae



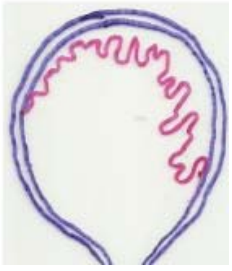
CORALLOID- a single cavity arises in the upper half of basidiocarp. This is enclosed above by tramal tissue which may unite with the inner side of peridium at the base of the cavity branching masses of hyphae grow upwards & outwards to form coral-like branched covered by a continuous hymenium;



MULTIPILEATE- a number of tramal branches grow rapidly reach the inner surface of the peridium and there spread out over it. Coral-like branches then grow from these main branches into the space; *Clathrus*



UNIPILEATE- a single tramal branch develops as a column of tissue from the base to apex & spreads out in a pileus-like manner on the inner surface of the peridium. Hymenium develops on the coralloid branches on the underside of the pileus-like structure; *Phallus*



AULEATE- a single central cavity arises within the basidiocarp & in the upper half of the cavity develops few inwardly growing tramal branches; *Hymenogaster*

Dispersal mechanisms of propagules among members of Subclass *Phyllomycetidae* are as follows:

i. Disintegration of gasterocarp followed by release of propagules by wind or animal trampling- ***Scleroderma, Pisolithus, Calvatia***

ii. Puffing through a pore after a raindrop hits the endoperidium ('bellows mechanism')- ***Lycoperdon, Geastrum, Astraeus***

iii. Splash cup dispersal following impact by a raindrop- ***Cyathus***

iv. Passive release into water- ***Nia***

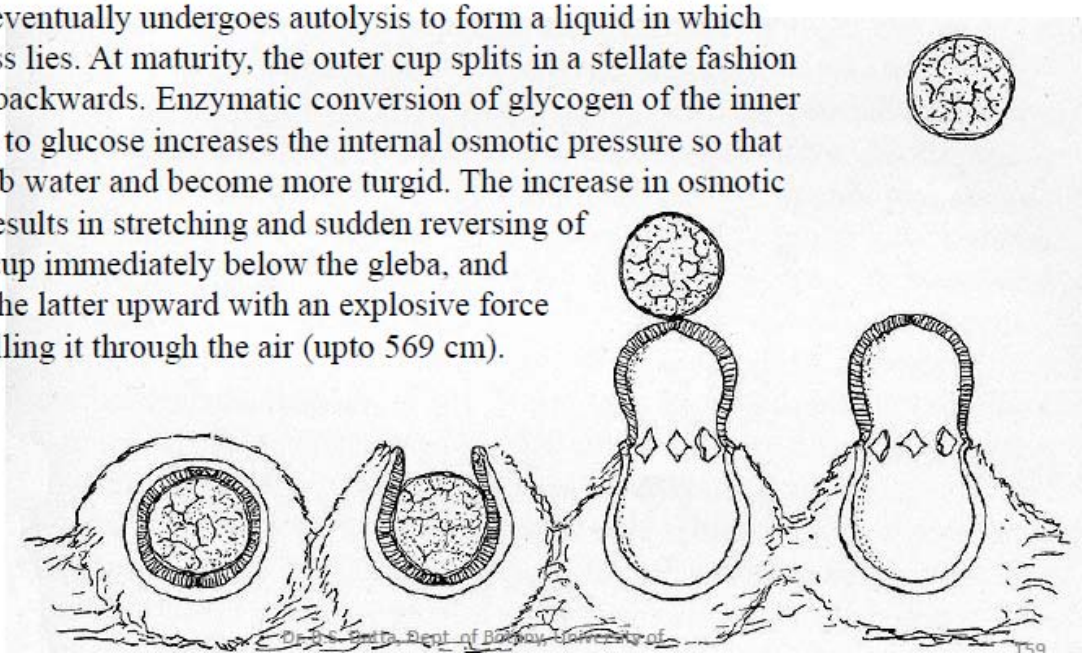
v. Distribution by burrowing animals and/or passive release into the soil following disintegration of the gasterocarp- ***Rhizopogon, Melanogaster***

vi. Insect dispersal following olfactory and visual attraction- ***Phallus, Clathrus, Mutinus***

Active discharge of peridiole by tension-snap or Eversion mechanism- ***Sphaerobolus***

Active discharge of peridiole by tension-snap or Eversion mechanism- *Sphaerobolus*

Basidiocarp contains only one peridiole and 6 layered peridium; when sporophore opens the 3 outermost layers become **outer cup** while next two layers form the **inner cup**. The innermost layer of peridium eventually undergoes autolysis to form a liquid in which glebal mass lies. At maturity, the outer cup splits in a stellate fashion and folds backwards. Enzymatic conversion of glycogen of the inner layer cells to glucose increases the internal osmotic pressure so that they absorb water and become more turgid. The increase in osmotic pressure results in stretching and sudden reversing of the inner cup immediately below the gleba, and thrusting the latter upward with an explosive force and propelling it through the air (upto 569 cm).



Active discharge of peridiole by tension-snap or Eversion mechanism- ***Sphaerobolus***

Basidiocarp contains only one peridiole and 6 layered peridium; when sporophore opens the 3 outermost layers become **outer cup** while next two layers form the **inner cup**. The innermost layer of peridium eventually undergoes autolysis to form a liquid in which

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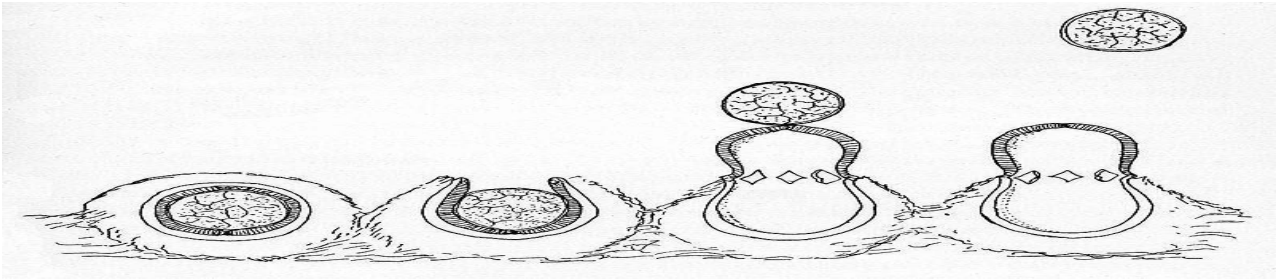
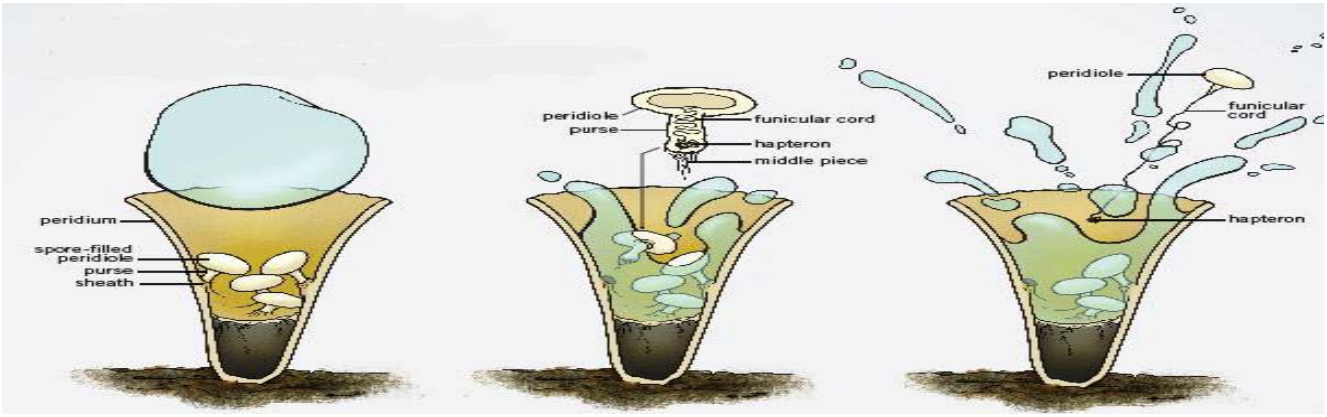


Splash cup dispersal following impact by a raindrop- *Cyathus*

When large drops of about 4 mm diameter, with a high velocity fall into the cup at right angle, splash action ejects peridiole that flies out. The hapteron sticks to plant stem as peridiole is carried forward by its own movement and funicular cord is extended by pull. The peridiole is jerked back when it reaches the limit of extension of cord. Jerk causes peridiole to swing around point of attachment and cord is wrapped around stem.



Auricularia auricula-judae, the Jew's ear fungus forms rubbery, ear-shaped fruit-bodies on branches of elder. The fruit body can dry to a hard brittle mass, but on wetting it quickly absorbs moisture and discharges spores. The basidia are cylindrical and become divided into four cells by three transverse septa. Each cell of the basidium develops a long cylindrical epibasidium which extends to the surface of the hymenium and terminates in a conical sterigma bearing a basidiospore.

Depending on the nutrient status, basidiospores are typically able to germinate in several different ways:-

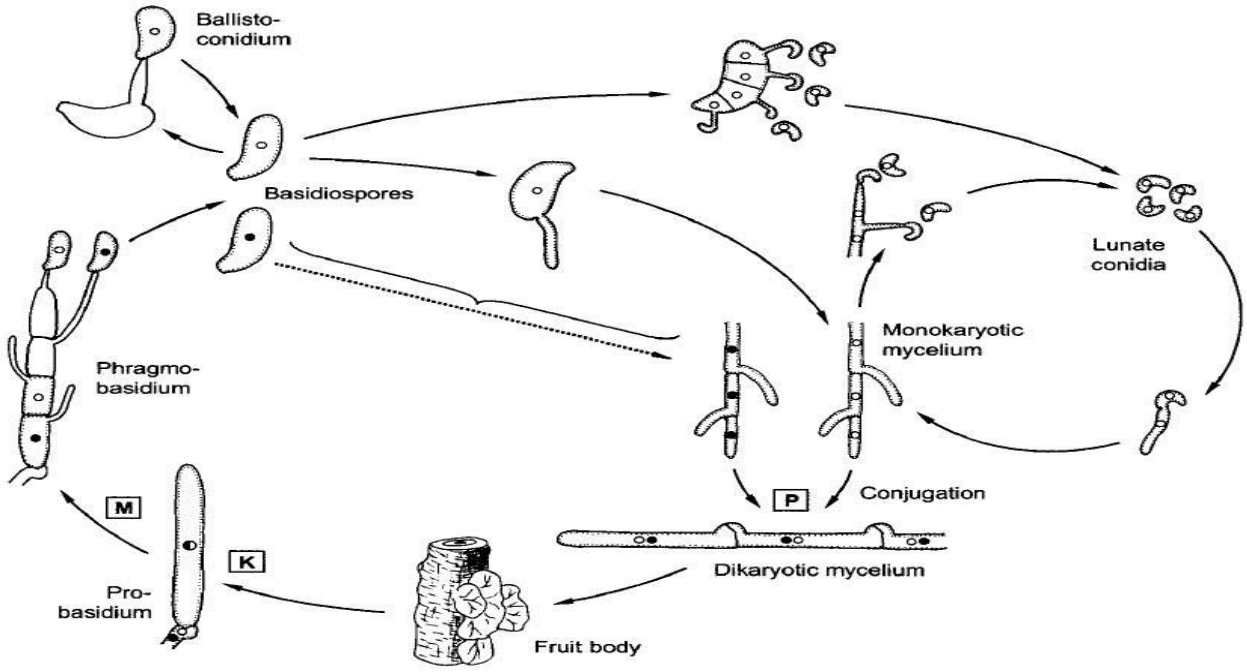
(i) On richer medium, they germinate directly by forming germ tube;

(ii) On nutrient poor medium, they undergo repetitious germination by means of a sterigma which produce another ballistospore;

(iii) On nutrient starvation they develop three transverse septa, each cell form lateral or terminal denticles which produce clusters of sickle-shaped (lunate) microconidia.

The latter may also be produced by monokaryotic hyphae. Conjugation leads to the establishment of a dikaryotic mycelium which may form basidiocarps. During direct germination, a septum is laid down on the basidiospore, the first one bulging backwards from the protoplast-containing germ tube towards the empty basidiospore. Such septa are interpreted as **retraction septa**.

Life cycle of *Auricularia*



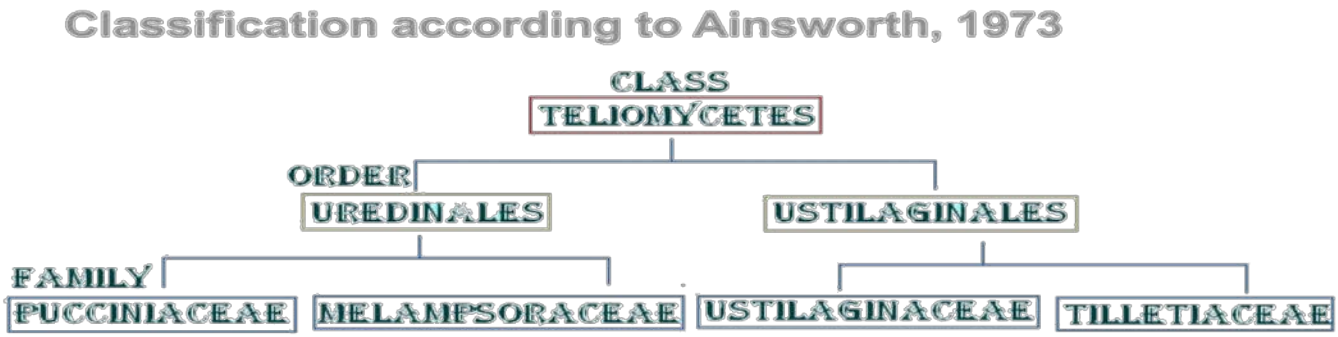
FAIRY RINGS

Naturally occurring ring of mushrooms produced by underground mycelia; 3 types:

1.No effect on vegetation; Lepiota morganii
2.Increased growth of vegetation; Tricholoma personatum
3.Damaging vegetation badly; Agaricus praerimosus

Class TELIOMYCETES
Characteristic features:
i.Absence of basidiocarp;
ii.Basidia transversely septate, with basidiospores produced laterally;
iii.Mycelia septate, with simple septa; dolipore-parenthosome complex absent;
iv.Clamp connection absent;
v.Occurrence of teliospores in their life cycle.
Terminology regarding teliospores
Teliospore (Gk. ***Telos*** = end) is a thick-walled resting spore where karyogamy takes place; hence it is a diploid spore.

It germinates to form promycelium where diploid nucleus undergoes meiotic division. Each promycelium becomes 4-celled by three transverse septa; each cell receives one haploid nucleus and produces basidiospore(s). These basidiospores are also known as **sporidia**. As karyogamy occurs in the teliospore, it is designated as the **probasidium** and the promycelium, where meiosis takes place is the **metabasidium**. Each cell of the teliospore is also known as the **hypobasidium** and the promycelium is the **epibasidium**.



Kirk et al. (2008) divided the members into two subphyla
1. **PUCCINIOMYCOTINA**. Rust fungi.
2. **USTILAGINOMYCOTINA**. Smut fungi.

Subphylum pucciniomycotina
Former order uredinales (rust fungi)
- They are named so due to the presence of reddish-brown spores, the **uredospores** (L. ***urere*** = to burn) which are dikaryotic (n+n) spores in their life cycle.
- They are obligate parasite producing haustoria within the host tissue and are responsible for rust disease.
- Promycelium, after germination from teliospore produces four sporidia, i.e., each cell of promycelium develops a single basidiospore.
- Basidiospores are sterigmatic, discharged violently.

The life cycle of *Puccinia graminis tritici* , causal organism of black stem rust of wheat involves five distinct stages; *viz*.

Hosts	Stage no.	Name of the stage	Respective spores
BERBERRY	0	Spermogonial / Pycnial stage	Spermatia / pycniospores (n)
	I	Aceial stage	Aeciospores (n+n)
WHEAT	II	Uredial stage	Uredospores (n+n)
	III	Telial stage	Teleutospores (2n)
	IV	Basidial / Sporidial stage	Basidiospores / sporidia (n)

Rusts can produce up to five spore types from corresponding fruiting body types during their life cycle, depending on the species.

1. P**ycniospores** (Spermatia) from Pycnidia. These serve mainly as haploid gametes in heterothallic rusts.

2. **Aeciospores** from Aecia. These serve mainly as non-repeating, dikaryotic, asexual spores, and go on to infect the primary host.

3. **Urediniospores** from Uredia (Uredinia). These serve as repeating dikaryotic vegetative spores. These spores are referred to as the repeating stage because they can cause auto-infection on the primary host, re-infecting the same host from which the spores were produced. They are often profuse, red/orange, and a prominent sign of rust disease.

4. **Teliospores** from Telia. These dikaryotic spores are often the survival/overwintering stage of life cycle. Later they germinate to produce basidia.

5. Basidiospores from Teliospores. These haploid spores often infect the alternate host in Spring.

Life cycle of rust fungi

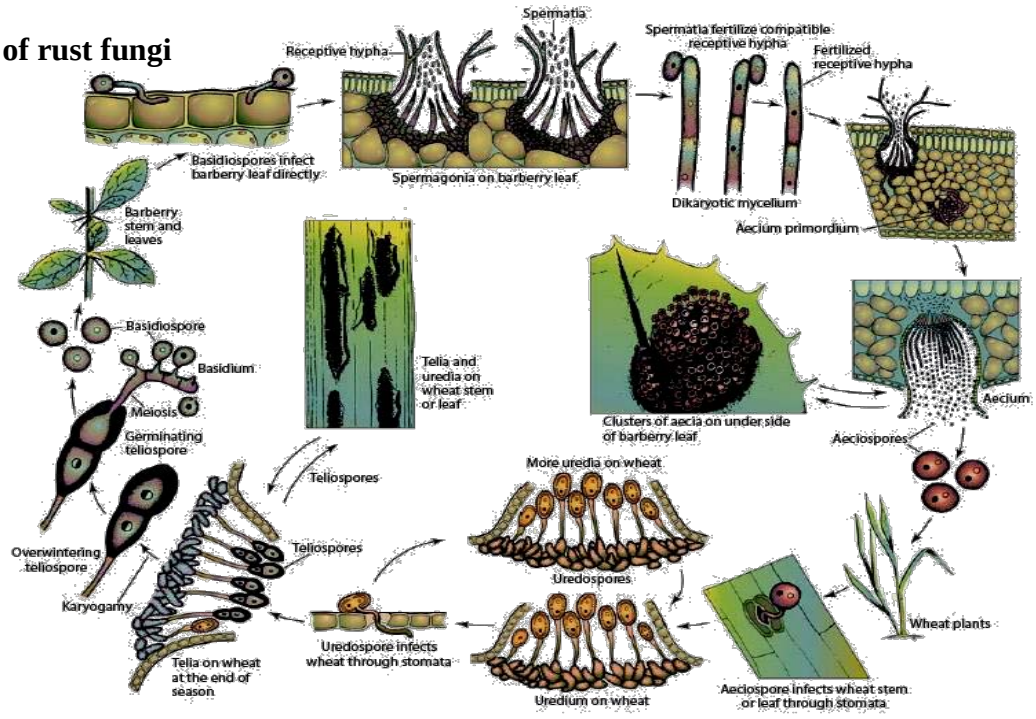


FIGURE 11-134 Disease cycle of stem rust of wheat caused by *Puccinia graminis tritici*.

FORMS OF RUST FUNGI

Based on life cycle

•Macrocyclic forms- exhibit all five stages;

•Demicyclic forms- lack uredinal stages;

•Microcyclic forms- lack both aecial and uredinal stages.

TYPES OF RUST FUNGI

BASED ON HOST

1. **Autoecious**- complete life cycle on a single host.
   - All microcyclic forms are autoecious-
     - *Puccinia malvacearum* on *Malvaceae*
     - *Puccinia thwaitessi* on *Justicia* sp.
     - *Uromyces ficariae* on *Ranunculus ficaria*.
   - Autoecious demicyclic form
     - *Xenodochus carbonarius* on *Sanguisorba officinalis*
   - Autoecious macrocyclic form
     - *Melampsora lini* on *Linum* sp.

2. **Heteroecious**- require two unrelated hosts to complete life cycle. Primary/Principal host produces stage II (Uredia) and III (Telia) and alternate host produces stage 0 (Spermogonia) and I (Aecia).

Examples of heteroecious rust	Primary host	Alternate host	Life cycle
Puccinia graminis tritici	Wheat	Berberry	Macrocyclic
Gymnosporangium juniper-virginianae	*Juniperus*	Rosaceae	Demicyclic

SUBPHYLUM USTILAGINOMYCOTINA [Former Order USTILAGINALES] (Smut fungi)

L. ustulatus or ustus = „to scorch or burn" in ref to the blackened appearance of infected plants; de Bary (1853) used the term „Brandpilze" to describe smut fungi

They are plant pathogenic fungi, parasitic on flowering plants, facultative saprophytes, can grow on specific culture medium.

The term „smut" refers to the mass of dark powdery teliospores formed in sori or smut balls. The term „bunt" is used for smut fungi infecting ovaries of their hosts

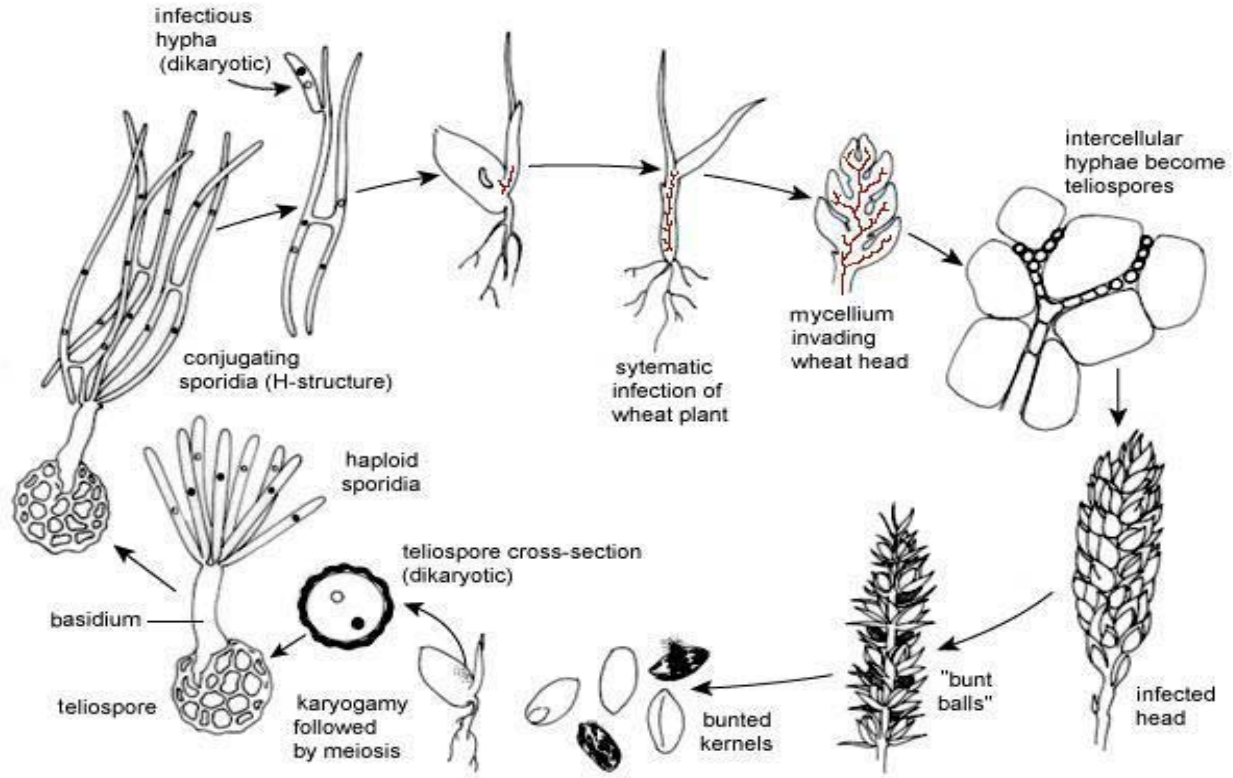
The smut can be either covered smut (spore masses remain covered in silvery membrane) or loose smut (membrane break up to expose the spores).

They never have alternate host and other types of spores as rust fungi.

Distinction between Rust and Smut fungi

Characters	Rust fungi	Smut fungi
Position of teliospore	Terminal	Intercalary
No. of sporidia in a promycelium	Definite (4)	Indefinite
Sterigmata in basidiospore	Present	Absent (Except some spp. of *Tilletia*)
Basidiospores discharge	Active (ballistospore)	Passive (Except some spp. of *Tilletia*)
Heteroecism	Common	Absent
Polymorphism (five types of spores)	Common	Absent (only teliospore & sporidia)
Parasitism	Obligate parasite	Facultative saprophyte

Smut of wheat (*Tilletia tritici*



SUBPHYLUM USTILAGINOMYCOTINA (3 classes)
Class Entorrhizomycetes (1 order)
Class Ustilaginomycetes (2 orders); Order Ustilaginales, Fam Ustilaginaceae Class Exobasidiomycetes (8 orders); Order Tilletiales, Fam Tilletiaceae

Ustilaginaceae	Tilletiaceae
Meiotic division of the diploid nucleus of teliospore occurs within promycelium after teliospore germination.	Meiotic division of the diploid nucleus of teliospore occurs within teliospore before it germinations to form promycelium.
Promycelium is prostrate, transversely septate, develops basidiospores both laterally and terminally.	Promycelium is either unicellular or aseptate, aerial and develops basidiospores terminally.
Both primary and secondary sporidia are morphologically identical, non-sterigmatic and not discharged violently.	Basidiospore develops two types of secondary spores- filiform spores and sickle-shaped spores. The later is produced on sterigma and is a ballistospore
Genera: *Ustilago, Sphacelotheca, Tolyposporium*	Genera: *Tilletia, Urocystis, Neovossia.*

Diseases caused by smut fungi

***Ustilago* spp. on Monocotyledonous hosts:**

Several species of *Ustilago* attack grasses and cereals of economic importance. *U. avenae* (oats), *U. nuda* (wheat), *U. hordei* (barley) are phylogenetically closely related and can hybridize with each other, so it has been proposed that they should be merged into one taxon, *U. segetum*. However, because the existing names are so well-established, it is preferred to use the original names for time being.

Name of the disease	Host	Causal organism
Smut	Corn (*Zea mays)*	*Ustilago maydis*
Loose smut	Wheat (*Triticum aestivum*)	*U. nuda* var. *tritici*
	Oats (*Avena* sp.)	*U. avenae*
Covered smut	Barley (*Hordeum vulgare*)	*U. hordei*
Whip smut	Sugarcane (*Saccharum officinarum*)	*U. scitaminea*
Grain smut	Jowar (*Sorghum* sp.)	*Sphacelotheca sorghi*
Smut	Bajra (*Pennisetum typhoides*)	*Tolyposporium penicillariae*
Bunt or stinking smut	Wheat (*Triticum aestivum*)	*Tilletia foetida* (smooth spored)
		T. caries (rough spored)
Bunt	Rice (*Oryza sativa*)	*T. barclayana*
Flag smut	Wheat (*Triticum aestivum*)	*Urocystis tritici*
Root-gall smut	Mustard (*Brassica* sp.)	*U. brassicae*
Karnal bunt	Wheat (*Triticum aestivum*)	*Neovossia indica*
Leaf smut	Rice (*Oryza sativa*)	*Entyloma oryzae*
Blister smut	*Sagittaria* sp. (Aquatic plants)	*Doassansia* sp.

ASCOMYCOTINA

SALIENT FEATURES

☐ Largest group, genera 3400 & species > 32,000 (Kirk et al., 2001)

☐ Gk. askos = sac; non-motile haploid spores, the ascospores are produced within a sac-like structure, the ascus (pl. asci)

☐ Thallus- holocarpic/unicellular (Saccharomyces), eucarpic/mycelial (Ascobolus), dimorphic (Candida);

pseudomycelia: mycelia-like structure produced by successive budding that remains joined for a period

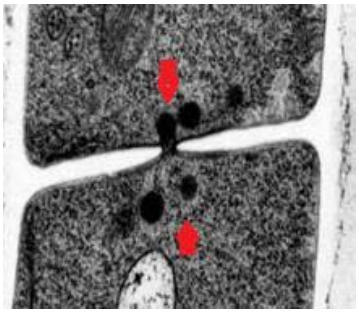
Mycelium septate; septation with simple pore; pores are plugged by various types of membrane bound structures-

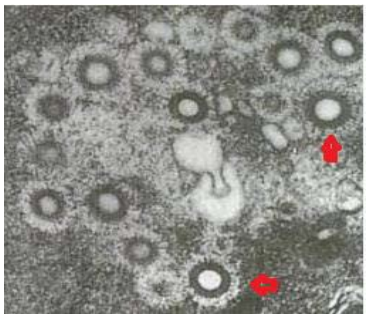
* Woronin bodies- globose, spherical, hexagonal proteineceous crystals, surrounded by a unit membrane; 150-500 nm in width and are sufficiently large to block the septal pore to separate aged or damaged hyphae from rest of the mycelium.

* Septal pore organelles- often shaped like pulley wheel, generally found at the ascus base, involved to isolate reproductive part from rest of the mycelium.

*Concentric bodies- occur primarily in lichenized ascomycetes & also Venturia inaequilis, causing apple scab,

have a translucent centre separated from a dense outer rim by a membrane like structure. The dense outer layer & its radiating filamentous structures are proteinaceous. They are arranged in clusters in an area of cytoplasm devoid of other organelles.





HABITAT

Terrestrial Saprophytic

Coprophilous (on dung)- *Ascobolus Corticolous* (in bark)- *Podospora ellisiana Lignicolous* (in wood)- Xylaria

Foliicolous (in leaves)- Leptosphaeria leucadendri Pyrophilous/Phoenicoid (on ash from burnt ground)-*Pyronema*

Keratin- Keratinophyton

Wall paint- *Aureobasidium pullulans*

Kerosene & aircraft fuel- *Amorphotheca resinae* Epigean- *Morchella*

Hypogean- Tuber

☐ Symbiotic with algae (Lichen)- Cladonia (40% members form lichen) with root of higher plants (Mycorrhiza)- Tuber, Elaphomyces with insects- *Ophiostoma*

☐ Parasitic

Plant pathogens:

powdery mildew- *Erysiphe* sp. peach leaf curl- *Taphrina deformans* apple scab- *Venturia inaequilis*

ergot of grain crops- *Claviceps purpurea*

chestnut blight- *Cryphonectria parasitica*

Mycoparasite- *Cordyceps capitata on Elaphomyces granulatus*

Human pathogens: *Pneumocystis carinii*

Insect parasites- *Cordyceps*

☐ Aquatic

☐ Marine: *Lulworthia*

BENEFICIAL ROLES

Edible fungi- *Morchella*, Tuber Medicines- Antibiotics Penicillin from *Penicillium notatum*, cephalosporin from Acremonium spp. Ergot, the sclerotia of *Claviceps purpurea* contains alkaloids such as ergotamine, ergometrine and are used as vasoconstrictor, to control bleeding of mother during child's birth. Industrial uses- production of ethanol, bread & cake *Saccharomyces cerevisiae* Decomposer- *Xylaria* Model organisms- *Neurospora crassa, S. cerevisiae*

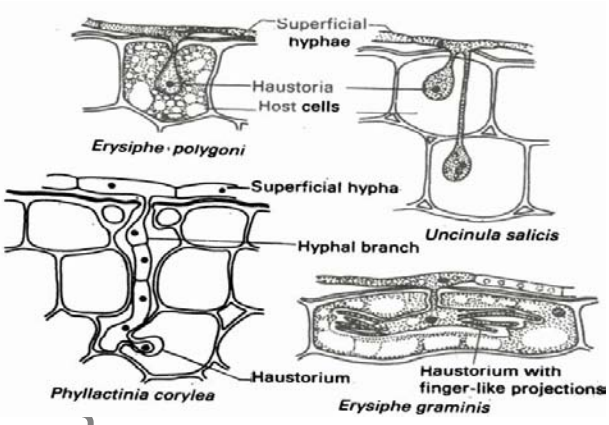
HYPHAL MODIFICATIONS

Mycelial plant pathogenic ascomycetes produce specialized structures associated with host infection. These include appressoria & haustoria. Several types of haustoria are found in Erysiphales:

☐ lobed *(Erysiphe polygoni),*

☐ finger-like *(Blumeria graminis),*

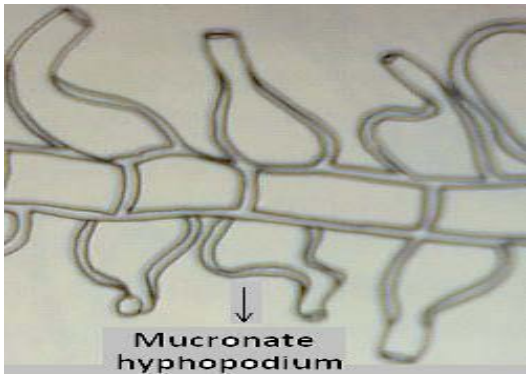
☐ branched *(Uncinula salicis),*



□ intracellular within mesophyll cells *(Phyllactinia corylea).*

HYPHAL MODIFICATIONS

Another modified mycelial structure is **hyphopodium**, which is of two types: **Capitate hyphopodium** produced by plant pathogens (*Gaeumenomyces graminis*) is two-celled, the terminal cell is swollen or lobed producing an appressorium to penetrate into the host surface. **Mucronate hyphopodium** is single-celled, flask-shaped, with pointed tip producing conidia that may function as spermatia; *Meliola*



Asexual reproduction

-fission

-budding

-fragmentation

-by formation of non-motile spores such as chlamydospores, sclerotia, **conidia**, papulospores, soredia & isidium

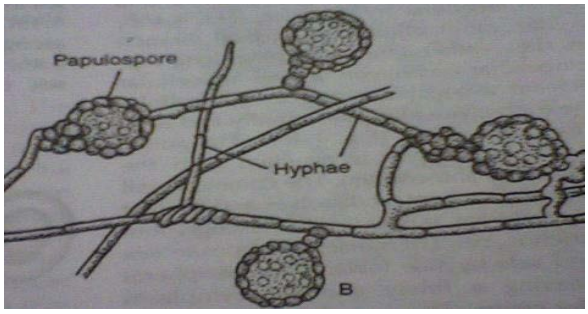


Fig. Papulospores in *Ascobolus scatigenus* with large central storage cells

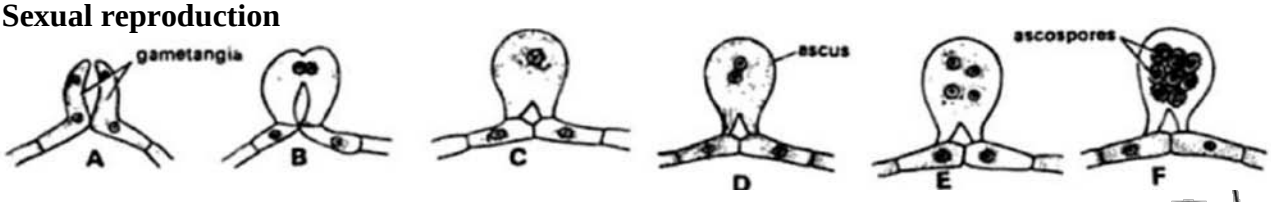
Sexual reproduction

This occurs following plasmogamy between cells of the two mating types where two compatible nuclei are brought together in the same cell.

Sexual reproductions of ascomycetes are of following types:

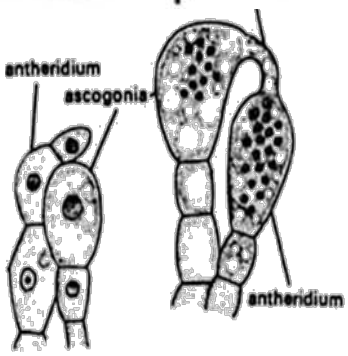
Gametangial copulation- two morphologically similar gametangia touch at their tips or coil around each other and fuse. The gametangia lose their entities during the process producing a unicellular zygote in which karyogamy follows immediately after plasmogamy. Thus, n+n condition is almost absent. The unicellular structure transformed into ascus, within which meiosis occurs producing four haploid nuclei.

Sexual reproduction



2. Gametangial contact-

Two morpholohically differentiated gametangia known as **antheridium** (male gametangium) and **ascogonium** (female gametangium) come in contact with each other and their entities are not lost during the process. No fusion cells are formed. Plasmogamy results dikaryotic condition that persists until ascus formation.



Four types of ascogonia are found:

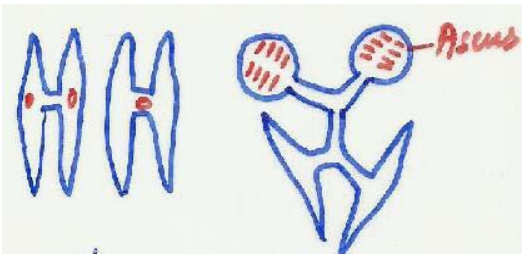
(i) Clustered, intermingled- *Ascodesmis*

(ii) Clusters of paired ascogonia & antheridia- *Pyronema*

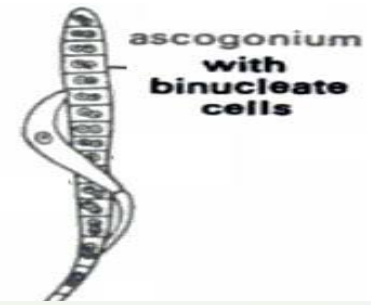
(iii) Stalked- *Scutellinia*

(iv) Beaded chains or ascogonial cells- *Ascobolus*

(v) Aplanogametic union-



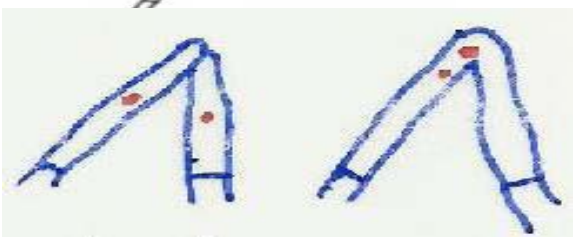
In *Spermaphthora gossypii*, two non-motile fusiform gametes come in contact with each other and unite by a conjugation tube within which two nuclei fuse to form diploid nucleus. From the conjugation tube rather limited branched septate hyphae of uninucleate cells are developed which behave as ascogenous hyphae. The tips of the ascogenous hyphae become spherical asci, each containing eight ascospores.



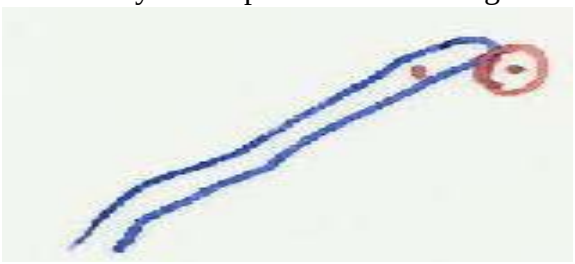
4. Autogamy-

When the tip of the antheridium comes in contact with the ascogonium the ascogonium nuclei arrange themselves in functional pairs or dikaryons. The antheridia do not donate nuclei to the ascogonium. eg. *Talaromyces vermiculatus* (anamorph *Penicillium vermiculatus*)

5. Spermatization-

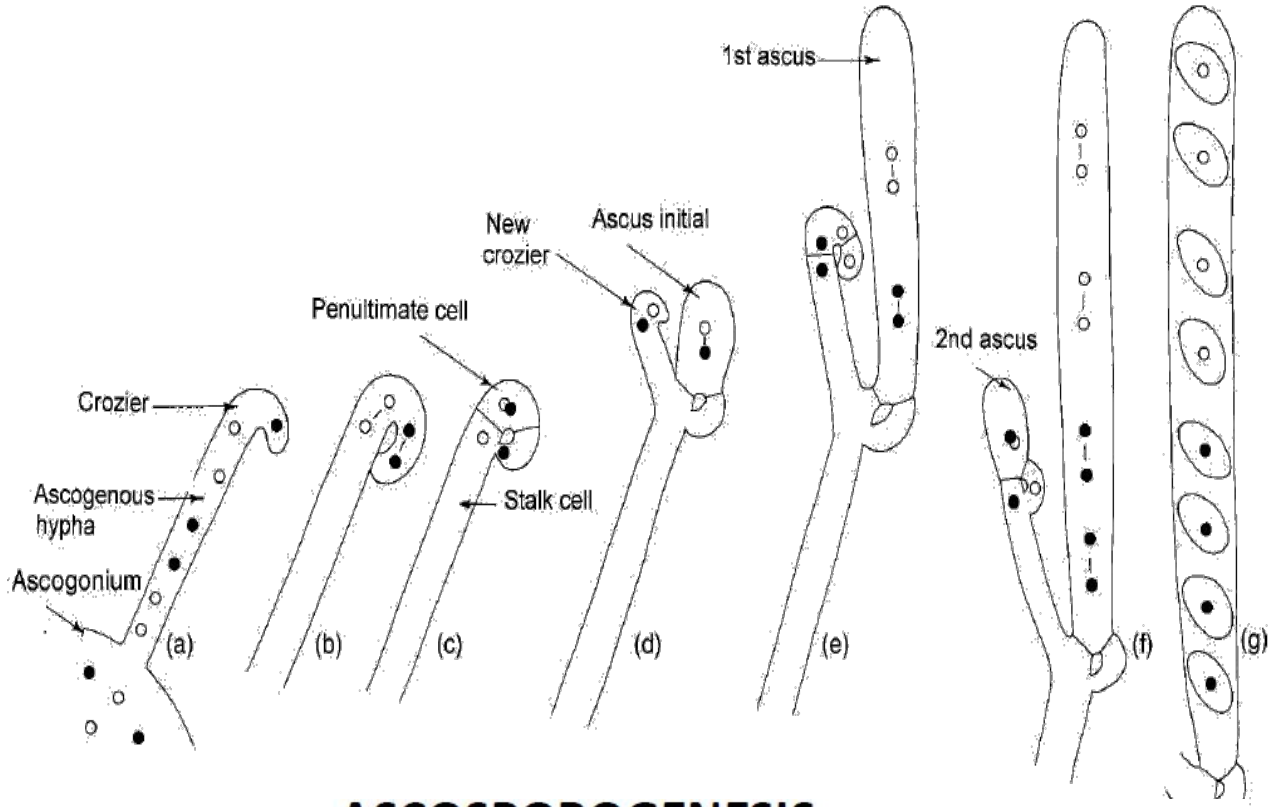


Instead of antheridium, specialized male sex cells, the **spermatia** are formed on the mycelium or in a specialized structure called **spermagonium**. They brought in contact with the trichogyne of the ascogonium or with hypha where there is no ascogonium, usually through some external agencies like insects, water, wind etc. Dikaryotic condition is achieved by dissolution of common wall, followed by karyogamy and meiosis. Fertilized ascogonium directly develops into an ascus. eg. *Neurospora crassa*, members of Laboulbeniales



6. Somatogamy- dikaryotic condition is attained by the union of somatic hyphae of opposite strains; nuclei migrate to the ascogonia through septal perforations; karyogamy and meiosis result the formation of ascospores within the asci. eg. *Coprobia granulate*

Cytological events during ascus development

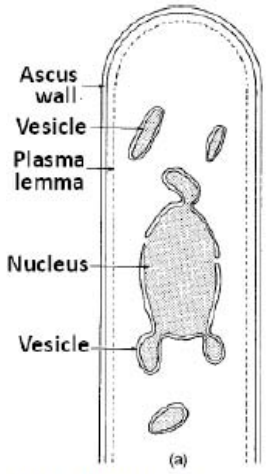


ASCOSPOROGENESIS

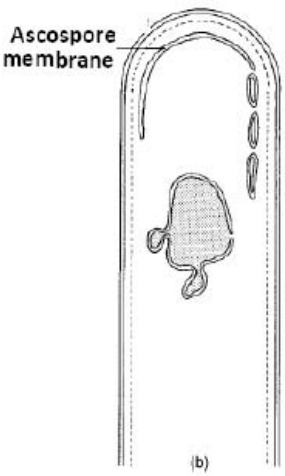
Ascospores are formed within the ascus as a result of 'free cell formation'.

The process takes place through two steps:

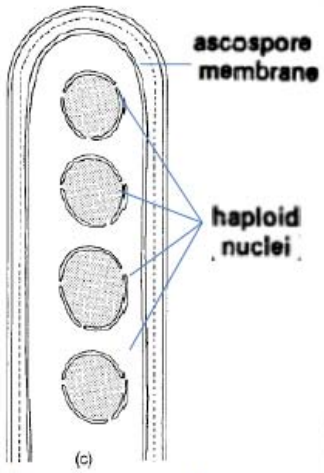
(I) Formation of enveloping membrane system (EMS)-



In young ascus, membrane-bound vesicles or EMS are formed from the nucleus and the ascus wall is lined by the plasmalemma.

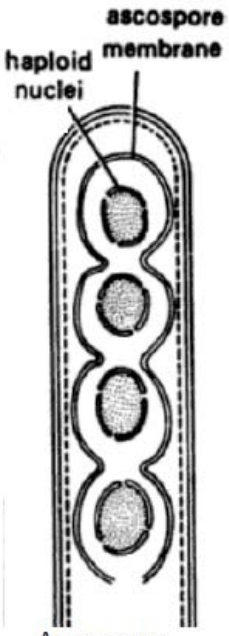


Ascospore membrane is appeared at the tip of the ascus and the vesicles are arranged along the periphery of the ascus.



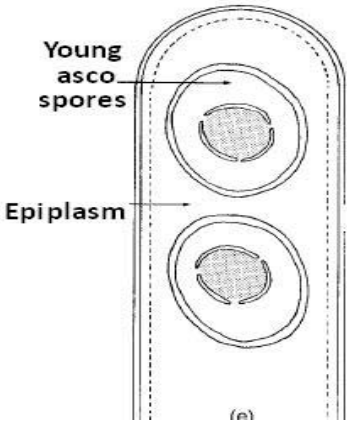
Ascospore membrane in the form of a peripheral tube opens at the lower end. The diploid nucleus has divided meiotically.

Cont...

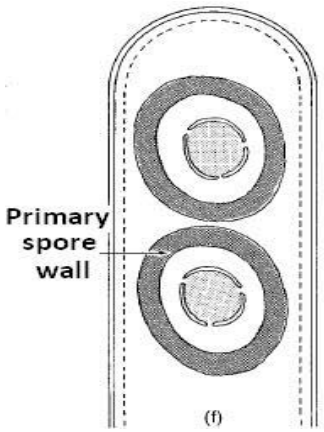


Ascospore membrane invaginates between the haploid nuclei.

(II) Deposition of ascospore wall-



Young ascospores are delimited by the ascospore membrane from the epiplasm

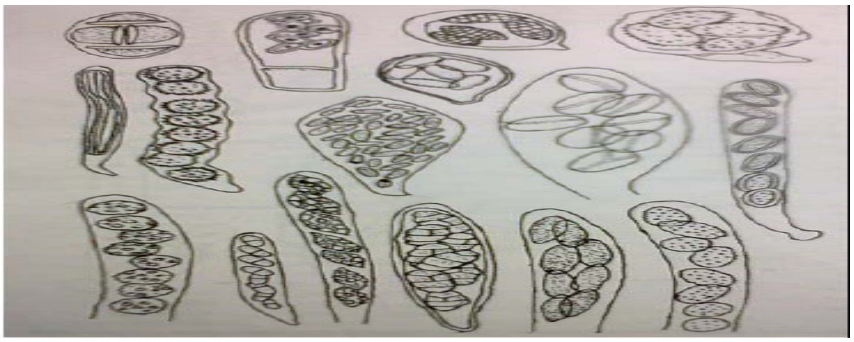


Young ascospores are delimited by the ascospore membrane from the epiplasm

In Archiascomycetes (*Taphrina*) and Hemiascomycetes (*Saccharomyces*), the nuclei within the ascus become enveloped by separate EMS formed by direct invagination of discrete parts of the ascus plasma membrane.

ASCUS (pl. asci)

It is a sac-like hypha containing definite number of ascospores formed by free cell formation after karyogamy & meiosis.



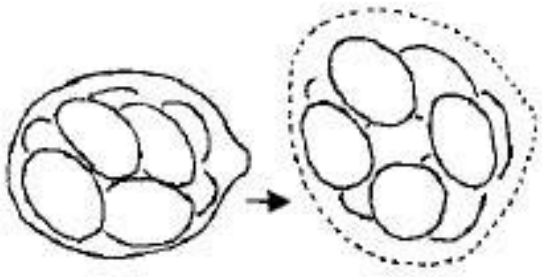
SHAPE: cylindrical / clavate / globose / ovoid;
sessile / stalked
(*Taphrina deformans*) Synascus or
multinucleate ascus (*Protomyces* sp.)

Types of asci based on wall

Prototunicate asci have thin, delicate & evanescent wall releasing spores by deliquescing the wall; hemiascomycetes (*Saccharomyces*),
plectomycetes (*Eurotium*)

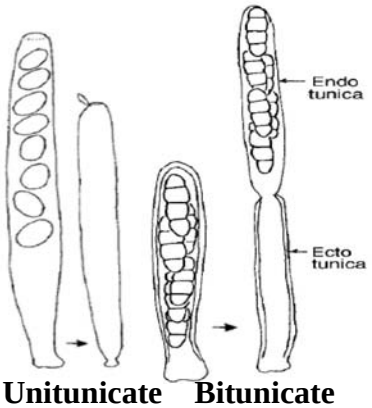
Unitunicate asci have two walls: outer exotunica and inner endotunica;
both are firmly attached to each other and remain together even during
spore release; pyrenomycetes (*Neurospora*), operculate discomycetes (*Ascobolus*)

Bitunicate asci also have two walls: the rigid exotunica and flexible endotunica



Prototunicate ascus

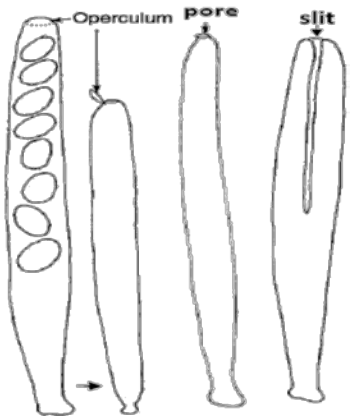
that can become considerably extended. During ascospore discharge exotunica
splits and sometimes slips downward to form a collar and the endotunica protrudes
through the opening and expands. Most of the bitunicate asci separate at ascospore
discharge into two functionally distinct layers, and such asci are termed **fissitunicate**. Loculoascomycetes (*Pleospora*). [**Jack-in-the-box mechanism**



Unitunicate ascus **Bitunicate ascus**

Apical apparatus-

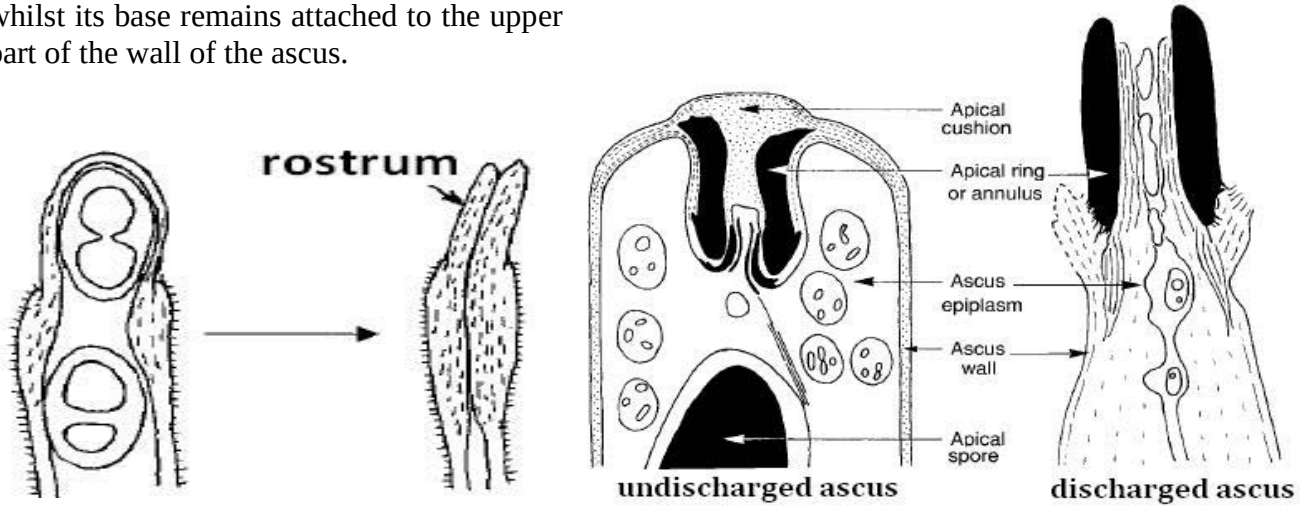
The apical dome of both the unitunicate & bitunicate asci are modified to form various structures for release of ascospores: Operculum (*Ascobolus*), Pore (*Claviceps*), Slit (Pertusaria), the apical ring or Annulus (*Xylaria longipes, Sordaria fimicola*), Rostrum (*Lecanora, Physcia*). The opening formed at the top of ascus is known as ascostome.



Annulus is a specially thickened inward extension of the apical wall of the ascus, arranged in the form of a cylindrical flange. During ascospores discharge the annulus is turned inside out like a sleeve & elastic to expand and contract as an ascospore passes through it.

It acts as a sphincter, minimizing the decrease in hydrostatic pressure inside the ascus as spore discharge proceeds.

In *Physcia stellaris* ripening ascospores push against the apical dome and on ascospore discharge the dome is extruded to form a **rostrum** (Lat. rostrum = beak) which extends upwards to the surface, whilst its base remains attached to the upper
part of the wall of the ascus.

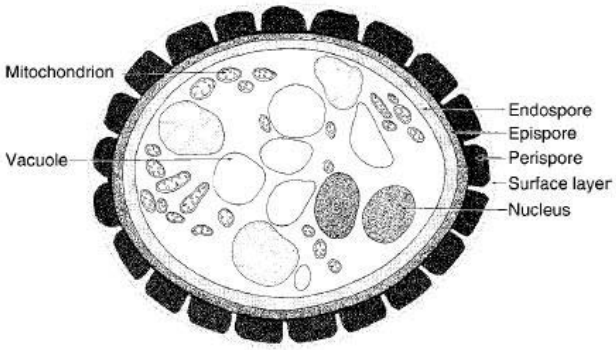


ASCOSPORES

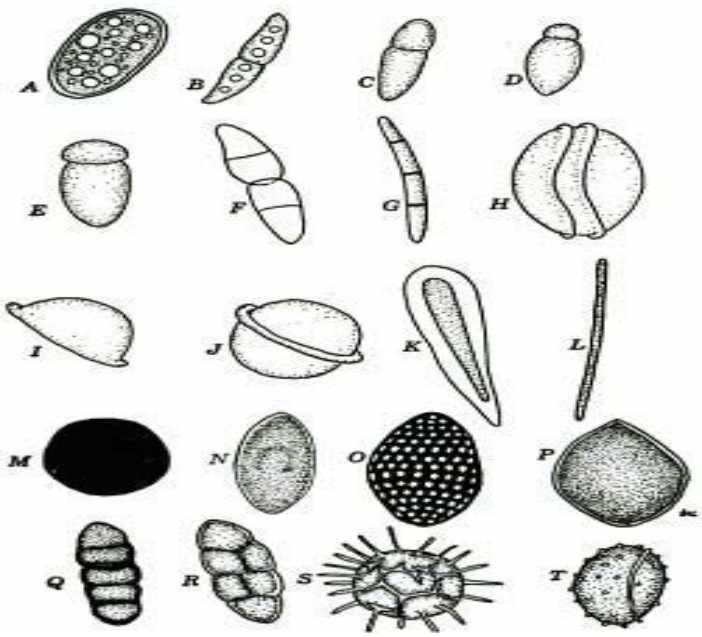
Unicellular (*Ascobolus, Neurospora*), bi-cellular (*Nectria, Hypocrea*), multi-cellular (*Pleospora, Cordyceps*); some multicellular ascospores break into fragments at septa, these are called **part spores**; each part behaves as an individual spores; *Cordyceps, Hypocrea*.

Size: 4-5 X 1 µm in *Dasyscypus* to 130 X 45 µm in *Pertusaria pertusa*

Shape: globose, oval, elliptical, lemon-shaped, hat-shaped (*Pichia*), filiform (*Cordyceps*, *Claviceps*) Colour: hyaline to variable colouredWall sculpture: smooth, sculptured- rough, spiny, reticulate, ornamented by ridges, with appendages (*Podospora*), mucilaginous outer layer extended to form simple or branched appendages especially in marine forms to help in bouncy & attachment; oil globules present in ascospores- **guttules.**



Various types of ascospores



The mechanism of ascospore discharge

Explosive release

In the young ascus, after the spores have been cut out, the epiplasm remains lining the ascus wall, and this surrounds a large central vacuole containing ascus sap, within which the ascospores are suspended. The epiplasm is rich in the polysaccharide glycogen which converts to osmolytes (mannitol) of lower molecular weight during spore ripening.

This brings about an increased osmotic concentration of the ascus sap, followed by increased water uptake. The resulting increase in turgor pressure causes the ascus to stretch and, eventually, to burst open, squirting out the ascospores.

In cup fungi (*Ascobolus*) tips of the mature asci are phototropic, project above the hymenium.

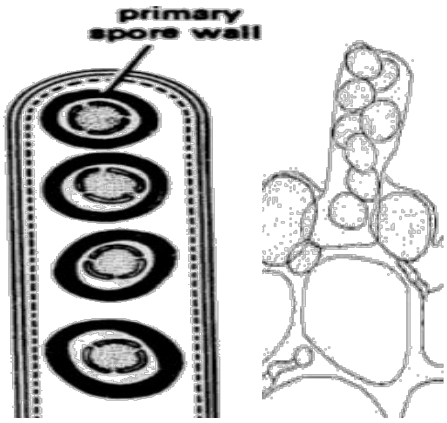
Members of operculate asci (*Ascobolus*) discharge large numbers of spores simultaneously, a phenomenon known as **puffing**.

Passive release

In many marine ascomycetes the ascus walls are evanescent and dissolve to release the ascospores passively. In ascomycetes with subterranean fruit bodies, e.g. in the truffle *Tuber* and its relatives, the ascospores are dispersed when the fruit bodies are eaten by rodents and other animals attracted by their characteristic odour.

In most ascomycetes asci are surrounded by or enclosed in an aggregation of hyphae to form a fruit body, known as ascocarp or ascoma. In *Taphrina*, ascocarp is absent; naked asci are borne on the leaf surface.

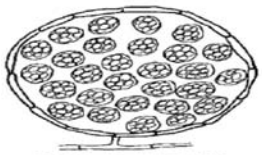
TYPES OF ASCOCARP



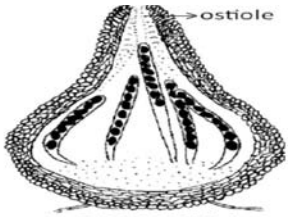
CLEISTOTHECIUM (Gr. *kleistos* = closed; *theke* = case) - A globose fructification; asci are totally enclosed; no special opening; plectomycetes (*Eurotium*)

APOTHECIUM (Gr. *apotheke* = storehouse) - An open saucer-shaped ascocarp, and the naked asci tips of the asci are freely exposed at maturity; sessile (*Ascobolus*), stalked (*Morchella*) & other discomycetes members.

PERITHECIUM (Gr. *peri* = around; *theke* = case) - A flask-shaped fruit body discharge spores through an narrow opening (**ostiole**); wall is formed from sterile cells derived from hyphae which surrounded the ascogonium during development and ostiole formation is schizogenous i.e. by pushing apart of tissue by the periphyses at the apex. Perithecia are often single (*Sordaria*, *Neurospora*), but in some genera (*Claviceps*) they are embedded in or seated on a mass of tissue forming a perithecial stroma.


CLEISTOTHECIUM

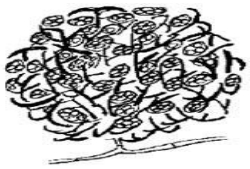

APOTHECIUM



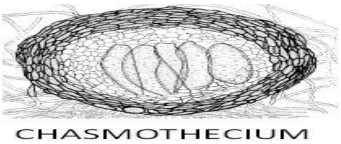
PERITHECIUM

GYMNOTHECIUM (Gr. *gymnos* = naked) - asci are mixed with loose open network of peridial hyphae; *Gymnoascus*

CHASMOTHECIUM (Gr. *chasma* = an open mouth) - A modified cleistothecium capable of cracking open along a line of weakness; *Erysiphe*


GYMNOTHECIUM

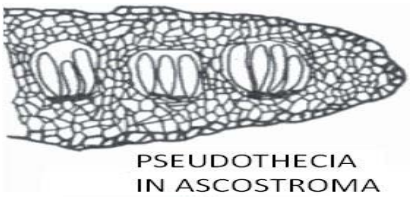
PSEUDOTHECIUM / ASCOLOCULE- Asci are formed within a single cavity (locule) that develops within an stroma. The locule is not surrounded by a distinct wall. Ostiole formation is lysogenous i.e. by lysis of a preformed mass of tissue. *Mycoshaerella*


CHASMOTHECIUM

ASCOSTROMA- A compact stomatic structure on which or in which ascocarps are formed or asci are directly formed in the locules.

Unilocular (*Venturia inaequilis*),

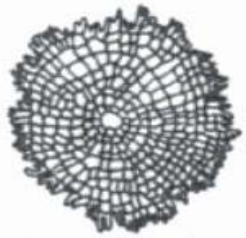
multilocular (*Myriangium duriae*);


PSEUDOTHECIA
IN ASCOSTROMA

sclerotial stroma (sclerotia) are compact hyphal structure, *Sclerotinia*;

substratal stroma are diffused form where medulla is made up of loose hyphae, *Rutstroemium*

THYRIOTHECIUM / CATATHECIUM- An inverted saucer-shaped ascocarp having wall more or less radial in structure, *Asterina*


Thyriothecium

HYSTEROTHECIUM- An elongated ascocarp enclosed when young, but opens at maturity by a long slit following a line of dehiscence parallel to the long axis extending almost the entire length of the ascocarp. *Glomium*

STEREOTHECIUM- A solid fleshy ascoma with asci which are either solitary i.e. scattered through the medullary excipulum or grouped in dispersed pockets, *Tuber*.


Hysterothecium

ASCOCARP DEVELOPMENT

Ascohymenial- ascocarp develops after plasmogamy & pairing up of two genetically dissimilar nuclei; Hymenomycetes, i.e. both discomycetes & pyrenomycetes **Ascolocular**- ascoma is already developed before the compatible nuclei are brought together & asci are produced in preformed locules; Laculoascomycetes.

CHARACTERS	Perithecium	Pseudothecium
Ascocarp development	Ascohymenial	Ascolocular
Ostiole formation	Schizogenous	Lysoginous
Asci	Unitunicate	Fissitunicate
Hamathecium	Paraphyses, periphyses	Absent, or if present pseudoparaphyses, periphysoids
Wall	Wall is formed from sterile cells derived from hyphae which surrounded the ascogonium during development	Locule is not surrounded by a distinct wall
Example	Pyrenomycetes	Laculoascomycetes

CLASSIFICATION

According to Ainsworth (1973)	According to M.E. Barr (2001); Kurtzman & Sugiyama (2001); Weber & Webstar (2007)
KINGDOM **MYCOTA**	KINGDOM **FUNGI**
DIVISION **EUMYCOTA**	SUBKINGDOM **EUMYCOTA**
SUBDIVISION **ASCOMYCOTINA**	PHYLUM **ASCOMYCOTA**
CLASS **HEMIASCOMYCETES**	CLASS **ARCHIASCOMYCETES**
CLASS **LOCULOASCOMYCETES**	CLASS **HEMIASCOMYCETES**
CLASS **PLECTOMYCETES**	CLASS **PLECTOMYCETES**
CLASS **LABOULBENIOMYCETES**	CLASS **HYMENOASCOMYCETES**
CLASS **PYRENOMYCETES**	CLASS **LOCULOASCOMYCETES**
CLASS **DISCOMYCETES**	

CLASS **ARCHIASCOMYCETES**
(150 species in 10 genera, two orders)
CHARACTERS:

1. Ascocarp & ascogenous hyphae lacking (apothecium in *Neolecta*);
2. Asci prototunicate and are formed singly by yeast cells or by converion of hyphal tips; asci may (*Taphrina, Protomyces*) or may not (*Pneumocystis, Schizosaccharomyces*) forcibly discharge their spores;
3. Enveloping membrane system (EMS) is associated with individual nuclei, no common EMS enclosing all nuclei;
4. In mycelial form, septal pore is simple; Woronin bodies absent;
5. Cell wall contain little amount of chitin;

Common genera: ***Taphrina-*** biotrophic parasite; mycelia n+n; ***Protomyces-*** contains synascus; ***Schizosaccharomyces-*** fission yeast; ***Pneumocystis*** - human pathogen.

Taphrina deformans
Leaf Curl of Peach

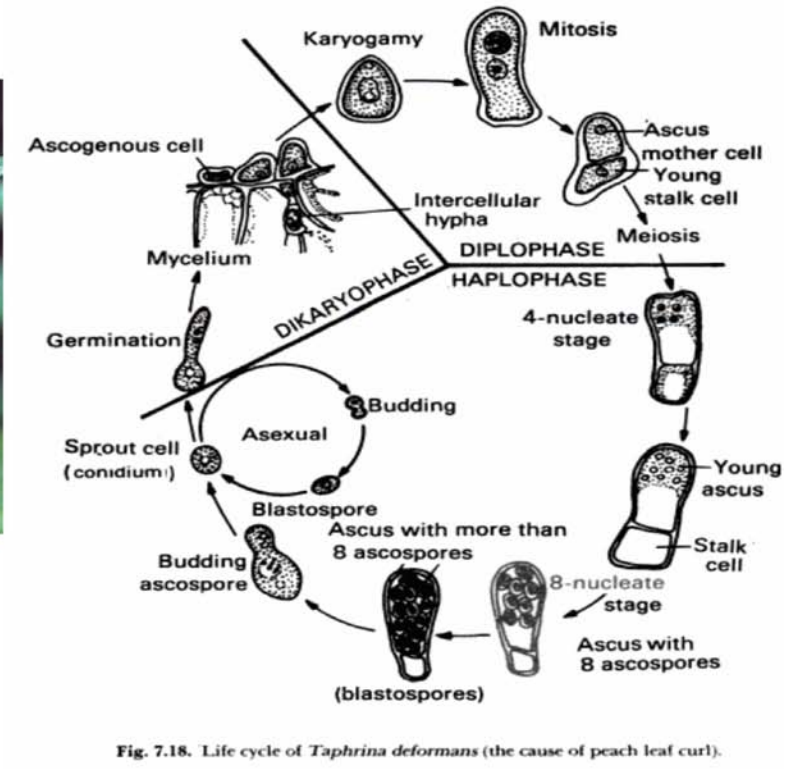


Fig. 7.18. Life cycle of *Taphrina deformans* (the cause of peach leaf curl).

CLASS **HEMIASCOMYCETES**
(one order, the Saccharomycetales, 11 families , 276 species)
CHARACTERS:

1. Ascocarp & ascogenous hyphae lacking;
2. Enveloping membrane system (EMS) is associated with individual nuclei, no common EMS enclosing all nuclei;
3. Thallus mycelial or yeast-like or pseudomycelial as in *Saccharomyces*
4. In mycelial form, septal pore is simple; woronin bodies absent;
5. Asci prototunicate and are formed singly with evanescent walls, release their ascospores passively;

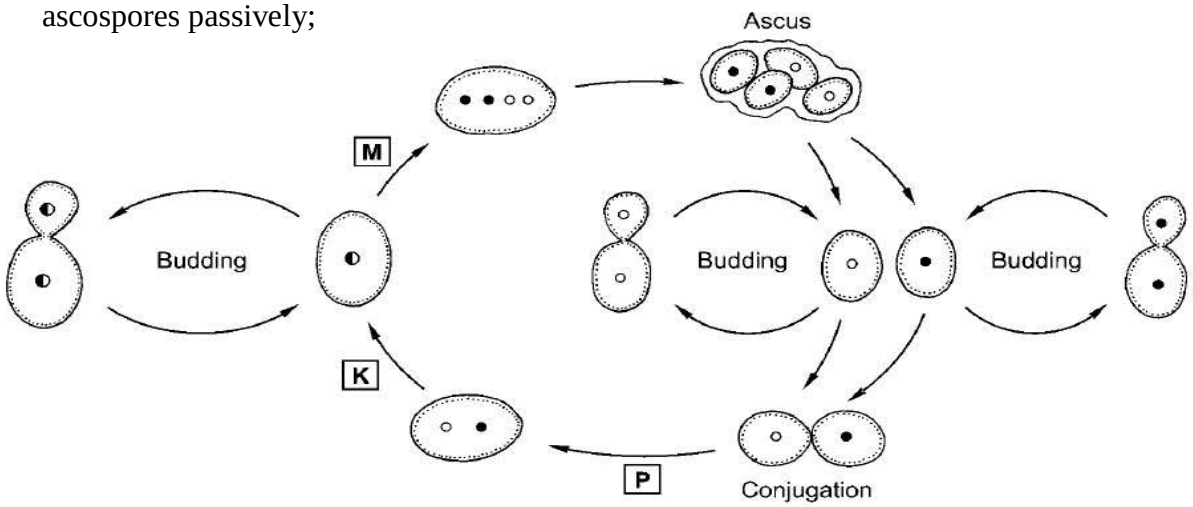


Fig 10.2 The life cycle of *S. cerevisiae*. Both haploid and diploid cells can reproduce by budding. Open and closed circles represent haploid nuclei of opposite mating type; diploid nuclei are larger and half-filled. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M).

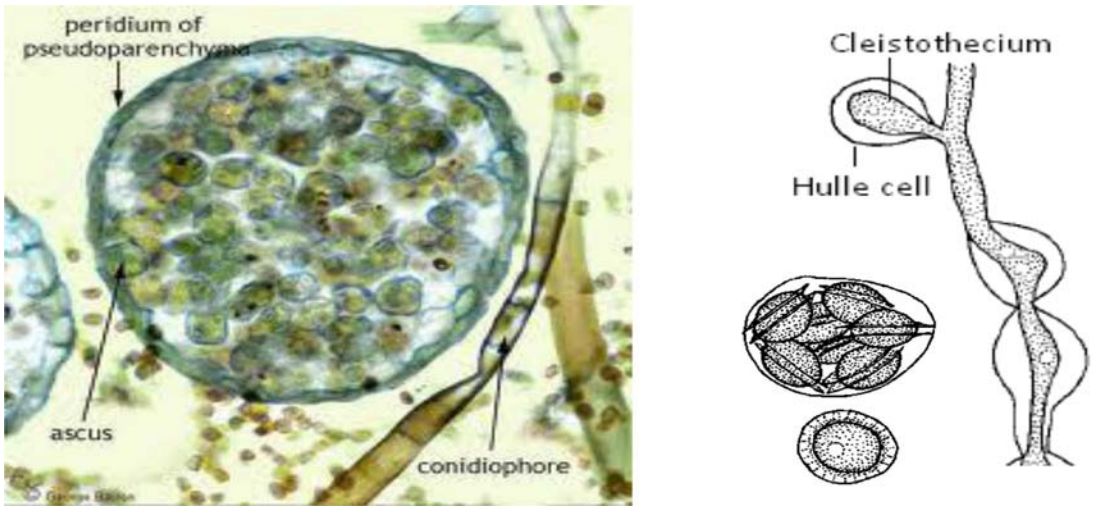
6. Cell wall contains very little chitin (only in bud scar);
7. No dikaryotic phase in life cycle; diploid stage may persist & multiply by budding (budding yeast}

Diploid strains of *S. cerevisiae* can be induced to form ascospores by suitable treatment, and this yeast is therefore termed an **ascosporogenous** yeast, in contrast to **asporogenous** yeasts in which ascospores have not been observed.

Killer yeasts are strains which produce toxins capable of killing other strains belonging to the same or to closely related species. Three important virus-encoded killer toxins (K1, K2, K28) are known to exist in *S. cerevisiae*; all three are polypeptides and are encoded by double-stranded RNA encapsulated in virus-like particles (VLPs).

CLASS PLECTOMYCETES
(900 species in 90 genera, three orders)
CHARACTERS:
1. Ascogonium & ascogenous hyphae present;
2. Ascocarp **cleistothecium,** rarely **gymnothecium** as in *Gymnoascus*; sometimes embedded in stroma;
3. Asci prototunicate, evanescent and scattered throughout the fruit body;
4. Ascospores aseptate.

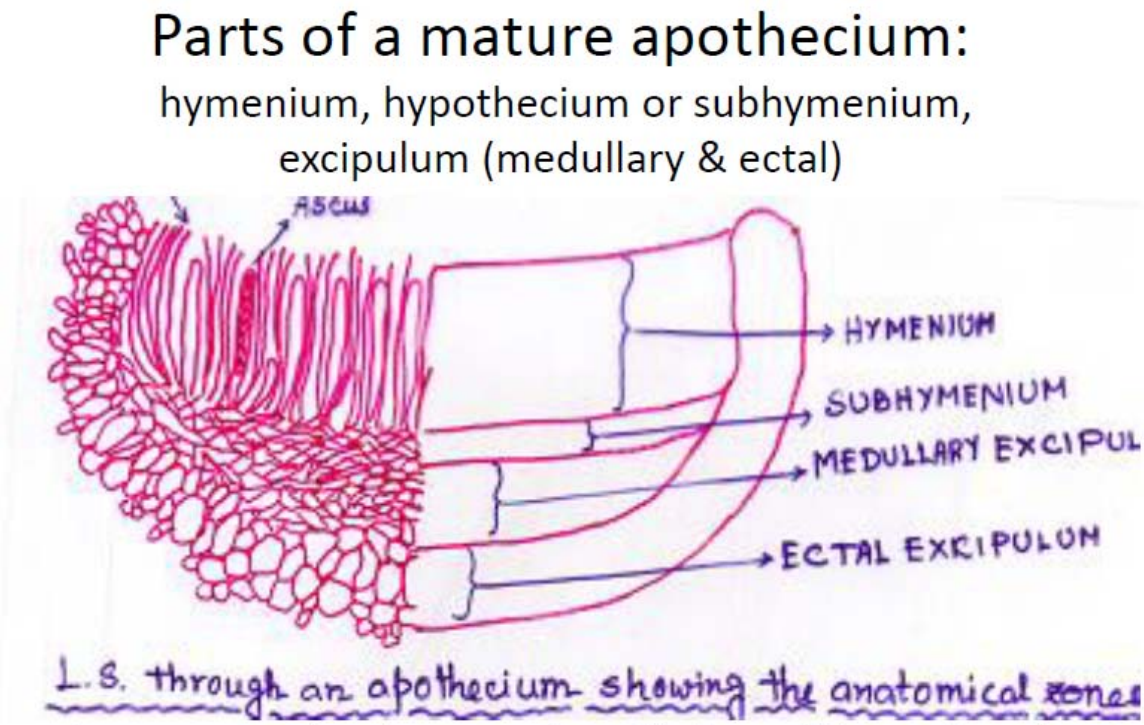
Genera: *Eremascus, Gymnoascus, Onygena, Arthroderma, Eurotium, Emericella, Talaromyces, Monascus*



Cleistothecia of *Eurotium* (left), *Emericella* (right)

CLASS **HYMENOASCOMYCETES**
CHARACTERS:

1. Asci are produced in a hymenium (fertile layer) around which the ascocarp develops;
2. Ascogonium & ascogenous hyphae present;
3. Ascocarps either apothecia (**Discomycetes**) or perithecia (**Pyrenomycetes**);



Epithecium- present above hymenium, formed by fusion of the tips of paraphyses

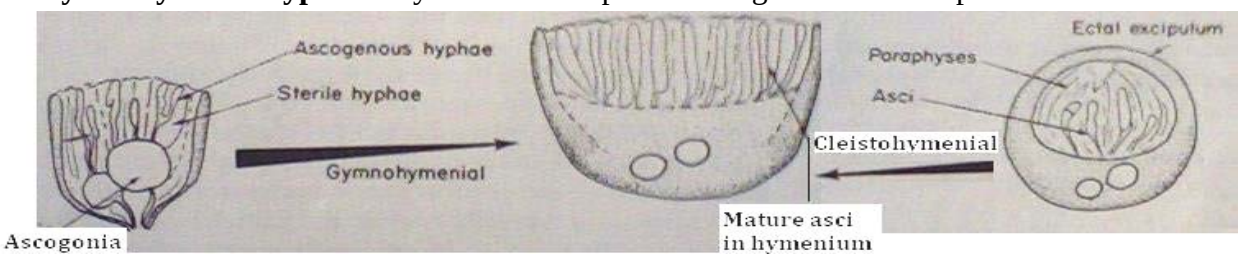
DISCOMYCETES

CHARACTERS:

- Ascocarp apothecium, macroscopic, epigenous or hypogeous; sessile (*Ascobolus*) or stalked (*Morchella*);
- Asci bitunicate, inopreculate (Order Helotiales) or operculate (Order Pezizales).

Development of apothecia

According to Van Brummelen (1967),
two basic types of apothecia on the basis of their development:
Cleistohymenial type- the hymenium is enclosed atleast during its early stage within the excipulum of the developing ascoma.
In hypogean spp. hymenium remains permanently closed.
In epigean form (*Ascobolus immerses*), hymenium is exposed due to rupturing of excipulum by the developing asci & paraphyses.
Gymnohymenial type- the hymenium is exposed throughout its development



PYRENOMYCETES

(640 genera, 6000 species)

CHARACTERS:

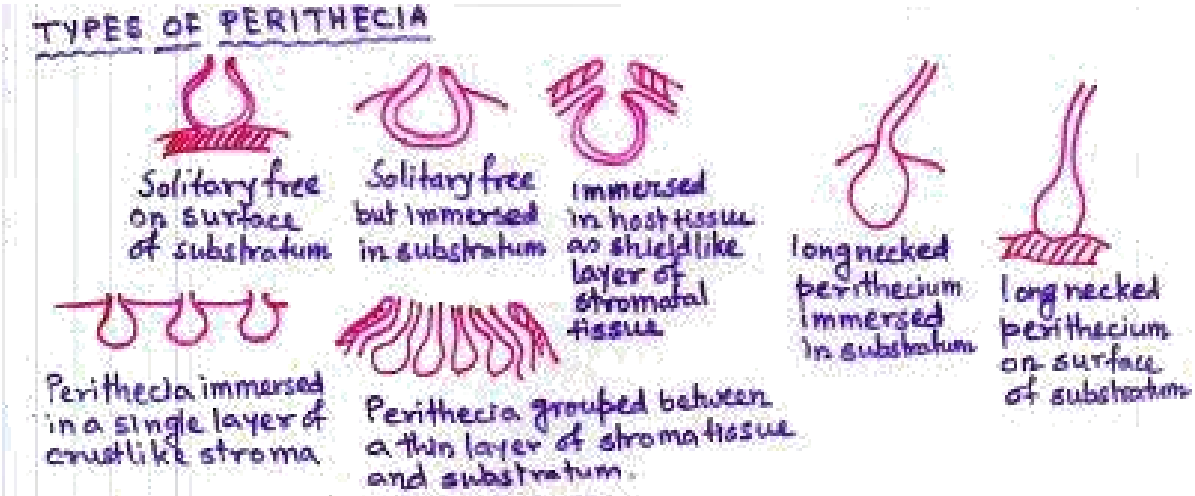
1. Ascocarps **perithecia**, often formed in a **stroma**; develop in ascohymenial ways;

2. Asci unitunicate, persistant, discharge ascospore forcibly with the ascospores oozing out of the perithecial ostiole as a tendril (**cirrhus**).;

3. **Hamathecia** consist of sterile hyphae (**paraphyses** growing up from the basal fertile region & **periphyses** lines inner surface of ostiole); ostiole development schizogenous, i.e. formed by pushing apart of tissue by the periphyses at the apex of perithecium.

4. All the tissues including asci & sterile hyphae within ascocarp are collectively called **centrum**.

Genera: *Neurospora* (without stroma), *Podospora, Chaetomium, Xylaria, Daldinia, Gibberella, Claviceps, Ceratocystis, Magnaporthe, Glomerella, Meliola*



Diaporthe
The centrum is formed due to disintegration of pseudo-parenchyma

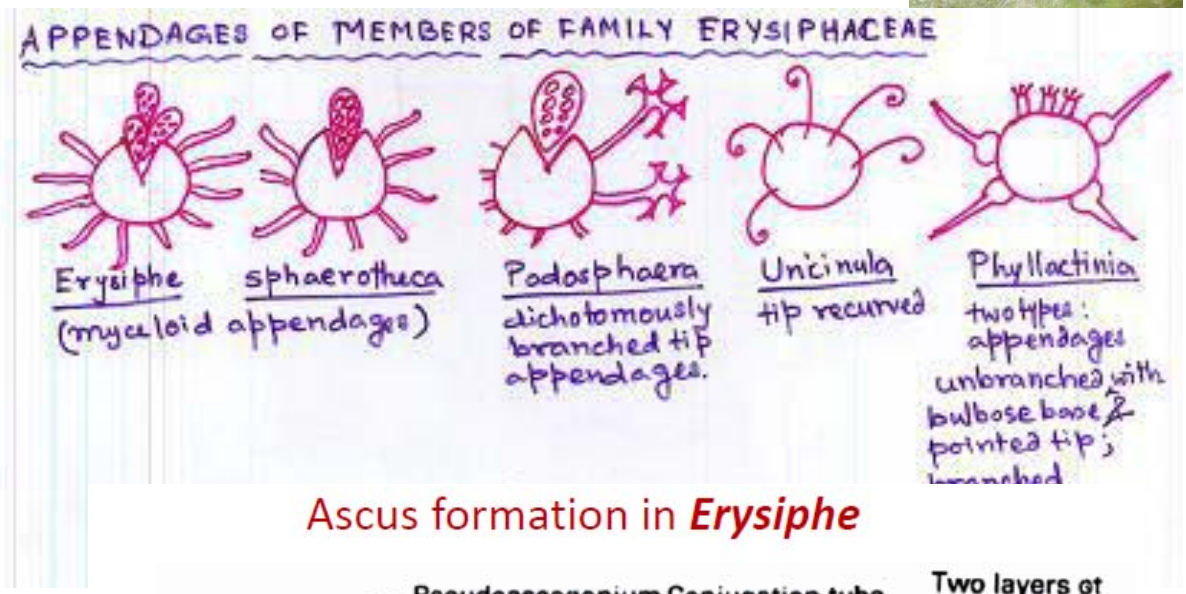
Xylaria
The centrum is formed due to pressure exerted by the growth of opposed paraphyses

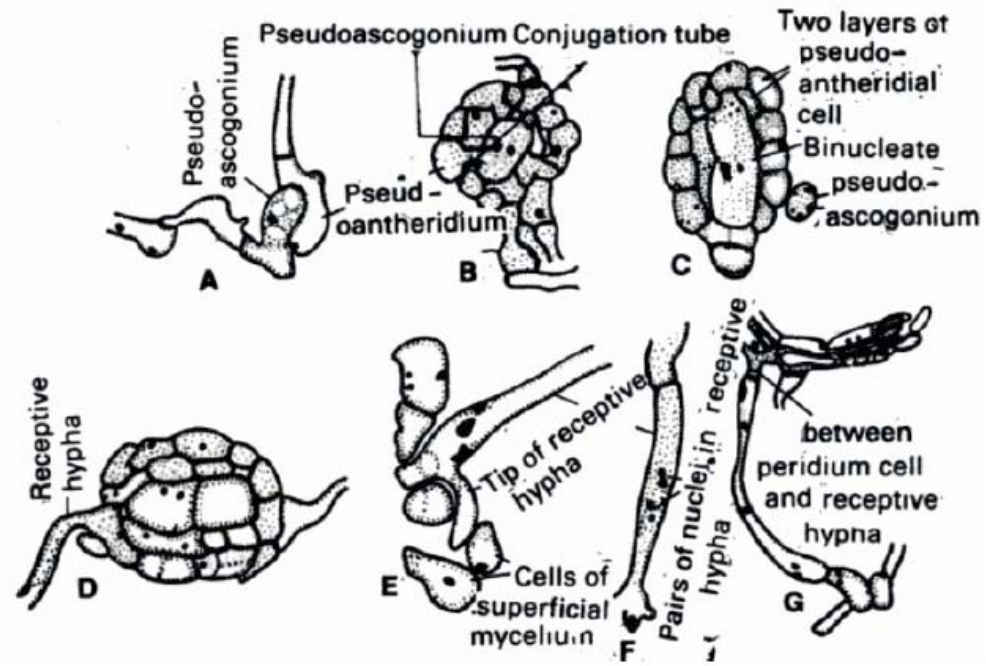
Sordaria
Differentiation of the innermost cells of the proto perithecium gives rise to the centrum

Nectria
Pressure exerted by the elongation of the **apical paraphyses**, accompanied by expansion of the wall, creates the central cavity within the perithecium

Order Erysiphales

Obligate biotrophs, cause powdery mildew; Ascocarp chasmothecium; bears different types of appendages;





Asci are not developed from ascogenous hyphae. Here, sexual union takes place between two superficial uninucleate hyphal branches that meet and encircle each other as functional gametangia. The central cell that receives a nucleus is called **pseudoascogonial cell** (because it does not seem to play any direct role in ascus formation). The **pseudoantheridial cells divide to form the peripheral cells of the ascocarp**. Some outer peripheral cells ('mother cells') develop short septate receptive hyphae with uninucleate segments. The tips of the hyphae make contact with vegetative hyphae on the host surface. Following plasmogamy, one nucleus is taken up by

the receptive hypha and divides in each segment of the receptive hypha until one nucleus derived from the vegetative hypha reaches the mother cell. The mother cell then divides repeatedly. At this stage, the immature ascocarp consists of a pseudoparenchymatous centrum composed largely of binucleate cells derived from the mother cells intermixed with some uninucleate cells, and surrounded by a peridium, some 4-6 cell layers thick. The peridium becomes darkly pigmented. Uninucleate and binucleate cells above the middle part of the centrum lyse. Karyogamy occurs only within certain of the binucleate cells which are more or less isolated from the surrounding cells by lysis. These cells then enlarge to form asci. The asci appear to grow at the expense of the uninucleate and binucleate cells of the centrum, so that eventually the asci (or a single ascus, depending on the genus) occupy almost the entire centrum. Meiosis of the fusion nucleus in developing asci is usually delayed until the centrum cells have all been absorbed.

CLASS **LOCULOASCOMYCETES**

- Asci are produced within locules in a preformed stroma (ascolocular development);
- Ascocarps- ascostromata, pseudothecia, thyriothecia, hysterothecia, unilocular (*Venturia*) or multilocular (*Myriangium*), no special wall around the centrum;
- Hamathecia absent or if present, consist of pseudoparaphyses (originate above hymenium & grow downwards) and periphyses (grow downwards but do not reach the base); ostiole formation lysogenous type i.e., by breakdown of preformed mass of tissues;
- Asci fissitunicate; ascospores multicellular.

Based on centrum, ascolocules are of three types:

Myriangium type- Globose asci occur singly in locules that are scattered or grouped in a fertile region in stromatal tissue. Hamathecial tissues are absent and ascus discharge occurs after the asci break through the stromatal tissue.

Dothidea type- Asci are produced in fascicles in small perithecium-shaped locules in ascostromata and do not produced pseudoparaphyses.

Pleospora type- Asci are developed among the pseudoparaphyses which originate above hymenial area and grow downwards, become attached at the bottom of the locule; they elongate to form a flask-shaped locule within the stroma.

DEUTEROMYOTINA

Salient features

- Second largest group; **2400 form-genera, 20,000 form-species**.
- '***Deutero***', means second; their status as second class members among sexually reproducing fungi.
- 'Fungi imperfecti' – lack of sexual reproduction.
- 'Anamorphic fungi' – production of asexual spores, only.

•'Mitosporic fungi' – asexual spores are produced by mitotic division.

•Somatic structure – unicellular or mycelial; mycelia septate; with either simple or dolipore septa; parasitic forms produce appressoria, haustoria, nematode traps, etc.

•Absence of motile cells in life cycle.

• Parasexuality as mechanism for genetic recombination.

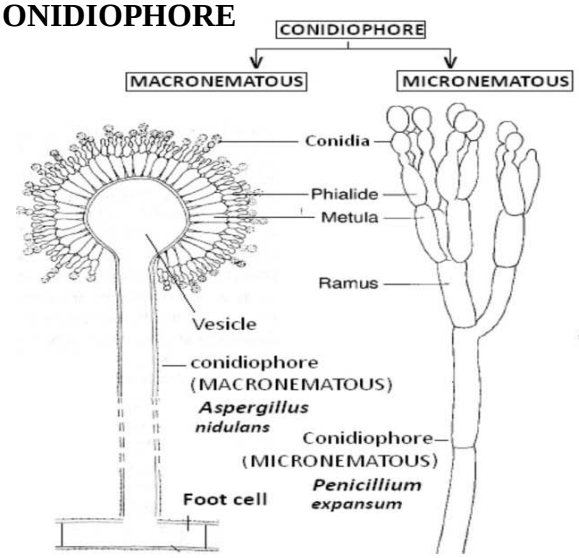
OCCURRENCE

- **PLANT PARASITES-** *Alternaria solani* (early blight of potato); *Colletotrichum falcatum* (red rot of sugarcane)
- *Fusarium oxysporum* f.sp. *udum* (vascular wilt of pigeon pea)
- **ANIMAL PARASITES-** *Candida, Aspergillus* on human beings *Beauveria, Metarhizium* on insects *Arthrobotrys* on nematodes
- **MYCOPARASITE-** *Trichoderma harzianum* on *Rhizoctonia solani*
- **AQUATIC**- *Alantospora*

ASEXUAL REPRODUCTION

- Unicellular forms reproduce by **budding**;
- Mycelial forms produce non-motile spores-**chlamydospores**, **conidia**;
- Conidium (Gk. *konidion*, small dust) is produced from a specialized fertile cell, called C**onidiogenous cell**;
- One or more conidiogenous cells are borne on a stalk, called **conidiophore**. Conidiophores are simple or branched;
- Conidiophores may arise singly (**monanematous**) as in *Aspergillus* and *Penicillium* or aggregate to form conidial fructification (**conidioma**).

CONIDIOPHORE



Conidiophores of *Aspergillus*

Uniseriate ***A. penicillioides***
conidiophore > phialide > conidium

Biseriate ***A. nidulans***
conidiophore > metula > phialide > conidium

Conidiophores of *Penicillium*

monoverticillate ***P. spinulosum***
conidiophore > phialide > conidium

biverticillate ***P. verruculosum***
conidiophore > metula > phialide > conidium

terverticillate ***P. expansum***
conidiophore > ramus > metula > phialide > conidium

quaterverticillate
conidiophore > ramus > ramulus > metula > phialide > conidium

CONIDIOMA: AGGREGATES OF CONIDIOPHORES

In the members of classes hyphomycetes and coleomycetes, the conidiophores aggregate to form a **conidioma** (pl. conidiomata) which are of different types: **Coremium** & **Synnema**- a cluster of conidiophores aggregate to form a parallel bundle (fascicle) and adhere to each other for part of their length, forming an elongated, bristle-like structure, called coremium (Gr. *korema* = a brush) or synnema (Gr. Prefix *syn* = together). Synnemata may be fleshy,

brittle or very hard in consistency. Conidia may be formed along the length of the synnema or only at its apex. The unattached apical portions of the conidiophores radiate outwards and frequently produce a mucoid slime in which conidia are borne. Seifert (1985) has distinguished several types of synnema (simple, compound, or made up of parallel conidiophores, or intricately interwoven hyphae). These types of conidiomata are found in ***Penicillium claviforme, Cephalotrichum*** *(Doratomyces)* ***stemonitis****.*

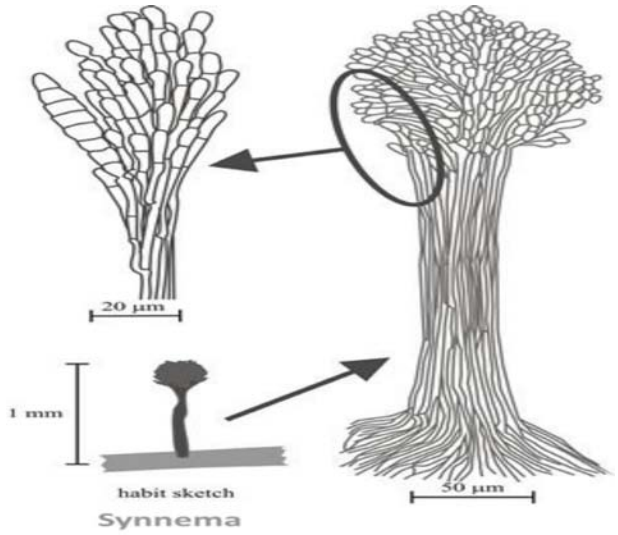


FIG. Synnema (bunched conidiophores) of *Podosporium elongatum*

Conidiostroma- In many ascomycetes the conidiophores develop on or in a stroma (Gr. *stroma* = bed, cushion), an aggregation of pseudoparenchymatous cells. In ***Xylaria hypoxylon***, powdery white conidia develop at the tips of the branches of the conidial stroma and, later, asci develop in flask-shaped perithecia at the base of the old stroma.

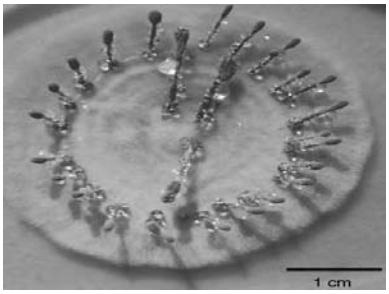


FIG. *Penicillium claviforme*

CONIDIOMA: AGGREGATES OF CONIDIOPHORES

Sporodochium (Gr. *spora* = a seed; *doche* = a receptacle)- a cluster of short conidiophores tightly clustered together to form a cushion-like conidioma, called sporodochium, which may be formed upon a stroma. Sporodochia conidiomata is characteristic of Form-family **Tuberculariaceae** of Class **Hyphomycetes (*Fusarium, Epicoccum*).** In ***Tubercularia***, the sporodochium is brightly coloured and shaped like a mushroom with a short stalk; in ***Volutella***, thick stiff bristles (setae) are present scattered over the entire sporodochium.

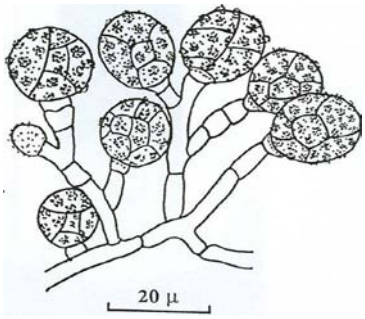


FIG. Sporodochium of *Epicoccum andropogonis*

Acervulus (L. *acervulus* = a little heap)- It is a saucer-shaped fructification made up of closely packed conidiophores and conidiogenous cells developing from a pseudoparenchymatous stroma. Acervulii may be cupulate or slightly pulvinate and may also bear long dark bristles (setae) as in ***Colletotrichum*** sp. Their position may be superficial or may develop inside the tissues of host plant as subcuticular (***Titaeosporina***), intraepidermal (***Psammina***), subepidermal (***Dolhistroma***), subperidermal (***Asterosporium***). When the conidia mature, they split epidermis and cuticle due to the increasing pressure, thus allowing the conidia to escape. The conidia are held together in slime and are chiefly dispersed by rain splash. Acervulii are characteristic of Form-order **Melanconiales** of Class **Coleomycetes.**

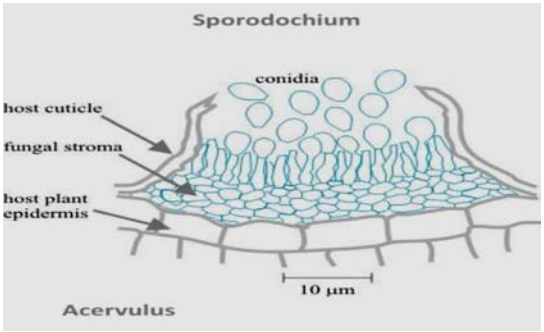


Fig. Acervulus of *Cryptocaline butulinum* and *Collectotricum botulnum*

CONIDIOMA: AGGREGATES OF CONIDIOPHORES

Pycnidium (Gr. diminutive of pyknos = dense, packed, concentrated)- It is a globose or flask-shaped pseudoparenchymatous structure bearing its conidiophores, conidiogenous cells and conidia within a cavity. The pycnidia may be entirely closed or may have an opening (ostiole); these may be provided with a small papilla, or with a long neck leading to the opening (beaked), or provided with setae (setose). These may be uniloculate or labyrinthiform. Pycnidia may be superficial or embedded in host tissue. Conidia formed from conidiogenous cells lining the inner wall of the pycnidium are held together in slimy masses which ooze out through the ostiole, sometimes as spore tendrils (cirrhus). They are generally dispersed by splash or in water films. These also vary in size, shape and colour and may be formed by loose mycelia or are stromatic. In some cases the pycnidia, instead of producing conidia with an asexual function, produce spermatia which are involved in fertilization.Pycnidia are characteristic of Form-order Sphaeropsidales of the Class Coleomycetes. e.g. Phoma acuta (anamorphs of Leptosphaeria acuta), Stagonospora nodorum (anamorph of Phaeosphaeria nodorum).

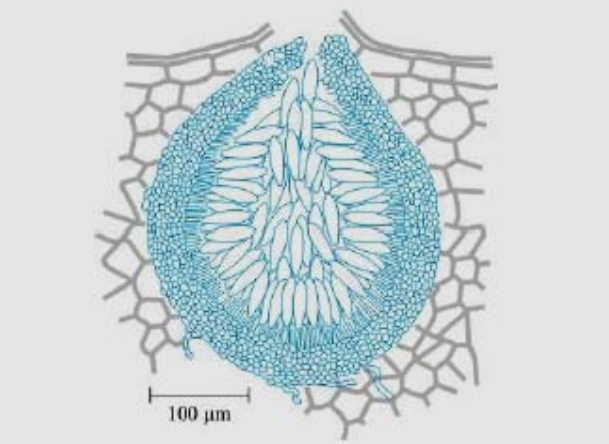
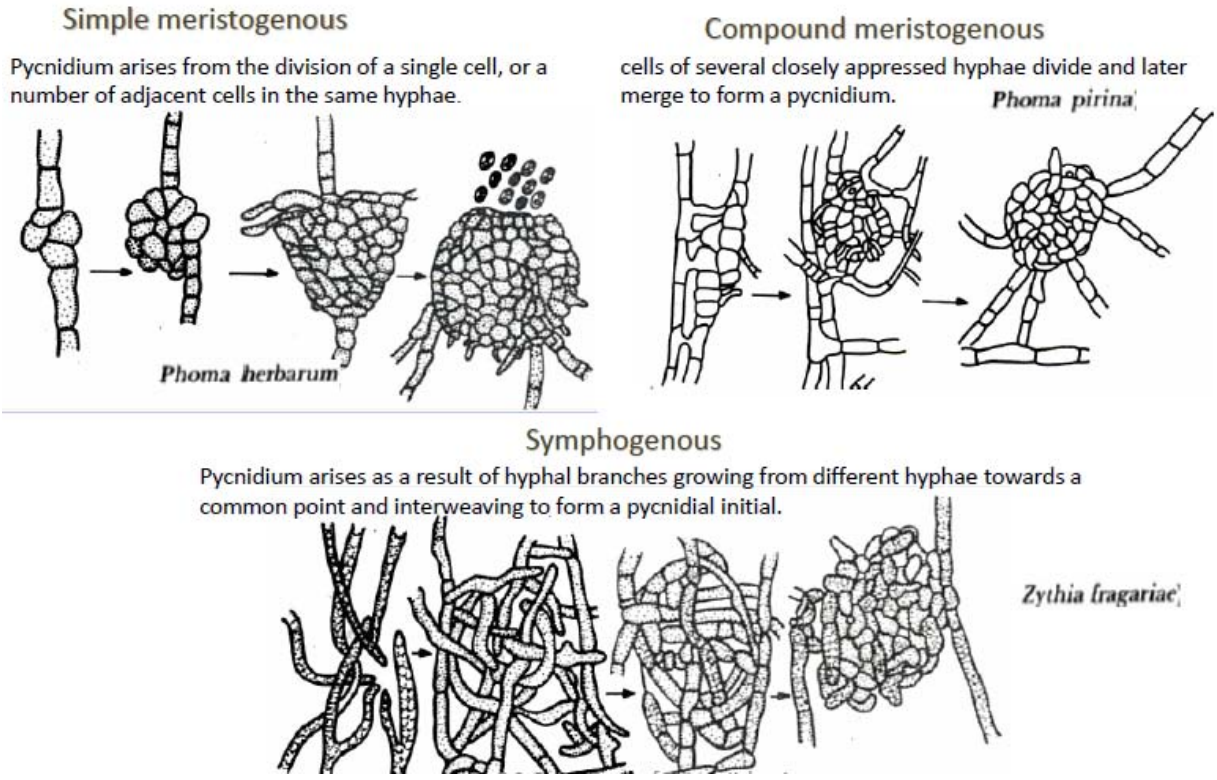


FIG. L.S. through pycnidium of *Macrophoma*

DEVELOPMENT OF PYCNIDIA (Kempton, 1919)



Conidia vs. other spores

Kirk *et al*. (2001) defined conidium as 'a specialized non-motile (cf. zoospore) asexual spore, usually caducous (i.e. detached), not developed by cytoplasmic cleavage (cf. sporangiospore) or free cell formation (cf. ascospore); in certain Oomycota produced through the incomplete development of zoosporangia which fall off and germinate to produce a germination tube'.

CONIDIATION

Macrocyclic conidiation. Most conidia germinate by germ tube formation to produce mycelium and then, eventually, conidia again;

Microcyclic conidiation. Some fungi may germinate by producing conidia directly from an ascospore or conidium; yeast-like buds or microconidia different in morphology from other

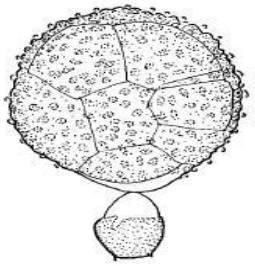
conidia in the life cycle of the organism may be produced as well. By this process, new conidia are produced very quickly.

Macrocyclic conidia disperse away from parent hyphae and microcyclic conidia remain near the site of their production.

DISPERSAL & GERMINATION

Conidia represent a means of rapid spread and colonization from an initial focus of infection.

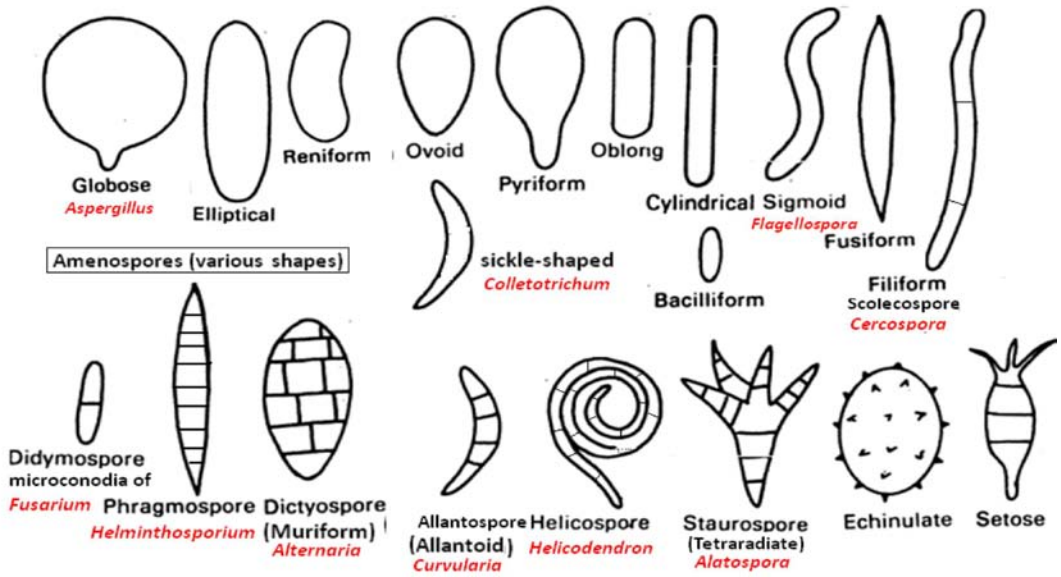
Gregory (1966) distinguished between **xenospores** (Gr. *xenos* = a foreigner) for spores which are dispersed from their place of origin and **memnospores** (Gr. *memnon* = steadfast, to persist), which stay where they were formed.



Conidia are dispersed passively, but in a few cases discharge is violent. In *Nigrospora*, the conidia are discharged by a squirt mechanism; In *Helminthosporium*, drying and shrinkage of the conidiophore is associated with the sudden development of a gas phase, causing a jolt sufficient to project the conidium;

In *Epicoccum*, discharge is brought about by the rounding-off of a two-ply septum separating the conidium from its conidiogenous cell.

Conidia types based on septation & shape



Steps for the production & release of conidia

Conidiogenesis, i.e. conidial initiation; maturation; delimitation; secession, i.e. separation from the conidiogenous cell; proliferation of the conidiogenous cell or conidiophore to form further conidia.

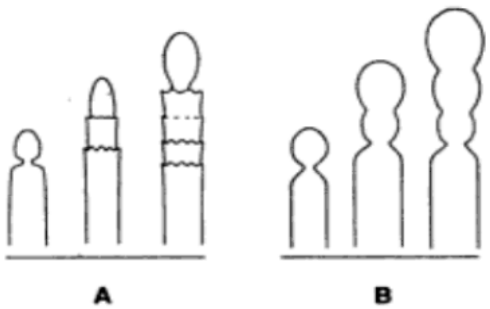
Successive development of conidial loci

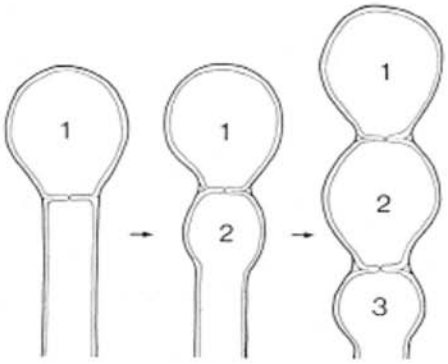
A. **Progressive**- conidia arise apically with conidiogenous cell growing out (proliferating) after each conidium is delimited apically;

B. **Stationary**- no change of conidiogeous cell is observed during conidiogenesis;

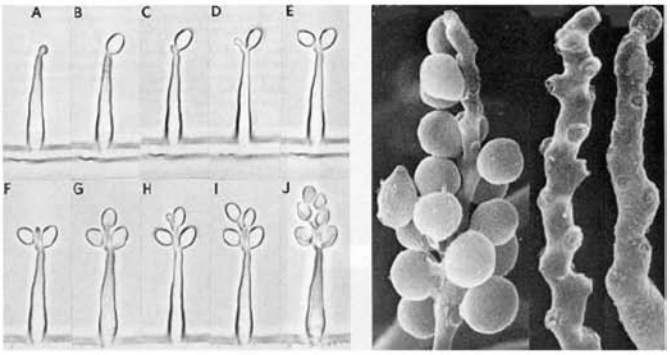
C. **Retrogressive**- conidiogenous cell shortens after each conidium is produced;

D. **Sympodial**- conidial locus is subapical and shifts to lateral as successive conidia develop.

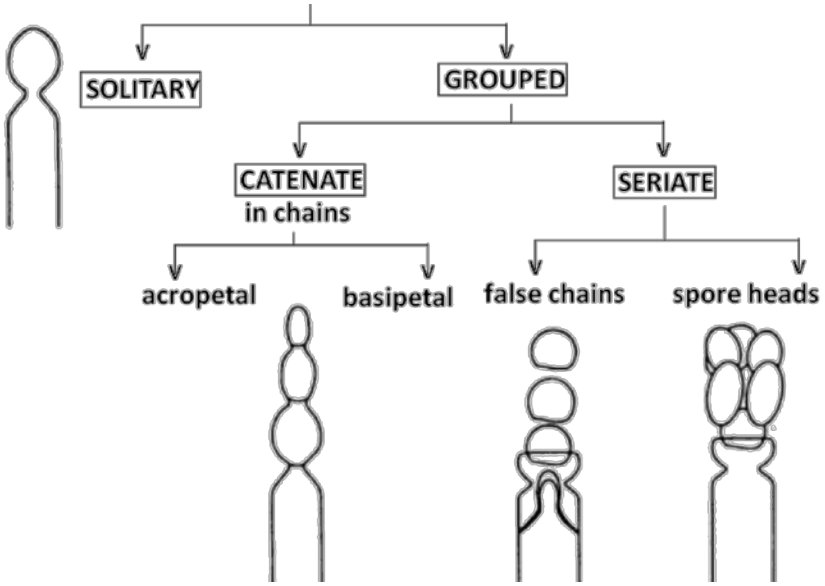




blastic-retrogressive development



Arrangement of Conidia at Locus

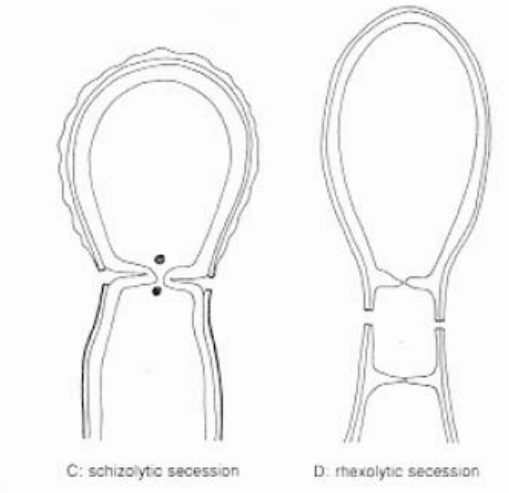


Conidium secession

Detachment of conidium takes place in one of two ways in relation to the septum delimiting the conidium:

Schizolytic secession- the two layers of the delimiting septum separate; eg. *Penicillium*

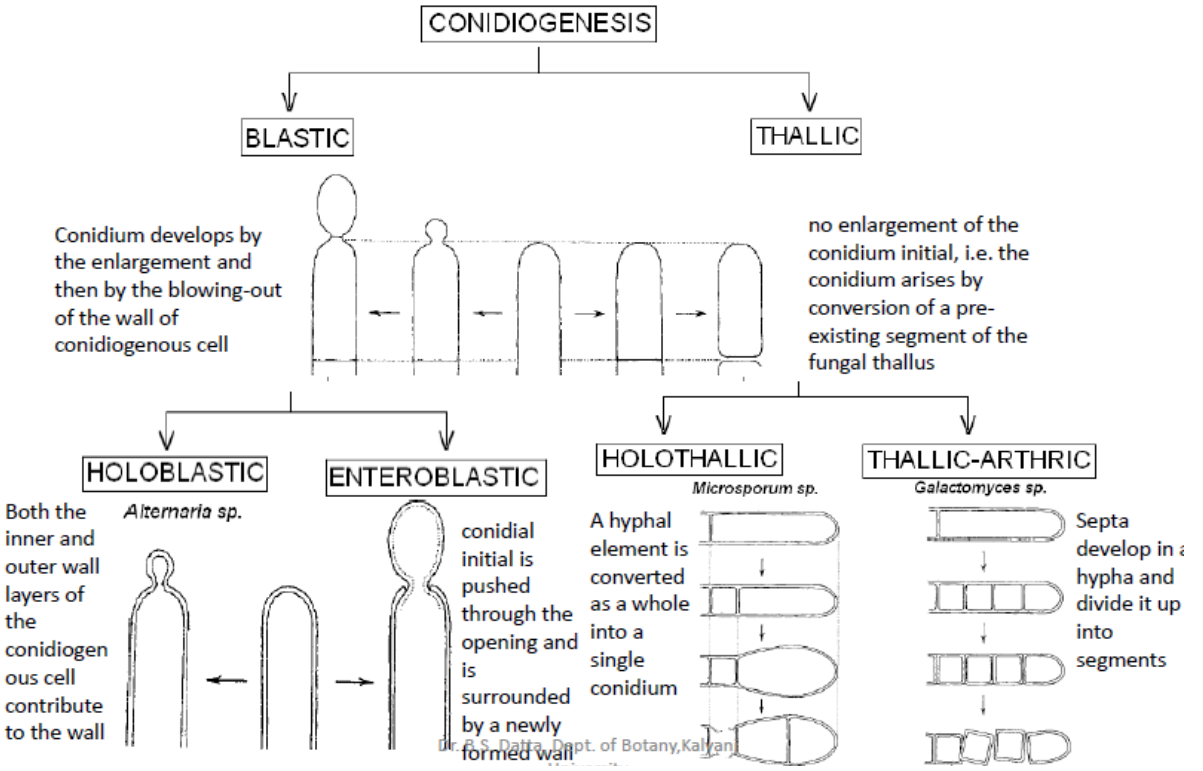
Rhexolytic secession- the entire septum separates with the conidium, often tearing the cell directly below; eg. *Onygenales*.

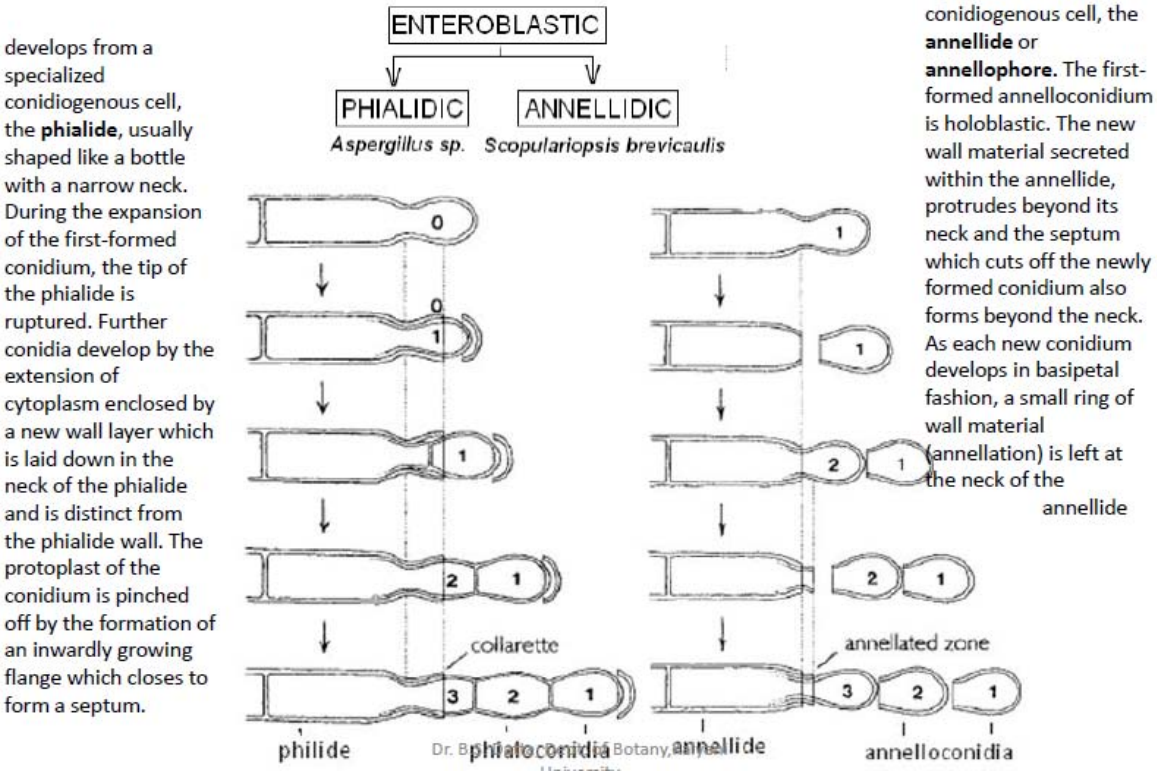


C: schizolytic secession D: rhexolytic secession

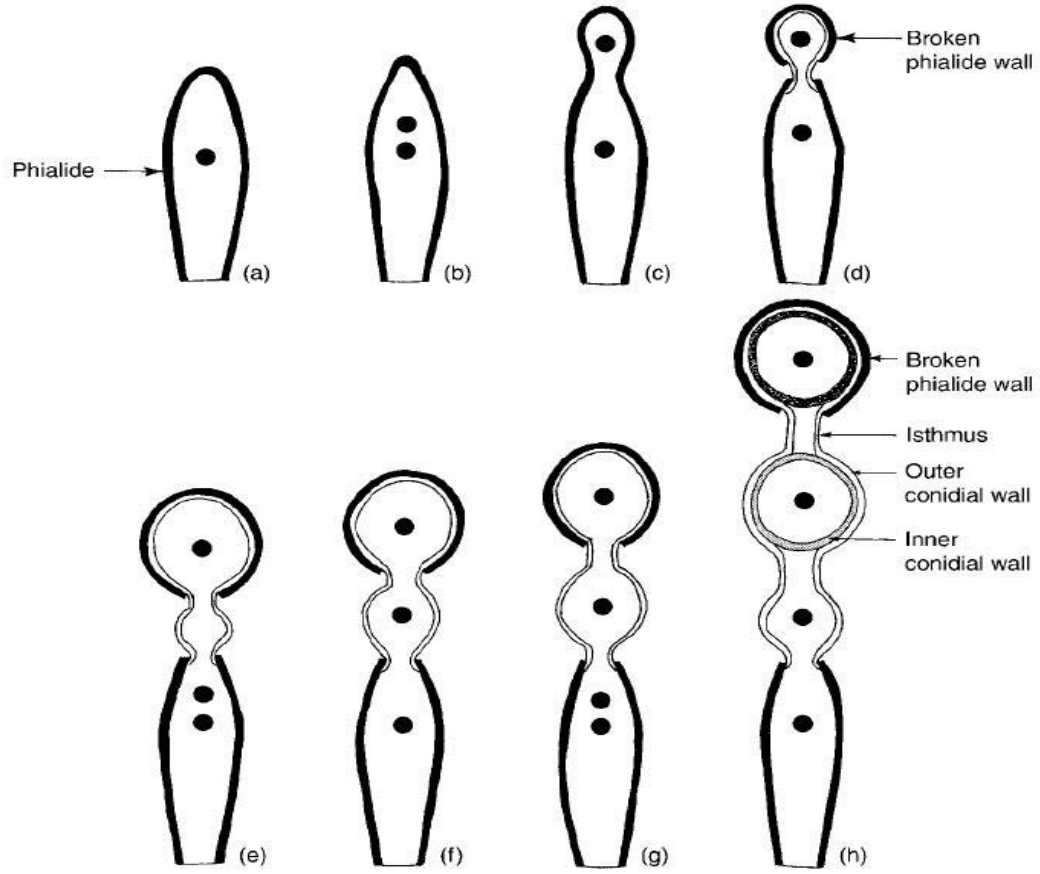
Blastic - Conidium develops by the enlargement and then by the blowing-out of the wall of conidiogenous cell.
Thallic - no enlargement of the conidium initial, i.e. the conidium arises by conversion of a pre-existing segment of the fungal thallus.

CONIDIAL ONTOGENY





Phialoconidium ontogeny in *Aspergillus niger*



Classification (Ainsworth, 1973)
Subdivision **DEUTEROMYCOTINA**

- Class **BLASTOMYCETES**
- Class **HYPHOMYCETES**
- Class **COELOMYCETES**

Class **BLASTOMYCETES**

Budding (yeast or yeast-like) cells with or without pseudomycelia
True mycelium lacking or not well-developed
Genera: ***Candida***, ***Sporobolomyces, Bullera***

Class **HYPHOMYCETES**

True mycelium may be sterile or bearing asexual spores (conidia) directly or on conidiophores which may be variously aggregated (synnema, coremium, sporodochium), but not in pycnidium or acervulus.

Form-order **Mycelia Sterilia -** conidia absent, mycelia sterile, may form sclerotia**;** ***Rhizoctonia, Sclerotium***

Form-order **Moniliales** - conidia borne on synnema, coremium, sporodochium

Form-family **Moniliaceae:** ***Pyricularia***
Form-family **Dematiaceae:** ***Nigrospora, Aspergillus, Penicillium, Verticillium, Botrytis, Trichoderma, Curvularia, Drechs*** (= ***Helminthosporium***), ***Cercospora, Alternaria, Stemphylium***
Form-family **Tuberculariaceae:** ***Fusarium, Epicoccum***

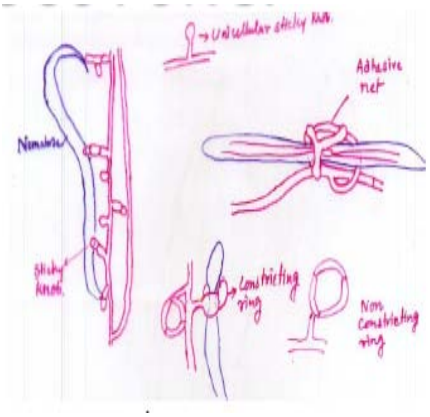
Class **COELOMYCETES**

True mycelia present, conidia formed in pycnidia or acervuli Form-order **Melanconiales -** conidia formed in acervulus

Form-family **Melanconiaceae:** ***Colletotrichum, Pestalotia***
Form-order **Sphaeropsidales -** conidia formed in pycnidium
Form-family **Sphaeropsidaceae:** ***Septoria, Stagnospora, Phoma***

NEMATOPHAGOUS FUNGI

Kill nematodes by various ways;
Three types:



1. **Predaceous fungi –** have several trapping mechanisms:

Adhesive knobs and lateral branches- stalked (*Dactylellina*) & unstalked (*Gamsylella*
Adhesive nets (*Arthrobotrys oligospora*); **Non-constricting rings** (*Dactylellina*);
Constricting rings (*Drechslerella dactyloides*)

2. **Endoparasites** - *Harposporium anguillulae*

3. **Egg and cyst parasites**- *Pochonia chlamydosporia, Paecilomyces lilacinus*

Nematocidal toxins (trans-2-decenedioic acid, linoleic acid) produced by *Arthrobotrys oligospora, Pleurotus* spp.
Telomorph vs. Anamorph

Telomorph- sexual/perfect/meisporic stage Anamorph- asexual/imperfect/mitosporic stage

Telomorph	Anamorph
Gibberella (Ascomycotina)	Fusarium
Filobasidiella (Basidiomycotina)	Cryptococcus

Holomorph = Telomorph + Anamorph

Pleomorphic fungi produce more than one form.

Orphan anamorphs- asexual forms having connections to sexual fungi with similar anamorphic states.

Synanamorphs- asexual fungi producing several types of asexual spores.

Andromorphs- purely sexual stage producing spermatia only.

9.Let's sum up

The third sections are related to fungi, in which diversity, different classification, systematic position, different reproduction methods and its biological control in relation to agricultural purpose and human welfare are discussed. General characters of major groups and subgroup are mentioned in details in several sections. Different aspects of biotechnological production are mention in different section including proper application.

10.Suggested Reading

1. J. Webster and R. Weber 2007. Introduction to Fungi, 3rd Ed
2. Dubey, H.C. An Introduction to Fungi (2nd ed.), 1990, Vikas Publishing House 23
3. Arora, D. (ed.). Hand book of Fungal Biotechnology (2nd ed.), 2003, Dekker, N.Y.
3. Sharma, P.D. Fungi & Allied Organisms, 2005, Narosa Publishing House
4. Deacon, J.W. Fungal Biology, 2006, Black Well Science Ltd.
5. Ingold, C.T. & Hudson, H.J. The Biology of Fungi 96th ed.), 1993, Chapman & Hall
6. Vashista, B.R. Fungi, Latesgt Ed., S. Chand & Company 8. Chopra, G.L. and Verma, V.A. Text Book of Fungi, Pradeep Publications
9. Mehrotra, R.S. and Aneja, K.R. An Introduction to Mycology, Wiley Eastern Ltd.
10. https://www.wikipedia.org/
11. http://www.biologydiscussion.com/

11.Assignment

1. Explain morden trends of classification in fungi.
2. What is the translocation in mycelia of fungi?
3. Distiguish between heterothallism, hetrokaryosis and paraseuality.
4. Describe reproductive structure of Basidiomyota.

5. Explain different spore dispersal mechanis of fungi.
6. What is dolipore septum?
7. Describe differeny type of thallic and blastic type of conidial ontogeny.
8. Give a short note on different types of ascocarp.

All the materials are self written and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - I

Course: BOHCT 1.2

(Biology and Diversity of Algae, Bryophytes and Pteridophytes)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

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Kalyani, Nadia

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SYLLABUS

COURSE–BOHCT 1.2

Biology and Diversity of Algae, Bryophytes and Pteridophytes
(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 1.1	Biology and Diversity of Algae	**Unit 1. Modern trends and Cyanobacteria**	1. Modern trends: Outline of principles and recent trends in algal systematics; endosymbiotic theory of chloroplast evolution and algal origin. 3. Cyanobacteria: Diversity of forms and habitats; cyanobacterial taxonomy; cyanobacterial evolution.	1
		Unit 2. General overview of algae	2. General overview & Resource utilization: Prochlorophyta; Glaucophyta; Dinophyta; Heterokontophyta: Bacillariophyceae, Xanthophyceae, Eustigmatophyceae; Bio-fertilizers and bio-molecules with commercial application.	1
		Unit 3. Rhodophyta and phytoplankton	4. Rhodophyta: Diversity of forms and habitats; evolutionary trends in red algae; ecology of red algae. 6. Phytoplankton: Types of phytoplankton; algal blooms; algal toxins.	1
		Unit 4. Chlorophyta	5. Chlorophyta: Diversity of forms and habitats; evolutionary trends of green algal lineages; salient features of different classes of chlorophytes.	1
	Biology and Diversity of Bryophytes	**Unit 1. General account of bryophytes**	1. General classification: Criteria, recent trends and outline of classification of the liverworts, mosses and hornworts.	1
		Unit 2. Phylogeny and evolution of bryophytes	2. Phylogeny: Evolutionary significance and interrelationships; recent concepts on evolution of the three lineages (liverworts, mosses and hornworts).	1
		Unit 3. Biogeography and ecological significance	3. Biogeography and Ecological significance: Diversity and distribution patterns; population and community dynamics; physiological ecology and adaptations; ecological roles of bryophytes.	1
		Unit 4. Economic significance and Conservation	4. Economic significance and Conservation: Economic importance; threats and vulnerability; conservation strategies; restoration ecology.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 1.1	**Biology and Diversity of Pteridophytes**	**Unit 1. General features, diversity and evolutionary trends of pteridophytes**	1. Introduction: A general account and an outline of recent system of classification of Pteridophytes upto order level. 2.1 Diversity in organography and the evolutionary trends in the members of Psilophyta, Lycophyta, Sphenophyta and Filicophyta - Early ferns, Eusporangiate ferns (Ophioglossales, Marattiales)	1
		Unit 2. Leptosporangiate ferns and gametophyte	2.2 Leptosporangiate ferns (Filicales, Marsileales, Salviniales). 3. Gametophyte: Patterns of spore germination; patterns of gametophyte development in pteridophytes; mating system in fern.	1
		Unit 3. Sporophyte, cytogenetics and speciations	4. Sporophyte: Variations in vegetative and reproductive structures and their evolution with special emphasis on shoot apex, stelar organization, and soral characters. 5. Cytogenetics and Speciations: Pteridophytes with chromosome number; polyploidy in microphyllous and megaphyllus forms; intergeneric and interspecific hybridity; obligate interbreeding forms.	1
		Unit 4. Antheirdiogen, habitat and conservation diversity of pteridophytes	6. Antheirdiogen in ferns. 7. Habitat diversity of pteridophytes and their conservation; endemic and endangered pteridophytes with special reference to India.	1

Director's Note

Open and Distance Learning (ODL) systems play a threefold role- satisfying distance learners' needs of varying kinds and magnitudes, overcoming the hurdle of distance and reaching the unreached. Nevertheless, this robustness places challenges in front of the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and other personnel involved in them. A dedicated team of the University of Kalyani under the leadership of Hon'ble Vice-Chancellor has put its best efforts, professionally and in unison to promote Post Graduate Programmes in distance mode offered by the University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 had been our endeavour and we are happy to have achieved our goal.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further suggestions from the learners' end would be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either at a conceptual level or at the operational level of the DODL of University of Kalyani.

Their concerted efforts have culminated in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials have been composed by distinguished faculty from reputed institutions, utilizing data from e-books, journals and websites.

Director
Directorate of Open & Distance Learning
University of Kalyani

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COURSE – BOHCT1.2
Biology and Diversity of Algae, Bryophytes and Pteridophytes
Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**
Group – A (Biology and Diversity of Algae)

Content Structure

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1. Introduction
2. Course Objectives
3. Modern trends: Outline of principles and recent trends in algal systematics; endosymbiotic theory of chloroplast evolution and algal origin.
4. General overview and Resource utilization: Prochlorophyta; Glaucophyta; Dinophyta; Heterokontophyta: Bacillariophyceae, Xanthophyceae, Eustigmatophyceae; Bio-fertilizers and bio-molecules with commercial application.
5. Cyanobacteria: Diversity of forms and habitats; cyanobacterial taxonomy; cyanobacterial evolution.
6. Rhodophyta: Diversity of forms and habitats; evolutionary trends in red algae; ecology of red algae.
7. Chlorophyta: Diversity of forms and habitats; evolutionary trends of green algal lineages; salient features of different classes of chlorophytes.
8. Phytoplankton: Types of phytoplankton; algal blooms; algal toxins
9. Let’s sum up
10. Suggested Readings
11. Assignments

1. Introduction

As you enter the world of plants, the first group of photosynthetic organisms that you conventionally study are the algae. The algae are an interesting group of organisms with tremendous variety and variability in terms of their morphology, metabolism, ecology and phylogeny. This module shall help you to understand the traditional and modern trends in algal systematics and the very important endosymbiotic theory for understanding the evolution of chloroplast and the algal groups. This module shall also guide you in comprehending and applying the knowledge of algal evolution in understanding the evolutionary pathways of other plant groups. The module shall introduce you to the general characteristics of some important algal groups, you will get an overview of their diversity in forms and habitats and conventional resource utilization patterns. The module also has an unit that introduces you to the concept of phytoplankton, their role in the systems they occupy and certain issues of ecological importance associated with the phytoplankton.

2. Course Objectives

After completion of the module you will be able to:

- provide an overview of algal systematics and theories explaining chloroplast evolution and algal origin.
- apply this knowledge in understanding the evolutionary significance of algae and use it as a basis for understanding the evolutionary pathways to other plant groups.
- describe the general characteristics of important groups of algae especially the phytoplankton and explain their ecology, role in environment and in human welfare;
- apply the knowledge and skills acquired to identify various algae species.

3. Outline of Principles and Recent Trends in Algal Systematics

3.1. Principles and Recent Trends

Algae are known to befound in nearly all habitats throughout the globe. The limits to their distribution are understandbly non-existent. We find the presence of algae even in the harshest of environments. **From this near universal presence we can conclude that algae are a particularly successful group.** A group that has managed to flourish under a variety of situations and inhabits the most inhospitable areas. Their widespread distribution can be attributed to their success as colonisers. **The broad range of habitats occupied by the algae also reflectsupon their broad evolutionary diversity.**

It is also necessary to understand that 'the algae' as a group is a cluster of organisms not united by a common ancestor. In phylogenetic terms the algae are polyphyletic, that is, derived from a number of ancestral lineages that are not closely related to each other. One cannot equate the algae with, other groups of the plant kingdom, which are widely acknowledged to have evolved from a single ancestor. **In its present use, the term 'algae' is applied to a phylogenetically artificial cluster of unrelated or distantly related groups of organisms**. Each group is internally consistent, their members being, as far as is known, related to one another, but for the algae as a whole group one cannot make the same claim.

So what is it that unites the algae? **Essentially, it is chlorophyll 'a' photosynthesis**. Combined with this is a **relatively simple level of organisation** and it defines 'algae'. But, the ability to photosynthesise has been acquired by a wide variety of unrelated organisms. For this reason **the term 'algae' can be regarded only as one of convenience, and discussing the phylogeny of the 'algae' as an entity by itself is very difficult**.

Defining algae

One of the most frustrating questions often asked in the study of algae is: What is an alga? Perhaps the **frustration arises fromthe inability to define concisely all that is presently encompassed by the term, without seeming exceedingly ambiguous**. Algae

(plural, from the Latin alga which means seaweed) both historically and currently have been variously defined.

Stearn (1992) gave the literal translation as ‘seaweed, a thing of little value’, a definition that is entertaining in its quaintness, but not exactly informative. **For the algae are both more and less than seaweeds.** More, in that the term is now also applied to a large number of unicellular as well as thallose organisms occupying a variety of habitats that include freshwater and terrestrial, as well as marine. Less, in that the term ‘seaweed’ sometimes encompasses marine angiosperms, *i.e.* the seagrasses that are more closely related to trees than they are to any of their photosynthetic co-inhabitants of the marine environment.

Authors have attempted to define the algae by exclusion:

Entwisle *et al*., (1997) have defined algae as ‘simple photosynthetic organisms not included among the mosses, liverworts (and other bryophytes) or the vascular plants’.

Ragan (1998) defines algae as ‘cyanobacteria plus photosynthetic eukaryotes and their immediate relatives, but excluding green plants’.
Some have adopted a vividly circular explanation, e.g. ‘those organisms studied by phycologists’ (Entwisle & Huisman, 1998), phycologists being those scientists who study algae!

Yet others take a historical view, essentially including those organisms traditionally regarded as algae and their close relatives.

Papenfuss (1955) defined the algae as, ‘...plants [in which]... reproductive organs lack a primarily produced sterile jacket of cells’, but qualified this by giving the Charophyta as an exception.

Bold & Wynne (1978) amended the definition to include organisms that, when unicellular, may function directly as gametes, when multicellular producing either unicellular or multicellular gametangia; in the latter case every gametangial cell is fertile. This definition separates the algae from other green plants, but only serves to define the group partially (excluding as it does the many asexual taxa).

Although an accurate definition of the algae appears to be elusive, the reason for our seeming inability to define what we are talking about is more straightforward, for the term is applied to an artificial cluster of unrelated or distantly related groups of organisms. In fact, it is imperative that this aspect of the 'algae' be stressed if there is to be any understanding of their place in the overall scheme of life. So what do we mean when we talk of algae? **In essence it is a term of convenience, one that describes a concept rather than a taxonomic group.** If pushed, we would describe the algae, and please note the ***intentional vagueness***, as **'mostly simply constructed, mostly photosynthetic, plant-like organisms and their close relatives'**.

Historical perspectives in position of algae:

Recognition of **protists** began in the late 19th and early 20th centuries and was linked to early microscopical work that, for the first time, revealed the diversity of the 'miniature' plants and animals (e.g. Kützing, 1834). Several formal kingdoms were proposed to include these micro-organisms, of which the kingdom Protista (Haeckel, 1866) was the most widely accepted. The early Protista included all microscopic organisms, including bacteria and blue-green algae, plus the fungi, slime moulds and seaweeds. Many of these were subsequently removed as a greater understanding of their relationships evolved. The recognition of the fundamental differences between prokaryotes and eukaryotes resulted in the erection of the kingdom Monera for the former, the green algae being placed in the Plantae in light of their ancestral relationship with the higher plants. The fungi were placed in their own kingdom, Fungi, by Whittaker.

For some time it has been recognised that the traditional 'plant or animal' separation does not apply to a vast number of organisms, including many of those included in the algae. Over the last thirty or so years there has been a resurgence of interest in the 'protists', the collective name for the large, heterogeneous group of (mainly) unicellular organisms – some plant-like, some animal-like, some with features of both kingdoms – that comprise the 'lower' eukaryotes. In many phylogenetic schemes the protists are regarded as ancestral to (or arising from the ancestors of) the higher plants, animals, and true fungi.

In 1969, Robert Whittaker published a proposed five kingdom system for classification of living organisms. **Whittaker's system placed most single celled organisms into**

either the prokaryotic Monera or the eukaryotic Protista. The other three kingdoms in his system were the **eukaryotic Fungi**, **Animalia**, and **Plantae**. Whittaker, however, did not believe that all his kingdoms were monophyletic.

The latest proposed classification scheme - the '**six kingdom' model** based on **molecular, ultrastructural** and **paleontological evidences by Cavalier-Smith (2004),** include the kingdoms of **Bacteria, Protozoa, Animalia, Fungi, Plantae and Chromista**, with the **algal taxa included within three kingdoms of the bikonts viz., Protozoa, Chromista and Plantae**. These include nine lineages of algal groups, viz., Chlorophyta, Rhodophyta and Glaucophyta under Kingdom Plantae; Euglenophyta and Chlorarachniophyta under Protozoa and Heterokonta, Cryptophyta, Haptophyta and Dinoflagellates under Chromista. The last four are grouped together as Chromalveolates (Chromista) or chromophyte algae because they contain various xanthophylls that make them appear yellow or brown, in addition to the light harvesting pigments chlorophyll a and c. Moreover, although a chromalveolate, lineage Alveolata has been included within the kingdom Protozoa. Another lineage, of the highly debated algal group Cyanophyta has been designated to the Kingdom Bacteria within this classification system.

Until relatively recently, all living organisms were conveniently (if not realistically) divided between the **two kingdoms proposed by Linnaeus (1753)**: the **Animalia** and **Vegetabilia** (the latter now generally known as Plantae). This enforced categorisation as plant or animal was rarely questioned by early biologists, and the algae (due to their photosynthetic nature) were placed in the Kingdom Plantae along with the seed plants, ferns, bryophytes and mosses.

Some argument ensued when flagellate, unicellular taxa displaying both animal and plant characteristics were discovered (e.g. certain euglenoids and dinoflagellates), but in general an uneasy consensus prevailed.

Recognition of protists in the mid- to late-19th century did little to upset the *status quo*. When Chatton (1925), then Stanier and others (Stanier & van Niel, 1962; Stanier et al. 1963) recognised and promoted the **fundamental differences between the prokaryotes and eukaryotes**, a revolution was set in motion. While not immediately affecting the classification of the algae, increased attention to the diversity of the lower eukaryotes

resulted in a more realistic appraisal of their evolutionary relationships, one consequence being a resurgence in acceptance of the Protista as apart from plants and animals (Corliss, 1984). Thus the revived, single kingdom Protista saw an early demise. Ragan (1998) suggested that the protists could be considered a phylogenetically coherent group only if all their descendants are included, which would mean including all eukaryotes. In those terms, 'protist' becomes meaningless. Corliss (1994) stated that 'a single kingdom Protista as such must be laid to rest', but the term 'protist' persists as a convenient and perfectly acceptable way of describing the 'lower' eukaryotes. In the words of Andersen (1998), 'The Protista represent, at best, a grade, not a clade, and there appears to be little hope that this group will ever again be considered to represent a natural taxonomic unit, i.e. the Kingdom Protista'. Despite this, there is little doubt that protistology will continue as a legitimate field of research.

So where does this leave 'algae', another epithet of convenience used to describe a diverse but overlapping group of organisms? The emergence of protistology and its 'global' view might seem to consign traditional terms such as 'algae' and 'protozoa' to the level of historical oddities, but for the moment, at least, this is not the case. The answer essentially remains one of historical precedent and practicality. Phycologists will undoubtedly continue to talk of the algae, protistologists of protists, and zoologists of protozoa. **As long as the concepts of all terms are understood, there is no particular reason to discontinue their use.**

Classically, algae were classified based on their color, imparted by the pigments present within and their distinct micro-morphological characters. The system of identifying algae on the basis of the same has not yet lost relevance and continues to be the most widely used technique of identifying and classifying them. This in fact, holds good for some of the best known classification systems as propounded by Fritsch (1945), Round (1973) and Bold & Wynne (1978).

A significant advancement in algal systematics took place with the advent of electron microscopy during mid 20th century, when phycologists began to characterize the ultrastructure of algal cells. A number of classification schemes have been proposed since then, based on ultrastructure of the algae, especially that of the plastids and the motile cells (flagellar roots and basal bodies) because these characteristics were found to be evolutionarily more conserved (Friedl, 1997).

By the end of the 20th century, molecular systematics had largely superseded ultrastructure based systematics as it was shown that the morphological and biochemical diversity of the algae results from their polyphyletic origins. Since then, phycologists have based their classification on phylogenetic system, widely accepted among them being van den Hoek *et al*., (1995) and Lee (1999). Major conceptual or methodological advances in addressing the problem of classification of algae came with the advent of electron microscopy and its use in the examination of the ultrastructure of algal cells, which led to major advances in our understanding of algal phylogeny and diversity. As highlighted by Andersen (1998), before electron microscopy there were 12 algal classes; at present there are over 30. Electron microscopy has had a significant impact on the higher-level classification and phylogeny of virtually all groups of algae. Examples include: the arrangement of the flagellar root assemblages and cell division processes in the Chlorophyta, which led to the erection of many new classes and the refinement of our understanding of the evolutionary pathway that gave rise to the higher plants (van den Hoek et al., 1995); The delineation of the orders of the Rhodophyta by examination of pit-plug ultrastructure (Pueschel & Cole, 1982); Recognition of several new classes of phytoplankton [the Haptophyceae Christensen ex Silva (1980), Eustigmatophyceae Hibberd & Leedale (1971), Dictyochophyceae Silva (1980), Chlorarachniophyceae Hibberd & Norris (1984), Synurophyceae Andersen (1987), Pelagophyceae Andersen et al. (1993), Phaeothamniophyceae Bailey et al. (1998), and Bolidophyceae Guillou et al. (1999)]; The recognition that the euglenoids were not closely related to the green algae (Kivic & Walne, 1984). Of the many important revelations through the study of ultrastructure of the algal cells, mention must be made of the **increased support for the concept of the endosymbiotic origin of chloroplasts as the major breakthrough**.

3.2. Endosymbiotic theory of chloroplast evolution and algal origin

At least twice during the evolution of the photosynthetic eukaryotes, prokaryotes have been incorporated into host eukaryote cells as organelles in a process known as serial endosymbiosis (Margulis, 1970, 1981a; Gray & Doolittle, 1982; Cavalier-Smith, 1982). Thus the chloroplasts and mitochondria of what we now know as phototrophic eukaryotes have had independent origins, the chloroplasts from cyanobacteria (blue-green algae) and the mitochondria from alpha-proteobacteria. Eukaryotes are, therefore, composite or chimeric organisms derived from several sources.

This theory was originally proposed by **Mereschkowsky (1905)** but was ignored for many years. Evidence for the symbiotic origin of organelles was initially derived from biochemical and ultrastructural observations, in particular the striking similarities between the Cyanophyta and the chloroplasts of the Glaucocystophyta and the Rhodophyta. All three produce chlorophyll a but not chlorophylls b or c, and their accessory pigments include phycocyanin and allophycocyanin, contained in phycobilisomes attached to the thylakoids.

Ultrastructural evidence for the endosymbiotic origin of organelles includes the multiple membranes surrounding them. In the Glaucocystophyta, Rhodophyta and Chlorophyta the chloroplasts are surrounded by a double membrane envelope and are considered the result of a primary endosymbiotic event of a cyanobacterium. In such cases the inner membrane is interpreted as derived from the plasmalemma of the original endosymbiotic cyanobacterium, whereas the outer membrane is thought to have originated from the ancestral host's food vacuole. In the other photosynthetic eukaryotes (the Euglenophyta, Heterokontophyta, Chlorarachniophyta, Haptophyta, Dinophyta and Cryptophyta), the chloroplasts are surrounded by a three- or four-layered membrane and are thought to be derived from the secondary endosymbiosis of a phototrophic eukaryote by a heterotrophic eukaryote.

In the case of a four-layered membrane, the additional layers are interpreted as the vestigial plasmalemma of the phototrophic endosymbiont plus the food vacuole membrane of the secondary host. Therefore, the initial ingestion would have been via endocytosis. Where only three layers occur (the Dinophyta and Euglenophyta), van den Hoek et al. (1995) and others suggested that the plasmalemma layer is missing due to the feeding habits of the host, which might have punctured the plasmalemma of the prey and sucked out the cytoplasm without ingesting the membrane.

Alternative hypotheses suggest that one of the membranes has been lost during evolution (Lee, 1977) or perhaps two have somehow fused to become one (Gibbs, 1970; also Saunders et al., 1997a and references therein). Given the overwhelming evidence for the endosymbiotic origin of organelles it is, therefore, no longer possible to discuss the evolutionary history of a given eukaryotic organism as though it were a single entity, since it is also important to consider the origins of the organelles with their individual ancestries. Fortunately, plastids have a genome (Ris & Plaut, 1962) that both betrays

their ancestry and enhances understanding of their phylogeny, while at the same time providing strong support for the endosymbiont theory of their origin.

Given the large variety of plastids and pigments in photosynthetic organisms, it was initially postulated that multiple symbiotic events must have occurred, each incorporating a different prokaryotic organism, i.e. a polyphyletic origin to plastids. The discovery of the green prokaryote *Prochloron* (Lewin, 1976, 1977) appeared to provide additional evidence, as it was postulated to be similar to the ancestral chloroplast. DNA studies of chloroplast and bacterial genomes have, however, shown the prochlorophytes to be unrelated to the plastids of green plants, and it is now believed that all plastids have arisen from a single symbiotic event, i.e. a monophyletic origin (Delwiche & Palmer, 1997).

The variety of pigments and plastids is considered, therefore, to be the result of evolution following an initial symbiotic event. The arguments for this are convincing, although the acquisition of plastids through separate endosymbiotic events involving closely related cyanobacteria cannot be discounted entirely (Stiller & Hall, 1997). While current evidence suggests that the primary endosymbiotic event occurred only once, secondary (and even tertiary) endosymbiotic events have occurred several times.

Thus the photosynthetic lineages of many algae arose through separate endosymbiotic events (Battacharya & Medlin, 1995; Medlin et al., 1997). This scenario is supported by the presence of numerous heterotrophic taxa that are closely related to photosynthetic algae which are believed to have arisen via secondary endosymbioses.

Plastids, as mentioned earlier, are believed to be monophyletic and derived from the cyanobacteria. The plastids of the Glaucocystophyta, Rhodophyta and green plants form discrete monophyletic lineages (Medlin et al., 1997), whereas those of the Heterokontophyta, Haptophyta and Cryptophyta, Dinophyta and apicomplexans are variously related to the red algae. This indicates that the plastids of the latter groups (but not the host cells) are derived from the endosymbiotic inclusion of a red alga, or at least something closely related (Medlin et al., 1997). Recent evidence suggests that the plastids of these groups all arose from a common endosymbiosis involving a red alga (see review by Palmer, 2003). Similarly, the plastids of the Euglenophyta and Chlorarachniophyta are related to green algal plastids, whereas their host eukaryotic cells are related to divergent flagellate and amoeboid species, respectively. A review of the origin of plastids was provided by Delwich & Palmer (1997) and Palmer (2003).

The development of methodologies that allow sequencing and comparison of genetic material has provided a powerful analytical tool. In theory, these methods allow us to view the blueprints by which organisms are constructed, and to compare them for slight or major changes. As a result, algal phylogenetic studies, in fact phylogenetic studies in general, are presently in the midst of one of their most exciting phases. For the first time it is possible to propose phylogenetic trees that are based on analysis of the genotype, rather than the easily misinterpreted phenotype. The impact of these new methods can be gauged by the flood of papers appearing in many scientific journals, and the widespread acceptance of sequence studies as the method for phylogenetic speculation.

Sequence studies have been undertaken, at least in part, on all major algal groups and have revealed many unexpected associations. Many of these are, in hindsight, logical; others have prompted the search for new or previously under-appreciated phenotypic characters to buttress the molecular results. The search for the origins of plastids was also greatly enhanced by the development of molecular methods that enabled portions of genes to be sequenced and compared. By sequencing plastid and host genes separately, phylogenetic trees can be inferred for the two entities.

Strongly correlated (even overlapping) concepts to taxonomy and systematics, are the words phylogeny, phylogenetics and phylogenetic systematics. Phylogeny can be defined as the evolutionary history of a group or lineage, the origin and evolution of higher taxa, or the natural process or repeated irreversible splitting of populations (see Lincoln *et al*., 1998; Wägele, 2005). Phylogenetics is the science of the reconstruction of phylogeny, and phylogenetic systematics is a method of classification based on the study of evolutionary relationships between groups of organisms, and the integration of proper names of groups of organisms into a hierarchical system which reflects their phylogeny.

Classification of algae: Robert Edward Lee (1989)

According to this system of classification, there are four distinct evolutionary groups.

1st Group: Includes the prokaryotic algae. Include the **Cyanophyta**&**Prochlorophyta**

2nd Group: Algae with a chloroplast surrounded only by two membranes of the chloroplast envelope. Include the **Glaucophyta**, **Chlorophyta**&**Rhodophyta**.

These algae evolved through an evolutionary event hat involved the capture of a prokaryotic algal cell in a food vesicle by a phagocytic non photosynthetic protozoan. Normally the prokaryotic algal cell would have been digested as a source of food by the heterotrophic protozoan. However, the prokaryotic algal cell was retained in the host

cytoplasm as an endosymbiont. Such an endosymbiont was of benefit to the host protozoan because the host received some of the photosynthatates from the endosymbiont. It was also of benefit to the endosymbiont because it resided in the more stable and protected environment of the cytoplasm of the host. Eventually, in the process of evolution, the plasma membrane of the endosymbiont became the inner membrane of the chloroplast envelope and the food vesicle membrane of the host became the outer membrane of the chloroplast envelope.

The Glaucophyta represent an intermediate stage in this process, in which the endosymbiotic alga has not completely evolved into a chloroplast. The endosymbiotic bacteria is called the **Cyanelle**, the host -**Cyanome** and the association between the two is called **Syncyanosis**. The algae in the Rhodophyta and the Chlorophyta represent the completion of this evolutionary pathway into mature chloroplasts.

3rd Group: Algae that have the chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum. Include the **Euglenophyta** and **Dinophyta**.

This evolutionary pathway as outlined by Lee (1977) resulted when a chloroplast from an eukaryotic algae was taken up into a food vesicle by a phagocytic protozoan. Normally the protozoan would have digested the chloroplast as a source of food, however, in this case the chloroplast was retained in the cytoplasm of the protozoa as an endosymbiont. The host protozoan benefitted from the association by receiving photosynthatates from the endosymbiont chloroplast. The endosymbiont chloroplast benefitted by the stable environment created by the cytoplasm of the host. Eventually the food vesicle membrane of the host became the single membrane of chloroplast endoplasmic reticulum surrounding the chloroplast.

4th Group: Algae with two membranes of chloroplast endoplasmic reticulum surrounding the chloroplast. Include the **Cryptophyta**, **Chrysophyta**, **Prymnesiophyta**, **Bacillariophyta**, **Xanthophyta**, **Eustigmatophyta**, **Raphidophyta** &**Phaeophyta**.

This evolutionary pathway started with a phagocytic protozoan taking up a red algae into its food vesicle. Instead of being digested by the protozoan, the red alga remained in the food vesicle as an endosymbiont. The food vesicle membrane of the protozoan was lost. The nucleus of the red alga became reduced to a nucleomorph as many of the functions of the red algal symbiont became controlled by the nucleus of the host symbiont (protozoan). This protozoan along with its algal symbiont as taken up by a second phagocytic protozoan into a food vesicle. The initial protozoan with its algal symbiont

was retained by the cytoplasm of the second protozoan as an endosymbiont. The nucleus of the first protozoan took over the functioning of the cellular apparatus and the nucleus of the second protozoan was lost. Also lost were the food vesicle membrane of the second protozoan and the outer nuclear envelope of the first protozoan. This resulted in the characteristic two membranes of chloroplast endoplasmic reticulum found in this group. The outer membrane of the chloroplast ER is derived from the plasma membrane of the first protozoan and the inner membrane of the chloroplast ER is derived from the food vesicle membrane of the first protozoan. The algae in the extant Cryptophyta have the cytology of the cell in this stage of evolution. There are two membranes of chloroplast ER. The outer membrane of chloroplast ER is continuous with the outer membrane of the nuclear envelope. The inner membrane of the chloroplast endoplasmic reticulum (CER) surrounds the chloroplast and the nucleomorph leading to the cytological characteristics of the rest of the algae with two membranes of CER.

This definitely does not imply that all of the algae with two membranes of CER evolved only once. Indeed, it is probable that the Cryptophyta and the rest of the algae with two membranes of CER evolved along two separate lines since there are considerable morphological, cytological and biochemical differences between them.

4. General Overview and Resource Utilization

4.1. Prochlorophyta

Named after the genus *Prochloron*, it is an artificial division with an assemblage of green Cyanobacteria. Belongs to the prokaryotic group Eubacteria. It is an interesting group since it was thought possible at one time that they could represent the prokaryotic ancestors of the chloroplasts of green algae and higher plants. The name *Prochloron* is also an expression of this speculation meaning ‘primitive green thing’. The division contains only one class the Prochlorophyceae.

Principal characteristics:

1. Habit: Unicellular coccoid forms or unbranched filamentous forms.
2. Cellular organization: Prokaryotic.

3. Photosynthetic apparatus: Thylakoids with bound photosynthetic pigments that lie free in the cytoplasm not enclosed within chloroplasts. The thylakoids are grouped into stacks (lamellae) containing two to several thylakoids as in case of chloroplasts of the green algae and higher plants. These differ from the cyanobacteria since the thylakoids in this group are single and equidistant.

4. Photosynthetic pigments: Chlorophylls a and b, thus again resembling the chloroplast of green algae and higher plants. Chlorophyll c is absent.

5. Accessory pigments: β-carotene and a variety of xanthophylls, principal one being zeaxanthin and cryptoxanthin. Phycobiliproteins and phycobilisomes are absent.

6. Reserve food: The reserve polysaccharide is starch like. Polyhedral bodies (carboxysomes) containing Rubisco is present both in cyanobacteria and the prochlorophyta. Monogalactolipid is synthesized as in cyanobacteria, but cyanophycin a nitrogen reserve characteristic of the cyanobacteria is not found in the prochlorophyta.

7. Genetic material: DNA is diffused, occurring throughout the cell in regions between the stacks of thylakoids that are rich in ribosomes. In cyanobacteria, it is concentrated in the centre of the cell.

8. Cell wall: Similar to cyanophyta and contains a peptidoglycan (murein) layer.

9. Genera: So far only three genera are known. Two marine forms – *Prochloron & Prochlorococcus* and one freshwater form – *Prochlorothrix hollandica* which is a filamentous form.

The genus *Prochloron* contains a number of related coccoid forms which live as extra-cellular symbionts within tropical and subtropical colonial ascidians (sea-squirts).

Recently coccoid prochlorophytes (*Prochlorococcus*) about 1 µm in diameter have been discovered to be abundant in the picoplankton of the dimly lit deeper water (at about 50-100m below sea surface) of the open oceans.

Prochlorothrix hollandica is a filamentous prochlorophyta, which gives rise to massive blooms in the shallow subtropical lakes of Netherlands, where it was discovered. The filaments are unbranched and contain gas vesicles.

Though the Prochlorophyta separated at an earlier date from the cyanobacteria on the criterion of presence of chlorophyll b but nucleic acid sequencing studies have shown that chlorophyll b has evolved a number of times within the cyanobacteria and the term Prochlorophyta was discarded, but the discovery of *Prochloron* was a matter of excitement as it was interpreted as the ancestor of the chloroplasts of green algae and

land plants sharing the presence of chlorophylls b & a and stacked arrangement of the thylakoids.

According to the endosymbiotic theory, the chloroplast of eukaryotic algae originated as prokaryotic algae, which were ingested but not digested by early heterotrophic eukaryotes. The chloroplasts of the Rhodophyta and Glaucophyta can be interpreted as descendents of ingested cyanophytes, because of the striking resemblances between them and the striking resemblances between them and the cyanobacteria in structure and photosynthetic pigments (equidistant thylakoids bearing phycobilisomes, the presence of chlorophyll a but not chlorophyll b and the presence of phycobiliproteins).

The chloroplasts of green algae and those of brown algae (mainly the Heterokontophyta) differ considerably from the chloroplasts of the red algae, in ultrastructure and pigment composition, and so in the early development of the endosymbiotic theory green and brown chloroplasts were suggested to have been derived independently from two hypothetical photosynthetic prokaryotes:

a green one (with chlorophylls a & b and stacked thylakoids) and

a brown one (with chlorophylls a & c, together with the brown accessory pigment fucoxanthin)

Thus when *Prochloron* was discovered it was thought to represent the missing link between prokaryotic algae and the green chloroplasts. But recent studies however show that *Prochloron* and *Prochlorothrix* are more closely related to the cyanobacteria.

Reasons for thinking that the prochlorophytes were ancestors of the chloroplasts of green algae and green plants include – they in common possessed chlorophylls a & b and a stacked arrangement of the thylakoids. Chloroplasts of Rhodophyta and Glaucophyta were interpreted to be descendents of ingested cyanophytes, because of equidistant thylakoids bearing phycobilisomes, presence of chlorophyll a but not chlorophyll b and phycobiliproteins. But chloroplast of the green algae and brown algae differ considerably from the chloroplast of red algae. As such, they were suggested to have been derived independently from two hypothetical photosynthetic prokaryotes: a green one (with chlorophylls a & b and stacked thylakoids) and a brown one (with chlorophylls a & c together with brown accessory pigment fucoxanthin). Thus when *Prochloron* was discovered, it was thought to represent the missing link between prokaryotic algae and the green chloroplasts.

But this did not prove to be true in the long run. Phylogenetic relationships have been determined between *Prochlorothrix* and a number of cyanobacteria and the results clearly establish that the *Prochlorothrix* belongs within the lineages of the cyanobacteria. The same is true for *Prochloron* although another important conclusion that is drawn from these studies is that within the cyanobacteria – *Prochloron* and *Prochlorothrix* do not cluster together.

Moreover, comparisons of nucleotide sequences of 16s ribosomal RNA and of the gene for a subunit of a ribosomal RNA polymerase in *Prochloron, Prochlorococcus* and *Prochlorothrix* indicate that these genera are unrelated except for the fact that all belong to cyanobacteria. This implies that Prochlorophyta is not a phylogenetically coherent group and that the division is an artificial one.

4.2. Glaucophyta

The glaucophytes belong to the 2nd group which includes algae with chloroplasts surrounded by only two membranes of the chloroplast envelope. The Rhodophyta and the Chlorophyta also belong to this group. The glaucophytes are algae that are said to have endosymbiotic cyanobacteria in the cytoplasm instead of chloroplasts. Because of the nature of their symbiotic association, they are thought to represent intermediates in the evolution of chloroplasts. In the endosymbiotic association that prevails the cyanobacteria is named cyanelle, the host is named cyanome and the association is named syncyanosis.

The division Glaucophyta belongs to Eukaryota and the cells have an organization accordingly, although the division is placed immediately after the Prokaryota because the chloroplasts of the Glaucophytes are in various respects like unicellular, coccoid cyanobacteria. The division contains one class – Glaucophyceae

Principal characteristics:

Habit: The three identified genera are all unicellular flagellates.

Habitat: All the genera are rare freshwater forms, each with one species.

Morphology: The flagellate cells are dorsiventral, with a rounded dorsal side and a flat ventral side, bearing 2 unequal flagella, inserted in a shallow depression just below the apex of the cell. These flagellate cells are surrounded by a superficial layer of flat

vesicles may be empty, or they may contain scales or fibrillar material. These resemble the thecal vesicles of the Dinophyta.

Photosynthetic apparatus: The chloroplasts are very similar to unicellular, coccoid members of the Cyanobacteria, surrounded by a thin peptidoglycan wall and lies within a special vacuole. The thylakoids are not stacked (as in the Prochlorophyta and in most eukaryotic algae) but are single and equidistant similar to the Cyanobacteria and in the chloroplasts of Rhodophyta.

There are a number of similarities between cyanobacteria and the chloroplasts that support The endosymbiotic theory. This theory was proposed by Mereschkowsky (1905) in his famous paper in Russian and German – "On the nature and origin of chromatophores in the plant kingdom". In 1920 he published another extensive paper on the endosymbiotic origin of chloroplasts.

1. They are about the same size.
2. They evolve oxygen during photosynthesis.
3. They have 70s ribosomes.
4. They contain circular prokaryotic DNA without basic proteins.
5. Nucleotide sequencing of rRNA or of DNA encoding rRNA show similarities.
6. They have chlorophyll a as the primary photosynthetic pigment.
7. The centre also harbours polyhedral bodies (Carboxysomes) typical of the cyanobacteria.

Inspite of all these similarities to the cyanobacteria, the cyanelles should be regarded as organelle rather than mere endosymbiotic cyanobacteria. The reasons that support this claim include the fact that the cyanobacteria have over 3000 genes, whereas the cyanelles have about the same number of genes as in plastids – about 200 genes. It is also clear that the cyanelles (and also plastid) genomes have undergone substantial reduction during endosymbiosis, and many of the missing genes eventually relocated to the nucleus, while other genes were lost or made redundant in the cyanelles new role as an endosymbiont. eg. Cyanobacteria have a respiratory electron chain, whereas plastids do not, the respiratory electron chain is coded by the nucleus in eukaryotic algae.

Photosynthetic pigments: The chloroplasts appear blue-green, since the green chlorophyll is masked by the blue pigments phycocyanin and allophycocyanin. These accessory pigments are contained in the phycobilisomes, which are attached to the thylakoids as in the cyanophyta and the chloroplasts of red algae. Carotenoids in the form of β-carotene, zeaxanthin & β-cryptoxanthin are present. Although 2 of cyanobacterial carotenoids – myxoxanthophyll and echinenone are absent.

Reserve food material: The reserve polysaccharide is starch. Starch grains are formed outside the chloroplast as is in the Rhodophyta. In the chlorophyta, the starch grains are formed within the chloroplast.

Flagella: The two unequal flagella are inserted in a shallow depression just below the apex of the cell. The flagella possess two rows of delicate hairs, which resemble the hairs on the flagella of some green algae but are different from the stiff tubular hair (mastigoneme) on the flagella of the Heterokontophyta. These have the typical 9+2 structure found in almost all eukaryote flagella, each one containing an axoneme containing 9 peripheral doublet microtubules, together with a central pair of single microtubules.

Evolution and origin of the Glaucophytes:

As such, although with many artificial similarities with the Cyanobacteria, Rhodophyta and Chlorophyta in different aspects, the organisms of the Glaucophyta are found to be very old and probably branched off the evolutionary tree long before the divergence of red and green algae.

Also the fact that has established over the years is that in the syncyanose one is dealing with a composite organism that exhibits features altogether new and no longer characteristic of any of the partners and the chloroplast cannot be cultured outside its host cytoplasm, the two being incapable of living as free or separate entities proves two things – (1) they belong to a separate phylum and (2) the cyanelle is a functional chloroplast and not an endosymbiotic cyanobacteria.

This does not mean that the glaucophyte chloroplast did not evolve from an endosymbiotic cyanophyte, it most likely did and the resemblances between the glauocophyte chloroplast and cyanobacterial cells are characteristics inherited from the

ancestral endosymbiont, but it must be appreciated that the organisms in the phylum are very old and that when evolving they were susceptible to change and undergoing a great deal of such changes in an attempt to reach the relatively stable level of a cell with a chloroplast. Such a dynamic group was formed consisting of a large number of organism not well suited to compete with their more highly developed progeny. Such a situation led to the death of many of the original members of the groups resulting in the existence of only a few extant members of the group.

4.3. Dinophyta

General characteristics

The term 'dinoflagellate' has its origin in the Greek word *dineo*, which means 'to whirl'. Habit & Habitat: Majority are unicellular flagellates, only a few coccoid or filamentous forms known, essentially a tropical group. Few interesting forms found in the highly specialized heterotrophic forms. A multicellular worm like parasitic dinoflagellate- *Blastodinium* occurs in the guts of marine copepods.About 90% of the known species are marine and the rest freshwater forms. Symbiotic dinoflagellate- *Symbiodinium* forms such associations with almost all species of tropical and reef-building corals, jellyfish and sea anemones.

Morphology: A typical motile dinoflagellate consists of an **epicone** and **hypocone** divided by the transverse **girdle** or **cingulum**. The epicone and hypocone are normally divided into a number of **thecal plates**, the number and arrangement of which are of taxonomic importance. A **longitudinal sulcus** runs perpendicular to the girdle. The **longitudinal & transverse flagella** (LF & TF) emerge through the thecal plates in the area where girdle & sulcus meets. The LF projects out of the cell, but the TF is wave-like & closely appressed to the girdle.

The cell surface has an outer plasmalemma, below which is a single layer of membrane sacs known as **alveoli**. These vesicles normally contain **cellulosic plates** and give characteristic structure to the theca. Many forms surrounded by a thin layer just beneath the thecal plates called **pellicle**. This collection of cell surface features is called **amphiesma,**it includes thecal plates, peripheral vesicles & microtubules.When the scales are absent from the alveoli the species are referred to as naked, unarmored or non-thecate dinoflagellates.

The thecal plates of armored cells usually fit very closely together, overlapping slightly to form a continuous surface. The zones between adjacent plates are called **sutures**. Cell

growth occurs by addition of materials along the margins of the plates, forming regions called **intercalary/ growth bands**. Each species has a characteristic **plate formula** that begins with the anterior and moves towards posterior of the cell.

Tabulating thecal plate organization (plate formula) is an important parameter in identification of dinoflagellates. Terminology used is shown above. Similar tabulation for alveoli is also used in case of unarmored dinoflagellates. The process requires breaking of cells & spreading the plates flat.

In many dinoflagellates, cell division involves sharing of the mother thecal plates between the daughter cells, with the daughter cells producing the new thecal plates that they lack. But in certain peridinoid genera the theca is completely shed (**ecdysis**) at cell division, followed by the formation of a thickened pellicle around the cell to form an **ecdysal cyst**. **Ecdysis** results in the loss of plasma membrane, thecal plates and vesicles. New vesicles & plates are formed under a thickening pellicle. When the amphiesma matures the cell escapes from the ecdysal cyst.

Dinoflagellate cells often contain different types of peripheral structures that can be discharged from the cell body into the environment as a defensive response –

a. Trichocysts or extrusomes

b. Mucocysts

c. Nematocysts

Trichocysts are ejectile rods that occur almost universally at the periphery of dinoflagellate cells. Found within the amphiesma. Generally oriented perpendicular to the cell surface. In armored species, they lie directly beneath pores in the thecal plates from where they are discharged. They develop within golgi apparatus inside sacs, consist of a protein rod few mm long & rectangular in cross section. The distal end consists of twisted fibres. When irritation occurs the sac ruptures, allowing the entry of water which changes the conformation of proteins and elongates the trichocyst nearly 8 times and an explosive release.

Mucocysts are relatively simple sacs that release mucilage to the cell exterior often in the form of rather thick rod-shaped bodies. eg. *Gymnodinium fuscum*.

Nematocysts produced by only a few dinoflagellates (*Polykrikos*&*Nematodinium*) are larger upto 20 µm long and even more structurally elaborate than the trichocysts.

Two different types of flagella:

1.**Transverse flagellum** (TF) that fits into transverse girdle

2.**Longitudinal flagellum** (LF) that projects out of the sulcus

Both the flagella are inserted into the cell in the area of intersection of the girdle and sulcus. The **LF** usually has a wide basal portion and a thinner apical portion. Fibrillar hairs may cover entire length of the flagellum. Mechanical stimulus causes the LF to be retracted and folded so that it lies along the sulcus.

The **TF** is 2-3 times as long as the LF & has a helical shape. It consists of -

(1) an axoneme whose form approximates a helix

(2) a striated strand that runs parallel to the longitudinal axis of the axoneme but outside the loops of the coil

(3) a flagellar sheath that encloses both (1) & (2).

Diel migration

The striated strand contains centrin,a Ca^{2+} modulated contractile protein. Contraction of striated strand leads to supercoiling of axoneme. The TF causes direct forward movement & at the same time makes the cell rotate, since the axoneme is coiled as a left-handed screw. The flagellar beat and cell rotation always proceeds counterclockwise when seen from the apex (leiotropic direction), with the fluid propelled in the opposite direction (dexiotropic direction). The dinoflagellates are fastest swimmers among algae. They swim from 200-500 µm/sec.

Marine dinoflagellates frequently move into deeper, nutrient-rich waters at night and better illuminated waters near the surface during the day. This movement which takes place over the time period of 24 hrs.is known as **diel migration**. The diel migration of dinoflagellates are 5-10m in relatively quiet waters. This process is best exhibited by species like *Ceratium*, *Prorocentrum* and *Peridinium*. The migration is probably brought about by an inherent endogenous rhythm of the organism and also found to be governed by +ve or –ve geotaxis. But alternation of day and night is found to have only a subsidiary modifying role in the process, since the migration has been found to go on in complete darkness in case of *Ceratium* sp. Mechanism of thrust generation by a TF.

a. Typical dinoflagellate cell, the arrow indicates the direction of wave propagation.

b. Cross section across the girdle of a typical dinoflagellate. The cell is swimming upwards and the flagellar wave is passing away from the viewer into the page.

1-4 positions of the TF. The broken line indicates the trajectory and the arrows the moving direction of the TF.

a. Overview of the swimming cell. The vertical arrow indicates swimming direction and the curved arrow the rotational directionof the cell. Neither end of the transverse flagellum is shown.

b. Directional terminology of *P. minimum* in the present study:**(i)** sutural view; (**ii)** valval view; **(iii)** anterior view. Open arrows indicate swimming direction.

Vacuoles and Pusules

Certain dinoflagellates possess a system of vacuoles collectively known as the vacuome. In addition to them there are usually two specialized vacuoles that arise from ducts that open at the flagellar bases, the pusules. Different morphotypes of pusules are known. A sac pusule can occupy a third or more of an episome, and a collecting pusule resembles a cluster of grapes.

The sac like pusules open by means of a pore into the flagellar canal and probably have an osmoregulatory function similar to that of a contractile vacuole. The pusule vesicles are lined by a double membrane. They are most developed in heterotrophic marine species.

Accumulation body: A large vesicle containing remains of digested organelles, probably similar to the Corps de Maupas of the Cryptophyta.

Chloroplast and Pigments

Most photosynthetic dinoflagellates have plastids that have originated from a secondary endosymbiosis with a red alga. These plastids are surrounded by three membranes (two membranes of chloroplast envelope and one membrane of Chloroplast Endoplasmic Reticulum). The major photosynthetic pigments include chlorophylls a&c and peridinin.

Some dinoflagellates are known to possess plastids acquired from variety of photosynthetic eukaryotes including haptophytes, cryptomonads, diatoms and green algae through tertiary endosymbiosis. This reflects an essentially phagotrophic lifestyle andkleptoplastidy. These plastids are surrounded by a singlemembrane of CER. Major photosynthetic pigments include the fucoxanthin characteristic of prymnesiophytes & marked absence of peridinin.

Tertiary endosymbiosis begins with the loss of the plastid (originally derived from a secondary endosymbiosis) from a dinoflagellate followed by endosymbiosis of an alga

from the Prymnesiophyceae. All of the protoplasm of the endosymbiont was lost, except for the chloroplast surrounded by one membrane of CER, which became the permanent chloroplast of the dinoflagellate. eg. *Karenia brevis.* A number of dinoflagellates contain short-term plastids stolen from their food source (**Kleptoplastids**) and it is sometimes difficult to distinguish between these plastids and the permanent plastids.

Dinoflagellates have transferred most of the plastid genome to the nucleus of the cells, making them the only eukaryotes that encode majority of the plastid genes in the nucleus. Most of the chlorophyll a and peridinin occur together in a water-soluble light harvesting protein complex called peridinin-chlorophyll-protein (**PCP complex**) which results in amazingly high efficiency in light capture & energy transmission in peridinin containing dinoflagellates.

Phototaxis & Eyespots

Less than 5% of dinoflagellates contain eyespots most of them being freshwater species. The eyespots of dinoflagellates when present are among the most complex in the group algae and of varied types:

1. **Cytoplasmic eyespot** – composed of aggregated lipid globules present freely in the cytoplasm and not membrane bound or within a plastid. eg. *Katodinium campylops* – a colourless dinoflagellate.

2. **Membrane bound, carotenoid containing lipid droplet type** – Species which have peridinin plastids have eyespots which are single or double layer of carotenoid-containing lipid droplets located between the 3-membrane plastid envelope & the outermost layer of thylakoids. eg. *Gymnodinium.*

3. **Three membranes bound carotene droplet type** – In species where photosynthetically active plastids were acquired from a diatom endosymbiont, the eyespots occur as carotene droplets surrounded by 3 membranes. eg. *Peridinium balticum.*

4. **Ocelli** – The most complex type of eyespots known as ocelli are found in a group of unicellular, predatory dinoflagellates known as the **Warnowiaceae**. The subcellular components of ocelli bear an extraordinary resemblance to metazoan eyes. The structure includes a lens-like refractile **hyalosome** (clear body) that is constructed within the ER. The hyalosome becomes surrounded by mitochondria and constricting fibers that can move, changing the shape of the lens. The ocelli also include an ocular chamber, backed

by a **cup shaped**, **darkly pigmented**, **retina**-like structure. This assembly allows formation of images upon the retinoid. This specialized visual system has evolved in response to the selective pressures of phagotrophic habit and allows the predator dinoflagellates to '***see***' their prey.

Nucleus

The nucleus of most dinoflagellates is unique and referred to as Mesokaryon/ Dinokaryon. The first evolved dinoflagellates although, had nuclei similar to other algae with a closed mitosis, histones associated with the nucleic acids and microtubules formed inside the nuclear membrane during mitosis. eg. *Oxyrrhis*. Histones were gradually lost during evolution and a type of mitosis evolved with the mitotic spindle outside the nuclear membrane.The Dinokarya lack nucleosomes,and the ratio of basic proteins to DNA in them is much lower than in any other eukaryote. Unicellular eukaryotic organisms usually have between 0.046 and 3 picograms of DNA per nucleus, whereas, dinoflagellates have 3.8 pg to 200 pg of DNA per nucleus corresponding to about 2,00,000 Mb, compared to only 3,180 Mb in haploid human genome. This implies that a large amount of the DNA is genetically inactive (structural DNA) in the dinoflagellates.

Chromosomes appear fibrillar because they remain continuously condensed during both interphase and mitosis, the 3–6 nm fibrils being packed in a highly ordered state (up to six levels of coiling) consisting of arches and whorls. A prominent nucleolus is also persistent. Most basic nuclear proteins in dinokarya are not histones. However, recent data suggests that histones do exist in dinoflagellate nuclei, though in very low quantities. In some dinoflagellates (*Noctiluca*, *Blastodinium*) chromosomes do decondense (to a degree) during interphase. Subsequently at some stage in their life cycle - usually gametogenesis - the chromosomes reassume a typical dinokaryotic appearance.Dinoflagellate mitosis is also unusual (*dinomitosis*). It is closed and the mitotic spindle is extranuclear. Spindle microtubules pass through furrows and tunnels that form in the nucleus at prophase. Some microtubules contact the nuclear envelope where the chromosomes also contact. The chromosomes usually have differentiated, dense regions inserted into the envelope. The genus *Oxyrrhis* exhibits closed mitosis with an internal spindle.

Life Cycle

Life cycle of a typical dinophyte whose vegetative cells are haploid. Diploid motile zygotes - planozygotes may be present following gametic fusion or following

germination of non motile hypnozygotes. Temporary asexual cysts are formed in some species. The vegetative stage of some dinoflagellates may be nonmotile and/or strikingly different in appearance from the typical dinoflagellate motile cell morphology. However, such organisms are recognizable as dinoflagellates due to presence of **dinospores**.

Cysts

Some dinoflagellates are known to produce nonmotile resting cysts by sexual or asexual processes. Cyst development usually begins with the formation of a colourless peripheral region of the cytoplasm of the planozygote, it stops swimming, sheds its flagella, thecal plates separate and pull away from the cell surface, followed by the formation of cyst wall with spiny outgrowths. Walls of these cysts contain cellulose, dinosporin and mucilage. A conspicuous red-pigmented accumulation body that occurs within these cysts is used to differentiate cysts of other algae from those of dinophytes.

There is a necessary dormancy period during which the cyst cannot excyst. Cyst germination is also inhibited by darkness & low oxygen. Excystment occurs in springs through an aperture known as archeopyle – which is a distinctive feature of fossil cysts. Within a few hours the resulting cells develop normal theca. Cysts can survive in storage for as long as 12 years. They are as toxic as the vegetative cells of the respective species. Formation of cysts is attributed to changes in nutrients, irradiance, photoperiod or temperature. In some case particular bacterial associations may promote cyst formation.

Modes of Nutrition

About half the known living dinoflagellate species lack chloroplast & are exclusively heterotrophic. In addition, many dinoflagellates that contain chloroplasts are capable of mixotrophy. The different modes of heterotrophy/ prey-capture adaptations include:

1. **Phagotrophy**: Process of feeding on prey/particles, three mechanisms by which phagotrophy takes place -

a. **Engulfment**: as in *Noctiluca*, which has a long tentacle that is covered by slimy exudates and two wing-like extensions of the cells at the base of the tentacle to form an oral pouch. At the bottom of the oral pouch is a cytosome that opens like a slit during ingestion of food organisms.

b. **Pallium feeding**: where the prey is engulfed by a cytoplasmic veil – the pallium – with digestion of the food taking place outside the the dinoflagellate cell. eg. *Protoperidinium*

c. **Peduncle feeding**: also known as myzocytosis, involving the uptake of intracellular material of the prey through a cytoplasmic extension – the peduncle, leaving the plasma membrane & extracellular material of the prey behind. eg. *Gymnodinium fungiforme*.

2. **Osmotrophy**: Process of uptake of dissolved substances.

Dinoflagellate Fossils

Chemical compounds specific to dinoflagellates – dinosteranes & dinosterols found in rocks belonging to the precambrian. Such chemical fossils suggest that dinoflagellates existed more than 600 mya. Fossil records also indicate the existence of many single-celled fossils (**acritarchs** – uncertain relationships) during the period, but these lack distinctive structural features that can definitely link them to dinoflagellates. Undisputed structural fossils of dinoflagellates are found about 200 mya. These are recent fossils known as **hystrichospheres**, which are very similar to resting cyst stages of modern dinoflagellates.

Dinoflagellates are known to produce an organic material dinosporin just beneath the outer cell wall of resting cysts. Dinosporin is resistant to decay and chemical treatments and thus aid the survival of dinoflagellate fossils of which a considerable diversity has been described till date. Thus, it can be inferred that though dinoflagellates represent a very ancient group, features such as dinosporin production have evolved more recently, suggesting a major diversification event in the jurassic. Modern dinoflagellates produce >35 types of sterols with chemical features linking to oil deposits, thus it is thought that past dinoflagellate blooms may have contributed to fossil carbon depositions.

4.4. Heterokontophyta

The algae in the Heterokontophyta usually have cells with an anterior tinsel and posterior whiplash flagellum. The plastids contain chlorophylls a & c along with fucoxanthin. The storage product is usually chrysolaminarin in cytoplasmic vesicles.

4.4.1. Bacillariophyceae

Bacillariophytes or Diatoms are microscopic unicellular algae that are mostly photosynthetic with some heterotrophic forms. Diatoms are one of the most distinctive and successful groups of unicellular algae, occurring throughout the world in marine, brackish and fresh waters, as well as in damp subaerial habitats, and represented by 50 – 200 thousand species depending upon one's species concept. In fresh water habitats, diatoms prosper throughout the year. Not only do diatoms exist in streams, lakes and other bodies of fresh water, but they can be found on the rocks, plants, and mud that are

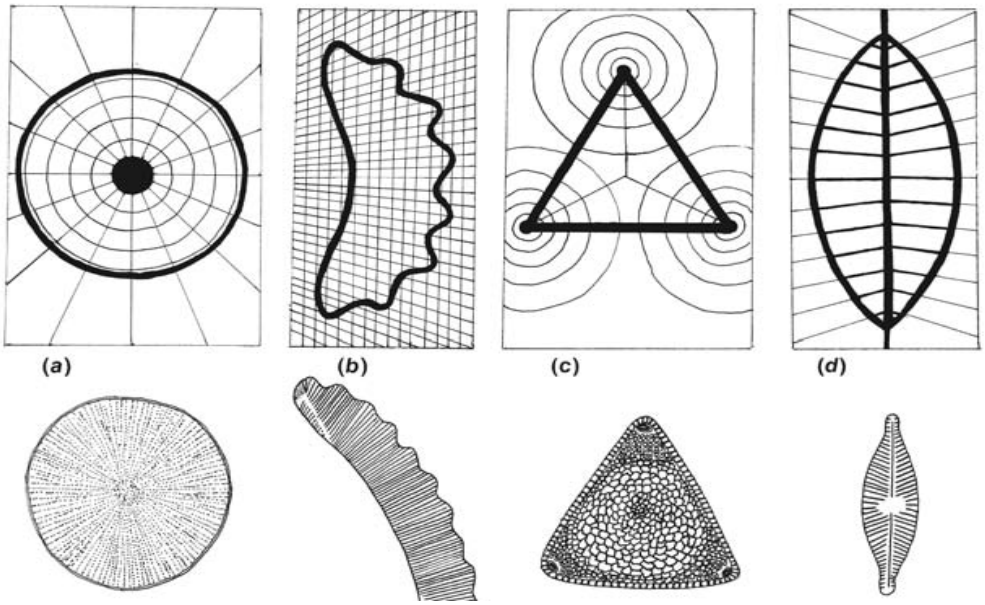
present within or at the borders of water. In marine environments, diatoms have the capacity to exist within animals' digestive tracts, in their shells, on macroalgae, and even on ice. Diatoms are found in both littoral (within the range of tides) and pelagic (water that covers the ocean bottom) regions. They may be simple or branched, filamentous, and even enveloped in a gelatinous envelope or tube. Individually, diatoms do not exhibit a strong, highly visible color, but when visualized in groups, there is a yellow to golden brown colouration that can be seen due to the presence of fucoxanthin, the most important accessory pigment of diatoms. When observed as larger and larger masses, the color takes on a darker color, often deep brown or black. The size of these very diverse micro-organisms varies greatly, spanning a large range of values (usually measured in micrometers). Diatoms can form small colonies, and filaments have been seen to measure over two feet.

With respect to shape, diatoms are grouped as either **centric** (with concentric markings on its valves) or **pennate** (markings are separated by a median). All diatoms are enclosed by a frustule that is made up of two valves fitted together by a connective zone called **girdle**. The diatom cell (frustule) is made of silica and consists of two overlapping halves, each composed of a more or less flat surface, the valve, to which are joined one or more hoop-like bands, the girdle and intercalary bands

- **Epitheca:** Larger, older valve with girdle elements (**epicingulum**).
- **Hypotheca:** Smaller, younger valve with girdle elements (**hypocingulum**).
- **Suture/ Connective Zone**: composed of the overlapping **girdle elements** of **epicingulum and hypocingulum**; connects valves, forming sutures allowing the valves to move.
- **Copulae**: cingula are divided into bands called copulae.
- **Septa:** partitions formed within valves;
- **Punctae:** small holes (pores) or thin, circular, sieve-like areas in the frustules that create ornamentation on the frustule.

The ornamentation of diatoms can be divided into four basic types: (1) **centric and radial,** where the structure is arranged according to a central point, *Coscinodiscus;* (2) ***trellisoid,*** where the structure is arranged uniformly over the surface without reference to

a point or line, *Eunotia;* (3) ***gonoid,*** where the structure is dominated by angles, *Triceratium;* (4) ***pennate,*** where the structure is symmetrically arranged upon either side of a central line, *Navicula.*



Ornamentation in Diatoms (Source: Lee, 2008)

Many **pennate diatoms** have a **raphe -** a longitudinal groove that helps gliding motility. Pennate diatoms without raphes are said to be **araphid.** In some araphid diatoms there is a **pseudoraphe**, a line running down the longitudinal axis of the cell. The raphe is also involved in mucilage secretion in some species, and can anchor the cell to the substrate. The raphe is probably homologous to the **labiate process** of **centric diatoms**. Labiate processes are also present in some araphid pennate diatoms. The labiate process may be a precursor to the modern raphe. In some diatoms, labiate processes are involved in mucilage secretion and some slow mobility.

Evolutionary Trends in Diatoms

Diatoms provide excellent material for phylogenetic studies as they are extremely well preserved as fossils because their silica wall is resistant to biological decay in natural settings. Fossil diatoms are known from deposits extending from recent times back to about 180 million years ago. Such fossil data are consistent with molecular clock evidence suggesting that diatoms appeared not before 240 mya. Fossil evidence suggest that first diatoms lived in marine waters but does not indicate on the immediate ancestors of diatoms or how they acquired their distinctive silica wall. But the early Cretaceous diatoms are noted for fairly uniform, robust, and highly silicified appearance of frustule walls. A highly silicified wall may have been a consequence of the extremely high concentrations of silicic acid in the Mesozoic ocean. Thinner valves in recent species

may reflect selection for more efficient use of silica in wall construction in response to decreasing silicic acid concentrations. The influence of climate change on frustule morphology of diatoms and silica cycleis an interesting arena of study. The findings highlight:

- There is some evidence that as large areas of the ocean are becoming more stratified there has been a decrease in chlorophyll standing stock and primary production.
- It seems likely that such an increase in stratification might result in decrease of silicate flux into the euphotic zone in these regions reducing availability of silicic acid.
- In the past millions of years decrease in nutrient availability have resulted in an increasing number of small-sized & lightly silicified, thin-walled diatoms, but it is unclear whether modern diatoms will continue to become smaller or less silicified in response to current warming.
- A time-series analysis from the Bermuda Atlantic Time Series Station in the Sargasso Sea found ~40% decline in particulate silica (decline in diatoms) associated with increasing water column stratification over the last 15 years.
- Further increases in warming and water column stratification may contribute to further decreases in silicic acid availability and more widespread decrease in diatom abundance over centuries.
- Molecular phylogenies indicate that the diatoms are most closely related to Bolidophyceae, a group of tiny photosynthetic marine flagellates that lack silica coverings.
- Photosynthetic marine non flagellates known as Parmales have walls composed of polygonal plates of silica and thus have also been suggested to be possible diatom relatives.
- Fossil and molecular evidence indicates that the earliest diatoms had frustules whose valves were radially symmetrical similar to modern diatoms.
- These centric diatoms have different forms like bipolar or multipolar centrics but neither centric diatoms as a whole nor the other subgroups form monophyletic groups which complicates diatom systematics further.

- A lineage of bipolar centrics gave rise to the pennate diatoms, with earlier araphid forms evolving into raphid forms some 50-60 million years ago.
- The new feature of possessing a raphe allowed the raphid diatoms to occupy new types of habitats. The evolution of raphe in diatoms has been compared to the evolution of flight in birds, keeping in mind its ecological impact.
- The evolution of rapid mobility via the raphe is thought to have led to other evolutionary changes in pennate diatoms. They have relatively few, larger plastids compared to many smaller plastids in araphid pennate and centric diatoms, helping them to acquire light as they cannot move rapidly.

4.4.2. Xanthophyceae and Eustigmatophyceae

Xanthophyceae

Most species are unicellular or colonial, coccoid algae. Many of the representative species also have thalli composed of multinucleate siphons and a few that consist of multicellular filaments. Only a few are flagellate unicells (monads) or amoeboid organisms. The zoids have flagella inserted close to the apex of the cell and not laterally. The class is also characterized by motile cells with a forwardly directed tinsel flagellum and a posteriorly directed whiplash flagellum. A typical photoreceptor apparatus is present in the zoids, consisting of a swelling on the short, smooth flagellum and an eyespot lying within a chloroplast. Chloroplasts are discoid and green or yellow-green, pigments include chlorophyll a and very small amounts of chlorophylls c_1& c_2. Principal accessory pigments include β-carotene, vaucheriaxanthin, diatoxanthin, diadinoxanthin and heteroxanthin. Chloroplast DNA is arranged in a ring shaped nucleoid. The principal storage product is probably a -1,3 linked glucan similar to paramylon, although lipids have been suggested as also being important. In most non-motile cells the wall is composed of two overlapping halves.

Molecular data have shown that the Xanthophyceae is most closely related to the Phaeophyceae. Although the class is commonly called the Xanthophyceae, the proper name is the Tribophyceae since there is no genus in the class that can lend its name to Xanthophyceae (Hibberd, 1981). Xanthophyceae are difficult to find since they do not occur as abundant species, exception being *Tribonema* and *Vaucheria*. They are widespread in freshwater and terrestrial habitats but rare in marine.

Eustigmatophyceae:

The name is derived from three Greeks root words that mean true or good (eu); spot (stigma); and plant (phyto), referring to the large orange-red eyespot (also called a stigma) in the zoospores.The Eustigmatophyceae is a very small class of yellow-green algae, separated from the coccoid Tribophyceae (Xanthophyceae) in 1971 by Hibberd & Leedale on the basis of cytological and ultrastructural evidence.

Unique features of the unicellular coccoids and flagellate zoospores:

- large size and special construction and position of the eyespot;
- typical location and structure of the flagellar swelling;
- absence of peripheral ring of DNA;
- chloroplasts without girdle lamellae;
- CER not continuous with the nuclear envelope;

Differences in chloroplast pigment composition:

- Unique combination of chlorophyll a, β-carotene & violaxanthin;
- Major xanthophylls – violaxanthin & vaucheriaxanthin.

All methods of reproduction known for the class are asexual, involving zoospores or autospores (non-flagellate spores having same morphology as the parent cell). A single order Eustigmatales with five families, distinguished on the basis of cell shape & size, presence or absence of zoospores and number of flagella on zoospores.Important Eustigmatophytes include *Ellipsoidion*, *Polyhedriella*, *Eustigmatos*, *Nannochloropsis*, *Chlorobotrys*, *Pleurochloris*and*Pseudocharaciopsis*.Most of the genera are freshwater or soil dwelling, rarely marine picoplanktonic forms (*Nannochloropsis*). One genera known to be a symbiont within a freshwater sponge *Corvomyenia everetti*. No known fossil records.

4.5. Bio-fertilizers and Bio-molecules with commercial application

Bio-fertilizers

Until the 20th century, agricultural production, and thus population growth, was limited by the availability of plant nutrients - namely nitrogen and phosphorus. Since early twentieth century, nitrogen synthesis was industrialized by reacting nitrogen gas with hydrogen gas to produce megatons of fertilizer and explosives. The fully developed system is called the Haber–Bosch process. Today, the process consumes more than 1% of the energy on Earth and is responsible for feeding roughly one-third of the world's population. Many countries have enacted policies that encourage the use of these

synthetic fertilizers and other modern farming technologies to boost crop yields and keep pace with population growth. Over time, these practices have led to a number of environmental problems and ironically, diminished crop yields. Aggressive synthetic fertilizer use and tillage is credited with increased soil erosion, degrading local ecosystems that fight pests and disease, increased water demand, and stunting crop productivity. Furthermore, the production of synthetic fertilizers is dependent on fossil fuels and contributes towards greenhouse gas emissions. In response to environmental concerns, sustainable organic agriculture has become an increasingly popular option. The application of biofertilizers has been shown to decrease soil erosion, pest infestation, and water requirements, and improve soil tilth.

The use of algae as a biofertilizer is particularly appealing for several reasons. Algae can be grown in arid areas that are unsuitable for traditional crops, can reclaim nutrients in waste streams, and can produce biofertilizer "crops" year round. Large scale growth of algae in accelerating research has lead to the development of cost-effective technologies to harvest and process algal biomass for use as animal feed or biofertilizer.

The Rise and Impact of Synthetic Fertilizers

Fertilizers are substances added to soil to improve its fertility, and thus, the growth and yield of plants. In the earliest times, people noted that the first yield on a plot of land qualitatively surpassed subsequent ones. Thus, there had to be a way of maintaining or even enhancing yield while staying on the same plot of land. With time, natural fertilization became more refined to keep pace with population growth. By the 20th century, it was understood that the core plant nutrients for optimal plant growth and yield are nitrogen (N), phosphorous (P), and potassium (K). Until the 20th century, agricultural production, and thus population growth, was limited by the availability of plant nutrients - namely nitrogen and phosphorus. Because of improvements in soil nutrient retention capacities, industrial agriculture has substantially increased crop yields parallel with population growth. The fertilizer consumption rate in 1920 was ~ 6.1 million tons. One hundred years and more than 4.8 billion people later, the fertilizer consumption rate is estimated to reach 200 million tons. By 2050, global population is projected to increase by 50% and global grain demand is projected to double. Given synthetic fertilizer's dependence on natural gas and rock phosphate, an alternate supply of fertilizer N:P:K is required to meet future global food demands.

Fossil Fuel Requirements

It is estimated that one percent of the world's energy consumption now goes toward fertilizer manufacture. On average, 5.5 gallons of fossil fuels per acre, per year are needed to fertilize soil for farming. But, it is estimated that crops actually absorb only one-third to one-half of the nitrogen applied to farmland as fertilizer. The excess nutrients are free to remain in the applied soil, or they are washed away as fertilizer run-offs.

Eutrophication of Waters

High-nutrient levels stimulate algal blooms, and when the bloom subsides, subsequent decomposition by bacteria consumes dissolved oxygen deep in the water column faster than it can be replenished from the surface. Low oxygen levels decimate immobile bottom dwellers and drives off mobile sea life such as fish and shrimp, which further impacts organisms further up in the food change and those whose livelihoods depend on the marketing of these organisms. Elevated levels of nitrate and pesticides are thus been found in shallow groundwater in agricultural watersheds. Water in most of the watersheds exceeds safe drinking water standards for nitrate, which is a potential risk factor for cancer and reproductive problems.

Impact on Air Quality

Nitrogen-based fertilizers contribute directly to global warming. Currently, one third of N_2O emissions and greater than half of the total global CH_4 emissions stem from anthropogenic sources including industry, fossil fuel acquisition and use, biomass burning, and agricultural practices.The IPCC estimated that the agricultural sector contributes 65% of global anthropogenic N_2O emissions, 40% of global anthropogenic CH_4 emissions, and 10-12% of global anthropogenic CO_2 emissions.

Impact on Soil Tilth

Tilth refers to the physical condition of a soil, including its texture and relative ability to hold moisture and nutrients. It is a key indicator of a soil's health. Soil in good tilth is a loamy nutrient-rich soil that has an appropriate mixture of sand, clay and organic matter that prevents severe compaction and promotes oxygen circulation. It takes more than 20 years for a centimeter of soil to form. The deterioration of soils is one of the most serious global challenges facing humankind as it attempts to feed a growing population. It has been estimated that since World War II, poor farming practices have damaged ~1.3 million acres, or about 38% of all farmland in use today. Chemical fertilizers decrease

soil fertility by stimulating the growth of microorganisms that thrive on nitrogen. Over time, these organisms can deplete the soil of organic matter, resulting in decreased soil tilth and crop yield. Simultaneously, many beneficial microbes may be displaced by synthetic fertilizers, further resulting in poor soil formation, a lack of decomposition of nutrients, and inadequate protection from parasitic and fungal growth. Excess fertilizer also causes substantial accumulation of major (K^+, Ca^{2+}, Mg^{2+}) and heavy-metal (Cd^{2+}, Zn^{2+}) ions in soil solutions and a decrease in soil pH, factors that may be inhibitory to plant growth.

Transitioning to a Sustainable Agricultural Economy with Biofertilizers

Sustainable agriculture can be defined as practices that meet current and future societal needs for food, healthy ecosystems, and healthy lives, and that do so by maximizing the net benefit to society. Additionally, sustainable agriculture also requires sustainability of energy use, manufacturing, transportation, and other economic sectors that also have significant environmental impacts. Despite the apparent unsustainability of synthetic fertilizers, it is also clear that in order to sustain the future world population, use of some form of fertilizers is necessary. Biofertilizers represent a promising alternative to synthetic fertilizers. Biofertilizers include microorganisms, such as bacteria, fungi, cyanobacteria, and algae and their metabolites that are capable of enhancing soil fertility, crop growth, and/or yield. Applying organic biofertilizers to agricultural land could increase the amount of carbon stored in these soils and contribute significantly to the reduction of greenhouse gas emissions by eliminating the requirement of fossil fuels for production through reclamation of N:P:K from wastewater streams. Furthermore, increasing organic matter in soils may cause other greenhouse gas-saving effects, such as improved workability of soils, better water retention, less production and use of mineral fertilizers and pesticides, and reduced release of nitrous oxide.

Nutrient reclamation

Microalgae grow suspended in nitrogen- and phosphorus-rich, CO_2-fertilized water. The microalgae feed upon these suspended nutrients to promote growth and conversion of CO_2 to O_2. As a result, incorporating microalgal systems into conventional wastewater treatment has the potential to improve the water quality of the effluent by reducing both nitrogen and phosphorous nutrient loads into freshwater ecosystems. The microalgae-rich Salton Sea in Southern California is an example of a potentially very large-scale

application of nutrient reclamation. Over one billion cubic meters of agricultural drainage waters flow annually into this body of water. These wastewaters contain approximately 1,000 tons of phosphate and 10-times this amount of nitrate. Nutrient removal from these drainage waters by microalgae cultures would avoid eutrophication of the Salton Sea while producing approximately 100,000 tons of microalgae biomass.

Biofertilizer nutrient transfer to crops

Plant roots take up plant-food elements from the soil in their ionic forms; potassium, Calcium, magnesium, iron, zinc, nitrogen, phosphorous, sulphur, Chlorine, etc. Given that that crops actually absorb only one-third to one-half of the nitrogen applied to farmland as fertilizer, there is a need to develop a suitable agricultural system which requires lower fertilizer input with higher fertilizer use efficiency. Recent studies show that an inoculation of a single algal biofertilizer significantly increased the biomass yield through increased nutrient uptake in plants. The studies predict that a lower concentration of biofertilizer would be required to produce the same yields promoted by conventional fertilizers.

Soil stability

Land degradation due to accelerated erosion is a serious global issue because soil resources of the world are finite and nonrenewable in the human - time scale. In general, background erosion removes soil at roughly the same rate as soil is formed. But “accelerated” soil erosion - loss of soil at a much faster rate than it is formed - is a far more recent problem primarily due to aggressive agricultural practices. There are different methods of reducing soil erosion, interestingly; microalgae also participate in the reduction of soil erosion by contributing soil - binding polysaccharides from their cell walls. The long-term influence of polysaccharides on aggregate stability may result from microbial mineralization of extracellular polysaccharides. Studies show that microalgae-supplemented soil significantly increased the % age of soil aggregates after six weeks of incubation as compared to soil without algae. These data, together with the prediction that lower amounts of biofertilizers are required for equivalent nutrient uptake by crops, may mean that soil erosion and consequent nutrient run-off may be much reduced through the use of algal biofertilizers. Common microalgal biofertilizers include: *Anabaena, Nostoc, Aulosira, Tolypothrix.* Till date, many studies have shown that algal biofertilizers have numerous benefits to soil quality and crop yield:

- increased nutrient transfer,
- comparable crop yield,
- increased beneficial microorganisms,
- stabilization of soil aggregates,and
- decreased reliance on fossil fuels.

Many individual reports suggest that microalgae could represent a promising alternative to commercial or alternative organic fertilizers:

- Microalgae have been shown to efficiently recycle N:P:K nutrients from wastewater streams and stabilize soil aggregates;
- Microalgae can be grown in environments that cannot support traditional land-based crops and therefore do not displace those crops;
- The production of microalgae requires no fossil fuel inputs;
- Algae can convert waste CO_2 to O_2; and
- Many pilot studies using processed microalgae as a biofertilizer show that crop yield exceeded that of chemical fertilizer applied at the same rate.

Bio-molecules with commercial application:

Macro- and micro-algae are currently mainly used for food, in animal feed, in feed for aquaculture and as bio-fertilizers. Biomass from micro-algae is dried and marketed in human health food market in form of powders or pressed in the form of tablets. Important species used for micro-algal biotechnology of biomass for human food belong to the groups of *Spirulina*, *Chlorella*, *Dunaliella*, *Nostoc* and *Aphanizomenon*. Aquatic biomass could also be used as raw material for co-firing to produce electricity, for liquid fuel production via pyrolysis (bio-oil), or for biomethane generation through fermentation.

1. Small molecules

Small molecules from algae include iodine, algin, mannitol, and L-fraction, the first three being commercial products. The L-fraction (a lignin-related fraction) was suggested as a feedstock or a component for making specialty plastics, adhesives and timed-release substances such as pharmaceuticals or pesticides. Some algal species accumulate high concentrations of proline under conditions of high salinity.

2. Hydrocolloids

Macro-algae have long been used for the production of group of phycocolloid polymers commonly termed hydrocolloids such as alginates, carrageenans or agars. These polymers are either located in cell walls or within the cells serving as storage materials. A characteristic of marine algae is the abundance of sulphated polysaccharides in their cell walls. In total these phycocolloids represent world market of some US$ 600 Mio y^{-1}. They make up the major industrial products derived from algae. The raw materials for the production of hydrocolloids are macro-algae (red and brown seaweeds). Hydrocolloids are polysaccharides that are not found in terrestrial plants, although polymers with similar properties can be produced by certain land plants, for example gum Arabic. Hydrocolloids possess a number of unique properties. The polymers are used in many food and industrial products to thicken, emulsify and stabilize. Hydrocolloids can be dissolved in warm water and will form a gel on cooling. The gel properties can be modified by varying the concentrations of metal ions present, the temperature and the pH, making them suitable for various applications.

Alginates are polymers from the cell walls of a wide variety of species of the brown algae, particularly species of *Laminaria*, *Macrocystis* and *Ascophyllum*. The alginates are extracted from the cell walls using hot alkali (sodium carbonate). Alginates are commonly used in the food and pharmaceutical industries as stabilisers for emulsions and suspensions, e.g. ice cream, jam, cream, custard, creams, lotions, tooth paste, as coating for pills. They are also used in the production of paint, construction material, glue and paper, the oil, photo and textile industry. Brown seaweeds for alginate production are harvested from the wild and not cultivated for this purpose. Although these seaweeds are cultivated to produce food in China, their cultivation to provide raw material for industrial uses would be too expensive.

Carrageenans are linear 1,3-α-1,4-β-galactans from cell walls of red algae that are substituted with one (κ-), two (ι-) or three (λ-carrageenan) sulphate groups. They are extracted from the cell walls with hot water. Carrageenans are used in the food, textile and pharmaceutical industry and function as a stabilizer for emulsions and suspensions. The bulk of carrageenan is now produced from cell walls of different species of the genus *Eucheuma*, and *Kappaphycus alvarezii* (cottonii), in addition smaller amounts are isolated from *Chondrus crispus* (Irish Moss) and *Gigartina stellata*. Most of the seaweed for carrageenan production is cultivated, because the demand for raw material cannot be

satisfied from natural resources. *Eucheuma sp.* and *Kappaphycus alvarezii* are most often cultivated with the fixed, off-bottom line or with floating rafts in the Philippines, Indonesia and Tanzania.

Agars are 1,3-α-1,4-β-galactans from cell walls of red algae that are substituted with sulphate groups. Like the carrageenans, the agars are extracted with hot water. The genera *Gelidium* and *Gracilaria* supply most of the raw material for agar production. *Gelidium* used for commercial agar production is harvested from the wild, whereas *Gracilaria* species have also been cultivated in Chile, China and Indonesia, in protected bays in the ocean, on lines ropes or nets, or in ponds on land. Like carrageenans, agars are used as stabilisers for emulsions and suspensions and as gelling agents. About 90% of the agar produced is for food applications and the remaining 10% is used for bacteriological and other biotechnological uses.

3. Ulvan

Ulvan is the name given to a group of polymers that can be extracted with water containing a cation chelator from cell walls of green seaweeds belonging to the family *Ulvales*, especially the genera *Ulva* and *Enteromorpha.* Yields of 8% to 29% of the algal dry weight have been reported. Ulvan samples from different species differ from each other in their composition. In general they are composed of variable proportions of different repeating sequences of rhamnose, glucuronic acid, iduronic acid, xylose and sulfate. So far these polymers have not been commercially used, however it has recently been proposed that Ulvan could be

i) a source of rare sugar precursors for the synthesis of fine chemicals;
ii) a source of oligosaccharides that could be used as pharmaceuticals and
iii) a gelling agent for designing gels with precisely controlled textures.

4. Pharmaceuticals and cosmetics

Many micro-algae produce bioactive compounds such as antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators. Antibiotics have been obtained from a wide range of algae and show great chemical diversity (fatty acids, bromophenols, tannins, terpenoids, polysaccharides, alcohols). The same holds for the neurotoxic and hepatotoxic compounds produced by algae. Some of these chemicals or compounds derived from them have potential applications as pharmaceuticals. Algae have also been investigated as sources of vitamins and vitamin precursors, most notably

ascorbic acid, riboflavin and tocopherols. Certain micro-algae, especially *Chlorella* and *Arthrospira* (*Spirulina*) are used in skin care, sun protection and hair care products. So far only a few hundred of the tens of thousands of micro-algal species have been investigated for potential pharmaceuticals and nutraceuticals. The huge biodiversity of the micro-algae makes the discovery of new metabolites very likely. There is therefore also potential for the discovery and production of high value compounds.

5. High value oils

The very long-chain poly-unsaturated fatty acids (vlcPUFAs) eicosapentaenoic (EPA), docosahexaenoic acid (DHA) and arachidonic acid (AA) are well known for their nutritional importance. As they confer flexibility, fluidity and selective permeability properties to cellular membranes they have been shown to be vital for brain development, beneficial for the cardiovascular system and for other important nutraceutical and pharmaceutical targets in human and animal health. For example, vlcPUFAs are found in many different product applications including formulas for infants, adult dietary supplements, animal feed, food additives and pharmaceutical precursors. These applications represent an extensive market for vlcPUFAs: the world wholesale market for infant formula alone is estimated to be about US$10 billion per annum. Animals lack the capability to synthesize vlcPUFAs and therefore these essential fatty acids must be obtained from food/feed. Typically sources of PUFAs are oil-rich fish such as eel, mackerel, herring, salmon and sardines. Due to concerns over declining fish stocks and the potential of the fish-oils being contaminated by a range of pollutants, possibilities of obtaining these fatty acids from other sources have been investigated. Interestingly the vlcPUFAs in the oil-rich fish originate from marine micro-algae that are eaten by the fish. Algal genes encoding relevant enzymes have been identified and recently several groups have reported progress on using these genes to produce DHA and AA in transgenic plants, including crops such as soybean, linseed, tobacco and the model species *Arabidopsis*. By adding additional genes to the ones that are needed to produce AA and EPA, production of DHA has been established in soybean, *Brassica juncea* and *Arabidopsis*.

An alternative approach is to use directly the algae that are the most efficient primary producers of the vlcPUFAs. Algae groups that contain vlcPUFAs include diatoms, chrysophytes, cryptophytes and dinoflagellates). High amounts of DHA, for example, are produced in the algae *Crypthecodinium cohnii*, *Thraustochytrium spp*., *Schizochytrium*

spp., *Isochrysis galbana* and *Crypthecodinium spp*. Several species have been suggested for the production of EPA including *Nitzschia spp*., *Nannochloropsis spp*., *Navicula spp*., *Phaeodatylum spp*. and *Porphyridium spp*. A slight inconvenience with using algal feedstocks directly for the production of vlcPUFAs is that in many species the accumulation of these fatty acids involves their presence in lipids other than triacylglycerides such as galactolipids. This makes their isolation more complicated.

6. Colourants

Micro-algae produce of wide variety of carotenoids, with over 40 carotene and xanthophylls isolated and characterized. The most simple is β-carotene, found in all algal species as well as other plants. In halophytic *Dunaliella* species it can accumulate to 14% of total dry weight, and several commercial facilities for β-carotene production operate in Australia, Israel, USA and China. The largest plant (800 hectares) is run by Cognis Nutrition and Health, and produces β-carotene extracts and *Dunaliella* powder for human use and for animal feed. Prices for these products range from US$ 300 - 3,000. Lutein, canthaxanthin, zeaxanthin, lycopene and bixin are also commercially produced carotenoids, but in much smaller amounts. The most interesting carotenoid is astaxanthin, which is produced in significant amounts (1.5 – 4% of dry biomass) by green micro-algae such as *Haematococcus pluvialis*. The synthetic equivalent (95% of the market) is used in aquaculture to give a pink colour to cultured salmon.

5. Cyanobacteria

Life on our planet was microbial for 3.2 billion years. During this long period, microorganisms have evolved an incredible diversity. Cyanobacteria and, hence, oxygenic photosynthesis evolved 2.7–2.2 billion years ago and therefore had ample time to diversify and adapt to newly evolving niches that emerged on the Earth. Through the means of oxygenic photosynthesis, cyanobacteria were responsible for oxygenation of the Earth’s atmosphere, thereby allowing the evolution of plants and animals 0.6 billion years ago and were eventually responsible for shaping the present biosphere.

Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis. The Cyanobacteria are also named as Chloroxybacteria or Cyanoprokaryotes or Cyanophyta

or Cyanophytes or Blue Green Algae. The term BGA was abandoned by many since their prokaryotic nature became apparent. They exhibit wide variety and range in habitat, in morphology and in physiology & metabolism.

5.1. Diversity of forms and habitats

Successfully colonized almost any illuminated environment on Earth, many of which are considered to be hostile for life. Play a prominent role in many of these extreme environments. Occupy all types of natural habitats - freshwater, marine and terrestrial habitats along with being symbionts. Successful survival strategies in such diverse habitats can be attributed to their characteristic features of structure and metabolism. Though photoautotrophy is their dominant mode of nutrition through oxygenic photosynthesis, some of the cyanobacteria are capable of switching from oxygenic photosynthesis to anoxygenic bacteria type photosynthesis when they occur in environments where hydrogen sulfide is present at relatively high concentrations.

Habitat	Common species	Environmental characteristics
A. Marine planktonic		
A1 Oceanic	*Prochlorococcus, Synechococcus, Trichodesmium*	May range from high light, oligotrophic to low light, higher nutrients. Environment ranges from tropical to polar.
A2 Coastal	*Oscillatoria, Phormidium*	May range from oligotrophic to eutrophic, depending on terrestrial inputs.
A3 Estuarine	*Oscillatoria, Scytonema*	More variable changes in salinity, pH, temperature and nutrients.
B. Freshwater planktonic		
B1. Non-bloom forming	*Synechococcus, Aphanothece, Chroococcus*	Waters are poorly buffered, lakes may stratify in summer favoring growth in high-light surface waters. Nutrients may range from oligotrophic to eutrophic

B2. Bloom-forming	*Anabaena, Aphanizomenon, Microcystis, Oscillatoria, Spirulina*	Gas vacuoles allow species to control buoyancy and exploit high-light water zones in summer. Stratification and mixing play important role in creating bloom conditions

Marine Cyanobacteria

Occurrence in open sea is more frequent than in fresh water.Mainly occur as phytoplankton though less important in terms of biomass & productivity of marine systems.Still of considerable importance in terms of being important contributors to marine vegetation. All cyanobacterial orders other than Stigonematales have marine representatives.

Estuarine Cyanobacteria

Adaptability to grow in freshwater and also marine environment. Grow as planktonic, epilithic, epibiontic and benthic forms in the littoral and intertidal zones, in mangrove areas and salt marshes.Represent a wide range of diversity of forms as well.

Freshwater Cyanobacteria

Common in freshwater lakes, rivers and other aquatic systems.Grow as planktonic, epilithic, epiphytic and benthic forms.May form dense planktonic populations in eutrophic systems.In tropical regions growth is continuous throughout the year.In temperate regions there is characteristic seasonal fluctuation attributable to differences in physico-chemical properties due to thermal stratification.

Terrestrial Cyanobacteria

Though more prevalent in aquatic systems, also found to be growing on the surfaces of moist soils and also beneath soil surfaces.In temperate regions are common in calcareous & alkaline soils but absent from acidic soils.Universal component of tropical soils, areas with high temperatures & high humidity provide habitat for luxuriant growth of cyanobacteria on sub-aerial systems as well – on rocks, barks of trees, walls & roofs of buildings, etc.Characteristic thick mucilage sheath present.

Attached Cyanobacteria

Epilithic: growing on surfaces of stones and rocks.

Epibionts: growing on surfaces of submerged plants (epiphytic) and also on animals (epizoics). Epipelic/ Benthic: growing on bottom sediments.

Symbiotic Cyanobacteria

Form symbiotic associations with non-photosynthetic eukaryotes as well as photosynthetic eukaryotes, occurring in such relationship with representatives of all groups of the plant kingdom and some invertebrates of the animal kingdom.They are always a productive partner in all the symbiotic associations.When in association with non-photosynthetic partners they function as primary producers of organic matter.When in association with a photosynthetic partner they engage in nitrogen fixation.Forms an integrated system with fungi in the lichens.

Symbiotic Cyanobacteria

Form Intracellular or Extracellular associations.

Intracellular association:

In Glaucophyta, Rhodophyta and Chlorophyta, studies have confirmed that the simple plastids therein have all originated from cyanobacteria through endosymbiosis. In case of marine organisms – 38 genera of sponges are found to form such associations with 4 cyanobacteria (*Aphanocapsa, Synechocystis, Oscillatoria* and *Phormidium*). Freshwater (*Denticula* and *Rhopaldia gibba*) and marine (*Climacodium*) diatoms contain cyanobacterial cells within their cytoplasm. The dinoflagellate *Amphisolenia* is also found to have intracellular cyanobacterial symbionts. The soil fungus *Geosiphon pyriformis* contains *Nostoc punctiformae* intracellularly.

Extracellular association:

With Ascidians: 5 genera of ascidians of the family Didemnidae form extracellular symbiotic associations with *Prochloron*. With Bryophytes: *Nostoc* is found to grow in symbiotic association with liverworts (*Blasia cavicularia*), 4-6 genera of hornworts (*Anthoceros*), where they inhabit mucilage-filled intercellular cavities within the gametophytic thalli. With Ferns: *Anabaena azollae* forms a symbiotic association with

the water fern *Azolla*, where the cyanobacteria grows within central cavity of the ventral side of the lobe which remains immersed in water.With Gymnosperms: *Nostoc* is found within the mucilage filled intercellular spaces of the coralloid roots of *Cycas*.With Angiosperms: *Nostoc* is found in swellings/ nodules of the stem near the leaf-bases of *Gunnera*.

Cyanobacteria of Extreme Habitats:

Such organisms are called Extremophiles.Can tolerate temperatures as high as 74°C & as low as -60°C. Can tolerate extremes of pH. Can tolerate extremes of light intensities.Can tolerate extreme hypersalinity.Are found to inhabit hot springs and thermal pools.Are found to inhabit subsurface spaces (cryptoendolithic) within porous crystalline sandstone and limestone rocks in hot, arid desert habitats.Are found to inhabit cold deserts of Antarctica and other polar regions. Cyanobacterial growths are found on frozen lakes, water columns, as mats on shorelines, on ice and also snow.Are not found to inhabit acidic hot springs, having pH<5, but Dominic & Madhusoodanan (1999) have reported cyanobacteria from Kerala growing at pH 2.8.

5.2. Cyanobacterial taxonomy

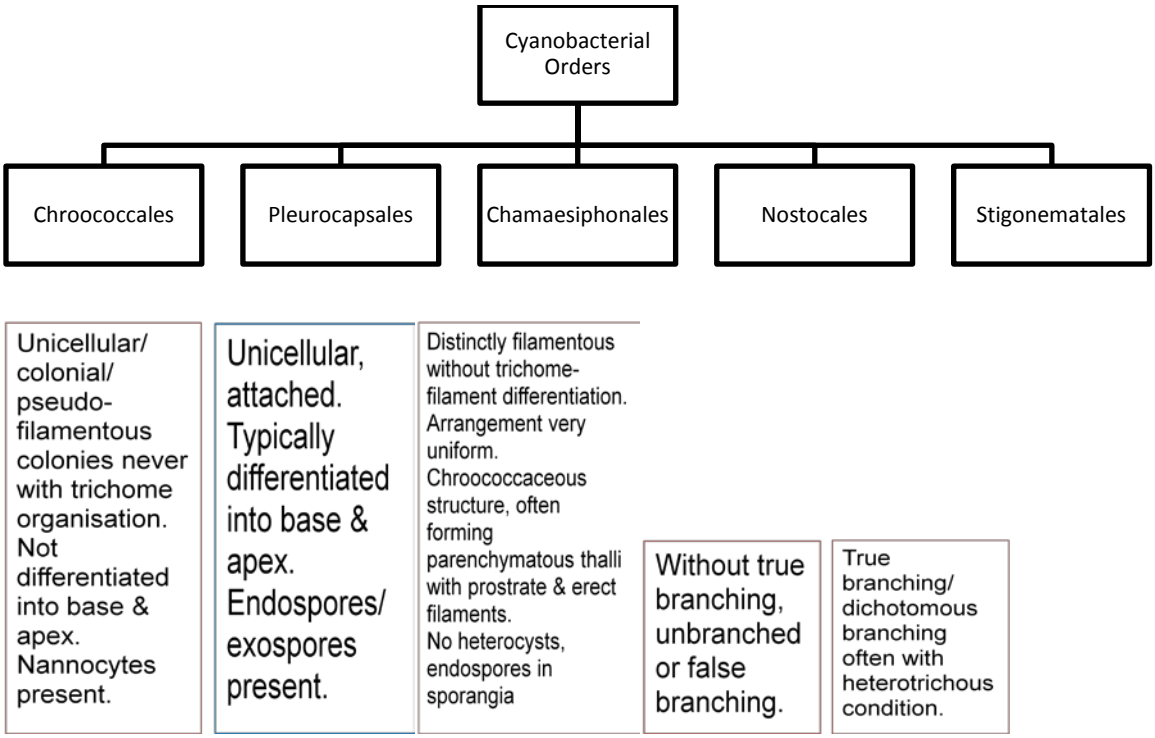
During the late 20th century there were several approaches that changed the taxonomic system of cyanobacteria. These approaches, listed below, incorporated new methods with characteristics used to classify cyanobacteria: Phenotype (morphological) approach; Paleobotanical approach; Ecological approach – (i) ecological demands are species-specific and must be a part of taxonomic evaluation; (ii) the geographic distribution of species depends on “distribution” corresponding to environmental conditions; Electron microscopy techniques; and Molecular approach.

It is generally accepted that the current system of Cyanobacterial taxonomy needs to use Bacteriological Code of Nomenclature (Rippka *et al*. 1979). However, the botanical name based on morphological characteristics remains the initial step in the new bacteriological approach. The main difference between the botanical and the bacteriological approach is practical.

The **botanical system** emphasizes a study of natural material, and so it reflects the knowledge of scientists working with field samples.

On the other hand, **bacteriological system** is based on genome information gained from clonal Cyanobacterial cultures. Bacteriological systematics allows an approximation of the real phylogenetic relationships among cyanobacteria, but it does not solve the question of the classification of such cyanobacteria, that have not been successfully cultured yet. Nevertheless, the two "schools", botanical and bacteriological, have approached one another over the last decade.

Botanical approach in Cyanobacterial Taxonomy:



In the framework of "the botanical school", a revised cyanobacterial system has been created by Anagnostidis & Komarek, which is a combination of the botanical & bacteriological approaches.

The classification is based predominantly on morphological & ultrastructural criteria, but information gained by biochemical and molecular methods applied on cyanobacterial isolates are also taken into account.

5.3. Cyanobacterial evolution

Modes of Propagation

- Binary fission
- Multiple fission
- Nanocyte formation
- Fragmentation of colonies
- Formation of necroids/ necrid cells
- Through non motile spores – endospores/ exospores

The first cyanobacteria evolved from two anoxygenic photosynthetic groups in earlier part of Precambrian – (i) Firmicutes and (ii) Chlorobi

During later part of Precambrian they were already ubiquitous and representatives of most present day Cyanobacterial orders have been recorded from fossil records of this period – often referred to as the age of cyanobacteria.Microfossil history also suggests that the present morphological diversity of the cyanobacteria had already evolved by the Precambrian.Diversification & speciation of cyanobacteria are yet to be satisfactorily explained. Recent studies indicate the main controlling factors of the cyanobacterial evolution as being the extracellular transfer of genetic material within related populations, combined with their great adaptability and very rapid changes in morphological and ecophysiological variability, continually over the ages.On the other hand, there are numerous stabilized morphotypes that have occurred unchanged for very long time periods under restricted and stabilized ecological conditions.

Two reasons for such stagnation can be explained:

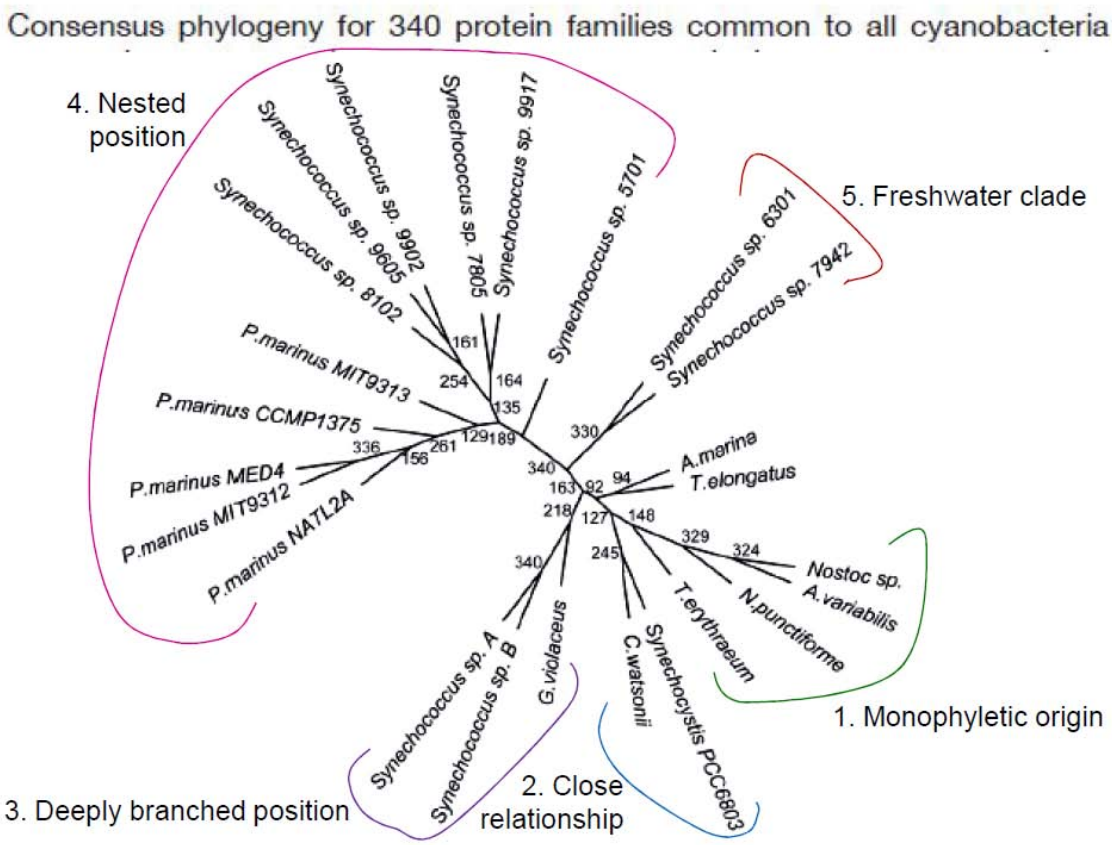
- Cyanobacteria are generalists and able to survive and grow under many varied conditions.
- Lack of sexual reproduction, as such genetic shuffling created by sexual reproduction does not occur in cyanobacteria, only variability is introduced by mutation.

So with little need to evolve and only limited means to do so, they have remained same over such a large span of time. Hypobradytely – extremely slow process of evolution.

- Historically, systematic methods of defining morphological traits in cyanobacteria have posed a major barrier in reconstructing their true evolutionary history.
- The advent of protein, then DNA sequencing and the use of 16S rRNA as a molecular marker helped overcome this barrier and now forms the basis of our understanding of the history of life on Earth.

Phylogenies generated from about 340 protein families of the cyanobacteria highlight a number of relationships that are suggestive of important events during cyanobacterial evolution:

1. **Monophyly** - and an apparently singular origin - of heterocyst forming cyanobacteria (*N. punctiforme, Nostoc sp., A. variabilis),* and their nested position within filamentous cyanobacteria (*Trichodesmium erythraeum)*
2. Relatively **close relationship** between *Synechocystis sp.* and *Crocosphaera watsonii*, despite marked differences in lifestyle
3. The **deeply branching position** of *Gloeobacter violaceus* and thermophilic *Synechococcus* in the rooted tree.
4. The **nested position** of the *Prochlorococcaceae* within the marine *Synechococcus*, and the apparently more basal position of *Synechococcus sp.* which may be an important modern analog of an ancient transition from freshwater to marine environments.
5. The **very deep radiation** of freshwater *Synechococcus* relative to the marine clade, and the close relationship of these two strains to one another.



Genomic variation in species belonging to the same evolutionary lineage cannot be explained by vertical descent alone. **Horizontal Gene Transfer** (HGT) is a major evolutionary factor to be considered. HGT is the acquisition of foreign genes by any genome either by transformation, conjugation or transduction. In cyanobacteria three major modes of gene transfer have been identified: natural transformation, conjugation and transduction.Natural transformation was first documented in *Synechococcus* sp. PCC 7942 (*Anacystis nidulans),* but later reported in other species of *Synechococcus, Synechocystis, Nostoc muscorum, Anabaena sp.* and recently in *Thermosynechococcus elongatus.*

Transformation typically involves several stages including development of competence, DNA binding, DNA processing, DNA uptake, and integration of DNA into the genome of the recipient cell. Exogenous DNA is taken up non-specifically during transformation and, depending on its length, may or may not be further cleaved for integration. Conjugation in cyanobacteria was first performed between several *Anabaena* strains with and without cyanobacterial donor. Conjugation allows for increased genetic manipulations in cyanobacteria than natural transformation, as in natural transformation DNA may be cleaved during uptake, limiting the length of DNA that can be transferred

and thereby decreasing efficiency of homologous recombination. The importance of transduction in freshwater cyanobacteria remains uncertain at this point in time, though freshwater cyanophages have recently been identified.

In the case of marine cyanobacteria, many cyanophages have been found and they frequently contain important photosynthesis genes as well as other genes from both archaea and bacteria. These cyanophages are thought to mediate transfer and recombination of these genes among the marine cyanobacteria. It has also been predicted that these cyanophages serve as reservoirs for hosts and play an important role in the evolution of cyanobacterial photosynthesis.

6. Rhodophyta

The red algae form a distinct photosynthetic eukaryotic lineage that consists of around 6,000 species including unicellular to large multicellular taxa. The red algae are unique among eukaryotes in lacking both flagella & centrioles during their entire life cycle. Distinguishing characters also include pit connections, pit plugs, and a triphasic life cycle. The plastid of red algae is bounded by two membranes and contains chlorophyll-*a*, phycocyanin, and phycoerythrinas majorphotosynthetic pigments. Pigment complexes, organized in phycobilisomes, are located on surface of unstacked thylakoid membranes. As a storage product, the red algae produce granulated floridean starch in the cytoplasm that is different from green algal starch.

6.1. Diversity of forms and habitats

Being an ancient lineage, the red algae have undergone a broad range of modifications in cellular organization. Even the spectrum of widest morphological possibilities, from unicellular forms to complex parenchymatous thalli, fails to convey the degree of cellular diversity that the red algae exhibit. Morphologically more diverse than any other group of algae, they range from single cells to large ornate multicellular plants. Uniquely they lack both flagella and centrioles and exhibit a remarkable, and exhibit peculiar range of reproductive strategies. Though, recent studies suggest that the rhodophytes have probably lost their flagella.

The rhodophytes exhibit a great range of morphological variations:

1. The simplest forms consist of **single cells** like *Porphyridium* or **thin filaments** of *Bangia*.

2. The habit of **expanded blades** is found in many genera, including some of the most spectacular such as *Delesseri*a, *Polyneura* and *Halymenia*. A widespread and economically important genus with blade-like habit is *Porphyra*.

3. The body of some red algae is formed by a crust which grows attached to the rocky bottom, eg. species of the order Corallinales - *Lithophyllum*, *Lithothamnion,* and *Phymatolithon.* In these species the cell walls accumulate calcium carbonate, conferring to their body a robust and coriaceous texture. They look like pink or red calcified crusts, very resistant to grazing and mechanical dislodgement; they often thrive on exposed rocky shores, where seaweeds with soft tissues would be easily dislodged by the violenc*e* of the wave*s.*

4. Many other species of red seaweeds have a branched plant like shape and look like small bushes or trees. Eg. *Polysiphonia, Chondrus, Gelidium, Gracilaria, Hypnea* and many others.

5. Early diverging rhodophytes occur as unicells, colonies, filaments or sheets.

6. In some multicellular forms, there is no specialized meristematic region and cell division may occur almost anywhere in the body and growth is thus diffused.

7. In contrast, the forms which are fundamentally filamentous grow via division of an apical cell located at the terminus of a filament, thus exhibiting apical growth.Apical cells cut off derivatives at their bases in a single linear series. Such divisions of the apical cells are responsible for increases in the number of cells in the filaments.

8. Delicate florideophyte bodies may be **uniaxial**, composed of a single branched filament.Commonly, subapical cells divide several times radially to form whorls of branch initial cells known as periaxial cells, which divide to form branch filaments, each with a terminal apical cell.Branches are typically determinate, meaning that only a genetically determined number of cell divisions occur, thereby limiting the length of the branch filaments. Subapical cells also generate rhizoids.

9. More robust and fleshy florideophytes are **multiaxial**, composed of multiple filamentous axes, each derived from a terminal apical cell.

10. These multiple axes arise during early development through the transformation of determinate branches into indeterminate axes. Meaning that the branch apical cells become less constrained in the number of cell division they can undergo giving rise to fleshy macroscopic thallus with considerable body and cellular differentiation formed by typical coalescence of the branched filaments making up a multiaxial body. Such bodies are called **pseudoparenchymatous.**

6.2. Evolutionary trends in red algae

Three patterns of Golgi association; Different patterns of achieving different states of nucleus (uni or multi) & ploidy levels during development; Several methods of establishing intercellular connections through cellular fusions and at least seven different types of plugs on the basis of ultrastructure; Five distinct patterns of mitosis, differing in details of microtubule number and mode and location of formation, number and disposition of gaps in the nuclear envelope, shape and size of the nuclear associated organelles; Three patterns of cytokinesis; Three distinct patterns by which the various reproductive structures are formed in the red algae.

Patterns of Golgi association

One of the most intriguing features of the red algal golgi apparatus is the consistent presence of other organelles in proximity to the cis (forming) face. In most eukaryotes, the cis-Golgi is associated with ER, but in red algae three different patterns are observed:

Association of mitochondrion with Golgi bodies (most common and conspicuous association) as in all florideophytes, some Bangiales, *Porphyridium*, etc.

Association of nucleus with Golgi bodies with no ER involvement as in *Rhodellacyanea*and*Rhodella reticulata*.

Association of only ER with cis face of Golgi bodies as in *Smithora* sp., *Cyanidium* sp. And *Compsopogon* sp.

Because this last state is most common to the eukaryotes it may represent the character state of the ancestral red alga.

Patterns of achieving multinuclearity & different ploidy levels in rhodophytes:

In a number of rhodophytes, the nuclei continue to undergo mitosis without cytokinesis giving rise to multinucleate cells. Red algal nuclei also undergo a process of **endoreduplication** – repeated replication of the entire nuclear genome without intervening mitosis giving rise to polyploidy. Such genome amplification is also known as **nuclear polygenomy**, **endopolyploidy** or **polyteny** and is a common phenomenon in rhodophytes. Such genome amplification is thought to serve as a buffer against mutation of essential genes. Variations exist in terms of number of nuclei and presence or absence of polyploidy in apical and other cells of the filament.

6.3. Ecology of red algae

Most red algae are marine and live attached to some substratum, eg. rocks, dykes, sea-walls, shells or on any other hard substratum within the tidal and intertidal zones of tropical, subtropical, temperate and boreal latitudes.More than 100 species grow as free floating forms or entangled in other vegetation (eg. *Phyllophora* sp.). Certain unattached corallines (eg. *Phymatolithon* sp.) form extensive beds of rhodoliths – slow growing, rounded nodules known as 'tumbleweeds of the sea'. Here calcification helps to prevent cell damage as the nodules roll on sea beds.Only a few species (about 150 species and 20 genera) are found as freshwater forms, eg. *Compsopogon* sp., *Batrachospermum* sp.A unicellular red algae *Porphyridium* is known to be a terrestrial red algae growing on moist or damp soil.Many red algae are found to grow on other animals as epizootics, (*Rhodochorton* sp. on crustaceans) or seagrasses and other algae as epiphytes (*Porphyra* sp. on kelps). A number of red algae are obligate epiphytes on other seaweeds (*Polysiphonia lanosa* on *Ascophyllun nodosum*) or non obligate epiphytes (*Achrochaetium-Rhodochorton* complex). A unicellular form of the genus *Porphyridium* is found to live as a photosynthetic endosymbiont within large foraminifera. Some red algae are nuisance growth formers and some are even regarded as invasive alien species (eg. *Acanthophora spicifera* in Hawaii introduced from Guam).

About 10% of the red algal species are semi or complete parasites of other red algae. The semi parasites show reduced photosynthetic pigments (eg. *Choreocolax* on *Polysiphonia*). The complete parasites show complete lack of pigmentation and reduced bodies (eg. *Harveyella*).Parasitic red algae can be either adelphoparasites or alloparasites. Adelphoparasites are closely related to, or belong to the same family as their hosts and constitute 90% of parasitic red algae. Alloparasites are not closely related to their hosts.

Among the rhodophytes we find the deepest growing photosynthetic eukaryote – a crust like coralline – this record setting rhodophyte lives on 210m deep sea-mounts near San Salvador, in the Bahamas, where irradiance is only 0.0005% of surface levels.Some red algae survive in Arctic & Antarctic waters, where they are covered by 2m of sea-ice for 10 months of the year.The cyanidophytes grow in acidic hot springs.Some red algal forms are found in hot (30ºC – 50ºC) and metal rich mine drainages.Though red algae are intolerant to salinity changes but most of them can tolerate high salinities of the marine environment.

Survival Strategies of Rhodophyta

A number of red algae are capable of producing halogenated terpenoids, alkaloids, and other secondary compounds that may function to inhibit herbivory or have antimicrobial properties. The red algae also exhibit toughness of the outer body which becomes a major hindrance to edibility of the same. Calcified red algae and low growing crusts appear to be more resistant to herbivory than non calcified and erect forms.Herbivory resistance and environmental persistance increase the capability of the calcified and crustose red algae to live very long. An individual of *Clathromorphum nereostratum* of Alaska was estimated to be 700 years old – the oldest living alga.

Responses to drought and osmotic stress

Many red algae are highly sensitive to dehydration and a number of growth habits have been interpreted as adaptations that favor maintenance of a well-hydrated state.Many rhodophytes are obligate understory species, living beneath a cover of larger brown seaweeds, which provides a high humidity habitat. Corallines are particularly sensitive to drought stress and tend to inhabit tide pools rather than nearby surfaces subject to drying during low tides.A number of red algae occupy shorelines including mangrove forests

where they are alternatively submerged or exposed or estuaries where they experience mixing of fresh and sea water creating severe osmotic stress.

Under such conditions of changes in salinity the organisms are capacitated to adjust the levels of ions like sodium, potassium and chlorides; carbohydrates like floridoside and digeneaside; and amino acids to restore normal turgor pressure. Osmolytes that contribute to such adaptations include mannitol, floridosides, D-sorbitol, dulcitol, etc. *Bangia atropurpurea* by use of floridosides can tolerate exposure to air for as long as 15 consecutive days and then revive rapidly upon resubmergence. *Bostrychia* – a common mangrove inhabitant uses D-sorbitol or dulcitol in warm waters as its active osmolyte. *Caloglossa leprieurii* – another mangrove inhabitant accumulates potassium and chloride ions and actively extrudes sodium to adjust osmotic conditions within cells.

7. Chlorophyta

The Chlorophyta or the green algae are a large and morphologically diverse group, including non-motile and motile unicells, colonies, branched and unbranched filaments, and blade-like thalli.Chlorophycean algae are especially abundant in freshwater but also occur in terrestrial habitats. The class is characterized by closed mitosis during cell division and diverse configurations of the flagellar apparatus of motile cells.The class is also characterized by the presence of chl. a and b, and forms starch in the chloroplast, usually in association with a pyrenoid. The Chlorophyta thus differ from the rest of the eukaryotic algae in forming the storage product in the chloroplast instead of in the cytoplasm.

7.1. Diversity of forms and habitats

Most of the part of the land is covered over either by fresh water or sea water. Besides, several other algae are found in somewhat drier conditions. They are found on the trunks of trees, on telephone wires, on rocks, on walls, in hot springs and in several other unusual habitats. Here some of the algae have been classified according to their habitats.

1. Hydrophytes:

They are more or less completely submerged or free floating on the surface of the water. The hydrophytes may be subdivided into following heads.

(i) Benthophytes:

Several fresh water and marine algae are found in attached condition. The fresh water algae such as *Chara, Nitella*, *Cladophora*, etc., are found attached to some substratum in the bottom of the water. Many marine chlorophytes are found in attached condition to some substrata in the sea.

(ii) Epactiphytes:

Such algae grow along the shores of lakes and ponds, and may be delimited from benthophytes with some difficulty. The most important fresh water forms are – *Oedogonium, Chaetophora*, some species of *Spirogyra* and *Mougeotia*.

(iii) Planktophytes:

The algae which float on the surface of the water are called 'planktophytes'. They may be of two types, 'i.e., (a) euplanktophytes (b) tychoplanktophytes.

(a) Euplanktophytes:

They are never attached, and from the very beginning are free floating, e.g., *Cosmarium, Closterium, Scenedesmus, Pediastrum, Chlamydomonas, Volvox*, other Volvocales and some members of Chroococcales. The above given forms are fresh water in habit.

(b) Tychoplanktophytes:

In the beginning, such algae are attached, but later on they become detached and free floating, e.g., some species of *Spirogyra, Zygnema, Cladophora, Oedogonium, Rhizoclonium, Mougeotia,* etc.

(iv) Halophytes:

The algae occur in saline waters are known as 'halophytes'. The most striking examples are *Dunaliella* and *Chlamydomonas* which occur in salt lakes, the species of Scenedesmus, Pediastrum, are found in saline waters; the species of Enteromorpha are found in inland estuaries; many species of Ulvales, Ulotrichales, Conjugales, are found near the sea in astuaries.

(v) Epiphytes:

Many algae are found upon other living plants and bigger species of algae. *Bulbochaete, Oedogonium* and *Microspora*, are found as epiphytes upon larger species of

Oedogonium, Cladophora, Rhizoclonium, Vaucheria and *Hydrodictyon* species. *Coleochaete nitellarum* is epiphytic upon species of *Chara* and *Nitella*.

Some of the species of *Coleochaete* are epiphytic upon some grasses grown on the banks of the ponds and the hydrophytes such as—*Vallisneria, Typha, Ipomoea* and several other aquatic plants.

(vi) Epizoophytes:

Certain algae are found on living aquatic animals such as turtles, mollusc shells, fishes, etc. Species of *Cladophora* grow upon mollusc shells. Protoderma and Basicladia occur on the back of turtles. *Characiopsis* and *Characium* occur on the posterior and anterior legs of Branchipus respectively.

2. Edaphophytes:

Such algae are also called terrestrial algae. They are found upon or inside the surface of the earth. They can be (i) saphophytes and (ii) cryptophytes.

(i) Saphophytes:

They are surface algae, *Oedocladium, Fritschiella* and many others are met with upon the surface of the wet soil.

(ii) Cryptophytes:

Such algae are subterranean in habit and occur inside the soil.

3. Aerophytes:

Such algae are aerial in habitat. They are found upon the trunks of trees, walls, fencing wires, rocks, and animals and so many other aerial substrata.

(i) Epiphyllophytes:

Such algae are epiphytic upon leaves of trees. Species of *Trentepohlia* are commonly found upon the bark of trees. They also occur upon rocks and fencing wires.

(ii) Epiphloephytes:

These algae grow on the bark of trees mixed with many mosses and liverworts.

(iii) Epizoophytes:

These algae are found even on the bodies of land animals. Certain Chaetophorales are found even on the hairs of sloth.

(iv) Lithophytes:

Many algae grow on the rocks and walls. The species grow on the walls in rainy season and the whole wall becomes black spotted. Many algae are also found on wet rocks.

4. Cryophytes:

These algae are found on ice and snow. These algal forms cause red snow, green snow, yellow snow, yellowish green snow and violet snow. In European countries, especially in arctic region the green snow is caused by *Chlamydomonas*, *Ankistrodesmus* and *Mesotaenium*; red snow is caused by species of *Chlamydomonas*.

5. Symbionts or endophytes:

Many algae grow in symbiotic association of other plants. The most striking example of symbiosis are lichens, here the algae are found in symbiotic association of fungi. Some green algae, e.g., *Chlorella, Palmella*, etc., are also found as symbionts in lichens.

Besides, several algae are endophytes in the tissue of other plants.

6. Endozoophytes:

Certain algae occur inside the body of animals. *Zooxanthell*a is found inside fresh water sponges; *Zoochlorella* is found inside Hydra viridis.

7. Parasites:

Certain algae are parasites upon other plants. The most striking example is *Cephaleuros virescens* which causes the havoc of tea foliage in Assam and neighbouring areas, called 'red rust of tea'.

8. Fluviatile algae:

Such algae are found in rapidly flowing waters; *Ulothrix* occurs in mountain falls.

7.2. Evolutionary trends of green algal lineages

Green Algal Relationships

A monophyletic group that has diversified into a wide variety of body types.Since long, green algae were classified according to their structural variations.Today we know that green algae have undergone extensive parallel evolution of body forms. As a result, species of the same body type are not necessarily closely related, and also close relatives may have diverse body structures.Relationship status of various forms are thus derived from life history studies, ultrastructural analyses, comparative biochemistry, and molecular features.

Earlier regarded to be close relatives of red algae.Recent multigene analysis did not find support for monophyly of the two groups and now it is suggested that the two might have independently acquired plastids whose genetic similarities arose via parallel adaptive processes.Now it is proposed that together with the land plants, the green algae form a monophyletic group that is known as Viridiplantae/ Chlorobionta/ Chlorobiota/

Chloroplastida.The green algae display both unifying features reflecting common origin and traits whose variation reflects evolutionary diversification.

Major Green Algal Lineages

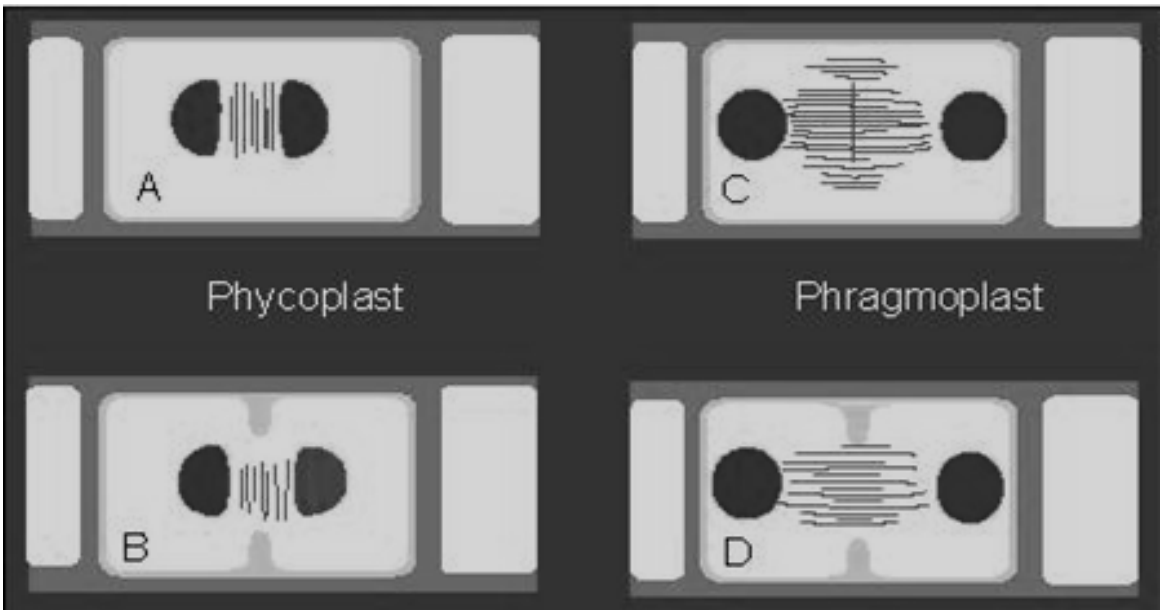
Despite their unifying features, green algae display substantial variations that reflect evolutionary diversification.Current hypotheses on green algal evolution posit the early divergence of two discrete lineages: the **Chlorophyta** and **Streptophyta**.Ultrastructural, biochemical, and molecular sequence evidence suggests that the earliest diverging modern green algae are unicellular forms known as **Prasinophytes**.The prasinophytes do not form a monophyletic group but rather consist of a number of clades.**Prasinophytes** are related to the two larger clades that include unicellular and multicellular forms – the **Streptophytes** (or charophytes) & the **Chlorophytes** (known as UTC clade).

Streptophyta

Informally known as streptophytes, formally Streptophyta, includes all land plants and their closest green algal relatives. Streptophyta is presently treated as infrakingdom within which there exists a phylum **Charophyta**. Charophyta also includes the land plants as a class – Embryophyceae along with related algae.The green algae most closely related to land plants are grouped under class **Charophyceae**.Modern charophyceans primarily occupy freshwater habitats, though some are terrestrial.They include the charalean, coleochaetalean and zygnematalean algae including the desmids.

Chlorophytes

Includes both unicellular and multicellular forms, formally called Chlorophyta, though the term chlorophyta in general includes all green algae. Chlorophyta includes classes Ulvophyceae (earliest diverged class), Trebouxiophyceae and Chlorophyceae, together referred to as **UTC clade** or the **Core Chlorophytes**.The core chlorophytes are characterized by a new mode of cell division that is mediated by a phycoplast (i.e., a system of microtubules that develops parallel to the plane of nuclear division), which was subsequently lost in the Ulvophyceae.Ulvophyceaens primarily occupy marine habitats & include common seaweed genera – *Ulva* & *Codium*.Trebouxiophyceans are mostly freshwater or terrestrial & include the common genera *Chlorella* and common lichen component *Trebouxia*. Chlorophyceans include *Volvox* and *Chlamydomonas* and many other freshwater green algae.



Core Chlorophytes/ UTC Clade

The UTC classes are species-rich and morphologically and ecologically diverse. Ecophysiological adaptations have most likely led to the success of the Chlorophyceae and Trebouxiophyceae in freshwater and terrestrial environments, while the Ulvophyceae mainly diversified in coastal ecosystems.Marine versus freshwater lifestyles also coincide with differentiations in life histories. Whereas the marine Ulvophyceae mainly have life cycles involving an alternation between a free-living haploid, gametophytic and a free-living diploid, sporophytic multicellular generation, most freshwater green algae have a haploid vegetative phase and a single-celled, often dormant zygote as the diploid stage. In terrestrial members of the core chlorophytes, sexual reproduction has rarely been documented.

Resolving the phylogenetic relationships among and within the UTC classes can provide important insights into the evolution of the ecophysiological, life history and morphological traits of the UTC classes, but molecular phylogenetic studies till date based on 18S sequences, chloroplast and mitochondrial multigene data have yielded ambivalent results. The monophyly of the Trebouxiophyceae and Ulvophyceae has not been unequivocally demonstrated. The unstable relationships exhibited among these three classes most likely result from their antiquity and the short time span over which they diverged from one another. The fossil record indicates the presence of the classes in the mid-Neoproterozoic and molecular clock estimates place the UTC divergence in the early Neoproterozoic.

7.3. Salient features of different classes of chlorophytes

	Mitosis	Cytokinesis	Habitat	Life History
Prasinophyceae	Variable	Furrowing	Marine	Zygotic meiosis
Ulvophyceae	Closed, persistent spindle	Furrowing, some with phragmoplast, cell plate	Marine or terrestrial	Zygotic meiosis/ alternation of gen./gametic meiosis
Trebouxiophyceae	Semi-closed, non persistent spindle	Furrowing	Freshwater or terrestrial	Zygotic meiosis
Chlorophyceae	Clased, non persistent spindle	Furrowing, phycoplast, some with cell plate	Freshwater or terrestrial	Zygotic meiosis
Charophyceae	Open, persistent spindle	Furrowing, some with phragmoplast, cell plate	Freshwater or terrestrial	Zygotic meiosis

8. Phytoplankton

Term first defined by Hensen. Comes from the Greek word ‘planktos’, which means to wander or to drift.Photosynthetic organisms that float freely with the current in oceans, rivers and in other water bodies on or near the surface of the water. Individual plankton are referred to as plankter and the study of plankton is termed as planktology. Primary producers of the aquatic ecosystems and the first link in the aquatic food webs. The most significant contribution to the primary productivity of the world comes from the microscopic, free-floating forms, collectively called phytoplankton, that are the dominant primary producers. They are capable of certain amount of independent movement and can swim large distances vertically in a single day (a behaviour called diel vertical migration). Their abundance and distribution are strongly dependent on factors such as ambient nutrient concentrations, the physical state of the water column, and the abundance of other plankton. Important phytoplankton groups include cyanobacteria,

cryptomonads, haptophytes, several heterokont groups, dinoflagellates, euglenoids and green algae.

8.1. Types of phytoplankton

Haeckel (1890) critically defined the term 'plankton' to encompass all drifting organisms including:

- plants - **phytoplankton**
- animals - **zooplankton**
- bacteria – **bacterioplankton**

These three groups are also called functional groups.

How do we classify Aquatic Organisms?

Based on their adaptation in the aquatic environment they are classified as

- **Nekton** - the organisms that actively swim in the water (aquatic mammals, fish, adult crustaceans)
- **Neuston** - the organisms that float on top of the water forming surface films (beetles, protozoans, spiders)
- **Benthos** - the organisms at the bottom of a body of water (adult sea stars, sea cucumbers)
- **Plankton** – the organisms that drift with the current

Based on their life forms these may be classified as

- Holoplankton – Planktonic for the entire life cycle (diatoms, dinoflagellates, copepods)
- Meroplankton – Planktonic for only a part of the organism's life cycle (larvae of sea urchins, sea stars, crustaceans, marine worms & most fish species)

Based on differences in terms of size

- Megaplankton (20-200 cm) jelly fish
- Macroplankton (2-20 cm) krills
- Mesoplankton (0.2mm – 2 cm) copepods
- Microplankton (20 – 200 µm) most phytoplankton, protozoans
- Nanoplankton (2 – 20 µm) small diatoms, small flagellates
- Picoplankton (0.2 – 2 µm), mostly bacteria
- Femtoplankton <0.2 µm, marine viruses

8.2. Phytoplankton Ecology

It is the study of the relationship of planktonic algae with the physical, chemical and biological factors that influence their growth, distribution and abundance.

Importance of Phytoplankton Ecology

- They provide the basis for much of the aquatic food chain
- They have a substantial impact on the global environmental balance
- Phytoplankton diversity is considered to be one of the determinants of water quality using biological means or obtaining data on the effect of pollutants.
- They can form nuisance blooms and sheer abundance can lead to
    - anaerobic conditions (algal blooms);
    - toxin production leading to different types of poisoning in aquatic organisms and also human being
- Provide an educational complement to terrestrial ecology.
- Are used to identify 'natural regions' of the oceans.
- May be used to trace climatic changes in different geological periods.
- Applied aspects of phytoplankton research have gained importance - Use as bioindicators.

Nutrient levels

Based on nutrient levels an area may be categorized into

Oligotrophic: Areas characterized by low primary productivity, low nutrient levels, low algal abundance and reasonably clear waters.

Mesotrophic: Areas characterized by intermediate levels of productivity. These areas commonly have clear water and beds of submerged aquatic plants with medium levels of nutrients and greater diversity of algae and plankton.

Eutrophic areas and Eutrophication Process: Areas characterized by nutrient loading and proliferous plant production, usually caused by excessive discharge of nutrients, specially phosphorus and nitrogen in the form of PO_4 and NO_3 primarily from agricultural runoffs, sewage discharges and fish farms.

Eutrophication Effects

- increased biomass of phytoplankton/ benthic & epiphytic algae
- toxic phytoplankton species
- changes in macrophytic species composition and biomass
- decrease in water transparency

- taste, odour and water treatment problems
- DO depletion
- increased evidences of fish kills
- loss of desirable fish species
- decrease in aesthetic values of water bodies

Three most important ecological effects of eutrophication:

- decreased biodiversity
- changes in species composition & dominance
- toxicity effects

8.3. Algal blooms

Harmful Algal Blooms: HAB concept

Phytoplanktons are normal components of all aquatic environments. When they bloom in significant numbers and produce toxins, these events are termed as HABs.

Cyanobacterial blooms and their controle

- Such blooms occur when cyanobacterial organisms that are normally present in a habitat grow gregariously.
- Mostly occur during summers & within a few days turn the water cloudy.
- Bloom usually floats on the surface in slow flowing waters that are rich in nutrients.
- Such blooms are indicators of -
- high nutrient load; low water acidity; relatively high temperatures; high light intensity; water column stability
- Organisms include – *Microcystis aeruginosa, Anabaena flos-aquae*, etc.
- Control measures include – keeping water bodies low in nutrients; increased aeration for maintaining preventive temperatures and avoid oxygen depletion.

Red Tides

- Red tide is a phenomenon caused by algal blooms during which algae become so numerous that they discolour coastal water bodies.

- Major factors influencing red tide events include warm ocean surface temperatures, low salinity, high nutrient contents, calm sea and rains followed by sunny days during the summer months.

- Red tide algae make potent natural toxins, some of these extremely harmful to larger organisms through the process of biomagnification and bioaccumulation.

8.4. Algal toxins

Some algae produce toxins that sicken and kill other organisms that prey on these algae. Probably this was the reason that these algae were selected for in the evolutionary process since it reduced predation by grazers. Filter-feeding shellfish can accumulate large quantities of these toxins as they filter the algae out of the water. Consumption of the shellfish by man, birds, and animals results in sickness and death.

The important algal groups known to produce phycotoxins are:

- Cyanophyta
- Dinophyta
- Bacillariophyceae
- Prymnesiophyceae

Cyanophyta:

A. Neurotoxins *anatoxin and saxitoxin*

- These block the transmission ofsignal from neuron to neuron.

B. Hepatotoxins *microcystin* and *nodularin*

- These are inhibitors of protein phosphatases.

Bacillariophyceae:

A. Neurotoxin *domoic acid*

- Causes Amnesic shellfish poisoning.
- This disease is caused by planktonic diatoms like *Pseudo-nitzschia pungens*.
- It is characterised by gastric and neurological symptoms including dizziness, disorientation and memory loss.

Dinophyta:

A. Saxitoxins causing Paralytic Shellfish Poisoning (PSP)

- This disease is caused by the dinoflgellate *Alexandrium* sp.
- Poisoning occurs when one ingests shellfish contaminated with PSP toxins causing disruption of nerve function & paralysis. Extreme cases may result in death by respiratory paralysis.

B. Okadaic acid, Macrolide toxins, Yessotoxin & related dinophysis toxins causing Diarrhetic shellfish poisoning

- These toxins are produced by *Dinophysis* sp.
- These are inhibitors of protein phosphatases.
- General symptoms include diarrhea, nausea, vomiting, abdominal pain and cramps. DSP is generally not lethal.

C. Ciguatoxin, Maitotoxin & Brevetoxin causing Ciguatera fish poisoning

- All of these compounds are ion-channel disrupters, increasing the permeability of the cell membrane to positive ions and causing membrane depolarization.
- Brevetoxins are also produced by Raphidophyceae

D. Spirolides, Gymnodimine, Pteriatoxin and Pinnatoxin causing Spirolide poisoning

- This group of toxins are “fast-acting toxins”, observed to cause death of mice within minutes after oral application.

Prymnesiophyceae:

Prymnesin that causes hemolysis of red blood cells.

9. Let’s sum up

- The term ‘algae’ is applied to a phylogenetically artificial cluster of unrelated or distantly related groups of organisms
- The chloroplasts and mitochondria of what we now know as phototrophic eukaryotes have had independent origins, the chloroplasts from cyanobacteria (blue-green algae) and the mitochondria from alpha-proteobacteria. Eukaryotes are, therefore, composite or chimeric organisms derived from several sources.
- According to Lee system of classification (1989), there are four distinct evolutionary groups.

1st Group: Include the Cyanophyta & Prochlorophyta

2nd Group: Algae with a chloroplast surrounded only by two membranes of the chloroplast envelope. Include the Glaucophyta, Chlorophyta & Rhodophyta.

3rd Group: Algae that have the chloroplast surrounded by one membrane of chloroplast endoplasmic reticulum. Include the Euglenophyta and Dinophyta.

4th Group: Algae with two membranes of chloroplast endoplasmic reticulum surrounding the chloroplast. Include the Cryptophyta, Chrysophyta, Prymnesiophyta, Bacillariophyta, Xanthophyta, Eustigmatophyta, Raphidophyta & Phaeophyta.

- Cyanobacteria are the only prokaryotes that perform oxygenic photosynthesis. It evolved 2.7–2.2 billion years ago and therefore had ample time to diversify and adapt to newly evolving niches that emerged on the Earth.

- The red algae are unique among eukaryotes in lacking both flagella & centrioles during their entire life cycle. Most red algae are marine and live attached to some substratum.

- The Chlorophyta or the green algae are a large and morphologically diverse group. The class is also characterized by the presence of chl. a and b, and forms starch in the chloroplast, usually in association with a pyrenoid.

- The most significant contribution to the primary productivity of the world comes from the microscopic, free-floating forms, collectively called phytoplankton, that are the dominant primary producers. Phytoplanktons are normal components of all aquatic environments. When they bloom in significant numbers and produce toxins.

10. Suggested Readings

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11. Assignments

1. Outline the similarities between cyanobacteria and the chloroplasts that validate 'The Endosymbiotic Theory'.
2. Justify the re-inclusion of Prochlorophyta in Cyanobacteria. Explain the importance of Horizontal Gene Transfer in Cyanobacterial evolution. Write a short note on different nitrogen fixing mechanisms in the Cyanobacteria.
3. Write a note on intracellular and extracellular associations of the cyanobacteria with suitable examples.
4. Describe the sequence of events in the evolution of two membranes of CER that was initiated with the phagocytosis of a eukaryotic photosynthetic algae.
5. Write an elaborate note on ultrastructural diversity of Rhodophytes.
6. Validate the separation of the Prymnesiophytes from Heterokontophytes.
7. How will current changes in climate influence the diatoms, their frustule morphology and the silica cycle?
8. What are kleptoplastids? Name the different modes of heterotrophy in the Dinophytes and explain their unique prey-capture adaptations with examples.
9. What is Silicification Index? What is the observed evolutionary trend of this Index? Give an account of the basic patterns of wall ornamentation in diatoms.
10. Give a classification of the phytoplankton based on size. Explain the causes and effects of Harmful Algal Blooms (HABs) and cyanobacterial blooms.

COURSE – BOHCT1.2
BIOLOGY OF ALGAE, BRYOPHYTES AND PTERIDOPHYTES
Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**
Group – B (Biology and Diversity of Bryophytes)

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1. Introduction

Bryophytes are an informal group consisting of three divisions of non-vascular land plants (embryophytes): the liverworts, hornworts and mosses. They are characteristically limited in size and prefer moist habitats although they can survive in drier environments. Bryophytes produce enclosed reproductive structures (gametangia and sporangia), but they do not produce flowers or seeds.

1. Course Objectives

After completion of the module you will be able to:

- Provide an outline of classification of the liverworts, mosses and hornworts..
- Evolutionary significance and interrelationships; recent concepts on evolution of the three lineages (liverworts, mosses and hornworts).
- Describe economic and ecological significance of bryophytes.

3. General classification: Criteria, recent trends and outline of classification of the liverworts, mosses and hornworts

Bryophytes (mosses, hornworts, and liverworts) can be found in all ecosystems of earth. The diversity of bryophytes increases at tropical and subtropical latitudes. A term commonly used for this group is nonvascular plants. Bryophytes lack xylem and phloem, the conductive tissues of vascular plants (= tracheophytes). Bryophytes and tracheophytes are monophyletic and collectively called embryophytes. As the name implies, there is an embryonic stage in these organisms as contrasted with their closest relative of green algae.

All embryophytes have a life cycle that involves an alternation between sporophyte and gametophyte generations. In vascular plants the sporophyte is the dominant, obvious stage; the grasses, flowering trees, ferns, and conifers we admire are all sporophytes. They are the generation of the plant that produces the spores that will develop into the inconspicuous gametophyte stage.

Bryophytes, on the other hand, have a conspicuous gametophyte stage (in most cases) that forms the green mats and tufts we see. Unlike tracheophytes, it is the gametophyte stage that is composed of either stem or leaves or flattened ribbon-like thallus. The leaves of bryophytes

are structurally very different from those of tracheophytes; they are generally one cell layer thick. The sporophyte of bryophytes is dependent on the gametophyte for water and nutrients and in most cases conspicuous. Unlike the multisporangiate indeterminate sporophyte of the tracheophytes, bryophyte sporophytes are unisporangiate and determinate; once spores are shed from the often elaborate sporangium.

Bryophytes, while very simple, exhibit a great diversity in growth form and habitat. There are bryophytes that can withstand extended periods of desiccation and others that are aquatic. One can find them on virtually every substrate: rocks, soil, tree bark, decaying wood, and even cars and other synthetic materials. Due to their diminutive stature and few human applications they have remained a largely overlooked group of organisms. Mosses, liverworts, and hornworts are the most ancient lineages of plants and can offer some clues about early plant evolution.

Bryophytes include three lineages:

Phylum Bryophyta – Mosses

Phylum Marchantiophyta – Liverworts Phylum Anthocerotophyta – Hornworts

Here is a brief outline of classification of Bryophyta

Phyllum Bryophyta

☐ Class Bryopsida

☐ Class Tetraphidopsida

☐ Class Polytrichopsida

☐ Class Oedoppodiopsida

☐ Class Andreaeopsida

☐ Class Andreaeobryopsida

☐ Class Sphagnopsida

☐ Class Takakiopsida

Phylum Marchantiophyta

☐ Class Haplomitriopsida

☐ Class Jungermanniopsida

☐ Class Marchantiopsida

Phylum Anthocerotophyta

Phylum- Bryophyta

Mosses (Phylum Bryophyta) may be found all around the world and inhabit diverse habitats. Habitats range from exposed rock types, shaded coniferous forests, to bogs. Mosses can be

distinguished from liverworts (Phylum Marchantiophyta) and hornworts (Phylum Anthocerotophyta) by a number of gametophytic and sporophytic features.

The gametophytic generation of mosses is characterized by its organization and morphology of its tissue. Much like tracheophytes (commonly known as vascular plants), mosses have leaves. Leaves are unistratose and radially arranged around a stem.

Mosses may be acrocarpous, with their shoots grow vertical in tufts with the sporophyte terminal on the shoot. Mosses may also be pleurocarpous, with their shoots grow laterally in mats with the sporophyte on specialized buds or branches. Species can be distinguished by means of the features of their leaves. A commonality to leaves is the presence of chloroplasts, and lack of oil bodies. The conducting tissues of Bryophyta are not lignified, thus are not tracheophytes. Nevertheless, mosses contain conducting tissues known as hydroids, for water transport. Mosses rarely contain leptiods; these cells are responsible for nutrient conduction. Hydroids and leptoids may be found in the stem or leaves, depending on the species.

The sporophytic generation is more complex than liverworts and hornworts and generally defines the phylum. Growth of the sporophyte is determinate and there are three main parts of the sporophyte known as the foot, seta, and sporangium. The seta supports and elevates the sporangium to aid in spore dispersal. The seta elongates to elevate the sporangium before the sporangium and spores are fully mature. The calyptra, a protective tissue around the sporangium arising from the venter of the archegonium, expands after seta elongation and falls off the sporangium when the sporangium is mature. The spores are released when the sporangium is mature and typically dry. An operculum, at the apex of the sporangium, falls off to expose peristome teeth that aid in the dispersal of spores. Peristome teeth arrangement and morphology.

Aid in distinguishing certain taxonomic categories. Finally, stomata are typically present on the walls of the sporangium. Stomata aid in gas exchange and are also found on tracheophyte.

Phylum Marchantiophyta

Phylum Marchantiophyta are commonly known as the - liverworts due to the shape of hepatic liverworts resembling the shape of the liver. According to an old medical doctrine, this resemblance indicated liverworts could cure illnesses of the liver. Liverworts are located on nearly every continent, inhabiting a diverse array of ecosystems. They may be found in harsh environments that many organisms find difficult to live and reproduce, however liverworts are not generally found in salty aquatic environments such as the ocean. Further, some species of Marchantiophyta rely on the photosynthate produced from other tracheophytes and

mosses, as well as form symbiotic relationships with different groups of fungi (typically located on their rhizoids). However, this symbiotic relationship is not specific to species of liverworts or fungi, thus the two groups did not co-evolve together.

The morphology of the free-living gametophytic generation varies within Marchantiophyta. There are many forms of gametophyte ranging from thalloid to leafy, and large to small. The isodiametric cells of the gametophore contain chloroplasts, and typically have oil bodies. The oil bodies are true membrane-bound organelles that vary in size, shape, number, color, and chemical composition among species. When liverworts are dried (such as for storage in a Herbarium) the oil bodies degrade at varying rates over time. The function of oil bodies is currently unknown, but they may serve to deter herbivores or aid in protection from the cold or UV rays.

There is not a well-developed conducting system composed of hydroids and leptoids, however, endohydric water conduction does occur in some groups. At the apical regions of active mitotic cell division, mucilage is produced to protect against dehydration. Rhizoids are smooth and unicellular and serve to anchor and conduct water and minerals.

The sexual reproductive structures are generally different from those of Bryophyta. The archegonia are either contained within a modified region of the thallus known as an involucre (in thalloid liverworts) or a modified leaf known as the perianth (in leafy liverworts). There are no paraphyses present in either the female or male gametophytic regions.

The sporophytic generation helps to classify Marchantiophyta. The unbranched sporophyte matures within the protective calyptra and female gametophyte. Once mature, the sporangium is elevated by the seta. Elongation occurs via water intake of thin-walled parenchymal cells within the seta. The increase in turgor pressure causes the cells of the seta to elongate (where as seta elongation in Bryophyta is due to cell division).

Within the sporangium there are two types of cells developing from sporanogeous tissues, spore and elater mother-cells. Elater mother-cells develop into a single diploid cell with spiral wall thickenings, known as an elater. Spore mother-cells undergo meiotic divisions to become many haploid cells, known as a spore. The sporangium does not contain stomata, a columella, or peristome teeth. The mature sporophytic generation is short-lived with a brief period of spore dispersal by longitudinal lines of dehiscence on the sporangium. Spores may persist in the harsh environments liverworts may inhabit. When the spores germinate, they develop into a new branched and free-living gametophyte.

Phylum Anthocerotophyta

Anthocerotophyta is the least diverse phylum of the bryophytes, however its distribution is widespread, with Antarctica being the only continent in which they are not found. Most species in this phylum typically grow on moist soil in shaded areas, but some are found growing in exposed sites, while a few others are epiphytic. Hornworts are considered to be ecological pioneers as they often grow in areas where there is little to no competition, such as on mineral soils. Although they may superficially resemble a thalloid liverwort, they have gametophytic and sporophytic features that separates them from other bryophytes seen thus far.

All species have a thalloid gametophyte that is flattened and can occur in the form of a rosette or ribbon. In most genera, the thallus is multistratose in the center and thins out closer to the margins. The cells on the upper surface of the thallus are generally chlorophyllose, while those found in deeper layers do not contain chloroplasts. Most genera of this phylum have a single large chloroplast per photosynthetic as well as a pyrenoid associated with the plastid. Hornworts cluster Rubisco in the pyrenoid, which is a trait that is also seen in algae. These features along with the absence of oil bodies aid in distinguishing these species with those in Marchantiophyta.

The surface of the thallus is generally cutinized; however this cuticle layer does not prevent gas exchange from occurring. The thalli are attached to the substrata by smooth, thin-walled, unbranched and unicellular rhizoids.

While the thallus is primarily composed of parenchyma cells and has little tissue differentiation, there are often intercellular cavities, which are filled with mucilage, on the ventral surface. These mucilage chambers, as seen in this picture, open to the environment due to the presence of stomata-like pores and are frequently invaded by small colonies of blue-green algae called *Nostoc*.

Nostoc are filamentous cyanobacteria that can easily fix nitrogen. They are able to form symbiotic relationships with hornworts in which the alga receives carbohydrates and a protected shelter while the hornwort receives the fixed nitrogen.

Most species within this phylum are bisexual, having both the male and female sexual organ embedded in the upper surface of the thallus. The antheridia, which develop in chambers within the thallus, are derived from a single initial. Furthermore, this initial is capable of giving rise to several antheridia within the same chamber, as seen in this picture. The archegonia, which are also embedded within the thallus, are derived from an initial cell.

While only the upper portion of the neck rises from the surface of the thallus, the lower portion of the neck as well as the egg cell remain beneath the surface. Because the cells that surround the archegonia do not differ from those of the thallus, the sexual organ is not considered discrete.

The Anthocerotophyta are characterized by their long, horn-shaped sporophyte, from which they get their name —hornworts. Although the sporophyte remains attached to its parent, like in other bryophytes, it differs significantly in that it possesses a meristematic region at its base. This feature allows the sporophyte to have indeterminate growth, giving it the ability to differentiate new sporogenous tissues throughout its life. Stomata similar to those found in vascular plants can be found on the sporangium, however, unlike those in higher plants, they do not open or close.

As the sporophyte grows, it not only becomes longer but the spores at different height within the sporangium differ in maturity. The younger spores at the base of the sporangium typically remain in a tetrahedral arrangement, while the mature spores near the tip are separated. Thesporophyte lacks a seta but has a columella, and dehiscence occurs along one or two longitudinal lines, thus the spores are released along the halves as they mature.

Not only are there spores within the sporangium, but there is also the presence of pseudo-elaters. These multicellular structures are filamentous and aid in the dispersal of the spores by changing their shape when dry. Their cells walls can either be thin, spirally thickened or even. Although they resemble the elaters seen in the liverworts, the difference in the cell division patterns sets them apart.

Although asexual reproduction is rare in hornworts, a few species produce marginal gemmae while others are capable of producing perennating tubers, which are able to tolerate some dessication. A simple and effective means of asexual reproduction is by having the older parts of the thallus die off, leaving the younger and disconnected parts to continue to grow.

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4. Phylogeny: Evolutionary significance and interrelationships; recent concepts on evolution of the three lineages (liverworts, mosses and hornworts).

Liverworts

The common ancestor to Marchantiophyta arose immediately after the conquest of land some 450 m.ya. Their evolutionary history is characterized by more recent diversification during the early tertiary period. Approx. 5000 extant species currently recognized, liverworts

compose a diverse lineage of land plants, represented on every continent and in nearly all ecosystems.

Gametophyte plant body thalloid or foliose. Sporangium enclosed by an epidermis lacking stomata and elevated above the maternal protective tissue on translucent seta that elongates after sporogenesis. Capsule lacks columella and sterile cells within the sporangium develop into elaters.

Except for the Blasiales, liverworts lack endophytic cyanobacteria, but most sp. Establish symbiotic association with mycorrhizal fungi.

Although inconspicuous at the landscape level, liverworts form diverse assemblage in moist habitats such as temperate and tropical rainforests, where they thrive on trunks and leaves.

Structure and Development:

Gametophyte:

The long evolutionary history of liverworts is characterized by subsequent modifications in the architecture of gametophyte, unmatched among land plants and the phyllogenetically more derived hornworts. The vegetative body of the liverworts is either flattened into a thallus with little or no elaborate internal differentiation or terete and lined with foliar appendages.

Growth:

Growth occurs through the mitotic activity of single apical cell. The shape of the latter varies and so does the number and orientation of the cutting faces that determine the arrangement of daughter cells contributing to the plant body. The geometry of the apical cell is conserved within the leafy (tetrahedral), complex thalloid (cuneate) and one lineage of simple thalloid i.e. in Metzeriopsida (lenticular), but variable with another lineage of simple thalloids, namely Pelliidae (tetrahedral, cuneate or hemidiscoid).

Gametophytes are branched either dichotomously, sympodially through subapical innovations or monopodially with multiple ramifications occurring along the stems. In some cases, branches are dimorphic, with ventral ones acting as anchoring organs.

Anatomiaclly thallus is lined by a unistratose epidermis, but otherwise lacks much differentiation. In Marchantia, and its relatives, the thalluis is composed of a ventral layer of hyaline storage cells, a dorsal layer of green cells and a unistratose epidermis. The photosynthetic layer includes of green cells and a unistratose epidermis. The photosynthetic layer includes air chambers that open through superficial pores. The reticulate pattern on the dorsal surface of the thallus marks the distribution of air chambers below. Simple thalloid

lacks such conspicuous differentiation. In most of the case a multistratose midrib separates the two unistratose wings. In some taxa strands of elongated water conducting cells which are absent from complex thalloid and leafy liverworts (except Haplomitrium) are formed. In others the thickness of the thallus tapers towards the margin.

The leafy liverworts compose the largest group, including 80% of the taxic diversity. Unlike mosses the leaves develop along three longitudinal parallel lines rather than spiral, and the stem bears two lateral and one ventral rows of leaves. The latter termed underleaves or amphigastria, are either similar (isophylly, e.g. *Haplomitrium*) ir different (anisophylly, e.g. Bazzania, Lejeunea & Porella) in size and from lateral leaves. Underleaves are lacking in many taxa. The insertion of underleaves is always transverse, whereas the lateral leaves are commonly inserted obliquely with the line forming either an acute or obtuse dorsal angle with the axis depending on the orientation of apical cell. In the former case, known as incubous insertion, the forward or upper edge of each leaf overlaps the rear or lower margin of the next higher leaf along the stem. As a result, the upper edge of each leaf is visible from above, whereas the lower edge is obscured by its older neighbouring leaf. By contrast, the front margin of a succubous leaf is hidden by the rear margin of the yonger leaf. Hence only the rear margin is visible from above.

Because leaves in leafy liverworts grow from two to three leaf initial cells (only one leaf initial cell in mosses) leaf morphology is highly variable. Lobed or finely segmented leaves which are unknown in mosses are common among liverworts. The division of the leaf lamina into two or more lobes triggered a vast array of modification of leaf form including functional divergence between the lobes. Unequal leaf lobing characterizes many taxa. In most lineages dorsal lobe is larger (e.g. *Porella*), whereas in others it is smaller than ventral one (*Scapania* or *Douinia*). In two independent lineages of leafy liverwort, one or two lobes of the divoided leaf have been modified into a structure that has been interpreted as a water sac. Since bryophytes are poikilohydric, this would allow the plant to remain physiologically active for a longer time. E.g *Frullania.*

Two microscopic features shared by nearly all liverworts: unicellular rhizoids and oil bodies.Rhizoids are lacking only in Haplomitrium, although they may lost in some aquatic forms of Riccia. Pluricellular in Plagiochila, but only after fungal infections. Rhizoids arise from cauline epidermal cells or from the specialized cells typically confined to the base of underleaves, they occur in fascicles or are scattered. The rhizoids of most complex liverworts are dimorphioc, smooth walled and pegged rhizoids bearing intracellular wall projections.

Whereas unicellular rhizoids are plesiomorphic features shared with hornworts, oilbodies are truly a unique featrure of liverworts among land plants.In contrast to the lipid bodies of other embryophytes oil bodies are true membrane bound organelles. They contain terpenoid oils suspended in a carbohydrate /and/or protein rich matrix. Ninety percent of liverworts develop oil bodies. Their size, shape, number, color, distribution and chemical composition vary among taxa. For instance, complex thalloid liverworts exhibit peculiar oil bodies which are found in scattered idioblastic cells.

Complex thalloid liverworts bear ventral scale s often arrange in two or more rows. These unistratose structures may create capillary spaces essential for external water conduction or prolonged water retention or may protect the apices. Liverworts also produce mucilage.

Multicellular sex organ /gametangia are formed from superficial cells and develop on the thallus surface. In thalloid liverworts they are often clustered in chambers. About two-thirds of liverworts, are dioecious: male and female sex organs occur at distinct individual. Bisexual plant typically carry male and female gametangia on different branches rather intermixed in a single cluster. Paraphyses or modified slime hairs are rarely mixed among gametangia.

Antheridia consist of a stalked and more or less spherical chamber which mostly lacks a differentiated zone of dehiscence. Upon hydration mature antheridia rupture at their apex and release the sperm cells. In some complex thalloid liverworts, the antheridia are concentrated in chambers on the upper surface of receptacles, which oin some species are elevated on a stalk. These stalks likely favour the dispersal of sperm to neighbouring female plants.

Archegonia consist of a venter that is stalked and mounted by a narrow neck. At maturity, the central canal cells disintegrate and the cover cells are released. The distribution of the archegonia parallels that of the male sex organs. The development of archegonia in leafy liverworts is initiated by the apical cell of either stem or branch a condition referred to as acrogyny. By contrast anacrogyny implies derivation of archegonia from lateral cells.The gametangial cluster are typically protected by modified lateral and underleaves, the bracts and bracteoles respectively or by leaf like outgrowth of the thallus.

Sporophyte: The organization of sporophyte into foot an unbranched seta and acapsule is highly conserved. Foot and seta are lacking only inRicciales wherein capsule develops within the thallus. The first division of the zygote is always transverse and yields a hypobasal and an epibasal cell. Subsequent growth never involves an apical cell or an intercalary, meristem as in other bryophytes. The significance of the two initial cells in the development of foot seta and sporangium varies among lineages, with the hypbasal cell typically forming foot and seta

in complex thalloid liverwotrts but solely a haustorium consisting of elongated cells around the foot in *Haplomitrium* and various simple thalloid liverworts.

Absorption of nutrients and water may be enhanced by modifications of the placental cell marking the junction of the two generations, in particularly by wall ingrowths on one or both sides which increases the surface area for exchange.

Cells of the seta are entirely thin-walled parenchymatous lackimng any differentiation. The seta elevates the capsule above the protective gametophytic tissue only after spore maturation.

In complex thalloid liverworts, which elevates their spores high above the thallus via archegoniophore substantial elongation of the seta is not necessary and consequentlky the seta remains very short. Similarly in Porellales the seta is generally very short as an adaptaion to the epiphytic habitat of the majority of this order and merely ensures that the capsule dehisces beyond the protective tissue.

The capsule consists of a wall derived from the amphetheciumand a sporangium of endothecial origin. A columella is absent. Each cell of the sporogenous tissue divides to yield a saprocyte (spore mother cell) and an elaterocyte (elater-mother cell).

The sporophyte is green and photosynthetic when young. The elongation of the seta pushes the capsule against the calyptras and causes it to tear. The calyptras thus remains completely attached to the maternal gametophyte and never form a hood covering the sporangium as mosses.

Asexual Reproduction:

Asexual propagation involes the development of specialized propagules. Many species develop propagules along the leaf or thallus margins. In others propagules arise from the leaf or thallus margins. In others propagules arise from the thallus surface. In rare cases, they are formed internally within the cauline parenchyma or simply by the development of a new likely resistant cell wall within existing epidermal cells. In Marchantia and Lunularia discoid gemmae aggregated in small cups on the surface of the thallus. The discs are stalked and dispersed by raindrops imploding the cups. Perennating tubers are also involved to aestivate the gametophyte.

Mosses

Mosses (Division Bryophyta) are small plants confined to humid habitats especially where short growing seasons limit plant growth, mosses may dominate the vegetation. In temperate and tropical rain forests, mosses compose luxuriant epiphytic communities that play

important ecological functions especially in terms of water and nutrient flow. About1200 sps. Are currently recognized reflecting a broad morphological diversity.

Gametophyte:

The architecture of the vegetative body of mosses is fundamentally modular: it is built from blocks of cells assembled into axes and multiple axes are joined to form a stem or branch system. The series of divisions that gives to all cells composing an axis typically begins with the single apical cell dividing along each of its three cutting faces to yield derivatives. These daughter cells then undergo a suite of divisions.The resulting blocks of cells then undergo a suite of divisions. The resulting blocks of cells also called metamers, are assembled into a module, which is the axis. The module is always terete and typically bears leaves. The gametophyte is neaver thalloid at maturity and only in Sphagnum and Andreaea are dorsi-ventral thalli or protonemal appendages developed.

The anatomy of the stem or branch is typically simple consisting of a mostly unistratose epidermis that always lack a stomata, a cortex of parenchymatous cells and in some cases, a centralstrand of water conducting cells. The epidewrmal cells are often narrower than the cortical cells and their wall is distinctly thickened, at least relative to the size of the cell lumen. The walls are typically pigmnetd giving the stem a reddish coloration. In a few mosses, primarily hygrophytes the epidermal cells are thin walled and somewhat inflated forming a so clled hypodermis, which may function in external water conduction. Many pleurocarpous mosses produce paraphyllia, slightly branched photosynthetic epidermal outgrowths they may also contribute to the movement of water over the stem surface. The complexity of these paraphyllia varies in terms of degree of branchging size and surface ornamentation, offering characters suitable for distinguishing species.Unbranched hyaline filaments are found only in leaf axils, hence their name, axillary hairs. They arise from mthe leaf initial secreting a mucilage ythat prevents the dehydration of developing organ. The hairs are deciduous and are best observed in the axil of juvenile leaves The epidermis also develops small photosynthetic appendages called pseudoparapohyllia that are confined to the periphery of the branch primordium that they protect. The axial cells are differentiated to form a central conducting strand. These cells are hydroids are highly elongated tapered end (except Takakia) and lack cytoplasmic content at maturity. Conducting strands are lacking in Sphagnum, Andreaea and Andreaeobrym and are vbest developed in Polytrichopsida. Photosynthates are distributed either through parenchyma cells or through leptoids and are best developed in Polytrichopsida.The gametophyte is anchored to the substrate by

mmulticellular and generally uniseriate rhizoids. These arise from the epiderma;l cells of the stem or branch or eveb from the costa. The rhizoids are absent in Takakia and restricted to the protonemal stage in *Sphagnum.*

The leaves of the mosses are always sessile they lack a petiole and the lamina is thus inserted directly on the stem at its base. Most mosses display however, heteroblastic leaf development, with the leaves at the base of the module often lacking bthe modifications characteristics of the mqature leaves found high up the axis. Dimorphism marks the leaves around the stem of a few species with either the ventral or dorsal leaves being smaller than the lateral ones.

Distribution of sex organs: Individual mosses may produce either both sexes or only one (dioicy). Sexes in hermaphrodite individuals occur in distinct clusters (autoicy) or are grouped together in which case the male sex organs may be surrounding (Paroicy) or be intermixed (synoicy) among the female gametangia.

Sexual dimorphism is uncommon, but extereme in phyllodioicous mosses in which the male plants are dwarf, consisting of only a few leaves around the sex organs and grow exclusively as epiphytes on the more robust female plants. Antheridia and archegonia originate at the apex of the stem or branch forming inconspicuous terminal clusters. In some moisses isolated archegonia are formed in the axils of leaves. Groups of gametangia are typically surrounded by differentiated leaves that protect the delicate sex organs and also the young embryo. Clusters of archegonia and their associated leaves are called perichaetia whereas similar male structures are referred to as perigonia.

The cylindrical antheridium is stalked and contains numerous sperm cells. Archegonium is stalked consists of a single egg and mounted by a narrow neck whose central canal cells disintegrate at maturity.

Sporophyte:

The fundamental architecture of the moss sporophyte is simple similar to that of liverworts: an unbranched seta anchored into the maternal gametophyte by foot and carrying a single terminal sporangium.

The seta serves to raise the capsule above the perichaetial leaves protecting the developing sporophyte. An intercalary meristem and thus actual seta is lacking only in two genera Sphagnum and Andreaea.In these lineages elevation of the cpsule is accomplished by gametophytic tissue into a stalk called pseudopodium.

Early in the ontogeny of the sporangium two tissues are differentiated. The four inner central cells form the endothecium. Its developmement lags behind that of amphithecium, which developes from the outer rings and undergoes repeated divisions yielding new layers of multiple cells. The amphithecium froms the capsule wall and the underlying parenchyma as well as in the region above the annulas, the teeth around the capsule mouth. The endothecium forms the columella and the spore sac although in Sphagnum this role is taken by amphithecium.

The columella is a sterile axis that extends through the sporogenous regfion and connect to the operculum except in basal taxa such as *Sphagnum* and *Andreaea* in which it is dome shaped or in *Archidium* which lacks a columella altogether.The capsule typically bears stomata dehisces subapically through the loss of a lid and bears one or two rings of teeth lining the mouth. Stomatal guard cells are rather elongated and kidney shaped. The pore is round or elliptical. Occasionally the mother guard cell fails to complete its division and the stoma is then defined by a single ring like cell. Single guard cells are found in members of the Funariaceae.

The peristome is a unique attribute of mosses but is not present in all sp.: this innovation was acquired after the divergence of the Andreaeobryopsida and subsequently lost in many taxa. The peristome consists of one or two concentric rings of teeth exposed following the loss of operculum. In Polytrichum and allied taxa the teeths are composed of entire elongated cells and the peristome is said to be nematodontous. In all other peristometae mosses, the cells contributing to peristome are partially degraded and teeth are built almost exxlusively from vertical cell walls. The teeths are articulate. They can bend inward or outward hence the name arthrodontous for this peristomial architecture. The movement of peristome is accounted for by differential forces acting upon hydration of cell wallsof different thickness. Each tooth is composed of two radial colums of plates an inner and an outer set. As water evaporates thick walls shrink and the collective movement along the columns results in the tooth bending towards that side.

There are two subtypes of arthrodontous peristome.

1. The first is termed **haplolepidous** and consists of a single circle of 16 peristome teeth.

2. The second type is the **diplolepidous** peristome fround in subclass Bryidae. In this type, there are two rings of peristome teeth—an inner **endostome** (short for *endoperistome*) and an **exostome**. The endostome is a more delicate membrane, and its teeth are aligned between the teeth of the exostome. There are a few mosses in the Bryopsida that have no peristome in

their capsules. These mosses still undergo the same cell division patterns in capsule development, but the teeth do not fully develop.

Spores are unicellular. In rare cases the spore undergoes divisions prior to dispersal. The wall of the spore is composed of sporopollenin, a compound that confers high resistance to mechanical and physiological stress. The outer layer is often patterned and the ornamentation may provide critical to distinguish the sp.

Following fertilization the archegonium acquires a protective function for the developing embryo. The venter and the cauline tissue immediately below form an epigonium, or sac enclosing the young sporophyte. The epigonium is of determinate size. The pressure resulting from the growth of the sporophyte causes the protective sac to tear. The basal section remains around the base of the seta and the remainder forms a hood or calyptra, covering part or all of the spoarangium.

Asexual Reproduction:

Through propagules: Propagules developed for clonal reproduction are diverse. They vary in their shape size and moide of abscission, longevity and origin on the plant and may occur on protonema, rhizoids stems and leaves. Ground dwelling mosses may be prone to breakage caused by animals and new individuals may be estsablished from detached leafy shoots.

Tubers occur in many lineages of mosses but bare conspicupously lacking among pleurocarpous mosses.

Hornworts

Hornworts (Division Anthocerotophyta) compose the least diverse lineage of bryophytes. Their name refers to the horn-like sporophyte that lacks a seta and dehisces along one or two vertical lines. They further differ from mosses by the lack of leaves. Their vegetative body is indeed thallois and rosette or ribbon-shaped and thereby resembles that of some liverworts. Allthough morphologically rather simple in architecture, hornwort may be physiologically elaborate organisms. Many species establish and maintain intimate symbiotic associations with endophytci nitrogen fixing cyanobacteria and endomycorrhizal fungi. Furthemore, hornworts have carbon concentrating mechanism lacking in other bryophyte. Phylogenetically, hornwrots appear more derived than mosses and liverworts too: recent studies suggest that they alone share a unique common ancestor with vascular plants.

Structure and Development:

The vegetative body of hornworts is a flattened bilaterally symmetric thallus. Although the thallus lacks organized externa; appendages, the margin can be deeply incised, giving the

appearance of irregular leaves emanating from a thickened midrib in some taxa. In some case, the margin may be recurved downwards, forming narrow capillary spaces that may function to hold excess water. The growth of the thallus results from the activity of the single celled apical meristem, which is located in thallic notches covered by mucilage secreted by epidermal cells. The thallus lacks conspicuous internal differentiation, except for the presence of cavities borne through schizogeny, which are essential for symbiotic cyanobacteris. Most parenchyma cells may synthesize, store and secrete mucilage a carbohydrate that may be essential for water retention. The upper surface of the thallus harbours the sex organs, and in some cases, small dissected flaps or lamellae. The lower surface of thalus bears smooth unicellular rhizoids that are ttypically unbranched, except at their tips in *Megaceros.*

In the lower rarely also upper epidermis small pores are found with two kidney shaped cells, resembling the stomatal guard cells, as seen in the sporophyte. Like the guard cells in the sporangial wall these cells lack the ability to open and close the pore once formed, remains open. The pore leads to a small chamber that may holdglobose colonies of Nostoc embedded in mucilage. The stomata are hence called mucilage clefts. Schizogenous cavities are frequent in hornworts and some are formed above the epidermal pore. They are however, filled with mucilage and hence are inadequate for gas exchange. Mucilage clefts in the gametophyte of hornworts are stomata that have been co-opted from the sporophyte to fulfil a new function, in which case their evolutionary history is connected. Indeed the pore served not for gas exchange but as the entry point for filamentous *Nostoc*, which will form the endophytic colonies characteristic of all hornworts. The photosynthetic cells including the epidermal cells of the gametophyte typically possess a large solitary chloroplast including a pyrenoid. Numerous small plastids characterize only a few taxa such as *Megaceros.*Most hornworts are monoicous, dioicy is less common and sexual dimorphism between unisexual thalli weakly pronounced. Gametangia are produced at the dorsal thallus midline. Archegonia are shaped like a vase, with a slightly swollen venter holding the single egg and a short neck capped by two cover cells. The female sex organ develops form an epidermal initial and remains mostly sunken at maturity with only the apex of the neck protruding from the surface. Sperm cells are protected by a unistratose jacket subtended by a short stalk. The development of antheridia is similar to that seen in othger bryophytes, except that the initial is borne immediately below the epidermis. Male gametangia always develop within a chamber and typically occur in aggregates with all antheridia derivd from a single initial. The epidermal cell above the antheridial initial give rise to the two-layered roof of the antheridial chamber.

A schizogenous cavity is forme following the separation of the two initials early in gametangial ontogeny. At maturity, the location of the endogenous antheridia is revealed by the convex ceiling of the chamber on the thallus surface.

Sporophyte: The plane of first division of the zygote is longitudinal, unlike in other bryophytes where it is transverse. Early in its development the embryo is divided into basal region that develops into a capsule. The peripheral cells of the foot form a haustorium that penetrates the surrounding gametophytic tissue to form the placenta, characterized by a large exchange surface between the two generations favouring the efficient nourishment of the sporophyte. A distinctive feature of the placenta of some hornwort sp. is the occurrence of abundant protein crystals.

The sporangium grows from the activity of a basal meristem. The cells deriving from the meristem follow two disatinct developmental patterns. The axial cylinder composes the endothecium, which will form the columella. The outer cylinder or amphithecium develops into the epidermis, the assimilative layer and unlike in other bryophytes(except *Sphagnum* and *Andreaea*) also the sporogenous tissue. The basal meristem remains active throughout the life of the sporophyte except in *Notothylus*. The continuous growth of the sporophyte among land plants,unique among land plants, results in a continuous and hence nonsynchronize productions of spores and an acropetal gradient of their maturation. In mosses and liverworts all sporocyte undergo meiosis more or leaas synchronously and at the time of dispersal all spores have completed their maturation. When young capsule is protected by a multilayered involucres, developed from the gametophytic tissue enclosing the archegonium. The involucres typically cease to grow when the involucres typiacally cease to gro when the first meiotic division occur in the sporangium. Through continuous basal growth, the sporophyte soon ruptures the involucre near its apex and emerges from the gametophytic cylinder.

Before sporogenesis, the cells of the sporogenous region undergo a division that will yield a spore mother cell and a pseudo-elaterocyte. Pseudo-elaterocyte consists of one or more elongated diploid cells. Multicellular pseudo-elaters are filamentous. The cell walls may be thin, evenly or spirally thickened. Spore and pseudoelaters are typically arranged in alternative layers inside the sporangium.

Sporophyte dehiscence occurs near the apex by splitting along one or two longitudinal lines with the valves remaining along one or two longitudinal lines with the valves remaining attached apically. Separation and dispersal of the spore is often facilitated by the twisting of the capsule wall pseudoelaters that disrupts the spore mass upon drying.

Asexual Reproduction: Most hornworts develop both sex organs on a single thallus and sexual reproduction is frequent for these taxa. Highly specialized means of axeual reproductionare uncommon. Some annual sp. develop perennating tubers, either along the thallus margin or on the lower surface. Others develop gemmae on the upper surface nd its edges. In *Megaceros aenigmaticus* a sp. known only from Southern Appalachians in the eastern united states. Male and female plants occur in different watersheds and are thus geographically isolated.

Locally the species is abundant, reproducing seemingly excusive through fragmentation along the thallus margin.

WHY ARE BRYOPHYTES IMPORTANT?

Bryophytes are a critical link between aquatic and land plants and they contain a number of adaptations that are characteristic of both land and aquatic plants. As mentioned above they have cuticles, gametangia and embryonic development which is all features of more advanced, land plants. On the other hand, they still require water for reproduction and lack vascular tissue. This linkage is vital in piecing together the evolutionary history of life on Earth.

Besides that, Bryophytes provide a number of important services that help maintain the integrity of a landscape. They are important for their role in water filtration, primary production and provision of habitat for insects and other invertebrates. They have also been utilized by humans for a number of purposes both historically and in the present day. Traditionally, they have been used for insulation, padding and as a fuel; more recently they are mostly used in the florist trade.

Bryophyte Ecology

Substrates

Most people will have seen bryophytes growing on soil, rocks and tree trunks. These are very common substrates throughout most of the world. You can also find bryophytes on the leaves of vascular plants, especially in the tropics. These are called EPIPHYLLOUS BRYOPHYTES. In humid forests you can even find bryophytes growing on invertebrates. Leafy liverworts in the genera *Lejeunea*, *Cololejeunea*, *Odontolejeunea* and *Microlejeunea*; the thallose liverwort genus *Metzgeria* and the moss *Daltonia angustifolia* have been found on beetles in New Guinea. The beetles often have ridges, depressions or hairy to bristly surfaces that aid in trapping propagules of bryophytes, lichens, algae and fungi. Similar species grow in the surrounding vegetation so the beetles are camouflaged by having growths

of bryophytes and other organisms listed above. The leafy liverwort *Taxilejeunea obtusangula* was found growing with several algae on the head of the Mexican rainforest lizard species *Corythophanes cristatus*. The lizard may stay still for hours, moves slowly and the top of its head is slightly concave, ideal for catching moisture dripping from above. These characteristics would also ensure that liverwort propagules are readily caught, as well as providing a site conducive to gametophytic growth. It isunlikely that the liverwort would ever reach sexual maturity since the lizards possibly shed their skins more than once a year. The liverwort is widespread in the Neotropical rainforests where it is commonly found on bark or living leaves.Glass, plastic, rubber, roof tiles, dung, bones and carcases – all can be found with bryophytes growing on them.

Water, erosion and nutrient recycling

Bryophytes can hold from just a few times to many times their own weight in water, depending on species. The best performers are found in the genus *Sphagnum* and are capable of holding between 20 and 30 times their own weight in water. Moreover, bryophytes can quickly absorb water which is then released over a much longer period. Alpine *Sphagnum* bogs may hold on to much of the water that results from the spring snow melt. Rather than all this water quickly flowing away in alpine streams, it is fed into streams over a much more extended period, resulting in a more even stream flow. Bryophytes on tree trunks absorb rainwater that's flowing down the trunk and those hanging like curtains absorb water, both from rainfall and from mists or fogs. The curtain-like mosses, because of their very large surface areas are very efficient exploiters of mists or fogs. A fist-sized clump of moss on a tree trunk can hold as much water as a similar sized sponge. Moist clumps or curtains, especially if they are abundant as they are in many tropical areas, help maintain a humid atmosphere and so greatly influence the micro-climate. Bryophytes will also trap any nutrients that are dissolved in the rain or mist droplets, in many cases trapping nutrients that would otherwise be washed away. Living bryophytes make use of the nutrients they trap and dead, decaying bryophytes release nutrients to the surrounding plants. In these ways bryophytes play an important role in nutrient recycling.

In arid areas extensive carpets of lichens, bryophytes and cyanobacteria play important ecological roles. Such cryptogam carpets are called BIOLOGICAL SOIL CRUSTS and one of their major functions is erosion control. Both lichens and bryophytes commonly have root-like structures (but not true roots) to anchor themselves to the soil. In arid areas rainfall often comes as sudden, intense downpours. As well as having root-like appendages which help

bind the soil, a biological soil crust also forms a physical barrier between the falling raindrops and the soil. This means that much of the raindrops' force is absorbed by the crust and this greatly lessens the erosive potential of such intense downpours. In addition, such a crust will often slow down the flow of water and thereby lengthen the time during which the water can soak into the underlying soil.

Colonisers

From what has already been said about bryophyte substrates and habitats, it's not surprising to learn that bryophytes are often among the first organisms to colonize bare surfaces. Examples of these are the bare soil expanses such as those exposed during road works or by landslides, volcanic ash deposits, the scorched soil and charred wood left after a severe bushfire, freshly exposed rock faces, new footpaths, freshly tiled roofs and the upturned soil in a newly ploughed field. Bare soil may often be colonized as readily by pioneering vascular plants as by bryophytes and other cryptogams. On the other hand surfaces such as bare rock and thick volcanic ash are initially barren, nutrient-poor and often exposed to sun and wind. Such surfaces are inimical to the seeds of vascular plants but are readily colonised by various non-vascular cryptogams, among which various bryophytes and the bulkier lichens are good at trapping windblown organic debris. Of course, the bryophytes and other cryptogams also contribute to the organic deposits when they themselves die and decay. Death may be of the whole or just a part of the organism. A good example of the latter involves those pioneering mosses that grow as individual, upright stems but close enough to one another to form cushions. In such a cushion, as the stems grow, the lower leaves lose their access to light and die, thereby adding to the organic debris. You can see the live, green leaves above a layer of dead material. So, in one way or another, bryophytes help build up a layer of organic matter. This provides homes for various micro-organisms and invertebrates, as well as potential seed beds for vascular plants. The next section discusses the complexity of the interactions between bryophytes and plant seeds. For the moment it will be enough to note that the pioneer bryophytes often help pave the way for the transformations of many bare surfaces into ones that will eventually support complex plant communities and intricate food chains. Of course some barren surfaces (such as footpaths and roof tiles) will never support complex vascular plant communities. However, they may still support complex communities of cryptogams, invertebrates and micro-organisms. Another thing to note is that over time there may be a change in the species present in any area. Certain species are quick colonizers of disturbed areas – but are then replaced by others.

5. Biogeography and Ecological Significance

5.1. Diversity and Distribution Pattern

Liverworts are found literally everywhere. They flourish on every continentand landmass, including Antarctica, and exploit remarkably diverse arrayof microhabitats. Except for the ocean and other salty aquatic habitats, liverwortscan be found in most extremely harsh environments.Some species initiate the process of colonization on bare volcanic rocks; others thrive beneath dense moss carpets. They are notcapable of photosynthesis and are, hence, dependent on green plants.

Thalloid liverworts in general tend to occur on soil, whereas leafy liverworts occur on a wider range of substrata, including rock, barkand living leaves. Therefore, thalloid taxa dominate in open environments, whereas leafy liverworts are best represented in closed vegetations. Leafy liverworts are particularly prevalent in moist and cool habitats. Theyare luxuriant in tropical rainforests, where they typically exhibit a higherspecies diversity than mosses owing to the contribution of some highly diverse families such as the Lejeuneaceae and Plagiochilaceae (Sillett et al. 1995, Holz et al. 2002, Holz & Gradstein 2005). Because leafy liverworts represent the bulk of species diversity among liverworts, they influence the global diversity patterns of the group as a whole. As a result, liverwort diversity is much higher in the areas of the world with a wet climate, such as the Andes, southern South America and New Zealand, than in those regions with globally much drier climates, such as northern Africa and Arabia.

Thalloid liverworts, by contrast, globally tend to occur in more xeric environments.They predominate in Mediterranean and xerotropical environments, where they may form dense, gregarious patches on bare, very thin soil layers.

Mosses (Division Bryophyta) are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight. Yet, an alert naturalist will quickly notice their presence in virtually every ecosystem. In parts of the world where short growing seasons limit plant growth, mosses may dominate the vegetation. Similarly, in temperate and tropical rain forests, mosses compose luxuriant epiphytic communities that play important ecological

functions, especially in terms of water and nutrient flow. Approximately 12,000 species of mosses are currently recognized, reflecting a broad morphological diversity.

5.2. Population and Community Dynamics:

Bryophytes are important in terms of species richness and cover in many habitats, and also for ecosystem functions. The basis for understanding population processes in a species is its life history. Life-history traits are characteristics that affect the transition between different stages of the life cycle, such as birth, reproduction, dispersal, and death. Life-history traits evolve as the organism faces a trade-off in the use of limited resources, so that, for instance, in disturbed habitats a genotype with a large number of spores has the highest fitness, whereas in other habitats fewer but larger vegetative propagules is a more viable strategy. Life-history strategies are co-evolved integrated combinations of traits and the most common scheme used to group bryophyte species based on life-history strategies is that by During. His classification is drawn on variation in lifespan (annual, few years, long-lived) and spore trade-off (many small or few large) in response to habitat duration and distances among suitable habitats.

a. Spore Production:

Sexual reproduction is a process that spansmanymonths and is vulnerable to environmental stress at all stages. A tremendous variation in spore production between years can be caused by weather variation. For example, in some boreal *Sphagnum* species gametangia are formed in the late summer, and if this period is dry the next year's sporophyte production will be reduced. In the next stage, spring precipitation has an effect on fertilization rates, and finally large numbers of developing sporophytes abort in dry summers.

Monoicous species (potentially selfing) produce spores more frequently than dioicous ones, which may explain why fewer monoicous mosses (compared with dioicous ones) are rare. In dioicous species the distance between male and female shoots may limit fertilization, and variation in spore capsule density will depend on the distribution and ratio of male and female shoots. Sexual reproduction may be completely lacking owing to the absence of male shoots in the population.

b. Cost of reproduction:

An important issue in life-history theory is the cost of reproduction. Such a cost appears when reproduction leads to increased mortality or reduced growth rate such that ultimately

reproduction in the future will decrease. A cost of reproduction has only recently been demonstrated in bryophytes. In *Dicranum polysetum* growth was lower in shoots that developed sporophytes than in those where sporophyte formation was aborted or experimentally terminated, and in *Anastrophyllum hellerianum* there appeared to be a higher mortality in fertile female shoots than in males. In many bryophytes the sporophytes are minute compared with the gametophyte, and intuitively a reproductive cost seems unlikely. It is not always that the sporophyte is the most costly part of reproduction. In the desert dioicous moss Syntrichia caninervis, the reproductive effort in male organs was larger than in female ones, and this has been proposed as one factor behind the strongly female-biased sex ratio in this species.

c. Dispersal

The density of spore deposition decreases rapidly with distance, such experiments can only reveal the shape of the dispersal curve up to a few meters. In *Sphagnum*, spore density fitted well to an inverse power function, i.e., a linear relationship after log-transforming spore density and distance.The proportion of spores that disappear beyond a few meters is somewhat dependent on spore size, but even in species with quite large spores most of the spores traveled beyond 2m. For epiphytes (which start their dispersal at some height above ground) a somewhat more realistic appreciation of spore densities at longer distances can be achieved with functions other than the commonly used inverse power or negative exponential. In particular, the log-normal function is considered useful and realistic, and gives a ‘‘fatter’’ tail (i.e. higher densities at larger distances). Dispersal experiments indicate that a single patch has a great impact on spore deposition at close range, but for dispersal at a larger scale (colonization of more isolated sites), spores produced by numerous sources further away play an increasingly important role.

At a larger geographic scale, colonizations depend on events that may occur with very low probability but over a much longer time. To study this, spore trapping is not a feasible method. Instead dispersal can be inferred from distribution patterns, and especially useful are habitats that are of known age and/or of known distance from dispersal sources.

We normally assume that bryophytes are wind-dispersed, but other vectors may be involved. In wetlands and along rivers, fragments and spores could easily be water-dispersed.

d. Germination and Establishment

Spore germination is of course dependent on moisture, and an example of the highly specific requirements during spore germination comes from studies of *Neckera pennata* (epiphyte on tree trunks) and *Buxbaumia viridis* (on decaying wood). The germination depends on an interaction between moisture and pH so that high water availability facilitates germination atsuboptimal pH, and *vice versa*. For *Neckera*, which normally occupies rather desiccation-prone tree trunks, this may explain its preference for host trees with high pH where it can germinate quickly and therefore exploit the short windows of opportunity with wet bark after rain events. Not only is spore germination difficult, but there is probably also high mortality in the protonema stage as an effect of desiccation.

Establishment from vegetative fragments has a much higher probability of success, but is a habitat-sensitive process, too A very practical example are the methods developed for re-establishing Sphagnum on peatlands after peat harvest. Here fragments are used, and for success the hydrology must be controlled to produce a wet peat surface and the fragments need initial protection by a layer of straw mulch. Hence, the technique highlights the fact that surface desiccation is the critical factor for the establishment of bryophyte fragments.

e. Diaspore banks

The presence of spores as a diaspore bank in the soil has been demonstrated for many species, and it also appears that gemmae can enter a state of dormancy.The ecological importance of the diaspore bank is difficult to assess, but it suggests at least a potential for secure and rapid colonization after disturbance. for secure and rapid colonization after disturbance.

f. Clonal expansion and population persistence

As noted above, many bryophytes have the capacity to expand and disperse by vegetative fragments or specialized propagules. Of particularecological importance is clonal expansion by branching, since the new shoots benefit from being physiologically integrated with the mother plant and hence have a higher chance of survival than detached propagules. Clonal species may be very persistent, and Sphagnum individuals can probably survive for centuries as they slowly expand clonally and at the same time avoid a respiratory burden by losing old tissue to peat formation.

Clonal growth potentially gives the plant an opportunity to explore the habitat, thereby reaching positions with favorable conditions. There are not many tests of such ‘‘foraging’’ in bryophytes, but species with high growth potential (*Brachythecium praelongum* and *Thuidium tamariscinum*) may have some ability to expand laterally from dark to light patches.

g. Density-Dependence in Bryophyte Populations

Intraspecific competition in vascular plants is often described by the negative effects (such as decreased growth or increased mortality) that follow from increasing shoot density. For example, if the reduced growth per individual exactly compensates for increase in density, the total biomass produced per unit area will be independent of sowing density as described by the law of constant final yield. In many cases, an increase in density is also followed by an increase in mortality. This is referred to as self-thinning, and it has often been found that the average size of the surviving individuals increases more rapidly than density decreases.

Grazing animals, parasitic fungi, and m ycorrhiza exert strong influences on populations and co mmunities of vascular plants, but in most c ir cu mstances these can be ignored in studies of bryophytes. With their low nutrient co ntent, and in many cases peculiar b iochem istry, bryophytes are gener ally avoided by grazers. However, in some northern habitats mosses may be heavily grazed. Examples are several goose species in the Arctic, and mice, voles and lemmings in alpine heath ecosystems and boreal forests (listed in Prins 1981) where much grazing can occur under snow c over during the winter. In years, when their populations are large, lemmings may severely reduce moss biomass in alpine snow beds (Virtanen et al. 1997, and references therein) and boreal forests (Ericson 1977), and s t rongly influence the species richness and composition. I n other ecosystems the m ost obvious effect of grazing is to reduce the light competition from vascular plants, and as secondary effects to decrease the amount of litter covering the bryophytes and to produce small-scale gaps for colonization. Additional effects could be trampling and fertilizing by grazers.

5.3. Physiological Ecology and Adaptation

Bryophytes are on average some two orders of magnitude smaller than vascular plants, and this difference of scale brings in its train major differences in physiology, just as many of the differences in the structural organization and physiology of insects and vertebrates are similarly scale-driven. Surface area varies as the square, and volume and mass as the cube, of linear dimensions. Hence gravity is a major limiting factor for vertebrates or trees, but trivial for insects or bryophytes. Bryophytes in general have much larger areas for evaporation in

proportion to plant mass than do vascular plants. Surface tension, which operates at linear interfaces, is of little significance at the scale of the vascular plant shoot but is a powerful force at the scale of many bryophyte structures. There are also major scale-related differences in the relation of bryophytes and vascular plants to their atmospheric environment. Vascularplant leaves are typically deployed in the turbulent air well above the ground. The diffusion resistance of the thin laminar boundary layer is small, so the epidermis with its cuticle and stomata in effect marks the boundary between (relatively slow) diffusive mass transfer within the leaf and (much faster) turbulent mixing in the surrounding air. By contrast the small leaves of many bryophytes lie largely or wholly within the laminar boundary layer of the bryophyte carpet or cushion, or of the substratum on which it grows. For these reasons it is important to approach bryophyte physiology from cell-biological and physicalfirst principles; preconceptions and concepts carried over from vascular-plant physiology can be grossly misleading.

Raven (1977, 1984 and 1995) has emphasized the importance of supracellular transport systems in the evolution of land plants, and the physiological correlates that we must read alongside the anatomical structures of fossil plants. But the highly differentiated supracellular conducting systems exemplified by xylem and phloem are really only a prerequisite for large land plants. In adapting to the erratic subaerial supply of water, vascular land plants evolved tracheids and vessels, bringing water from the soil to meet the needs of the above-ground shoots and leaves. Bryophytes in generaladopted the alternative strategy of allowing free water loss (poikilohydry) and evolving desiccation tolerance, photosynthesizing and growing during moist periods and suspending metabolism during times of drought. Thesetwo patterns of adaptation are in many ways complementary. Bryophytes may appear to be limited by their lack of roots, but their poikilohydric habit means that they can colonize hard and impermeable surfaces such as treetrunks and rock outcrops, impenetrable to roots, from which vascular plants are excluded. Bryophytes typically take up water and nutrients over the whole surface of the shoots. They efficiently intercept and absorb solutesin rainwater, cloud and mist droplets, and airborne dust. This ability underlies both their conspicuous success in many nutrient-limited habitats and the vulnerability of many species to atmospheric pollution. The vascular-plant pattern of adaptation is undoubtedly optimal for a large land plant; there is much reason to believe that the poikilohydric pattern of adaptation is optimal for a small one. The divergence of bryophytes and the various vascularplant groups goes back to the early history of plant life on land – certainly 400 million years, and probably longer (Edwards et al.

1998, Goffinet 2000). Mosses, Hepaticae, and Anthocerotae may well have been evolutionarily independent for equally long. Physiologically, bryophytes are neither simple nor primitive. They should be seen not as primitive precursors of vascular plants, but as the diverse and highly evolved representatives of an alternative strategy of adaptation, prominent in the vegetation of such habitats as subpolar and alpine fell-fields and tundra, bogs and fens, and the understorey of many forests from the boreal zone to the ‘‘mossy forests’’ of tropical mountains. They are challenged at their own scale only by the comparabl adapted lichens.

5.4. Ecological Role of Bryophytes

(a) Pioneer of the land plants. Bryophytes are pioneer of the land plants because they are the first plants to grow and colonize the barren rocks and lands.

They prevent soil erosion by:

(i) Bearing the impact of falling rain drops

(ii) Holding much of the falling water and reducing the amount of run-off water.

b. Bryophytes are soil Producers, soil binders and also add organic matter to soil hence are important in Pedogenesis too. Mosses and lichens are slow but efficient soil formers. The acid secreted by the lichens and progressive death and decay of mosses help in the formation of soil.

c. Bog succession. Peat mosses change the banks of lakes or shallow bodies of water into solid soil which supports vegetation e.g., *Sphagnum*.

d. Rock builders. Some mosses in association with some green algae (e.g., Chara) grow in water of streams and lakes which contain large amount of calcium bicarbonate. These mosses bring about decomposition of bi-carbonic ions by abstracting free carbon dioxide. The insoluble calcium carbonate precipitates and on exposure hardens, forming calcareous (lime) rock like deposits.

e. They hold moisture & need little droplets of water for reproduction.

f. The liverworts, mosses and lichens are supposed to be the pioneers in establishing vegetation where other vegetation seems to be practically impossible.

g. However the *Sphagnum* plants are of great ecological importance. When these plants establish themselves in some lake or other areas full of water, sooner or later they cover the whole surface of the water. Due to deposition of plant debris the surface may be raised.

h. Formation of Peat:

Peat is a brown or dark colour substance formed by the gradual compression and carbonization of the partially decomposed pieces of dead vegetative matter in the bogs.

Sphagnum is an aquatic moss. While growing in water it secretes certain acids in the water body. This acid makes conditions unfavorable for the growth of decomposing organisms like bacteria and fungi. Absence of oxygen and decomposing microorganisms slows down the decaying process of dead material and a large amount of dead material is added year by year. It is called peat (that is why *Sphagnum* is called peat moss).

i. Acts as pollution indicator. Mosses are reliable indicators of air pollution risks to ecosystems, because they get most of their nutrients direct from the air and rain, rather than the soil.

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6. Economic Significance and Conservation

6.1. Economic Importance

a. Packing material:

Most of the mosses are used as packing material after being dried. They make a fairly good packing material in the case of glass ware and other fragile goods. Especially the dried peat mosses (*Sphagnum* spp.) are used to pack bulbs, cuttings and seedlings for shipment.

b. Used in seed beds:

Since the peat mosses have remarkable power to absorb and hold water like a sponge, they are extensively used in seed beds and green houses to root cutting. The peat mosses are also used to maintain high soil acidity required by certain plants.

c. As a source of fuel:

The peat is also a potential source of coal. Dried peat may be used as fuel. In Ireland, Scotland and other European countries the peat is used for fuel. In colder parts of the world where peat reaches its greatest development, the lower layers of peat become carbonized and after the ages have passed, become available to human kind in the form of coal.

d. Absorbent bandages:

The *Sphagnum* plants are slightly antiseptic and possess superior absorptive power. On account of these properties they may be used for filling absorbent bandages in place of cotton, in the hospitals.

e. Asmedicines:

Some Bryophytes are used medicinally in various diseases for e.g.,

(1) Pulmonary tuberculosis and affliction of liver—*Marchantia* spp.

(2) Acute hemorrhage and diseases of eye—Decoction of *Sphagnum*.

(3) Stone of kidney and gall bladder—*Polytrichum commune*.

(4) Antiseptic properties and healing of wounds—*Sphagnum* leaves and extracts of some Bryophytes for e.g., *Conocephalum conicum, Dumortiera, Sphagnum protoricense, S. strictum* show antiseptic properties

f. As Food:

Some Bryophytes e.g., mosses are used as food by chicks, birds and Alaskan reindeer etc.

g. In Experimental Botany:

The liverworts and mosses play an important role as research tools in various fields of Botany such as genetics. For the first time in a liverwort, *Sphaerocarpos*, the mechanism of sex determination in plants was discovered.

6.2. Threats and vulnerability

Bryophytes have biological properties that make them more vulnerable to environmental changes than many other plant groups. These properties include a dependence on high humidity microclimate, an intrinsic low competitive ability, and a reproduction process and population establishment heavily dependent on and contingent upon the combined environmental conditions of high moisture and cool temperature.

Today, many people are aware that the landscape on most continents has been modified in such a way that the land and water have become hostile for plants and animals to live in, and that this detrimental modification of landscape appears to continue, especially in densely populated regions (Vié et al. 2009). This is the main threat factor that is causing both a decline in the number of bryophyte species and the contraction of the geographic range of many species.

Habitat loss is the fastest-growing threat to the survival of individual species and this will probably continue to be the dominant threat factor in the decades to come (Brooks et al. 2002; Fahrig 2002). This negative impact has gone already too far, especially in tropical

lowlands with fertile soil, where forested land is cleared continuously for human population expansion and only small pieces of natural lowland vegetation that can harbour bryophytes are left (Hallingbäck & Hodgetts 2000).

One newly documented threat for some bryophyte species is the harvesting of living plants for commercial purposes (Muir et al., 2006). In forest, large mats of mosses are peeled off from tree trunks and boulders and sold in the market in United States, India, Japan and China (Peck 2006). Harvested bryophytes are used, for example, in decorating plant pots, packing bulbs for transport, making moss-sticks and moss- bags, etc. Large quantities of mosses are harvested, irrespective of the status of the species in the country. This trade is often not controlled by the governments and can result in considerable ecological damage to the plant group (Muir 2004).

6.3. Conservation Strategies

Interest in biodiversity and conservation biology of bryophytes is increasing. Bryophytes have been successfully introduced into the IUCN system, and the protection of threatened species and their habitat, although still limited, is gaining attention worldwide.

Increased field activities in several countries in Asia have resulted in the identification of bryological hot spots (Tan & Iwatsuki 1996). Bryophyte species and their habitats have been part of legislation in Europe (Porley et al. 2008). Practical conservation has received new tools to design and manage the network of conserved areas for bryophytes. Likewise, promising new methods of ex situ conservation are being developed (Rowntree et al. 2010).

New guidelines for bryophyte harvesting have recently been proposed in a number of countries, which include standardizing the reporting of requirements before a harvesting permit be given and creating incentives for the buyers and the participating harvesters to protect and conserve the natural resources of bryophytes.

In 2000 The UK Country Agencies in partnership with Royal Botanic Gardens, Kew, launched the ex situ project for the conservation of threatened bryophytes, the first such project of its “kind” in the world (Ramsay & Burch 2001; Rowntree & Ramsay 2005). The Target 8 of GSPC (Global Strategy for Plant Conservation) states that 60% of threatened plant species should be preserved in accessible, ex situ collections. Recognizing the fact that ex situ collection of live plants can not be a time-limited project, but requires time for its development, the partnership between Natural England and Royal Botanic Gardens, Kew, has been re-established recently.

There are several "centres of endemism" of bryophytes identified today, and almost all such centres lie in geographically isolated, geologically or climatically unique regions (Schofield 1985, 1992; Tan & Pócs 2000). The characteristic patterns of endemism in bryophytes do not appear to be the same as what has been shown in higher plants or animals. Various lines of evidence suggest that the different dispersal capacity and the geographic isolation of this group of non-vascular plants over the geological time period may provide the clue to our understanding of their present day distribution patterns.

6.4. Restoration ecology

Extending *ex situ* programmes

Ideally all species should be given a chance to exist and grow in the wild. However, for those species at the brink of extinction, exsitu preservation may be the most effective rescue solution (Ramsay & Burch 2001, Rowntree & Ramsay 2009). The above mentioned Kew Garden program should be extended and expanded to cover globally endangered species (not only those found in Great Britain). Ex situ storage of bryophyte spores and propagules is also fundamental for future translocation and reintroduction of species. It provides living materials for use in new experimental studies (Sarasan et al. 2006). The possibility to keep the species alive in labs and man-made storing facilities offers, in addition, the good opportunity to study the population biology and genetic conservation issues using newly developed laboratory techniques and instruments (Rowntree et al. 2010) and this can play a decisive role in the discovery of the still unknown underlying biological developmental processes in bryophytes (Rowntree et al. 2007).

Species or Habitat Approach?

Bryophyte plants are too small to be suitable as a flagship species for conservation and they sometimes are difficult to see in the field, which make it hard for the public to appreciate their beauty and unique morphological features. Therefore the protection of bryophytes is best achieved by protecting the habitats (Sastre & Tan 1995). However, even if most bryophytes are too small and inconspicuous to serve as the functional flagship plant, still, the protection of a certain species of moss or liverwort maybe necessary since some rare and unique species need species-specific protection measures. The work to identify these species includes setting a priority system to make sure that cost-effective measures are applied. The focus on rapidly declining and extremely rare species must be a top priority. Once we lose the

species, we lose them forever. How, then, do we select species of the highest priority for protection? The IUCN has long elaborated an assessment system for estimating the extinction risk of a species (IUCN 2001). This system has been applied recently to bryophytes with slight modifications.

Increasing of knowledge

Without scientifically sound basic knowledge we may choose the wrong target species to manage, identify the wrong site as a hot spot, undertake incorrect protective measures, and adopt inappropriate strategies and priorities of conservation (Bisang & Hedenäs 2000). Compared to vascular plants, our knowledge about bryophyte biology, ecology and distribution is relatively little. The shortage of knowledge is a serious problem when evaluating what appropriate actions to take, including prioritizing the actions to be taken. Information on actual hot spots and the species distribution, the population size of species, as well as its susceptibility to anthropogenic environmental changes are all crucial to the development of efficient and effective conservation measures. The building of awareness must start with the identification of threatened habitats/species/ genotypes, continue with the analyses of threats, and finally result in an effective action plan to ensure the long-term survival of the species (Söderström 2006). Bisang & Hedenäs (2000) described a phylogenetic approach to identify the most distinctive species in terms of the genetic information content, thus, providing a different rational basis for selecting the priority species among the rare and declining taxa for conservation. There are still large gaps in our knowledge about bryophytes, which must be filled before the conservation measures can be totally effective. These include preparation of species checklists for less known parts of the world, research on bryogeography, and the study of habitat requirement, natural dynamics and capability of dispersal, population structure and the genetics of endangered species. Also needed are handbooks on how species can be recognized (floras), what are their present ranges, and the information of narrowly endemics in a region (Scott et al. 1997).

Raising public awarness

Today, a serious problem in plant conservation is still the general lack of public awareness. Bryophytes are not well known to the general public, even among some conservationists. It is necessary to continue our effort to highlight the importance of their presence in nature and their beneficial role in ecosystem.

7. Let's sum up

- Bryophytes can be found in all ecosystems of earth. A term commonly used for this group is nonvascular plants. Bryophytes lack xylem and phloem. Bryophytes and tracheophytes are monophyletic and collectively called embryophytes.
- Bryophytes are divided into three phyla, Bryophyta, Marchantiophyta and Anthocerotophyta.
- The common ancestor to Marchantiophyta arose immediately after the conquest of land some 450 m.ya. Their evolutionary history is characterized by more recent diversification during the early tertiary period.
- Mosses are small plants confined to humid habitats especially where short growing seasons limit plant growth, mosses may dominate the vegetation. mosses compose luxuriant epiphytic communities that play important ecological functions especially in terms of water and nutrient flow.
- Hornworts compose the least diverse lineage of bryophytes. Their name refers to the horn-like sporophyte that lacks a seta and dehisces along one or two vertical lines. They further differ from mosses by the lack of leaves.
- Liverworts are found literally everywhere. They flourish on every continentand landmass, including Antarctica, and exploit remarkably diverse array of microhabitats. Mosses are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight.
- Bryophytes are pioneer of the land plants because they are the first plants to grow and colonize the barren rocks and lands. They are used as packing material, in seed beds, source of fuel, absorbent bandages, medicines, Food and in experimental botany.
- Bryophytes have been successfully introduced into the IUCN system, and the protection of threatened species and their habitat, although still limited, is gaining attention worldwide.

8. Suggested Readings

1. Parihar, N.S. Introduction to Embryophyta (Vol. 1 Bryophyta), Central Book Distributors

2. Shaw, A. Jonathan and Goffinet Bernard, Bryophyte Biology, 2009, Cambridge university Press

3. Rashid, A. An Introduction to Bryophyta, 1998, Vikas Publishing House

4. Chopra, R.N. & Kumar, P.K. Biology of Bryophyta, Latest Ed., Wiley Eastern

5. Puri, P. Bryophyte, Latest Ed., Atmaram & Sons 6. Vashista, B.R. Bryophyta, Latest Ed., S. Chand & Company

9. Assignments

1. Give an outline idea on classification of hornworts .
2. Explain recent concepts on evolution of the three lineages of bryophytes.
3. Write a short note on ecological roles of bryophytes
4. What is peristome teeth?
5. Describe restoration ecology of bryophytes.
6. What are the conservation strategies for bryophytes.

COURSE – BOHCT1.2
BIOLOGY & DIVERSITY OF PTERIDOPHYTES
Hard Core Theory Paper **Credit: (Groups A+B+C) = 3**
Group – C (Biology and Diversity of Pteridophytes)

Content Structure

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1. Introduction
2. Course Objectives
3. Introduction: A general account and an outline of recent system of classification of Pteridophytes upto order level with characteristic features.
4. Diversity and Evolution: Diversity in organography and the evolutionary trends in the members of Psilophyta, Lycophyta, Sphenophyta and Filicophyta - Early ferns, Eusporangiate ferns (Ophioglossales, Marattiales), Leptosporangiate ferns (Filicales, Marsileales, Salviniales).
5. Gametophyte: Patterns of spore germination; patterns of gametophyte development in homosporous and heterosporous pteridophytes; mating system in fern.
6. Sporophyte: Variations in vegetative and reproductive structures and their evolution with special emphasis on shoot apex, stelar organization, and soral characters.
7. Cytogenetics and Speciation: Pteridophytes with low and high chromosome number; polyploidy in microphyllous and megaphyllus forms; intergeneric and interspecific hybridity; obligate interbreeding forms.
8. Antheirdiogen in ferns.
9. Habitat diversity of pteridophytes and their conservation; endemic and endangered pteridophytes with special reference to India.
10. Let’s sum up
11. Suggested Readings
12. Assignments

1. Introduction

Ferns are very significant component in ecosystem as they can help to control erosion, to stabilize soils and slopes, to build soils where none exist and also to phytoremediate environment by accumulating heavy metals. They are now facing highest threat to extinction because of their strict habitat specificity. So, it is high time to give them the attention and protection they deserve as an indispensable member of the ecosystem. They are associated with such ecosystems that are particularly sensitive to degradation, some of which are considered natural habitats. Some of the taxa are included in common interest listings species and require a strict protection. As such, it is required to include the topics on 'Biology and diversity of pteridophytes' in Post Graduate degree course in Botany to make the students focused on this important plant group.

2. Course Objectives

After completion of the course the learners will be able to:

- describe a general account and recent system of classification of Pteridophytes;
- describe diversity in organography and the evolutionary trends in the major groups;
- explain patterns of gametophytic and sporophytic generations in both homosporous and heterosporous pteridophytes and can apply this knowledge in understanding the evolutionary significance among them;
- elucidate the chromosome numbers, polyploidy, and hybridity in pteridophytes;
- explainhabitat diversity of pteridophytes, and Indian scenario of endemic and endangered pteridophytes;
- apply knowledge for conserving the pteridophytic flora in their surroundings

3.1 Introduction: A general account and an outline of recent system of classification of Pteridophytes upto order level with characteristic features

General Idea

Pteridophytes are considered as first land vascular plants on Earth which appeared in the Late Silurian (~400 million years ago) as evinced by the discovery of fossil *Cooksonia*, an earliest plant with conducting system. They flourished during Devonian to Carboniferous (Late

Palaeozoic) with varied forms like arborescent lycopods, giant horsetails, tree ferns and thus the Late Palaeozoic age is straightforwardly considered as "Age of Pteridophyta".

Today, this plant group is represented approximately by 13,600 named species of ferns and lycophytes, mostly dominated by ferns (more than 10,000 species) and reached at second highest position among land plant groups after angiosperm. However, the statures of most of the present-day forms are herbaceous instead of arborescent as was in ancient ages.

Pteridophytes, especially the ferns, are not only worthy in forming a balanced ecosystem, but they attract common people for their exquisite lacy foliage and so are valued as horticultural plants since long. The plants are also explored worldwide as natural resources for food, fibre, handicraft, construction material, bio-fertilizer, medicine etc. for human benefit. Ferns and lycophytes have even been used by tribal people in many countries since ancient ages as ethno-medicine and in traditional rituals and ceremonies for spiritual purposes. Recently researchers are paying attention to utilize various terrestrial and aquatic ferns for heavy metal accumulation from the environment by proving their potential in phytoremediation. The existing knowledge from many places across the world gradually enriches the understanding that easy growing common ferns have abilities to absorb unamenable soil pollutants particularly heavy metals/metalloids. As such, the multifaceted usages of ferns and lycophytes have bound people for considering this group of plants as unequivocal component in their life.

Habitat

- Generally, grow in tropic, mesic forest
- Also occur in temperate region
- Even they grow in alpine region (*Lycopodium*)
- May grow along the mangrove region at sea side (*Acrostichumaureum*)
- Some ferns grow in desert region
- Some grow at road side in polluted area

Habit

- Generally, herb and shruby (e.g. *Oleandra*)
- Tree fern (e.g. *Cyathea*, *Dicksonia*)
- Climbers (e.g. *Lygodium*)

General Characters

- The pteridophytes are vascular plants (plants with xylem and phloem) that produce neither flower nor seeds. Instead, they reproduce and disperse only via spores.

- Plants characterised by a very distinct alteration of generations with independent inconspicuous and sort-lived gametophyte (sexual) and conspicuous and dominant sporophyte (asexual) stages (Fig. 1).

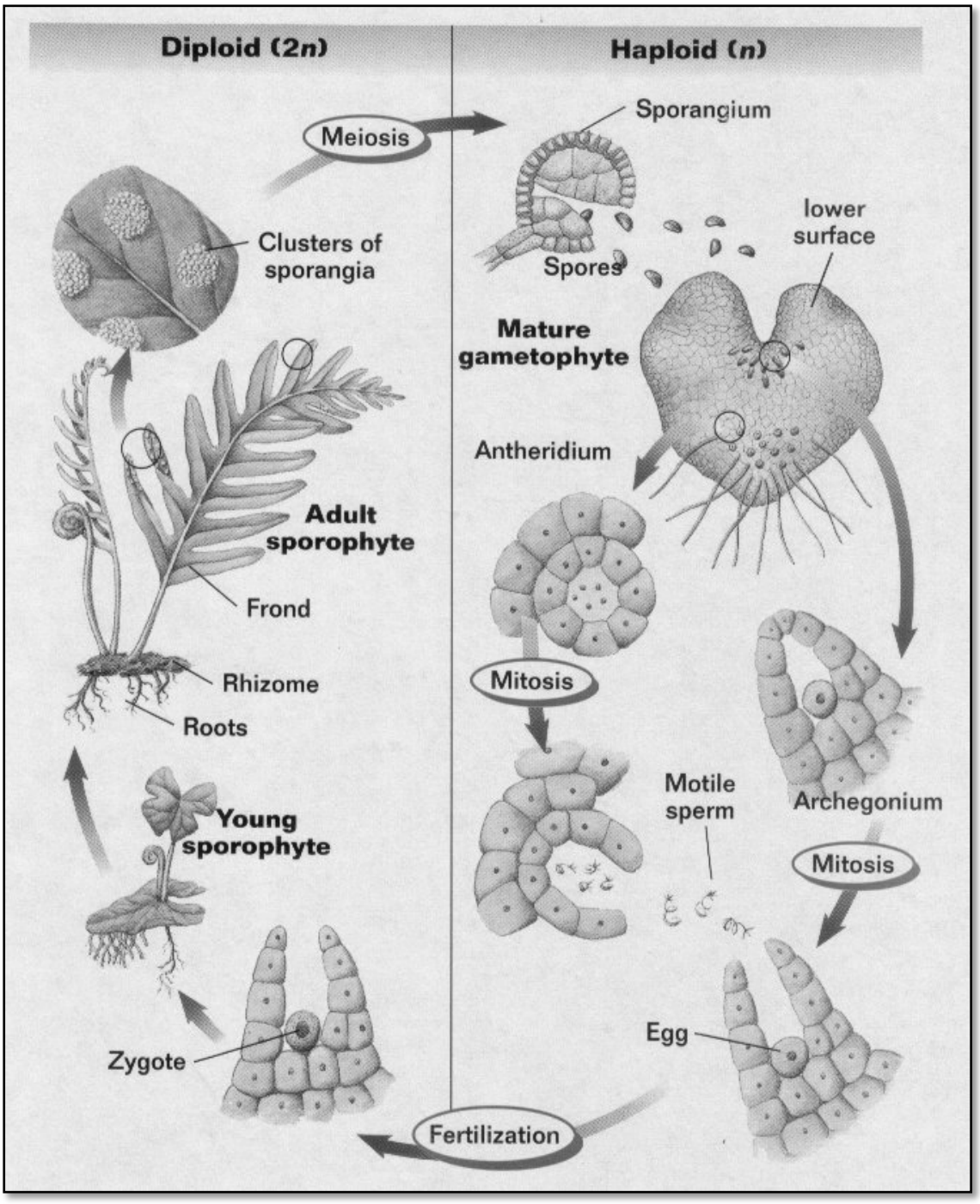


Fig. 1: Fig. Alteration of generations with sexual and asexual stages

(Source: https://www.bioexplorer.net/plant-life-cycle.html/)

- Sporophyte vascular herbs, shrubs (*Oleandra*), some with limited secondary thickenings (*Botrychium*, *Isoetes* root), mostly terrestrial, rupestral (lithophytes) or epiphytic, climbers (*Lygodium*), sometime floating, submerged or emergent aquatic; mostly soft or delicate, sometimes harsh or stout; rarely arborescent (*Cyathea*, *Dicksonia*) (Fig. 2).

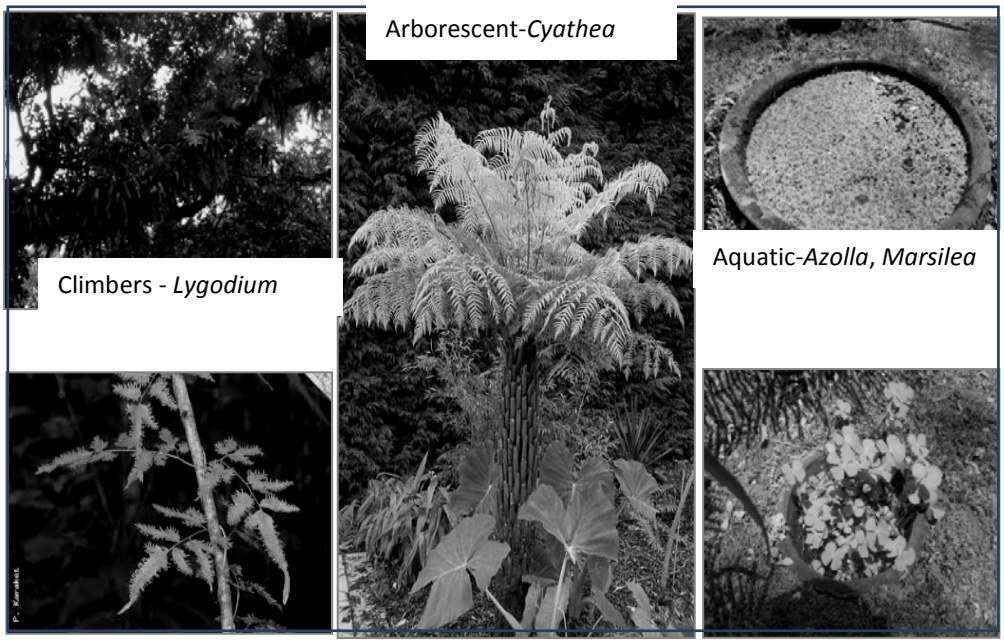


Fig. 2: Diversity of Pteridophytes

- Sporophytic plants with leaves, axes and roots (the latter missing in Psilotaceae) and well developed conducting tissue.
- Stem often rhizomatous, radial or dorsiventral, erect, prostrate, climbing, or subterranean; often with scales and/or hairs; stele simple to complex i.e. protostelic, solenostelic, dictyostelic and polystelic.
- Leaves either small, simple and bract-like or linear with a simple vein, straight in bud, or a broad frond with branched or divided veins (Fig. 3a), simple to several times pinnately divided, conduplicate or mostly circinate in bud (Fig. 3b); bearing sporangia (Fig. 3).

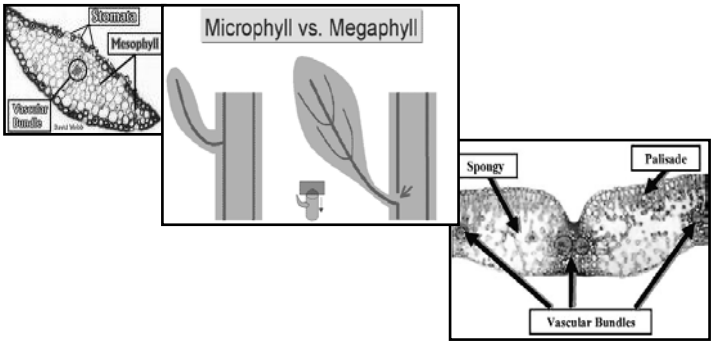


Fig. 3a: Microphyll and megaphyll

Fig.3b:Circinate vernation

- Leaf stalk or stipe is mostly present; stipules are mostly lacking.
- Sporangia thick or thin walled, homosporous or heterosporous, mostly grouped in sori or fused in synangia, or present as special structures viz., spike or sporocarp or solitary in axils or sporophylls which may be grouped into strobili (Fig. 4); sori naked or often protected by an indusium.
- Spores monolete or trilete; germinating to form an avascular autotrophic or mycotrophic prothallus (gametophyte stage) bearing flagellated male gamates (antherozoids) in antheridia and/or female gamates (egg cells) in flask shaped archegonia
- Gamete transfer and fertilization by water, producing a new plant (sporophyte stage) which obliterates the prothallus.

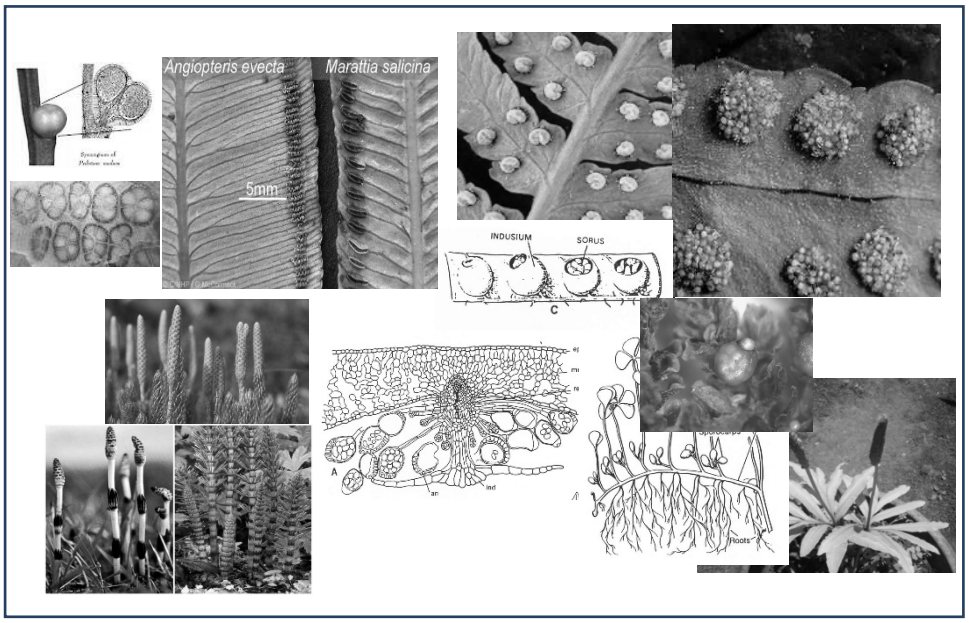


Fig 4: Diversity in reproductive structures

Fern ally

Fern ally is a general term covering a somewhat diverse group of vascular plants that are not flowering plants and not true ferns. Like ferns, these plants disperse by shedding spores to initiate an alternation of generations (*Lycopodium*, *Selaginella*)

True Ferns

True Ferns are vascular plants differing from lycophytes by having true leaves (megaphylls). They differ from seed plants (gymnosperms and angiosperms) in their mode of reproduction—lacking flowers and seeds.

Outline of recent system of classification of Pteridophytes upto order level with characteristic features

Based on morpho-anatomical characters, numerous classification schemes *(Bower 1928, Eames 1936, Tippo 1942, Holtumm 1949, Smith 1955, Bold 1957, Zimmermann 1959, Mehra 1961, Sporne 1962, Nayar 1970, Pichi Sermolli 1970, Kato 1983, Gifford & Foster 1989 etc.)* have been proposed for pteridophytes

PTERIDOPHYTES [based on Sporne 1962]				
CLASS	**ORDER**	**CLASS**	**SUB CLASS**	**ORDER**
PSILOPSIDA	Rhyniales	PTEROPSIDA	Primofilices	Cladoxylales
	Zosterophyllales			Coenopteridales
	Trimerophytales		Eusporangiatae	Marattiales
PSILOTOPSIDA	Psilotales			Ophioglossales
LYCOPSIDA	Protolepidodendrales		Osmundidae	Osmundales
	Lycopodiales		Leptosporangiatae	Filicales
	Lepidodendrales			Marsileales
	Isoetales			Salviniales
	Selaginellales	PROGYMNOSPERMOPSIDA		Aneurophytales
SPHENOPSIDA	Hyeniales			Protopityales
	Sphenophyllales			Archaeopteridales
	Calamitales			
	Equisetales			

Bower (1928) recognized 12 families, and divided Polypodiaceae into 15 subfamilies. His classification was based on the structure and development of the sorus. He recognized 3 groups – Simplices. Gradatae, Mixtae

Simplices	*Gradatae*	*Mixtae*
Coenopteridaceae	Hymenophyllaceae	Polypodiaceae
Marattiaceae	Cyatheceae	(divided into seven tribes)
Osmundaceae	Protocyatheaceae	
Gleicheniaceae	Plagiogyriaceae	
Schizaeaceae	Dixoniaceae	
Matoniaceae	Loxosoniaceae	

Ching (1940) divided Polypodiaceae into 33 families

Earlier classifications were based primarily on:

- External morphology of the shoot
- Apical meristem and its further segmentation
- Architecture and venation
- Vascular anatomy of stem and leaf
- Dermal appendages
- Position and structure of the sorus
- Indusial protection
- Characters of the sporangium
- Nature and form of sporangia
- Spore output per sporangium
- Mode of spore germination & type of prothallus
- Features of gametophyte
- Position and structure of sex organs
- Cytology
- Palynology

Cytological parameter is based on chromosome base numbers

- Chromosome base numbers have been used for classification purposes
- Commonly the base number is uniform for a genus or family, or it ranges around a given number
- More rarely, the number varies drastically in some genera: as in *Thelypteris*, which has x numbers ranging from 27 to 36

Recent Classification is based on molecular systematic studies in addition to morphological data.

- Smith *et al.* (2006)

- Rothfels *et al.* (2012)
- Christenhusz and Chase (2014)

Classification of Smith *et al.* 2006

This classification was based on both morphological and molecular data that was mainly obtained from

- Six chloroplast markers *(rbcl, atpA, atpB, accl, 16S rDNA, ITS)*
- One nuclear gene *(18S rDNA)*
- Three mitochondrial genes *(atp1, nad2, nad5)*

This classification divides ferns into four Monophyletic classes; 11 monophyletic orders, 37

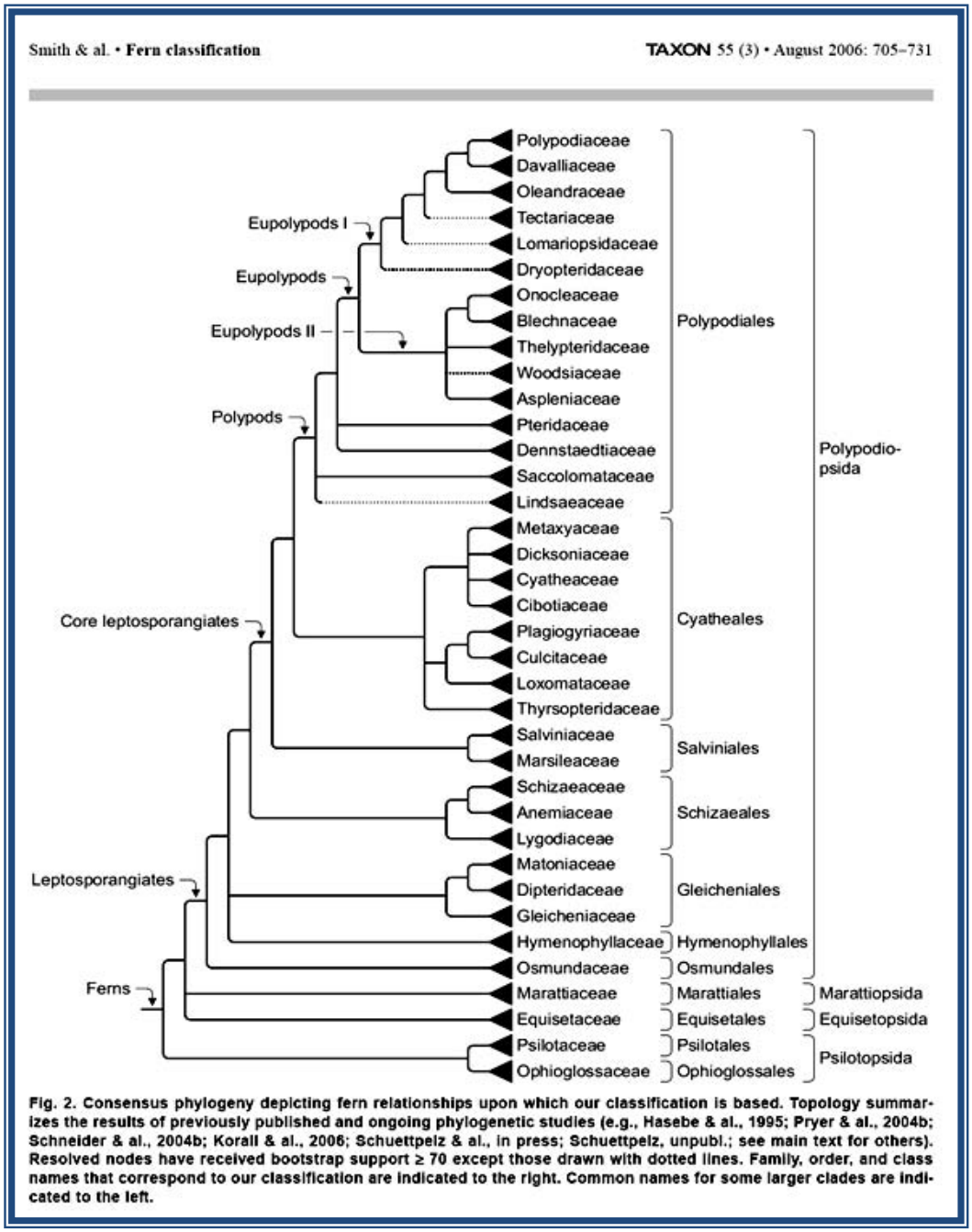


Fig. 2. Consensus phylogeny depicting fern relationships upon which our classification is based. Topology summarizes the results of previously published and ongoing phylogenetic studies (e.g., Hasebe & al., 1995; Pryer & al., 2004b; Schneider & al., 2004b; Korall & al., 2006; Schuettpelz & al., in press; Schuettpelz, unpubl.; see main text for others). Resolved nodes have received bootstrap support ≥ 70 except those drawn with dotted lines. Family, order, and class names that correspond to our classification are indicated to the right. Common names for some larger clades are indicated to the left.

Table 1a: Complete classification scheme proposed by Smith et al. (2006)

families of which 32 are monophyletic.

The classes are- Psilotopsida, Equisetopsida, Marattiopsida ,Polypodiopsida

The complete classification scheme proposed by Smith et al. (2006)-Table 1a, b

CLASS	ORDERS
Polypodiopsida	Polypodiales
	Cyatheales
	Salviniales
	Schizaeales
	Gleicheniales
	Hymenophyllales
	Osmundales
Marattiopsida	Marattiales
Equisetopsida	Equisetales
Psilotopsida	Psilotales
	Ophioglossales

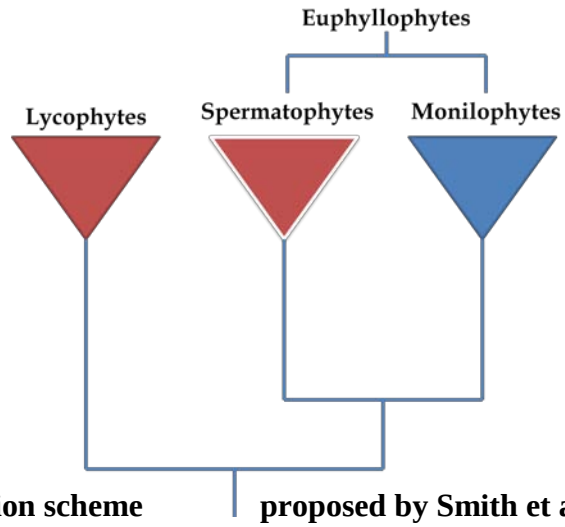


Table 1b: Classification scheme proposed by Smith et al. (2006) showing the orders

Christenhusz and Chase (2014) proposed classification based on molecular analysis

- They have aimed for a stable family and generic classification of ferns, and the current understanding of relationship of ferns and Lycopod clades.
- They are of the view that due to its vague circumscription and now evident non-monophyly, the term fern ally should be avoided.

- They also recommend that the term eusporangiate should be dispensed with because this includes ferns that are not leptosporangiate, and just describes the pleisomorphic state and the taxa included do not form a clade.
- The term ‘moniliophytes’ is unclear and has never been published as a formal taxon and more over its etymology is obscure.
- The leptospoangiate ferns are a natural group forming the majority of extant ferns

A comparison in two systems that are based on molecular data & morphology

	Smith et al. 2006	**Christenhusz & Chase 2014**
Families	37	38
Sub families	0	16

Terms to be dispensed with: Eusporangiate and fernally

- Although Sphenophytes share a common ancestors with fern there exact placement within fern is uncertain
- Ferns in the past include Equisetaceae & Psilotaceae
- Sphenophytes, Psilotophytes, Marattiophytes could be treated as independent lineages

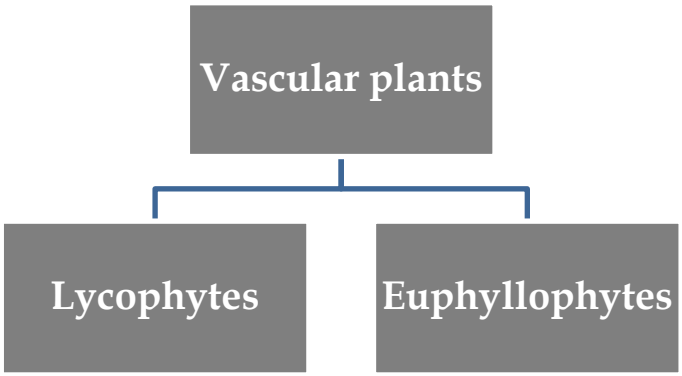
A revised family level classification for Eupolypod II Ferns (a clade of ferns in the order Polypodiales, under the class Polypodiopsida-Polypodiidae: Polypodiales) by Rothfels et al. 2012

- A family level classification for the Eupolypod II clade of leptosporangiate ferns (comprising over 2500 species), has been given on the plea that these were not well understood at the time of publication of the fern classification by Smith et al, 2006
- The composition and particularly the relationship among the major clades of this group have historically been controversial and challenged phylogenetic resolution until very recently
- Their classification reflects the current available data, largely derived from published molecular phylogenetic studies
- They provide circumscription for each family, which summarize their morphological, geographical, and ecological characters, as well as a dichotomous key to the eupolypod II families

By the pteridophytic classification it is assumed that vascular plans have a basal dichotomy separating:

Lycophytes: vascular plants having lycophylls or leaves without leaf gaps (microphylls), vein single medium, meristem intercalary

Euphyllophytes: vascular plants having euphylls or leaves with an associated leaf gap in the vascular stele (megaphylls), veins branched, and with marginal or apical meristem



This dichotomy occurred in the early – mid Devonian ca. 400 million years ago

Monilophytes (ferns) are characterized by-

- Lateral origin of the root in the endodermis
- mesarh protoxylem in shoot
- A pseudo- endospore
- Plasmodial tapetum
- Sperm cell with 30-1000 flagella

Euphyllophytes (or "Eu-phyllo-phyte" – true leaved plant): it includes most living plants except bryophytes and lycophytes

Features: Plant with upright, 3-dimensional branching

Stems: Upright growth of stems with lateral branches (pseudo-monopodial growth)

Branching in 360^0 (spiral branching off a main axis)

Leaves: True leaves in most derived forms ancestral members are leafless

Roots: True roots

Reproductive structures: Ancestral member spore-bearing; derived members are seed bearing

Geologic age: 420 my (Silurian Period) to Present

Lycophytes: include *Selaginella*, *Lycopodium*, *Isoetes*

- They are the oldest living vascular plants
- Reproduce through spores
- Have a single vascular vein through their leaves

There are about 1,000 species of Lycophytes worldwide

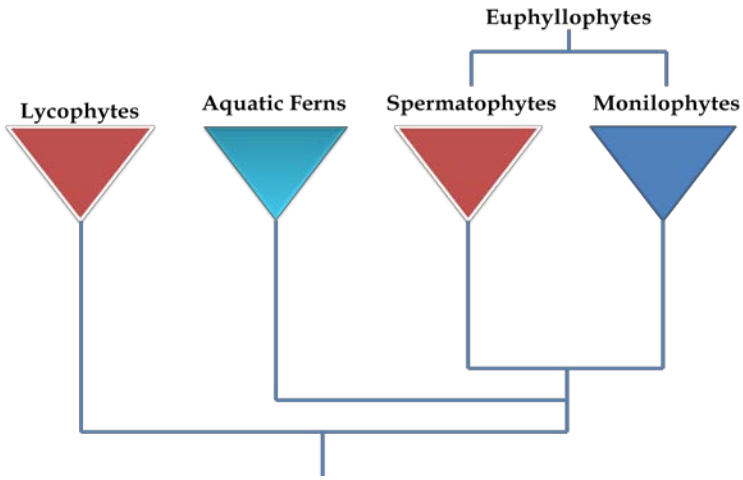
Evolution form homospory to heterospory

- The evolution of homospory to heterospory – in which a megaspore develops into a female gametophyte that includes one or more egg cells
- and a microspore develops into a male gametophyte that includes sperm – is among the most important transitions in the evolution of plants, with profound effects on plant reproduction and the life cycle.
- However, the nuclear genome of a homosporous vascular plant has yet to be sequenced

Divorce the heterosporous ferns from the homosporous extant ferns:

The aquatic ferns at first sight do not physically resemble other homosporous ferns

- They are heterosporous; remaining extant ferns are homosporous
- Their gametophytes are endosporic and entirely different
- Morphology of the sporophyte is different
- Habit and habitat are different
- Aquatic ferns are quite different and should be treated as separate from the rest of the ferns and perhaps be treated on an equal par as the Psilotaceae, Equisetaceae etc.
- Chromosome number in Marsileaceae is rather low compared to the homosporous ferns



A suggestion given by Khullar 2015

ORDINAL FEATURES

<u>Lycopodiales</u>

- Living and extinct plants
- Sporophyte with primary growth only, no vascular cambium
- Leaves eligulate

- Majority have definite strobili

Selaginellales

- Living & extinct plants with primary growth only, no vascular cambium
- Microphylls with ligule
- Definite strobili found
- Heterosporous
- Gametophytes endosporic
- Sperms biflagellate in living members

Isoetales

- Living and extinct plants
- Sporophyte with cormlike stems
- Sc. Growth present in root
- Ligulate microphylls
- Heterosporous
- Endosporic gametophytes
- Sperms multiflagellate in living members

Ophioglossales

- Species mostly terrestrial (a few epiphytic), temperate and boreal, but a few pantropical.
- Vernation nodding (not circinate)
- Rhizomes and petioles fleshy
- Root hairs lacking
- Aerophores absent
- Fertile leaves each with a single sporophore arising at the base of, or along, the trophophore stalk, or at the base of the trophophore blade (several sporophores per blade in *Cheiroglossa*)
- Sporangia large, with walls two cells thick, lacking an annulus
- Spores globose-tetrahedral, trilete, many (>1000) per sporangium
- Gametophytes subterranean, non-photosynthetic, mycorrhizal; $x = 45$ (46)

Psilotales

- Roots absent
- Stems bearing reduced, un-veined or single veined
- Euphylls

- Sporangia large, with walls two cells thick, lacking an annulus;
- Two or three sporangia fused to form a synangium, seemingly borne on the adaxial side of a forked leaf
- Spores reniform, monolete, many (>1000) per sporangium;
- Gametophytes subterranean (*Psilotum*), non-photosynthetic, mycorrhizal; $x = 52$.

Equisetales

The spermatozoids of *Equisetum* share several important features with other ferns that support their inclusion in this clade (Renzaglia & al., 2000). Kato (1983) adduced additional morphological characters, including root characters, supporting a relationship between horsetails and ferns.

- Stems whorled, lacunate
- Leaves whorled, connate
- Sporangia with helical secondary wall thickenings (Bateman, 1991), borne on peltate sporangiophores that collectively comprise strobili
- Sporangia large, lacking an annulus, many (>1000) per sporangium
- Spores green, with circular aperture and four paddle-like, coiled elaters
- Gametophytes green, surficial; $x = 108$.

Marattiales

- Roots large, fleshy, with polyarch xylem
- Root hairs septate
- Roots, stems, and leaves with mucilage canals
- Rhizomes fleshy, short, upright or creeping, with a polycyclic dictyostele
- Vernation circinate
- Leaves large, fleshy, 1–3-pinnate (rarely simple in *Danaea*, or 3–5-foliate in *Christensenia*) with enlarged, fleshy, starchy stipules at the base and swollen pulvinae along petioles and rachises (and sometimes other axes)
- Petiole and stem xylem polycyclic
- Stems and blades bearing scales pneumathodes (lenticels) scattered all around petioles and/or rachises
- Sporangia free or in round or elongate synangia (fused sporangia), lacking an annulus, enclosing 1000–7000 spores
- Spores usually bilateral or ellipsoid, monolete
- Gametophytes green, surficial; $x = 40$ (39)

Osmundales

- Temperate and tropical
- Stem anatomy distinctive, an ectophloic siphonostele, with a ring of discrete xylem strands, these often conduplicate or twice conduplicate in cross-section
- Stipules at bases of petioles
- Leaves dimorphic or with fertile portions dissimilar to sterile
- Sporangia large, with 128–512 spores, opening by an apical slit, annulus lateral
- Spores green, sub-globose, trilete
- Gametophytes large, green, cordate, surficial; *x*= 22.

Hymenophyllales

- Terrestrial and epiphytic; pantropical and south-temperate, but gametophytes survive in north-temperate regions as far north as Alaska.
- Rhizomes slender, creeping, wiry, or sometimes erect and stouter, protostelic
- Vernation circinate
- Blades one cell thick between veins (a few exceptions)
- Stomata lacking
- Cuticles lacking or highly reduced
- Scales usually lacking on blades, indument sometimes of hairs
- Sori marginal, indusia conical (campanulate), tubular, or clam-shaped (bivalvate), with receptacles, usually elongate, protruding from the involucres
- Sporangia maturing graduately in basipetal fashion, each with an uninterrupted, oblique annulus;
- Spores green, globose, trilete;
- Gametophytes filamentous or ribbon-like, often reproducing by fragmentation or production of gemmae; *x* = 11, 12, 18, 28, 32, 33, 34, 36, and perhaps others.

Gliecheniales

- Root steles with 3–5 protoxylem poles
- Antheridia with 6–12 narrow, twisted or curved cells in walls

Schizaeales

- Fertile-sterile leaf blade differentiation
- Absence of well-defined sori
- Sporangia each with a transverse, subapical, continuous annulus

Salviniales

- Fertile-sterile leaf blade differentiation
- Veins anastomosing
- Aerenchyma tissue often present in roots, shoots, and petioles
- Annulus absent
- Plants heterosporous
- Spores with endosporous germination
- Monomegaspory
- Gametophytes reduced.

Cyatheales

- Some of the species have trunk-like stems but others have creeping rhizomes
- Some have only hairs on the stems and blades, others have scales
- Sori are abaxial or marginal, either indusiate or exindusiate
- Spores are globose or tetrahedral-globose, with a trilete scar
- Gametophytes green, cordate

Polypodiales

- Indusia laterally or centrally attached (indusia lost in many lineages)
- Sporangial stalks 1–3 cells thick, often long
- Sporangial maturation mixed
- Sporangia each with a vertical annulus interrupted by the stalk and stomium
- Gametophytes green, usually cordate (sometimes ribbon shaped in some epiphytes), surficial

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4. Diversity and Evolution: Diversity in organography and the evolutionary trends in the members of Psilophyta, Lycophyta, Sphenophyta and Filicophyta - Early ferns, Eusporangiate ferns (Ophioglossales, Marattiales), Leptosporangiate ferns (Filicales, Marsileales, Salviniales).

Phylogeny:

Of the pteridophytes, ferns account for nearly 90% of the extant diversity. Smith et al. (2006), the first higher-level pteridophyte classification published in the molecular phylogenetic era, considered the ferns as monilophytes, as follows:

- Division Tracheophyta (tracheophytes) - vascular plants

- Sub division Euphyllophytina (euphyllophytes)
    - Infradivision Moniliformopses (monilophytes)
    - Infradivision Spermatophyta - seed plants, ~260,000 species
- Subdivision Lycopodiophyta (lycophytes) - less than 1% of extant vascular plants

Where the monilophytes comprise about 9,000 species, including horsetails (Equisetaceae), whiskferns (Psilotaceae), and all eusporangiate and all leptosporangiate ferns. Historically both lycophytes and monilophytes were grouped together as pteridophytes (ferns and fern allies) on the basis of being spore-bearing ("seed-free"). In Smith's molecular phylogenetic study the ferns are characterised by lateral root origin in the endodermis, usually mesarch protoxylem in shoots, a pseudoendospore, plasmodial tapetum, and sperm cells with 30-1000 flagella. The term "moniliform" as in Moniliformopses and monilophytes means "bead-shaped" and was introduced by Kenrick and Crane (1997) as a scientific replacement for "fern" (including Equisetaceae) and became established by Pryer et al. (2004). Christenhusz and Chase (2014) in their review of classification schemes provide a critique of this usage, which they discouraged as irrational. In fact the alternative name Filicopsida was already in use. By comparison "lycopod" or lycophyte (club moss) means wolf-plant. The term "fernally" included under Pteridophyta generally refers to vascular spore-bearing plants that are not ferns, including lycopods, horsetails, whisk ferns and water ferns (Marsileaceae, Salviniaceae and *Ceratopteris*), and even a much wider range of taxa. This is not a natural grouping but rather a convenient term for non-fern, and is also discouraged, as is eusporangiate for non-leptosporangiate ferns.

However both Infradivision and Moniliformopses are also invalid names under the International Code of Botanical Nomenclature. Ferns, despite forming a monophyletic clade, are formally only considered as four classes (Psilotopsida; Equisetopsida; Marattiopsida; Polypodiopsida),11ordersand37families,without as signing a higher taxonomic rank.

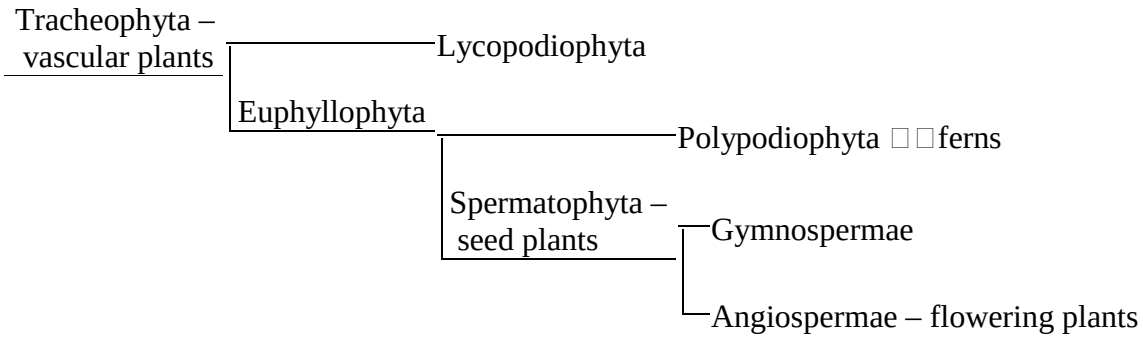
Furthermore, within the Polypodiopsida, the largest grouping, a number of informal clades were recognised, including leptosporangiates, coreleptosporangiates, polypods(Polypodiales), and eupolypods (including Eupolypods I and Eupolypods II).

In 2014 Christenhusz and Chase, summarising the known knowledge at that time, treated this group as two separate unrelated taxa in a consensus classification;

- Lycopodiophyta (lycopods) 1 subclass, 3 orders, each with one family, 5 genera, approx. 1,300 species
- Polypodiophyta (ferns) 4 sublasses, 11 orders, 21 families, approx. 212 genera, approx. 10,535 species
    - Subclass Equisetidae Warm.
    - Subclass Ophioglossidae Klinge
    - Subclass Marattiidae Klinge
    - Subclass Polypodiidae Cronquist, Takht. & Zimmerm.

These subclasses correspond to Smith's four classes, with Ophioglossidae corresponding to Psilotopsida.

The two major groups previously included in Pteridophyta are phylogenetically related as follows:



Subdivision

Pteridophytes consist of two separate but related classes, whose nomenclature has varied. The terminology used by the Pteridophyte Phylogeny Group (2016) (with some synonyms) is used here:

Classes, subclasses and orders

- Lycopodiopsida (lycophytes)
    - Lycopodiidae (clubmosses)
    - Selaginellidae (spikemosses and quillworts)
- Polypodiopsida (ferns)
    - Equisetidae (horsetails, single genus *Equisetum*)

- Ophioglossidae (Psilotidae)
    - Psilotales (One family, whisk ferns)
    - Ophioglossales (One family, grape ferns)
- Marattiidae (six genera, marattioid ferns)
- Polypodiidae (leptosporangiate ferns, largest subclass, seven orders)

Modern studies of the land plants agree that all pteridophytes share a common ancestor with seed plants. Therefore, pteridophytes do not form a clade but constitute a paraphyletic group.

Diversity and the evolutionary trends

Pteridophytes, understood as taxonomic group containing ferns, horsetails, and clubmosses, are especially diverse and abundant in the tropical mountain ranges of South America. My work aims to understand better the diversity of these plants, how they have evolved, and how they function in the ecosystems. Tree ferns are conspicuous elements of tropical montane rainforest. About 200 species of scaly tree ferns of the family Cyatheaceae occur in the Neotropics, ranging from small trunkless ferns of rocky outcrops to almost 20 m tall giants in dense forests. My work on these plants led to the recognition of 19 new and several formerly synonymized species in the genera Alsophila and Cyathea. The confusing nomenclature of the family was partially clarified by the correction of the typification of Cyathea pallescens (Sodiro) Domin. A checklist with keys to the Bolivian tree ferns was also accomplished during these studies. The revision of the genus Melpomene revealed 27 species with 10 varieties, most of them small ferns with deeply pinnatifid fronds, which are mostly epiphytes in upper montane forests or characteristic terrestrial elements of treeless páramo vegetation. The phylogenetic analysis based on morphology and chloroplast DNA shows that this genus as currently morphologically circumscribed is monophyletic and originated in South America. The radiation of the core group is apparently directly connected with the uplift of the northern Andes, which is the center of diversity of this genus. My ecologically orientated field studies of fern communities in southern Ecuador initiated several projects that aimed on different aspects. I found 248 different species of pteridophytes in our main study area, the Reserva Biológica San Francisco (RBSF), Prov.

Zamora-Chinchipe, but this number is likely to increase in the future. The study area is part of the Amotape-Huancabamba zone, a stretch of low elevation in the Andes located a the overlap of several biogeographic subunits and thus rich in endemic and widespread species alike. I found that the upper limit in the elevational distribution of most of the widespread ferns follows this dent in the mountain range, indicating that probably a downward shift of all

vegetation belts may be found. The mountain ridges in the RBSF support a unique heath forest dominated by the otherwise rare tree Purdiaea nutans Planch, but these peculiarities are not reflected in the fern composition. Ridge habitats in the study area, including two comparative sites close to the RBSF, are less diverse than adjacent slopes, and there was no higher representation of localized species on ridges. Overall, widespread species were weakly but significantly more abundant than localized species, and terrestrial – but not epiphytic – species were more abundant on ridges compared to slopes.

The observed influence of soils on the distribution of ferns in the Ecuadorian study area, where terrestrial and phylogenetically more derived taxa increase in diversity along a nutrient gradient caused by a downhill flux from the ridges to the gorges, encouraged me to look at the soil preferences of pteridophytes worldwide and see if it contains a phylogenetic signal. It seems that more derived lineages are better represented on rich soils, but have also a higher percentage of epiphytes. Looking at the mediator between soils and plant roots, the mycorrhizal fungi, I found that the published reports cover only a small fraction of the fern diversity and often give contradicting results. Focusing on neglected taxa, like the epiphytic Hymenophyllaceae, grammitid ferns (Polypodiaceae), and the genus Elaphoglossum (Dryopteridaceae), the investigation of root samples gathered in the Ecuadorian study area increased the known number of fern species with ascomycete infection considerably. The finding of this more derived type of mycorrhiza is in concordance with the phylogenetic position and life form of the host plants. Terrestrial and especially phylogenetically basal groups of pteridophytes have predominantly vesicular-arbuscular mycorrhiza, which is a very common and supposedly old form symbiosis.

Eusporangiate Ferns: Marattiales, Ophioglossales

Marattiales – Marattiaceae

This group of plants more closely resembles the Filicales than the Ophioglossales. Many of them possess large pinnate fronds with sporaniga n their lower surfaces. It is an ancient group with a fossil record that extends back to the transition of Lower to Upper Carboniferous. It is a tropical order and is generally known in temperate zones.

There are six genera and perhaps 200 living species in the order. The two better-known genera are ***Angiopteris*** (about 100 species) and ***Marattia*** (about 60 species). *Angiopteris* is distributed throughout the tropics of southeastern Asia, the *Marattia* is pantropical.

In growth habit the two genera typically have upright, unbranched, fleshy tuberous stems or short trunks bearing large, pinnately compound leaves (circinate in vernation) and thick fleshy roots.

A pair of clasping fleshy stipules is present at the base of each leaf, covering part of the stem; they persist along with leaf bases, even after the fronds abscise. New plantlets can arise from the stipules.

On the lower surface of the fronds, which may be up to 5 – 6 meters long, sporangia occur along veins. Venation in the ultimate segments is of the open dichotomous type.

In *Angioptris* a sorus consists of sporangia crowded together in two rows along a vein; each sporangium dehisces by a longitudinal slit on the side facing the other row of sporangia.

In *Marattia* the two rows of sporangia are united into a compact soral group surrounded by a common wall. This structure is termed a synangium. At maturity the synangium opens, much like a clamshell, exposing the sprangia, which dehisce by longitudinal slits.

In most other genera the stems are trailing, dorsiventral rhizome. The fronds may be simple to once pinnate (*Danaea*), or palmately compound with reticulate venation and scattered circular or ring like synangia (*Christensenia*).

Anatomy of the shoot

The apical meristem of a mature plant has a group of apical initials or an apical cell that is not regular or precise in its division.

The stem is protostelic at the base of the plants. Higher up the stele is an amphiphloic siphonostele with overlapping leaf gaps. At even higher levels in older plants the stele is a complex polycyclic dictyostelic consisting of two or more concentric vascular cylinders. The inner cylinders are continuous with the outer cylinders at lower levels in the stem.

In the petiole the leaf trace becomes subdivided in the formation of concentric cylinders of vascular bundles; the cylinders decrease in number higher up in the frond rachis.

Root primordia have their origin in the pericycle of stem vascular bundles of the outer and inner vascular cylinders. After their initiation, the large roots ‘bore’ their way through the stem cortex and then grow between the leaf bases.

Leaves

The leaves are circinate in vernation. The ultimate frond segments in most species have a single midvein with lateral dichotomous veins. The mesophyll in most forms is differentiated into an adaxial palisade and an abaxial spongy mesophyll. Stomata occur on the abaxial

surface. Mucilage cavities, hypodermal sclerenchyma, or collenchyma are often present in the petiole.

Roots

In the primary root and first formed roots on the stem there is a definite apical cell; later formed roots have a group of about 4 equivalent initials.

Roots become large and fleshy and contain mucilage cavities. Typically the vascular cylinder is polyarch – a feature not generally found in other ferns.

Sporangium

Sporangia are of the eusporangiate type and commonly originate from mounds of tissue paralleling the veins of developing fronds. At maturity each sporangium has a broad base and a sporangial wall that consists of several layers of cells. When mature the sporangia may be separate from each other (*Angiopteris*), or the sporangial walls may become confluent during development so that each sporangium is actually a pocket or loculus in a compact structure, the synangium.

Dehiscence of individual sporangia in a synangium is brought about by the drying out of wall cells, which results in longitudinal splitting of each sporangium (after the halves of the synangium separate in *Marattia*), or by the formation of a pore at the tip of each sporangium as in *Danaea*.

Spore output is large; spore numbers range from a minimum of 1,000 up to a maximum of 7,000 spores formed by each sporangium. In *Marattia* the spores are small, bilateral (monolete); those of *Angiopteris* are tetrahedral (trilete).

Gametophyte

The gametophyte is a large, green, dorsiventral ribbon shaped or heart shaped structure with a prominent ventral midrib or cushion shaped and thinner, ruffled, lateral wing like extensions. Te gametophyte, which may be 2 cm or more in length, is slow growing, long lived and has an endophytic fungus, which, however, must play only a minor role in the nutrition of the gametophyte because of the presence of chlorophyll. There are absorbing rhizoids along the ventral midrib. Gametangia of *Angiopteris* show the following pattern of distribution: antheridia are on the ventral surface but may occur on the dorsal side; archegonia are restricted to the projecting ventral midrib. Both antheridia and archegonia are sunken. Mature sperms are multiflagellate.

Embryo

The first division of the zygote is transverse, resulting in a two-celled embryo. Details of embryogeney are not too well known, Marattiales exhibit only endoscopic polarity. The future shoot apex and first leaf have their origin form the epibasal cell directed away from the neck of the archegonium. The hypobasal cell toward the archegonial neck gives rise to a multicellular foot. The root meristem, appearing late in embryogeny, is endogenous in origin. With subsequent growth of the embryo the young shoot grows up through the gametophyte, emerging from the upper surface. In some species of *Angiopteris*, suspensor is present. The first vascular bundle of the embryo is continuous between the root and first leaf. The vascular bundle of the next leaf is joined to the first vascular bundle.

Ophioglossales - Ophioglossaceae

Some botanists treat the Ophioglossales as an order in the Filicophyta along with other ferns; however, others consider the members of the Ophioglossales to be sufficiently different from other ferns to warrant making them a separate division. Some of the important attributes for consideration are:

a. ***The peculiar fertile segment***
b. ***Collateral vascular bundle***
c. ***Roots with endophytic fungus and without root hairs***
d. ***Non circinate vernation of leaves***
e. ***Lack of sclerenchyma in the plant body***
f. ***Subterranean gametophytes with an associate fungus***

The family comprises three recognized genera – *Ophioglossum, Botrychium* and *Helminthostachys*. *Botrychium* (grape fern, moonwort) with 35 or more species is nearly worldwide in distribution. *Ophioglossum* (adder's tongue) having about 45 or more species is widely spread throughout the world but is more abundant in the tropics. Although members of the Ophioglossaceae are typically terrestrial, there are some tropical, epiphytic species. The other genus *Helminthostachys* (monotypic) is native to the Indo-Malayan region. Commonly the stem is short and erect except in a few epiphytic species of *Ophioglossum* and in *Helminthostachys*, where it becomes a horizontal rhizome as the plant grows larger. Where the stem is erect, the leaves arise in a spiral sequence, but in temperate regions it is normal for only one leaf to be produced each year. In *Helminthostachys*, the leaves are borne in two ranks along the rhizome; they are large and ternately compound, but in other two genera they

are usually much smaller. Those of *Botrychium* are pinnately compound; those of *Ophioglossum* are simple or lobed and, unlike those of the genera, have a reticulate venation. At the base of petiole there is a pair of thin stipules which enclose the apical bud; and the next leaf, when begins to grow, has to break its way through the thin sheath covering it. Unlike all other living ferns their leaves are not circinately coiled when young.

In all three genera, the fertile fronds have two distinct parts, the fertile part being in the form of a spike which arises at the junction of the petiole with the sterile lamina, on its adaxial side. The fertile spike is pinnately compound in those genera with a compound lamina and simple in *Ohioglossum*, where the lamina is simple. The morphological nature of spike is thought to represent basal pinnae which have become ontognetically fused, face to face (i.e. it is believed that some early ancestor of the group had two fertile basal pinnae, whose primordial became fused during subsequent evolution).

The roots are peculiar in being completely without root hairs, a feature which is possibly connected with their mycorrhizal habit.

Growth of the stem apex is from a single apical cell, and its products are characteristically soft and fleshy, for they are without sclerenchyma. The stem of the young plant is protostelic, but soon becomes medullated. Later on, the stem of *Botrychium* become solenoxylic i.e. there are leaf gaps in the xylem, but not in the single external endodermis. *Botrychium* is the only genus of living ferns to show secondary cambial activity, and in some species it may give rise to a considerable thickness of secondary wood, composed of tracheids and wood rays.

Ohioglossum varies considerably in is internal anatomy, according to species. Some possess an outer endodermis, but in most species is absent, even in the young stages. The leaf gaps in the xylem overlap one another, giving rise to a network of meristeles, which form a rudimentary kind of dictyostele. The xylem is endarch in *Botrychium* and *Ophioglossum*, but mesarch in *Helminthostachys*. A pronounced feature of all three genera is the distinctly bordered circular pit in the metaxylem tracheids.

The sporangia in all three genera are marginal in origin. In Botrychium they are borne in two rows along the ultimate pinnules of the fertile spike and each receives its own separate vascular supply from a vein running into the pinnule. In *Helminthostachys*, the axis of the fertile spike bears numerous ‘sporangiophores’ in several rows, each bearing several sporangia and a few tiny green lobes at the tip. The spike of *Ophioglossum* bears two rows sporangia fused together, beyond which the axis projects as a sterile process. A number of

vascular bundles run longitudinally up the middle, anastomosing occasionally and giving off lateral branches to the sporangia.

Dehiscence of the sporangium is transverse in *Botrychium* and *Ophioglossum*, but longitudinal in *Helminthostachys* and large number of spores is released (more than 2000 in *Botrychium* and as many as 15000 in *Ophioglossum*.

Chromosome counts show a surprising range within the group, for *Botrychium* has a haploid number n=45, *Helminthostachys* n=46 or 47, while in *Ophioglossum vulgatum* n=250-260 and in *Ophioglossum reticulatum* n=631+10 fragments.

There can be little doubt that the three genera of the Ohioglossales are fairly closely related, nor that they represent an ancient and primitive group of ferns. The reticulate venation of *Ophioglossum*, its consolidated fertile spike and its complete lack of a suspensor together suggest that it has reached a more advanced stage of evolution than either of the other two genera.

Regarding the relationships between the Ophioglossales and the Marattiales, it is not easy to decide which characters are significant. Of the many characters common to the two groups, most indicate merely that they have reached roughly the same stage of evolution, rather than that they are closely related. These may be briefly listed as :

1. Basically erect axis 2. Stipules at the base of the petioles 3. Absence of sclerenchyma 4. Sporadic endodermis 5. Massive sporangium wall with stomata, the sporangia showing a tendency to fusion 6. Large spore output 7. Prothallus long lived 8. Massive antheridium 9. Suspensor present in some, absent in others.

Characters which suggest that the two groups are only distantly related are the circinate vernarion of the Marattiales and their superficial sori, contrasting with the absence of circinate vernation from the Ophioglossales and their marginal sori.

Filicales

Geographical distribution and growth habit

There are approx. 10,000 species in the Filicales and about 300 genera. The number of recognized families varies widely depending upon the morphological characters used to define families. The Filicales reach their greatest numbers and are most diversified in the tropics, where both epiphytes and tree ferns are common. The ferns are not restricted to the tropics, but their number decrease with increasing latitude and decreasing moisture. In temperate region ferns are largely terrestrial with a short, erect stem or commonly a prostrate rhizome with no aerial stem, the leaves being the only structure visible above ground.

Morphology and anatomy of the shoot

If the aerial stem of rhizome of a fern is short and erect, the plant appears to be a collection of leaves. If, on the other hand, the rhizome is prostrate, the leaves tend to be somewhat farther apart, and nodal and intermodal regions can be more easily seen. Branching of a prostrate rhizome in some ferns is dichotomous, but it is usually irregular by the activation of preformed buds at varying distances from the rhizome apex, often as a result of injury to the main axis. In *Hypolepis repens*, buds formed on the petiolar bases may develop into rhizomes. This type of branching results in the rapid occupation of favourable environments.

The majority of filicalean ferns have pinnate leaves, but many have simple ones.

Members of all families of ferns possess epidermal appendages on the leaf and stem and frequently on the root. These appendages may be of simple hairs, or develop into large chaffy scales called paleae.

Roots arise endogenously from the stem. Depending upon the taxon, roots may occur along a rhizome, be restricted to the nodes, or arise at the bases of leaves or at the base of lateral buds.

Size, form, and texture of the leaf

The majority of ferns have pinnatified, pinnate, or simple leaves. In size frond may vary from the enormous, almost branchlike leaves of tree ferns to the small leaves of certain water ferns. In living forms the large, compound, pinnate leaves are considered more primitive, whereas the small, simple leaves have been reduced in size during evolution. The latter type is therefore interpreted as a derived form. There is considerable variation in the texture of fern leaves: they may be thick and leathery, crisp or very delicate.

Two principal trends in the evolution of leaf form are suggested: 1. the evolution of a simpler leaf form from a more complex one and 2. The evolution of all other principal leaf forms from the pinnate-determinate type. Approximately 85% of the species and genera of ferns have the pinnate-determinate type. Specialization has led to the helicoids, radiate and furcated types.

Venation of the leaf

The stipe, rachis and axes of frond subdivision have prominent vascular bundles. The smaller veins of the lamina often have an open dichotomous type of venation. In some ferns the venation pattern is a reticulate type. Vein fusions are considered an evolutionary advancement. The first step toward vein fusions is expressed by the appearance of marginal

loops which connect the tips of vein dichotomies, or by the formation of a series of meshes adjacent to the midrib or throughout a leaf segment.

Anatomy of the leaf

The typical fern leaf is dorsiventral. Epidermal cells have thickened, outer tangential walls and usually contain chloroplast, a feature not shared by most other vascular plants. Stomata generally occur on the abaxial surface of leaves. The mesophyll may be uniform in organization consisting of homogenous parenchyma with chloroplast, or the cells may be organized into definite adaxial palisade and abaxial spongy parenchyma layers. Considerable evolutionary importance is attached to the form and arrangement of the vascular tissue in the stipe and rachis. There may be simple arc or the vascular bundle may be convolvulated, or the vascular tissue may consist of a cylinder of separate bundles. The last arrangement is considered to be derived. As an added complication, the pattern of the vascular tissue may change in passing from the stipe through the rachis to the axes of the pinnae and pinnules.

The sorus

In the vast majority of ferns the foliage leaves serve in both photosynthesis and reproduction. In some species there may be distinct separation of these functions: certain leaves function in photosysnthesis, whereas other are strictly ‘sporophyll’ and non photosynthetic. In other species there is an intermediate arrangement. For example, sporangia may be restricted to certain specific portions of a photosynthetic leaf. For the most part sporangia are crowded into compact groups on leaves, each group being termed a sorus. Sori may be circular or linear in outline. If sporangia are not grouped into definite sori they may form marginal tassels along narrow, reduced leaf segments or be scattered over the lower surface of expanded leaves, along and sometimes between veins. In those species having definite sori the sorus is along or near the frond margin or on the abaxial side of the frond.

Organization of the sorus

Both marginal and superficial sori are most commonly found over a vein or at the terminus of a vein. That portion of the leaf surface to which sporangia are attached is termed as receptacle. It may be a slight protuberance, a definite bulge, or an elongated cone. It is from the superficial cells of the receptacle that sporangia originate while the leaf is still in a very young developmental stage. Undoubtedly circinate vernation provides protection for the delicate sporangia during their ontogeny.

Sporangia may or may not be protected by a covering termed an indusium. If a sorus lacks an indusium it is a naked sorus termed as exindusiate. If an indusium is present it may be formed by adaxial and abaxial extensions of the lamina; this result in the formation of a cup or pouch like structure. In some species a reflexed marginal portion of the lamina itself is associated with an indusium, forming a pouchlike structure. In forms that have sori on the abaxial surface some distance from the leaf margin, the indusium is an outgrowth from the epidermis of the lamina or of the receptacle. The form of the indusium is variable: it may be a delicate, linear flap attached along one side only (unilateral indusium); it may be horseshoe shaped or circular and elevated (peltate); it may be cup shaped; it may be a collection of scale like structures overarching the sporangia; or the leaf margin may be turned back upon itself (the so called false indusium), with the sorus borne on it.

Structure of sporangium

The two features that are regularly considerable in comparing sporangia are final length of the stalk and the number of cells (or rows of cells) making up the stalk. Short, thick stalks are considered primitive, and long, delicate stalk (frequently consisting of three rows of cells or even one) are derived.

The sporangial wall is typically one cell in thickness at maturity. The main point of interest, however, is in the means of dehiscence. In the Filicales there are various method of dehiscence, depending on the position of the annulus (thick walled cells). In the Osmundaceae the annulus, located to one side, is responsible for the formation of a cleft that runs over the top of the sporangium and down the opposite side. The sporangium opens like a clam. In other ferns the annulus may form a cap at the distal end of the capsule, be obliquely places, or run over the top of the capsule in line with the stalk (in a vertical or longitudinal position). These three positions result in longitudinal, oblique, and transverse dehiscence respectively.

Maturation of sporangia within a sorus

The simplest way in which fern sporaniga are borne is singly along the margins of leaf segments – each with a vascular bundle leading to its base (as in some extant ferns such as *osmunda*). This arrangement is a primitive condition. In the majority of Filicales, sporangia are aggregated to form sori.

A sorus in which all of the sporangia originate, grow, and mature at the same time is termed a ‘simple’ sorus.

If sporangia are initiated over a period of time in a definite sequence the “gradate” sorus is produced. The order of sporangial initiation and development is basipetal; the oldest sporangium is near the summit of a receptacle with successively younger sporangia toward the base. When the fossil record is considered and compared with that of living ferns having the simple type of sorus, the gradate maturation is clearly a derived type. It must be emphasized that not all ferns with gradate sori are necessarily closely related. Gradate maturation represents an evolutionary level of specialization that has been achieved by different species.

The most advanced evolutionary level of development is achieved in the sorus that has intermingles sporangia, all in different stages of growth. This is the ‘mixed’ sorus. The more highly specialized and evolved families and subfamilies have this mode of soral development.

Sporangial dehiscence

The dehiscence of the fern sporangium is ingenious. The annulus is the structural feature associated with dehiscence and the forceful ejection of spores. As a sporangium matures, water is lost from the cells of the annulus by evaporation. There is a powerful adhesion between the cell walls and water. The continuous loss of water from each cell of the annulus results in the thin outer tangential wall of each cell being drawn inward, while the ends of the radial walls are pulled toward each other. This results in the tearing open of the sporangium on the weak side and eventually in the complete inversion in the position of the annulus. The annulus is now under tremendous tension. Water continues to evaporate. Eventually the cohesive force of the water in cells of the annulus is exceeded and the annulus returns suddenly to approximately its original position. In the process the spores are thrown out forcefully for a distance of a centimeter or so. The tensions built up prior to dehiscence are equivalent to about 300 or more atmosphere of pressure.

Soral type

Variation in the soral morphology are endless. At best only a few of the more common soral types that illustrate a generalized type or represent steps in a possible evolutionary series can be described.

Sori on fern leaves may occupy

1. A ‘marginal’ position - here the receptacle must originate strictly from the margin of the developing pinnae or pinnules. An indusium, if present, is formed by submarginal outgrowths

around the receptacle. The indusial may be funnel shaped or two lipped. e.g. *Hymenophyllum*, *Trichomanes*

2. An 'intramarginal' (near the margin) position - here sporangia originate near the marginal position. Cells at the margin suddenly or gradually lose their meristematic activity and become parenchymatous. Submarginal cells on the adaxial side of the developing lamina near the margin continue their meristematic activity-forming the receptacle. e.g. *Pteris, Cryptogramma, Pteridium* etc.

3. The abaxial or 'superficial' position in which sori are at some distance from the leaf margin on the lower or abaxial side of a frond. During leaf development the origin and final position of a sorus are correlated with the activity of marginal meristems and their derivatives. Here, a young receptacle has its inception from submarginal cells on the abaxial side rather early during marginal growth of the lamina. However, in contrast to the intramarginal type of development, the margin of the lamina remains actively meristematic and adds new tissue to the lamina. Therefore a growth of a younf leaf segment proceeds, the young sorus occupies positions progressively farther from the margin. An indusium, if characteristic of the species, is formed from superficial cells near the developing receptacle and eventually overarches it. The indusium varies in shape from elongate and attached along one of the longer sides (unilateral), to half moon shaped or reniform and attached at the sinus (*Dryopteri*s). In some species the indusium is an outgrowth from the top of the receptacle, resulting in the formation of a stalk and a radially symmetrical cap (peltate type).

Anatomy of the stem

A variety of structure and tissues take their origin from the meristamatic derivative cells of the apical cells: leaves, the protoderm of the leaves and the stem, epidermal hairs and dermal appendages, ground meristem, and procambium.

Throughout the Filicales there is no indication of cambial activity resulting in the formation of secondary vascular tissues. There is however, development of considerable sclerenchyma in the axes of some ferns. The radial maturation of xylem in a stem vascular bundle is typically exarch or mesarch. The large tracheids of the metaxylem generally have tapered ends and scalariform pitting. It has been found that certain filicalean ferns (*Pteridium aquilinium*) possess vessels; scalariform perforations instead of pits are present in the oblique end walls between two vessel members. Vessels also occur in the roots and rhizomes of certain water ferns.

The length of tracheary elements varies with location in the plant, age of the plant, length of an internode, polyploidy, and habitat. In spite of these variations the following changes appear to be correlated with specialization from the primitive condition for each feature: 1. Shortening of tracheary elements 2. Increase in the occurrence of a modified type of scalariform pitting (opposite or alternate) 3. Increase in the occurrence of slightly oblique to transverse end walls and 4. Sporadic occurrence of vessels e.g. *Pteridium* and *Marsilea*.

Osmundaceae

The Osmundaceae consisting of three living genera, *Osmunda, Todea and Leptopteris,* is represented in rocks of the Permian. Other relatives (e.g. *Osmunda caulis*) were common in the Mesozoic. For more than 100 million years the family has displayed a remarkable constancy in characters. *Osmunda* as a genus has probably existed for 70 million years, and the family was probably more abundant in the past and more widely distributed than at present.

Extant species are terrestrial, generally stand erect, and have simple trichomes. Some individual plants of *Osmunda* may be over 100 years old. In *Osmunda cinnamomea* there are two types of fronds – fertile and sterile. In other species the fronds consists of sterile and fertile regions. In *Osmunda* large sporangia are attached along the margins of narrow leaf segments often in clusters, but without a definite soral-type organization. In *Todea* the sporangia occur along veins on the abaxial side of the lamina. The origin of a asporangium is not always easily referable to a single superficial initial, and the mature sporangium has many eusporangiate features. A sporangium is large with a lateral annulus; upon dehiscence the sporangium opens much like a clam shell, liberating a large number of spores (up to about 512). A chromosome number of spores of n=22 is uniform in the family.

A t.s. of an *Osmunda* stem reveals a large number of closely packed leaf bases and the vascular cylinder. The stele is an ectophloic siphonostelic with overlapping leaf gaps. Some fossil genera from the Permian were strictly protostelic or protostelic with mixed pith (tracheids and parenchyma). Enough fossil forms are known that a stelar evolutionary sequence can be traced from the protostele to the *Osmunda* type of today.

The gametophyte is large, green thallus with a conspicuous midrib. Long lived gametophyte may reach 4-5 cm in length. Members of the Osmundaceae combine more eusporangiate and leptosporangiate characteristics in their morphology than do members of any other family in the Filicales. This is reflected in the establishment by some pteridologists of a separate order,

Osmundales, for the group. Many morphologists consider that no other group of ferns has been derived from the Osmundaceae.

Gleicheniaceae

The ancient family Gleicheniaceae may hae been derived from certain coenopteris ferns. There is some evidences for the existence of the family in the Carboniferous. The fossil genus Gleichenites has been reported from the Jurassic; more typical forms of the family have been described from the Upper Cretaceous.

Modern members are pantropical to subtropical in distribution. They are terrestrial with long creeping rhizomes and leaves that may clamber over other vegetation. They often form dense thickets at the edge of forests. Leaves have a rather unique architecture. They fork repeatedly, and a foliar bud is present in the sinus between the two axes at the level of branching. The bud of the main axis may continue to grow periodically, and more pinnae are formed. Leaves of some species may become 3-10 mts. Long. Pinnae grow in a similar fashion but the leaf buds remain permanently arrested. The ultimate portions of the branch system develop laminar segments.

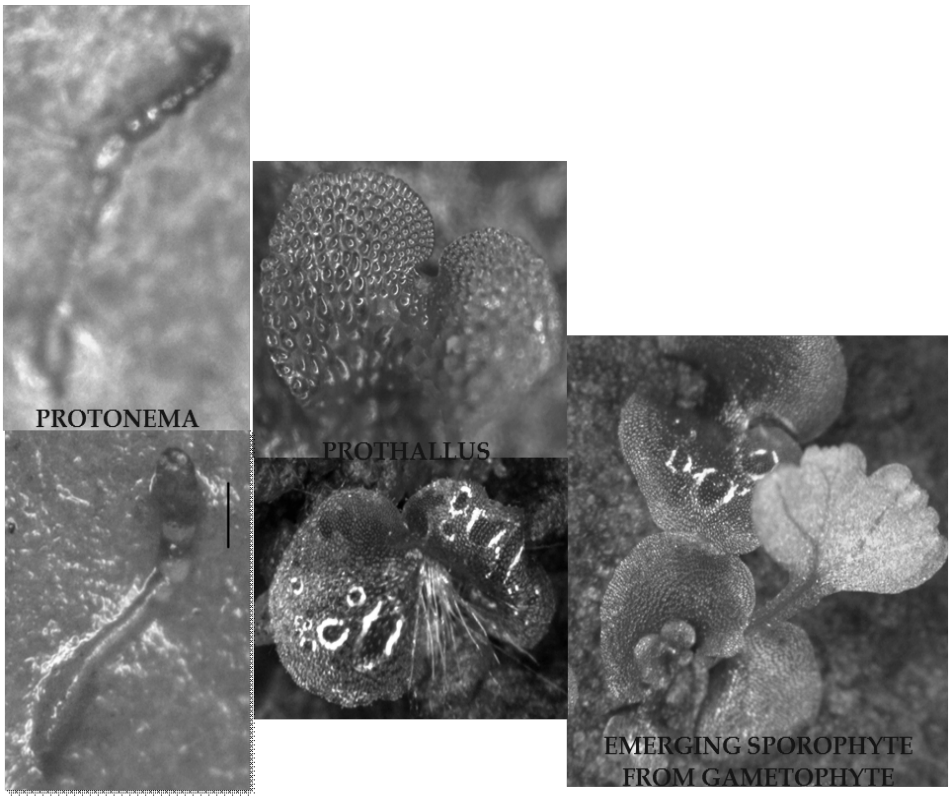
In some system of classification two genera, *Gleichenia* and *Dicranopteris*, are recognized. In *Gleichenia*, the ultimate lateral veins of leafy segments are unbranched or dichotomize once. Sori, consisting commonly of 2-4 large sporangia, are in two rows, one on each side of the midrib; there is no indusium. The ultimate veins in *Dicranopteris* dichotomize more than once, and sori consist of 8-15 sporangia. Dehiscence of a sporangium is brought about by the functioning of a transverse, to a transverse-oblique, girdling annulus. Each sporangium produces a large number of spores that are trilete or monolete depending on the species. Stems of most species are protostelic of the mixed-pith type.

Gametophytes are large, green and dorsiventral with a conspicuous midrib. An antheridium is large and generally produces several hundred sperm. In considering all the morphological characteristics, one can say that the family has retained many primitive features.

5. Gametophyte: Patterns of spore germination; patterns of gametophyte development in homosporous and heterosporous pteridophytes; mating system in fern.

- The gametophyte phase begins with a spore
- Upon germination, a spore gives rise to a green, thread-like tissue, called a protonema
- The protonema develops into a prothallus, a small, green, multicellular tissue that is rarely seen in nature. The prothallus has numerous subterranean rhizoids to anchor it to the substrate and absorb nutrients
- Light and other environmental factors control the development of gametophytes
- In many species, gametophytes kept in darkness do not develop beyond the thread-like protonemal stage
- However, illumination with blue or ultraviolet radiation causes the protonema to develop into a heart-shaped prothallus
- This is an example of photomorphogenesis, the control of development by light

SUCCESSIVE PHASES OF GAMETOPHYTE DEVELOPMENT



Gametophyte (Prothalli) Morphology

- Exosporic gametophytes: Germination of spore and development of gametophytes are under the control of external conditions e.g. Homosporous ferns
- Endosporic gametophytes: Independent of external controls e.g. Heterosporous ferns- *Marsilea*, *Salvinia*
- Radially symmetric form: Some sp. of *Lycopodium*, *Ophioglossum*, Psilotales and archegoniophores of filamentous gametophytes in Trichomanes
- Dorsiventrally symmetric form: Majority of pteridophytes
- Homosporous Filicales: Typically cordate shaped (Exceptions strap shaped, ribbon shaped, more or less filamentous, typical filamentous)
- Radial (primitive) to Dorsiventral (advanced) change can be due to adaptation to light conditions. No such relationship in the male prothalli of *Isoetes*, *Marsilea* and *Salvinia* where the prothalli develop in dark

Mycorrhizic gametophytes in many pteridophytes: common in gametophytes lacking chlorophyll, e.g. Ophioglossaceae, and some *Lycopodium* spp. forming subterranean gametophytes

Homosporous pteridophytes: No special contrivances for holding water, except the formation of lobes in the gametophytes of *Lycopodium* and *Equisetum*

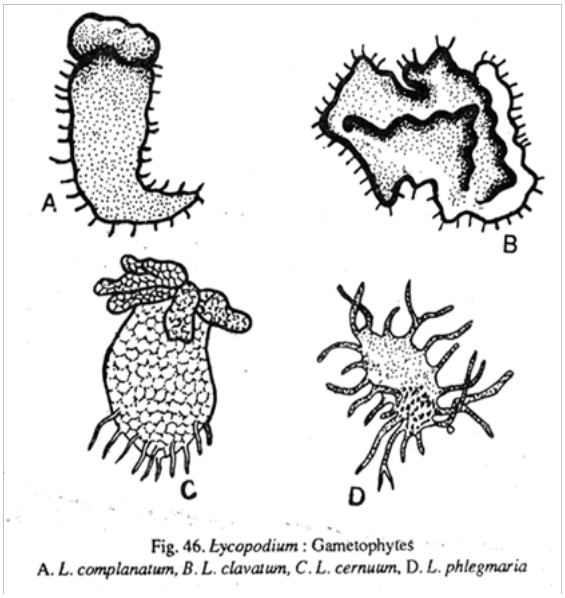
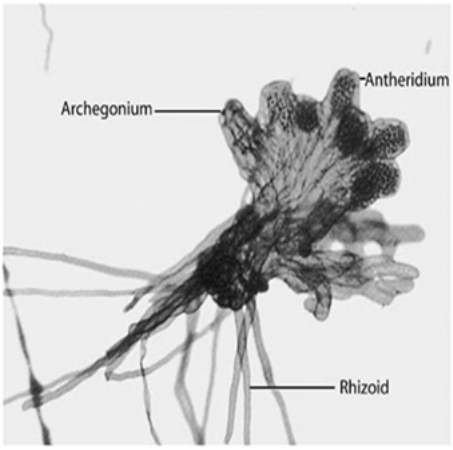


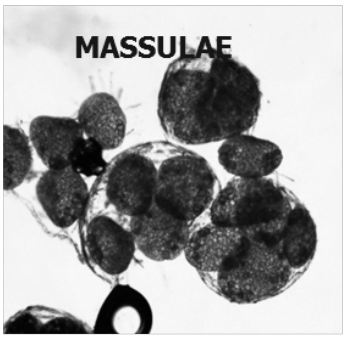
Fig. 46. *Lycopodium* : Gametophytes
A. *L. complanatum*, B. *L. clavatum*, C. *L. cernuum*, D. *L. phlegmaria*

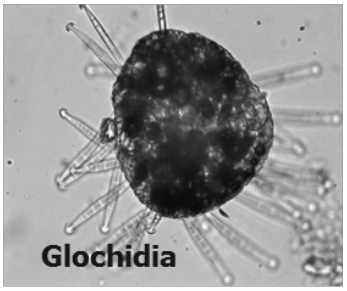
Equisetum gametophyte (hermaphroditic)

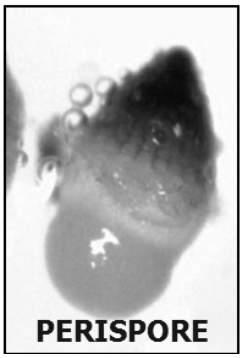


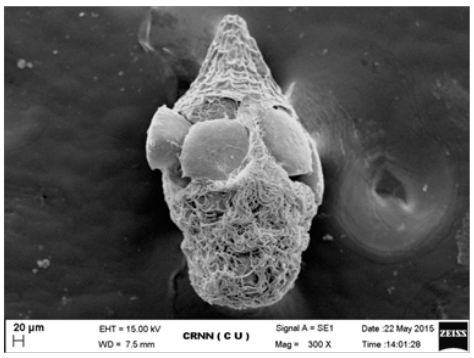
Heteroporous Pteridophytes: Male gametophyte of *Salvinia*, *Azolla*: No contrivances exist so that there are chances of being washed away if not shed in a mass, the massulae;
In some species of *Azolla*: Massulae have anchoring structure named as glochidia to effect fertilization;

Female gametophyte is surrounded by an irregular special wall – perispore









Gametophytic phase

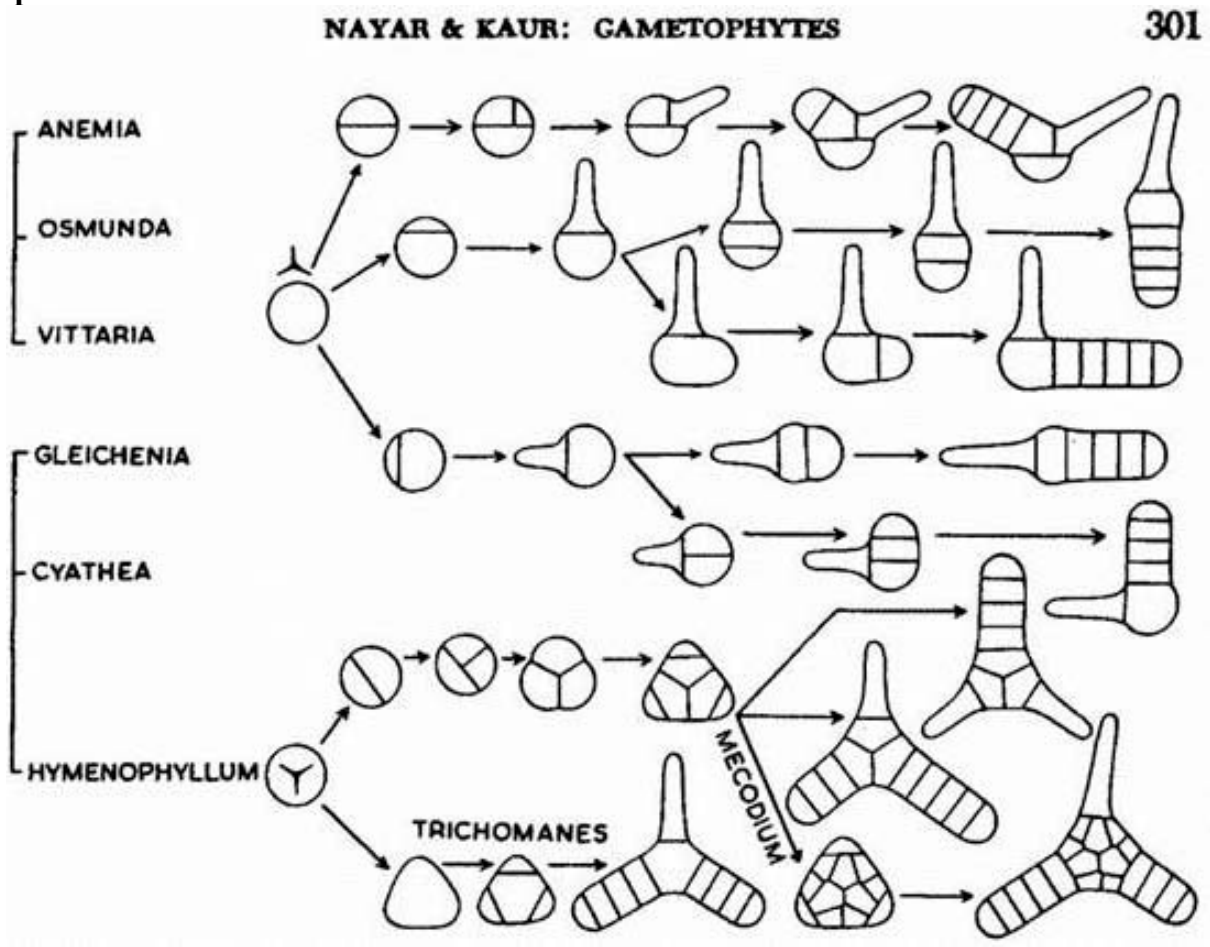
- Considerably reduced
- Distinct shortening of the life span of gametophytes is noticed from homosprous to heterosporous forms
- Homosporous: Relatively long lived structure and in certain *Lycopodium* spp. it may require years to attain maturity.
- Heterosporous: Gametophytes span ranges from a few hours to few days. Besides span, reduction form of gametophyte is also noticed. *Selaginella* and *Isoetes*- male gametophytes are extremely reduced consisting of single prothallial cell and an antheridium
- Rhizoids are altogether absent in male prothalli and also in female prothalli of *Salvinia* and *Azolla*

Vegetative propagation of gametophyte (very frequent in homosporous form)

- By adventitious branching
- Special structures of vegetative propagation i.e. gemmae occur in *Psilotum*, *Lycopodium* spp., *Hymenophyllaceae*, *Vittariaceae*
- Prothalli of *Gymnogramma*: form tubers to perennate
- Some forms like *Vittaria*: remain permanently in gametophytic phase

- Gametophytes can be maintained indefinitely in cultures by routine subculture and prevention of fertilization
- Gametophytes of *Trichomanes*: it remains independent and produces gametangia but fail to form sporophyte, instead reproduce vegetatively by forming gemmae (tolerant to desiccation and freezing).
    - These gametophytes grow in deep crevices of rocks at very low light levels and because of their long existence in these conditions they have possibly lost the capacity to produce sporophytes

Spore Germination



Patterns of spore germination

The mature germination patterns (on the basis of division in relation to polarity):

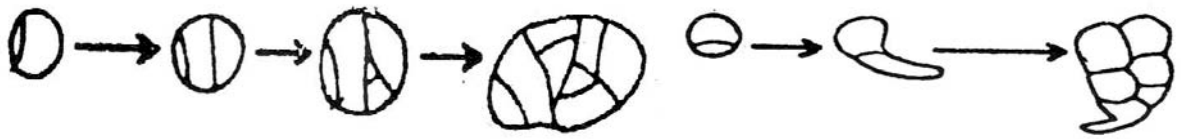
Bipolar germination

Tripolar germination

Amorphous germination

- The germination of spores and establishment of gametophytes in psilotales remain to be elucidated

- The germination of spores in *Lycopodium* and *Equisetum* is bipolar, resulting in the formation of a small rhizoidal cell and a larger prothallial cell
- The rhizoidal cell fails to form rhizoid in *Lycopodium*
- By regular divisions the prothallial cell gives rise to the gametophyte in *Lycopodium* and by irregular divisions in *Equisetum*

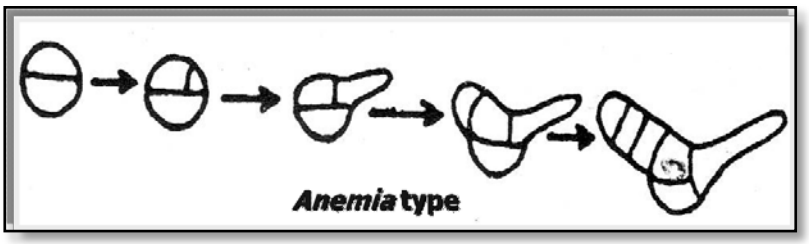


Lycopodium ***Equisetum***

Bipolar germination: *Anemia* type
Osmunda or *Gleichenia* type
Vittaria type
Cyathea type and

***Anemia* type**

- The first division is equatorial forming two equal daughter cells
- The anterior cell remains quiescent, while the posterior one cuts off a lateral rhizoidal initial and a large prothallial cell
- The prothallial cell divides by a series of walls parallel to the first wall and develops into the germ filament



***Osmunda* or *Gleichenia* type**

- The first division results in a small rhizoidal cell and a large prothallial cell
- A series of divisions in the prothallial cell leads to the formation of a uniseriate protonemal filament

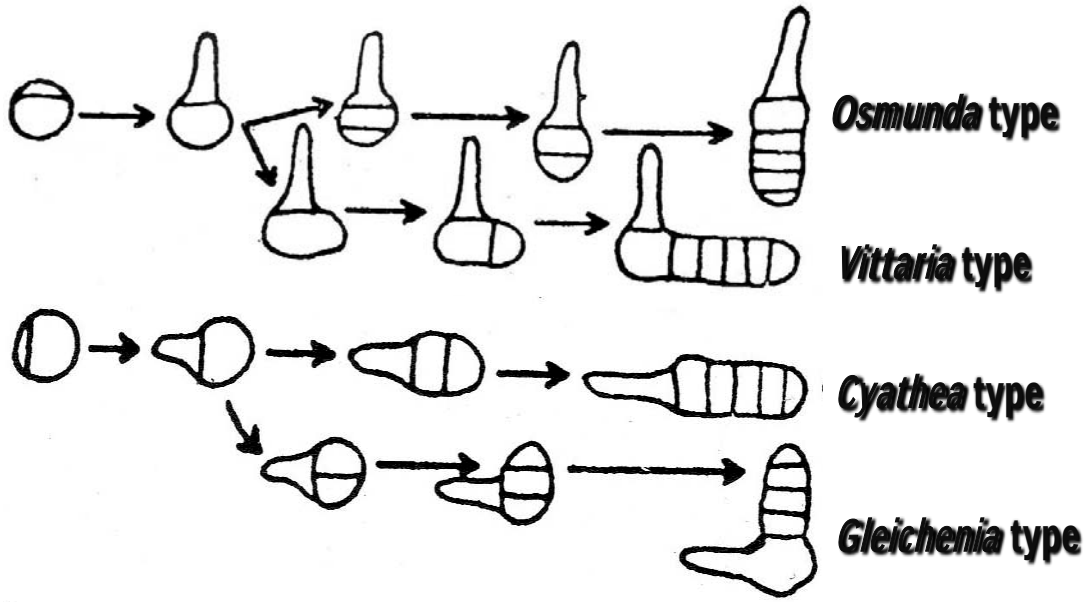
***Vittaria* type**

- First division is to form rhizoidal and prothallial cell
- The prothallial cell divides by a a wall perpendicular to the first wall.
- Of the two daughter cells one remains quiescent and the other, by a series of divisions parallel to the second wall, forms the germ filament

***Cyathea* type**

- Here also, rhizoidal and prothallial cell are formed

- But, the prothallial cell divides longitudinally and forms the germ filament



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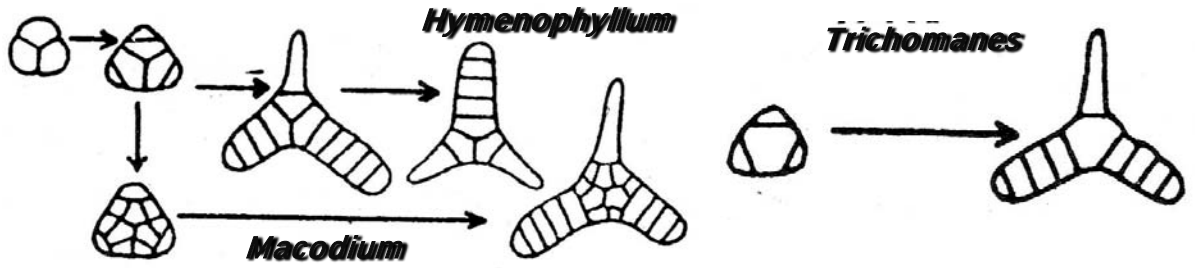
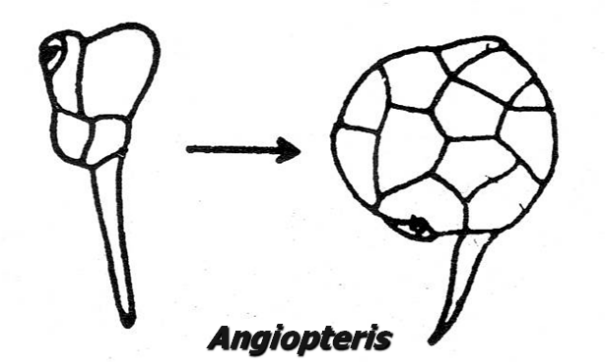
Tripolar germination (characteristic of Hymenophyllaceae)

- The initial divisions result into an equatorially expanded plate of three equal cells
- In each of these cells a lens shaped daughter cell is cut off towards the periphery
- The lens-shaped cell grows out either into a rhizoid or germ filament.
- In *Macodium*, the divisions occur in each of the three primary cell resulting into a triangular one cell thick plate of 9-12 cells.
- In *Trichomanes*, three lens shaped cells are formed simultaneously at the periphery of triangular spores



Amorphous type (Rare type, restricted to primitive ferns like *Angiopteris* and *Marattia*)

- This type is characterized by irregular cell division and irregular direction of growth
- A mass or plate of cells is formed
- At a later stage, one of the marginal cells becomes meristematic, further growth of the prothallus is in the direction of meristemetic cell



Patterns of gametophyte development in homosporous ferns

Seven different patterns

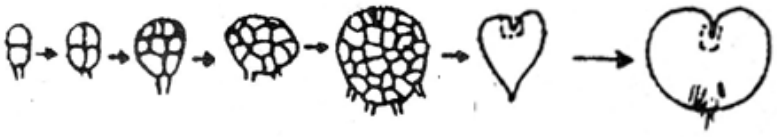
- *Osmunda* type
- *Marattia* type
- *Adiantum* type
- *Drynaria* type
- *Kaulinia* type
- *Ceratopteris* type
- *Aspidium* type

***Osmunda* type (characteristic of Osmundaceae)**

- A plate of four cells in the anterior cell of the germ filament
- Repeated divisions result in a circular prothallial plate
- One of the marginal cells in the anterior quadrant becomes meristematic (new cells arising from it make the developing prothallus an asymmetric structure)
- Ultimately the thallus elongates, develops a notch at the meristematic region and assumes a symmetrical cordate form

***Marattia* type (restricted to ferns having amorphous type of spore germination)**

A symmetrical cordate prothallus is derived by a pattern similar to *Osmunda*



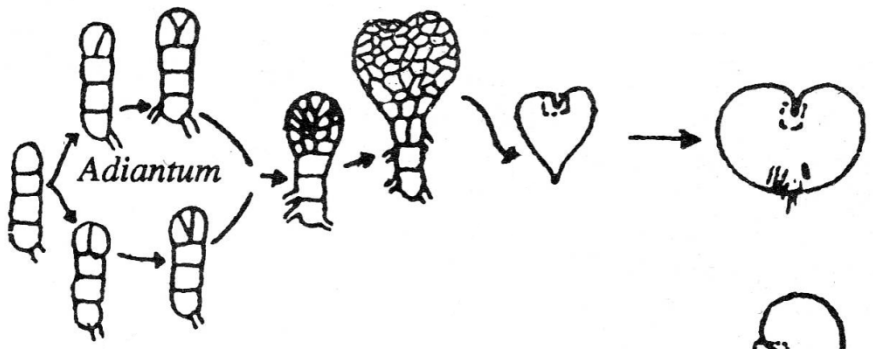
In rest of the ferns the spore germination results in an elongate uniseriate protonema of a few cells, and one or more rhizoids at the basal end

A change in plane of cell division occurs when the protonema comprises two to ten cells and with it initiates two dimensional growths

Exceptionally, in *Schizaea* and *Trichomanes* no change occurs and the prothalli remain filamentous. In Grammitidaceae also, uniseriate stage is more extensive

***Adiantum* type**

- The development is characterized by an early establishment of an apical cell
- The terminal protonemal cell divides by a wall parallel or oblique to the longitudinal axis
- The 2nd division is oblique to first division forming a wedge shaped meristematic cell, the activity of which leads to one cell thick obovate prothallial plate
- Later, the thallus apex at the meristematic region becomes notched and cordate shape is achieved



***Drynaria* type**

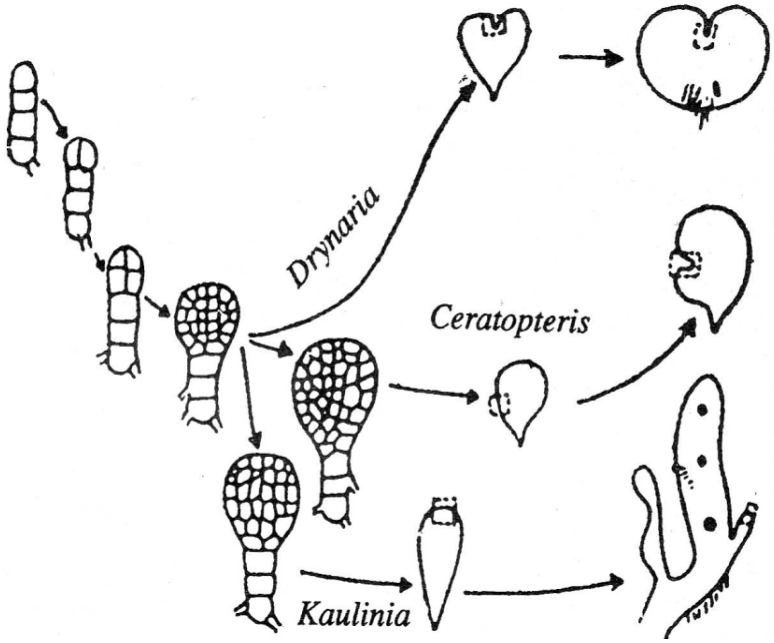
- The establishment of an apical cell is delayed
- A broad spatulate prothallial plate is formed
- When the prothallus is five to ten cells broad, by two oblique divisions in one of the anterior marginal cells, differentiates an obconical meristematic cell
- Further growth is as in *Adiantum* type-symmetrical cordate thallus

***Kaulinia* type**

- An ameristic prothallial plate is formed and no meristem is ever initiated
- The thallus elongates and becomes ribbon shaped
- Branches arise from a group of marginal cells
- Although no meristem is formed but towards maturity irregularly scattered small circular cushions, two to four cell thick, develop in the middle region of thallus

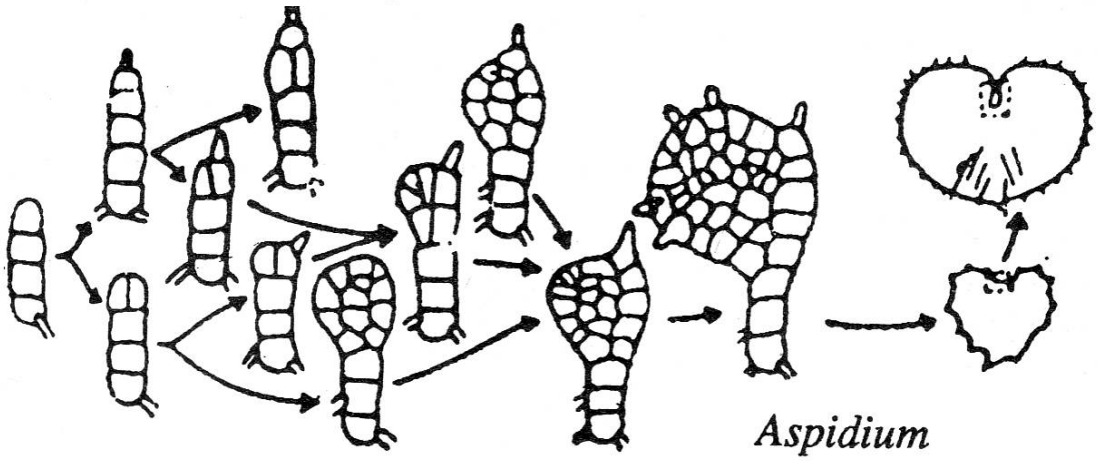
***Ceratopteris* type**

- The juvenile prothallus is a broad ameristic plate of cells
- The growth of male prothallus ceases soon after the differentiation of antheridia
- The prothallus destined to become female however, continues to grow and in it the meristematic activity gradually becomes restricted to a group of laterally situated cells
- This lateral meristematic region soon differentiates into a pleuricellular meristem and gives an asymmetric shape to the prothallus in that one wing is larger than the other



***Aspidium* type**

- characterised by an early hair formation and there is often great variability in development
- Normally the terminal cell of the protonemal filament produces a unicellular papillate hair
- This apical cell as well as one or two lower cells remains inactive and a broad plate is formed by posterior cells and it is usually lopsided
- In some cases the terminal cell divides longitudinally into a larger and a smaller cell
- The former develops the hair and remains inactive and from the latter by active divisions arises the prothallial plate
- As a variation, hair formation is delayed until after plate formation is initiated by longitudinal division in the anterior cell of germ filament
- One of the daughter cells of the terminal cell later develops a hair. (All these variations may occur in the same species)
- The prothallial plate formed is lopsided and one of the marginal cells on the more expanded side differentiates as meristematic cell
- Further development is as in *Adiantum* type



Pa
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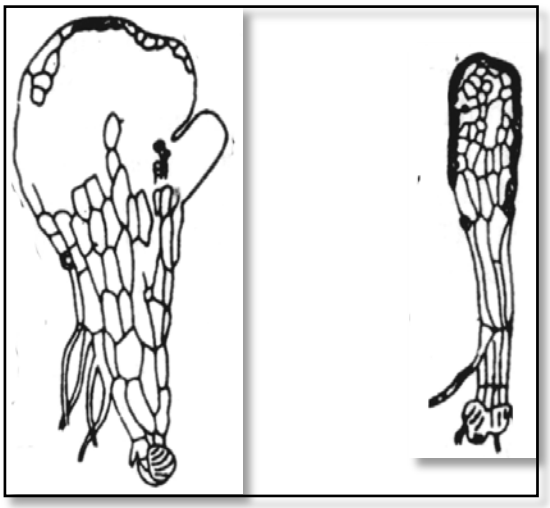
gametophytes

- However, male as well as female gametophytes are exosporous- this is in contrast to heterosporous pteridophytes. Moreover, female gametophyte develops antheridia if fertilization is delayed
- *Ceratopteris* - an interesting homosporous form shows incipient heterospory
- Produces male and female gametophytes and the female gametophytes becomes hermaphrodite, if fertilization is delayed.



MALE GAMETOPHYTE IN HETEROSPOROUS FORMS

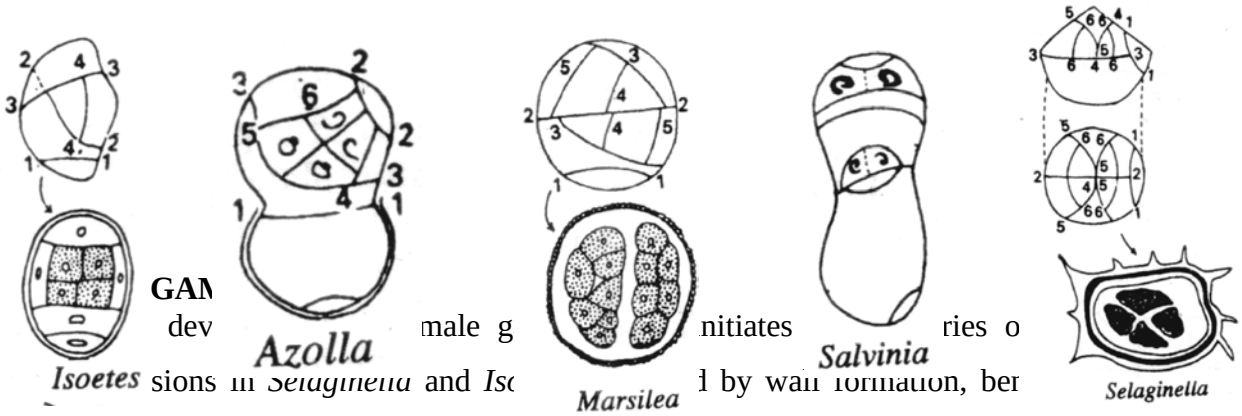
- The germinating microspores in all genera, except *Platyzoma*, produce a lenticular cell and the larger cell
- From the latter, by a series of divisions, spermatogenous cell (s) and a variable number of sterile jacket cells are formed

Variation in spermatogenous cells

- One spermatogenous cell- *Isoetes* [4 sperms]
  Azolla [8 sperms]
- Two spermatogenous cells- *Marsilea* [32 sperms]
  Salvinia [8 sperms]
- Four spermatogenous cells- *Selaginella* [128 or 256 sperms]

The entire male gametophyte in *Selaginella*, *Isoetes*, *Marsilea* and *Azolla* is referable to an antheridium

- In *Salvinia* each of the two spermatogenous cells represents an *antheridium* as it is separated by sterile cell



GAM

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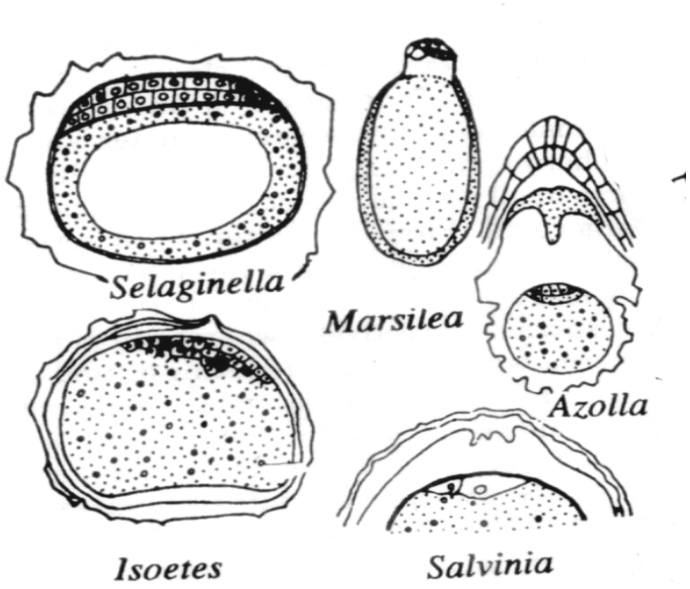
Isoetes sions in *Selaginella* and *Isc* l by wall formation, ber

ridge, and on this tissue differentiate archegonia

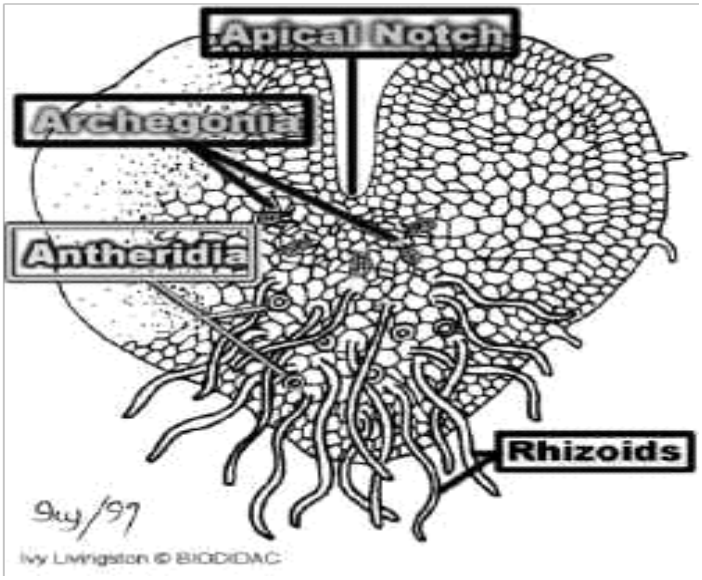
- The wall formation is slower in *Isoetes*

- In some species of *Selaginella*, the gametophytic tissue is separated from the free nucleate mass by a diaphragm
- The megaspores in *Marsilea*, *Salvinia* and *Azolla* divide to form a small papillate cell and the larger cell occupies the rest of spore storing abundant food material
- The papillar cell forms a tissue and on this region differentiate archegonia
- In *Marsilea* there is only one archegonium per gametophyte

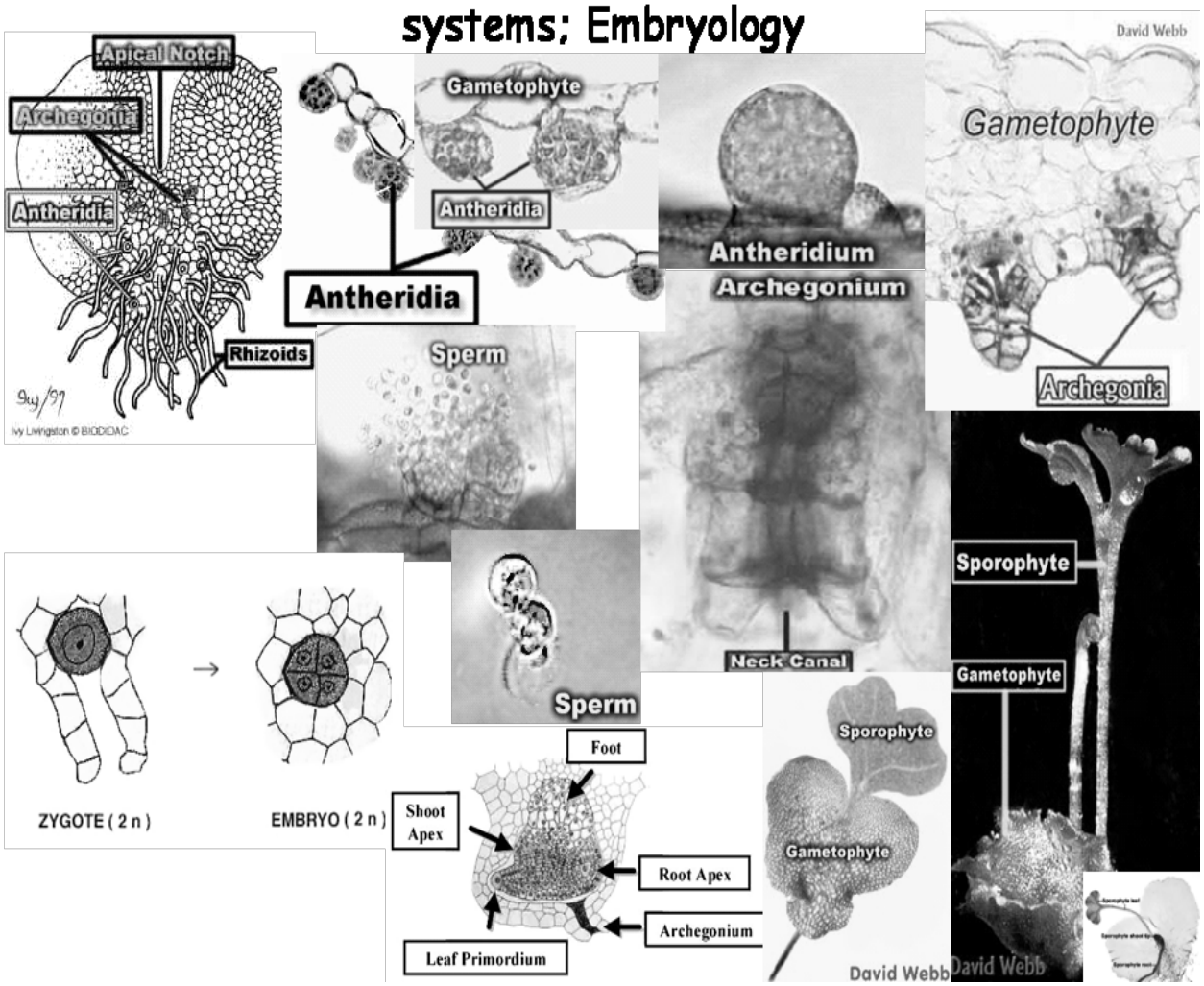


Gametangia: Antheridia & Archegonia

- Exosporic gametophytes of homosporous pteridophytes: monoecious (bisexual)
- Endosporic gametophytes of heterosporous forms: dioecious (unisexual)
- The spatial arrangement of sex organs is variable on axial gametophytes of Psilotales and Ophioglossales. Normally, antheridia and archegonia are intermingled and distributed all over the surface. In some species of *Lycopodium* intermingling of sex organs occur (*L. lucidulum*) but in other species the antheridia and archegonia form distinct patches.
- In more primitive ferns (*Marattia* and *Angiopteris*) archegonia are restricted to central cushion and antheridia occur on both surfaces.
- On dorsiventral, cordate type gametophytes of ferns the antheridia and archegonia generally are restricted to the ventral side. Archegonia are present near the notch on the so called archegonial pad that is more than one cell in thickness. Antheridia are generally formed toward the posterior end of the gametophyte, situated among rhizoids, but they may occur among on the wings toward the notch.



Gametangia; Gametes; Fertilization process; Mating systems; Embryology

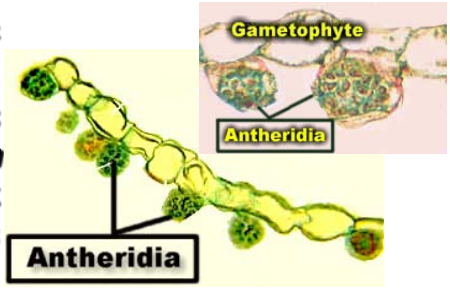


- **Archegonium:** always an embedded structure at its venter in both homosporous and heterosporous forms

- **Antheridium:** position is less consistent; it is embedded in some forms (*Lycopodium*, *Equisetum*, *Marattiaceae*, *Ophioglossaceae*, *heterosporous* ferns, *Selaginella* and *Isoetes*) and emergent in others (Psilotales and leptosporangiate ferns)

- **Antheridia: A well defined wall, the sterile jacket enclosing spermatids**

  In embedded antheridia the jacket is mostly one cell thick but in *Botrychium* and *Helminthostachys* it is two cell thick except at the centre where several cells remain undivided



The mature antheridium of a specialized homosporous leptosporangiate ferns is composed of
Three jacket cells (a basal cell, a ring cell, a cap cell) enclosing the spermatids
Not all filicalean ferns have this type of antheridial organization. There are variations in the number of jacket cells, and spermatids

Dehiscence of Antheridia: One or more cells- the opercular cells, of jacket breakdown and help release the sperms

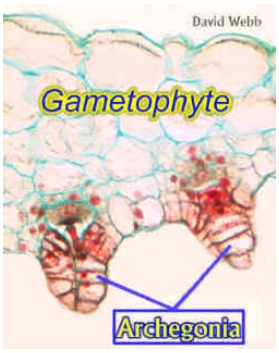
The dehiscence is possibly due to swelling of mucilaginous substances on the opercular cells
The dehiscence of antheridium in polypodiaceous ferns takes place by the extrusion of intact cap cell.
The cap cell remarkably lacks cellulose toward maturity of antheridium.
In all cases there is hole or pore in the outer membrane of antheridium.

Number of sperms per antheridium
❖Varies considerable in different groups.
❖*Isoetes*, produces only four spermatozoids per gametophyte.
❖Normally sperm output may reach up to one hundred and exceptionally to few thousands in *Ophioglossum*.

Biciliate pteridophytes: *Lycopodium*, *Selaginella*

Pleuriciliate pteridophytes: *Psilotum*, *Temispteris*, *Isoetes*, *Equisetum*, ferns

Archegonia



Archegonia: a protruding neck (functionally equivalent to the jacket of antheridium) and low embedded venter comprising four rows of five to seven tiers of cells that enclose

neck canal cell
ventral canal cell
egg cell

NECK CANAL CELL

- The neck canal cells are usually superposed row of cells
- In some species of *Lycopodium*, due to anticlinal division a double series of cells is formed or
- If wall formation fails to occur a single row of binucleate cells results.
- Some species of *Equisetum* also have two laterally placed neck canal cells

Based on number of neck canal cells the pteridophytes can be arranged in a reductional series

beginning with *Lycopodium*-many neck canal cells and
ending in leptosporangiate ferns-single binucleate neck canal cell

Largest necks:
In *Lycopodium* spp. forming subterranean gametophytes
Short necks:
Quick maturing prothalli of *Lycopodium*, *Isoetes* and *Selaginella*
Very short scarcely projecting necks:
Characteristics of leptosporangiate ferns

Ventral canal cell

It is well-defined cell in lower forms but is difficult to demonstrate in Ophioglossales
Ontogenetically ventral canal cell is the sister cell of the egg with a capacity of function like an egg.

From the functional point of view, the most significant and variable part is the axial row of cells in which the lowermost cell (the egg)-differentiates as the female gamete

All members of axial row except the egg disintegrate and produce a canal through which motile sperms can pass

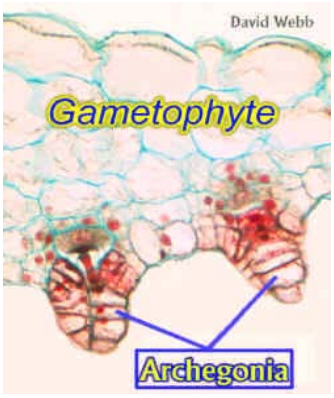
Fertilization and embryogeny

- A gametophyte is flooded with water
- An antheridium shows signs of an increased internal pressure followed by the
- Loosening of the cap cell and the escape of the spermatids
- In a few seconds, or at the most of few minutes, the spermatids are metamorphosed into flagellate swimming sperms.

Sperms

- A sperm resembles a short corkscrew with the flagella attached to a lamellar band in the anterior gyres of the helix. The flagella beat in a coordinated wavelike fashion, thrusting the sperms forward and also causing it to rotate.

- When a gametophyte is immersed in water, the opening of an archegoinum is occurred by the enlargement of the distal end and the separation of special neck cells.

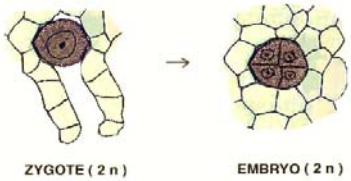


- This allows a tiny stream of mucilaginous material or slime to be released, which is flowed by the forceful release of cytoplasmic bodies-presumably they are entities of the axial row except for the basal egg cell or neck cells split apart.

- Swimming sperm are at first not attracted to archegonia, but later when the slime is diluted, they change direction chemotactically and swim toward an archegonium.

- In *Phlebodium aureum* from three to five sperms may swim into an open archegonium and occupy the ventral cavity at the same time. However, only one sperm fertilizes the egg.

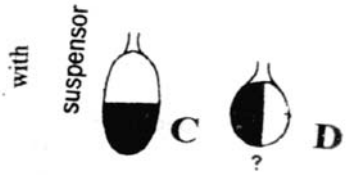
- The first division of the zygote is reported to take place anywhere from one hour to ten days after fertilization. In *Phlebodium aureum* it is five days



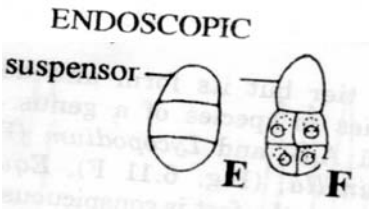
- Even before the zygote divides cell of the surrounding gametophyte divide and form a partially ensheathing calyptra

- The first division wall of the zygote is generally parallel to the long axis of the archegonium

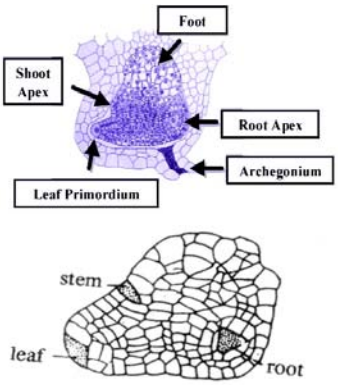
- This initial division separates an anterior cell that is directed toward the notch of the gametophyte and a posterior cell that is directed away from the notch



- The former cell is the apical pole or epibasal cell, and the later is the basal pole or hypobasal cell



- The zygote in the Filicales is said to be prone in orientation. Each of the cells divides, and the new cell wall in each is perpendicular to the original wall, resulting in the development of a four celled embryo or quadrant stage of embryogeny
- Each of the four cells then undergoes usually by synchronized divisions
- According to the classical descriptions of fern embryology, the primary organs may be traced back to specific segments of the quadrant stage
- The outer anterior quadrant or cell reportedly gives rise to the first leaf, the inner anterior quadrant to the shoot apex.
- The primary root originates from the posterior quadrant, the foot from the inner posterior quadrant.



Mating System

- Intragametophytic: fusion of gametes produced by the same gametophyte (only in homosporous ferns)
- Intergametophytic: fusion of gametes from two different gametophytes (common to both homosporous and heterosporous forms)
    - Intergametophytic selfing-if the different gametophytes contributing the gametes originate from spores produced by the same sporophyte (bisexual homosporous forms)

      Intergametophytic crossing-if the different gametophytes contributing the gametes originate from spores produced by the different sporophyte

Possibility of this phenomenon depends on certain gametophytic adaptations: morphological, populational and genetical)

Morphological: chronology of gametangial initiation e.g. Formation of antheridia followed by hermaphrodite phase

Orientation of archegonial neck in all directions (toward the antheridia is normal)

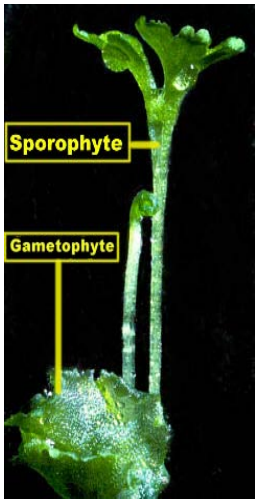
Populational: in a populations hormonal relationships or other factors regulate towards a particular mating

Genetical: Self incompatibility and due to prevalence of intragametophytic selfing

- After whatever manner the organs are delimited, the first root and the leaf begin to grow rapidly and pierce the calyptra

- The first leaf eventually grows forward and upward through the notch

- After the first leaf has unfolded more leaves are formed by the shoot apical meristem, and roots are produced on the developing stem axis



- Sooner or later, the gametophyte degenerates and dies

- Leaves of young sporophytes often do not resemble those of adult plants

6. Sporophyte: Variations in vegetative and reproductive structures and their evolution with special emphasis on shoot apex, stelar organization, and soral characters

Shoot apex:

- Apical meristem and the subjacent regions of expansion and maturation
- Regulates the nature of lateral organs, leaves and buds (branches on aerial axis)

The classical model shows characteristics and identifiable apical cell which is described to be active in young shoot tip whereas apical cell in older and mature plant has been described as quiescent

On histological basis the shoot apices of pteridophytes can be classified to several types-

- A single conspicuous apical cell, to which all cell lineages can be traced e.g. *Equisetum*
- Several definite but rather less conspicuous cells (*Lycopodium*). During dichotomy the central cells (x) cease to divide and other cells divide to form dichotomy
- Inconspicuous but definitely superficial initial cells *Dryopteris*, *Matteuccia;* An inconspicuous nature of an apical cell in ferns apices has led to the concept of apical zone

Shape and Size of Shoot Apex-Vary greatly

Shape is determined by relative rates of growth in vertical and transverse planes

In some species the growth in longitudinal direction is greater than in transverse

- In others transverse component is more conspicuous (Marattiaceae)

Organogenesis on the shoot apex of a fern

a. bilateral or lenticular apical cell, the leaf initial (may be tetrahedral in primitive ferns but more commonly it is 3 sided with two cutting faces)
b. preformed bud primordia which give rise to shoot buds

Organization

The apical and sub apical regions lack fully differentiated vascular elements-xylem and phloem.

- The ascending materials, therefore, are envisaged as diffusing laterally into developing cortex and pith, and upwards in the apical meristem and newly formed primordia.
- However, in ferns, incipient vascular elements are seen at the apical region - termed as pre-vascular tissue.

- This tissue is traceable upwards, from the mature vascular elements to a point immediately below the apical cell group, associated prism shaped cells, and youngest leaf primordial.
- Although, the tissue has an indistinct differentiation but it gives incipient pathway of translocation and is important in organogenesis.

Detached Meristem

- In *Matteucciastruthiopteris*, *Onocleasensibilis* :
- If the apex of a horizontal rhizome was excised the lateral buds always originated at quite definite positions or sites along the rhizome
- These sites are occupied by a small region of quiescent superficial prism-shaped cells resembling the cells of shoot apical meristem, protected by overarching peripheral cells
- These potentially meristematic regions, bud rudiments or detached meristems, originally constitute part of shoot apical meristem and occupy interfoliar positions at the apical region

Steles

- The vascular elements of the primary axes of practically all pteridophytes consist of a solid core of xylem surrounded by phloem. This simplest possible organization, a prototype, is termed as protostele.
- Archaic psilophytales such as *Rhynia*, and *Horneophyton* and present day Psilotales, *Psilotum*, and *Tmesipteris*: retained this organization even in adult form of subterranean stems.
- Therefore, phylogenetically as well as ontogenetially potostele is considered to be most primitive types of stele.
- A protostele of circular outline is termed as haplostele
- Star shaped configuration is actinostele (characteristic of *Asteroxylon*, aerial axes of *Psilotum* and certain species of *Lycopodium*).
- However, in certain other species of *Lycopodium*- central xylem breaks up into plates and each is surrounded by phloem. This specialized form is termed as plectostele.
- To keep pace of overall increasing diameter of stem axis, the stellar tissue in the central region undergoes parenchymatous development.
- This is best exemplified by *Psilotum*, the basal region of aerial axis has a central medulla or pith and the ultimate branches are without it.

- Medullated protostele is termed as siphonostele and represents advancement.
- The stele also has two zones of phloem that border the xylem internally as well as externally and is, therefore, termed as amphiphloic siphonostele or solenostele.
- During the departure of leaf traces in megaphyllous forms the solenostele undergoes parenchymatous developments and forms leaf gaps.
- Due to overlapping of leaf gaps a solenostele gets dissected and results in a dictyostele.
- A dictyostele in transection appears as a scattered series of bundles; each bundle is a meristele consisting of a central strip of xylem surrounded by phloem followed by a pericyclic and endodermal layer. In the axial form these strands are inter connected and form a tubular network. A dictyostele is therefore, an amphiphloic tube with overlapping leaf gaps.

Origin of Pith (controversial)

Stellar

- The pith has been considered to results from degradation of tracheary elements and, therefore stellar in origin. Forms with mixed pith serve as support examples of this view point.

Extrastellar

- In contrast, the other view suggests extra stellar origin of pith i.e. migration of cortical cells into the stellar axis. Support for this view point is derived from forms having solenostele that has two endodermal layers; one delimits the stele from cortex and other delimits it from the pith.

Stellar organization in Ferns

- The protostele still persists in filicales- in *Gleichenia*, *Lygodium*, Hymenophyllaceae and some Pteridaceae (*Lindsaea*).
- The protostele of Filicales is however, of special type as seen in *Gleichenia*. The central column is essentially a primary xylem in which tracheids of metaxylem are interspersed with parenchyma cells.

Four stages of specialization have been recognized in steles of filicales:

- 1st stage - complete differentiation of pith
- 2nd stage - differentiated a small and variable amount of internal phloem with or without an internal endodermis. It can be described as a prelude to solenostele.
- Both these stages in varying degrees are seen in some *Osmunda* spp and *Schizaea* spp.

- 3rd stage - complete solenostele; with internal and external endodermis, phloem regions and well defined leaf gap. e.g. *Adiantum*.
- If the gaps overlap the result is a dictyostele. Varying degrees of expression of this stage in Cyatheaceae, Pteridaceae, Anemiaceae, Platyzomaceae, Dipteridaceae, Davalliaceae, Aspleniaceae, and Blechnaceae.
- 4th stage - much finer network of steles. It is accompanied by multiplication of leaf traces at their origin on the stele as seen in most Polypodiaceae.Polycyclic stele is an additional specialization at the 3rd stage (*Matonia*) as well as 4th stage (*Platycerium*, Polypodiaceae).

Specialization of tracheary element in Ferns

- Shortening of tracheary elements
- An increase in occurrence of a modified type of scalariform pitting (opposite and alternate)
- An increase in the occurrence of slightly oblique or transverse end walls
- Finally sporadic occurrence of vessels

Occurrence of vessels and secondary growth

True vessels occur in:

- *Selaginella*
- *Equisetum*
- *Ophioglossum*
- *Helminthostachys*
- *Botrychium*
- *Pteridium*
- *Woodsia*
- *Notholaena*
- *Marsilea*

Interestingly, vessels occur in rhizome and root of pteridophytes and are absent from shoot and frond. Of the living pteridophytes, *Isoetes*, *Stylites*, *Botrychium* and *Regnillidium* show unique types of secondary growth

Root

- Embryonic root in pteridophytes is short lived and the roots on adults plants are adventitious structures.

- The lateral roots originated from endodermis.
- Similar to shoot apex, growth centres can be visualized to exist, but nothing is known about these centres.
- In leptosporangiate ferns e.g. *Polypodium*, and *Marsilea*, the root apex has a conspicuous tetrahedral apical cell from which by orderly sequence of divisions, originate root cap or calyptra, epidermis, cortex and stele.
- In eusporangiate ferns, several initial cells are present at the root apex.
- The roots of Pteridophyta exemplify a correlation between size and structure in their steles. The small roots of such ferns as *Marattia*, *Angiopteris* or *Acrostichum* are diarch or triarch structures while the large root from the same plant may have up to 15 or more xylem plates.

Leaf

Types (basis of structures)

Microphylls:

- Simple form, mainly small leaves but may attain considerable dimensions as in *Isoetes*
- An un-branched vein which is not accompanied with the formation of leaf gap in the stem stele (Exception: fossil arborescent lycopods with two veins in leaf blade and species of *Selaginella* with a branched vein)
- The microphyllus forms may be ligulate and eligulate based on the presence or absence of ligule

Megaphylls:

- Mainly large and usually pinnatified with a complex series of veins
- The origin of leaf trace is accompanied with the formation of prominent leaf gap in the vascular cylinder (Exception: In forms like *Lygodium*, *Gleichenia* and members of Hymenophyllaceae, the leaf trace is not accompanied with the formation of leaf gap in the vascular cylinder).

Fern Leaves

A fern leaf is an apically growing system. In a compound leaf the lamina or pinna are formed as lateral outgrowths on a peg like primordia that forms petiole and rachis. The leaf blade is formed by the activity or marginal meristem

Majority of ferns have pinnate but some with simple leaves

Size: Frond may vary from the enormous; almost branch like leaves of tree ferns to the small leaves of certain water ferns.

Texture: There is considerable variation: may be thick and leathery, crisp or very delicate**.**

Evolution of leaves:

Two principal trends in the evolution of leaf form are suggested:

1. Evolution of a simpler leaf form from a more complex one
2. Evolution of all other leaf forms from the pinnate-determinate type.

Approximately 85% of the species and genera of ferns have the pinnate-determinate type. Specialization has led to the helicoids, radiate and furcated types.

In living forms the large, compound, pinnate leaves are considered more primitive, whereas the small, simple leaves have been reduced in size during evolution. The latter type is therefore interpreted as a derived form.

Origin and Development of Fern Leaf

Many features of fern leaf morphology and development are suggestive of the shoot because of

a) autonomy of leaf in its development
b) long continued apical growth
c) the regular formation of lateral appendages
d) the general acropetal sequence of maturation

ORIGIN

Traditional concept of origin of leaf: From a single cell

Dryopteris, Osmunda cinnamomea: The primordium originates from a group of cells of prismatic layer or pro-meristem

Pteridiumaquilinium and *Marsilea*: Leaves originate from a single cell

DEVELOPMENT

Irrespective of its origin, from a single cell or group of cells at an early stage in the leaf primordium, finally a single cell named as an apical cell serves the function for the growth of leaf

- The leaf primordium, due to rapid growth, soon becomes a protuberance overtopping the apical cone.
- Early in its development, an increased cell division on the adaxial side than on abaxial side makes the apex curved adaxially. This is the first manifestation of dorsiventrality in a primordium.
- Later it becomes evident in the adaxial coiling of rachis to form the characteristic crozier as well as in the marginal meristems which grow to produce both basal wings and pinnately divided lamina.
- The derivatives of marginal meristems result in the formation of characteristic lamina of each species.
- An uncoiling of crozier takes place on equalization of adaxial imbalance in cell members.
- In *Osmundacinnamomea*, full development is completed in five growing seasons and is spread over four years

Anatomy of the leaf

- Dorsiventral Leaf
- Epidermal cells have thickened outer tangential walls and usually contain chloroplast (a feature not shared by most other vascular plants).
- Stomata generally occur on the abaxial surface of leaves.
- The mesophyll may be uniform in organization consisting of homogenous parenchyma with chloroplast, or the cells may be organized into definite adaxial palisade and abaxial spongy parenchyma layers.
- Considerable evolutionary importance is attached to the form and arrangement of the vascular tissue in the stipe and rachis.
- There may be simple arc or the vascular bundle may be convolvulated, or the vascular tissue may consist of a cylinder of separate bundles. The last arrangement is considered to be derived.
- As an added complication, the pattern of the vascular tissue may change in passing from the stipe through the rachis to the axes of the pinnae and pinnules.

Venation of the leaf

- The stipe, rachis and axes of frond subdivision have prominent vascular bundles.
- The smaller veins of the lamina often have an open dichotomous type of venation
- In some ferns the venation pattern is a reticulate type

Vein fusions are considered an evolutionary advancement

The first step toward vein fusions is expressed-

- by the appearance of marginal loops which connect the tips of vein dichotomies, or
- by the formation of a series of meshes adjacent to the midrib or throughout a leaf segment

Sporangium and Sporophyll

- The pteridophyta are polysporangiate
- In archiac Psilophytales, *Horneophyton*, *Rhynia* and *Cooksonia* etc. sporangia were cauline (growing from stem) and in some instances *Psilotum* also has been described to bear terminal sporangia.
- The sporangia, therefore, possibly antedated leaves (originated earlier than leaves).
- In rest of the pteridophytes the sporangia are associated with leaves- Sporophylls
- In lower pteridophytes the sporophylls aggregate to form compact cone like structures-Strobilli

Origin of sporangium

Sporangia are of two types on the basis of their development-

Eusporangiate sporangia:

•Sporangium originates from a group of cells and is characteristics of lower pteridophytes and some primitive ferns named as eusporangiate ferns

• Have a multilayered wall during their ontogeny. The multilayered wall is retained even in adult form in *Psilotum*, *Tmesipteris* and *Botrychium* but in other genera like *Lycopodium*, *Selaginella* and *Equisetum* the wall of mature sporangium consists of single layer of cells as the inner walls degenerates during maturation.

• The spore output is variable and is also correlated with homosporous or heterosporous condition. Massive microsporangia of *Isoetes* are recorded to have maximum number of spores in plants

Leptosporangiate sporangia:

•Sporangium originates from a single superficial initial and is characteristics of higher ferns (leptosporangiate ferns)

• Mature sporangium has a wall consisting of single layer of cells

• The spore output is less in a leptosporangium as compared to eusporangium

Transitional forms:

• Structure and development of sporangia are transitional type in Osmundaceae

Nourishment of sporocytes – The Tapetum

During ontogeny of sporangia a special nutritive tissue, the tapetum, differentiates on the periphery around the sporocytes

- Plasmodial type of tapetum: Characterized by the breakdown of cell walls and the intrusion of protoplasts between sporocytes and spores. This is characteristics of Psilotales, Equisetum and Ophioglossales.
- Secretory type of tapetum: nourishes the developing spores by its secretary action and without the breakdown of its cell walls. This type is found in *Lycopodium* and *Selaginella*.

The origin of tapetum is variable:

- In *Lycopodium* the tapetum is the innermost wall of sporangium
- In allied genus *Selaginella* it develops from outermost sterilized cells of sporogenous tissue.
- In leptosporangiate forms the tapetum is derived from sporogenous cells

From Eusporangium to Leptosporangium (Primitive vs Advanced)

Primitive structure

- The presume ancestral sporangium is considered to be:
  - Size large
  - Large no. of spore output
  - with a massive wall
  - a short and thick stalk
  - without an opening apparatus
  - eusporangiate in its development

Evolutionary occurrence

The archaic sporangium under specialization-

- Reduced in size
- Greatly reduced number of spores
- Thinning of wall to a single layer
- Development of long slender stalk
- Development of efficient dehiscence mechanism – which is caused due to presence of specialized cells (annulus) in the wall of sporangium

(structural feature associated with function, dehiscence of sporangium and dispersal of spores)

- Leptosporangiate type of origin

Sporangia in Ferns

- The sporangia in ferns are aggregated in groups known as sori
- If sporangia do not form sori they are either scattered over the lower leaf surface forming a felt (acrostichoid condition named after *Acrostichum*, and *Platycerium*) or
- Form marginal tassels over the surface of reduced leaf segments as in *Osmunda* or
- They are solitary, along or close to the margins of narrow leaf segments as in *Schizaea* and *Anemia*
- In some higher ferns the sporangia are produced in specialized structures, the sporocarps

Sorus

- In the vast majority of ferns the foliage leaves serve in both photosynthesis and reproduction.
- In some species there may be distinct separation of these functions: certain leaves function in photosynthesis, (vegetative leaves) whereas other are strictly 'sporophyll' and non photosynthetic (fertile leaves) -this results in dimorphic condition.
- In other species there is an intermediate arrangement. For example, sporangia may be restricted to certain specific portions of a photosynthetic leaf.
- Sori may be circular or linear in outline.
- In those species having definite sori, the sorus is along or near the frond margin or on the abaxial side of the frond.

Soral Diversity and Position

- The sori are most diverse in form: but they are commonly circular, reniform, or linear.
- Sori are also variable in size, the large ones result due to fusion, the coenosori.
- Coenosori may be broken up into segments as in *Blechnum* and Woodwardia. The sori occur over a vein or at the end of a vein.
- On the basis of their origin the sori are classified into following types:
    - » Marginal
    - » Intramarginal orsubmarginal
    - » Superficial

- A ‘marginal’ position - here the receptacle must originate strictly from the margin of the developing pinnae or pinnules. An indusium, if present, is formed by submarginal outgrowths around the receptacle. The indusial may be funnel shaped or two lipped. e.g. *Davallia, Hymenophyllum, Trichomanes*
- An ‘intramarginal’ (near the margin) position - here sporangia originate near the marginal position. Cells at the margin suddenly or gradually lose their meristematic activity and become parenchymatous. Submarginal cells on the adaxial side of the developing lamina near the margin continue their meristematic activity-forming the receptacle. e.g. *Pteris, Cryptogramma, Pteridium* etc.
- The abaxial or ‘superficial’ position in which sori are at some distance from the leaf margin on the lower or abaxial side of a frond. During leaf development the origin and final position of a sorus are correlated with the activity of marginal meristems and their derivatives. Here, a young receptacle has its inception from submarginal cells on the abaxial side rather early during marginal growth of the lamina. However, in contrast to the intramarginal type of development, the margin of the lamina remains actively meristematic and adds new tissue to the lamina. Therefore a growth of a young leaf segment proceeds, the young sorus occupies positions progressively farther from the margin. An indusium, if characteristic of the species, is formed from superficial cells near the developing receptacle and eventually overarches it.

Phyletic slide of a sorus (F. O. Bower 1936)

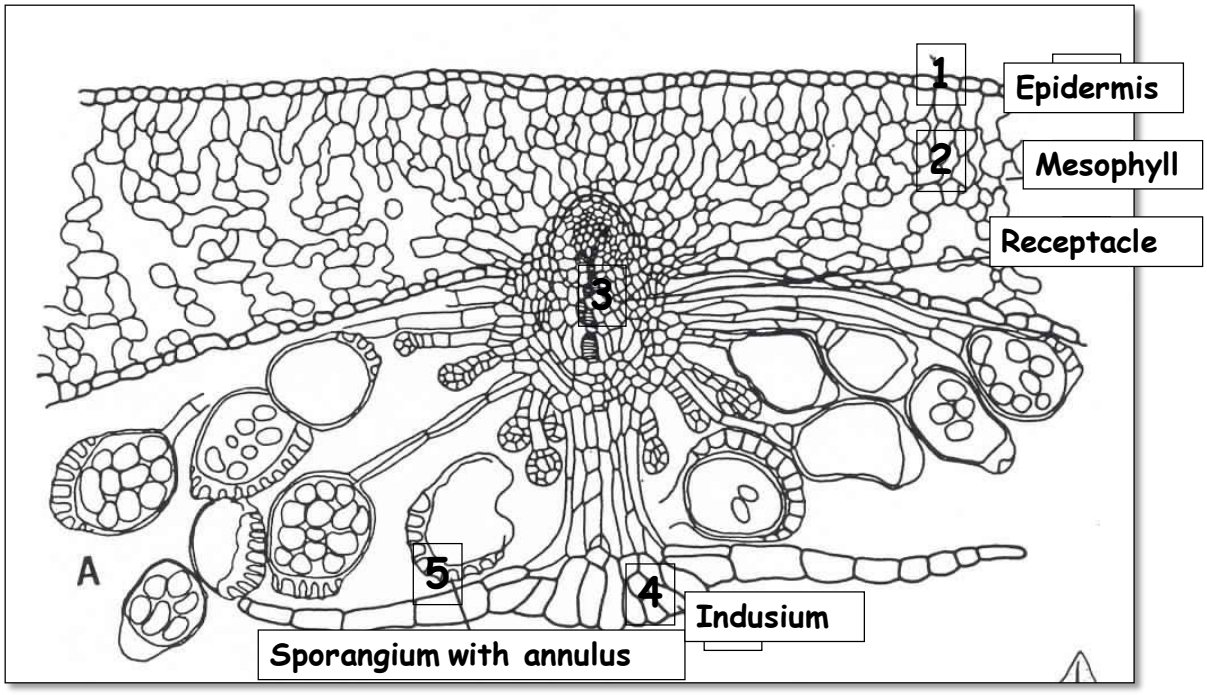
- The sporangium is primitively terminal upon a leaf segment or marginal, from this position superficial position has been acquired due to migration on the lower or abaxial surface.
- That this change has taken place in different groups at different times is supported by evidences from forms in which it is in the process at the present time.
- This change has been explained and the hypothesis has been named as phyletic slide.
- A truly marginal sorus can be seen in genus *Davallia*; the adaxial and abaxial flaps together form a cup shaped structure. Then, with the change to superficial position one lip of the indusium was lost being transformed into leaf margin or margining with expanding blade.

Organization of the sorus

- Both marginal and superficial sori are most commonly found over a vein or at the terminus of a vein.

- That portion of the leaf surface to which sporangia are attached is termed as receptacle. It may be a slight protuberance, a definite bulge, or elongated.
- Sporangia originate from the superficial cells of the receptacle while the leaf is still in a very young developmental stage. Undoubtedly circinate vernation provides protection for the delicate sporangia during their ontogeny.
- Sporangia may or may not be protected by a covering termed an indusium.
- The indusium is an outgrowth from the epidermis of the lamina or of the receptacle.
- If a sorus lacks an indusium it is a naked sorus termed as exindusiate (e.g. *Polypodium, Gleichenia*).
- If an indusium is present it may be formed by adaxial and abaxial extensions of the lamina; this result in the formation of a cup or pouch like structure.
- In some species a reflexed marginal portion of the lamina itself is associated with an indusium, forming a pouchlike structure (e.g. *Dicksonia*).

Vertical Section through Sorus of Typical Fern



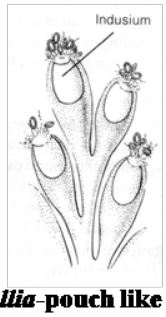
The form of the indusium is variable:

- a delicate, linear flap attached along one side only (unilateral indusium) e.g. *Asplenium, Nephrolepis*
- horseshoe shaped or circular and elevated (peltate e.g. *Polystichum, Matonia*)
- cup shaped e.g. *Cyathea*
- a collection of scale like structures overarching the sporangia

- or the leaf margin may be turned back upon itself (false indusium)

Variation in position and form in fern sori

The indusium varies in shape from elongate and attached along one of the longer sides (unilateral), to half moon shaped or reniform and attached at the sinus (*Dryopteri*s). In some species the indusium is an outgrowth from the top of the receptacle, resulting in the formation of a stalk and a radially symmetrical cap (peltate type).

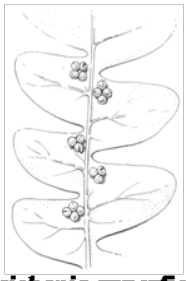


***Davallia*–pouch like indusium joined with lamina, open at laminal margin**

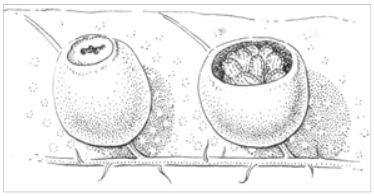
***Trichomanes*–marginal, receptacle elongate**

***Nephrolepis*–indusium attached at one side**

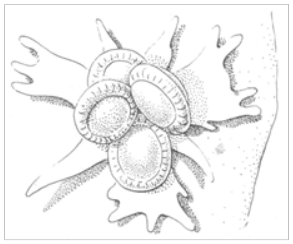
***Lygodium*–Laminal flap**



***Gleichenia*–superficial position, no indusium**



***Cyathea*– cup shaped indusium**

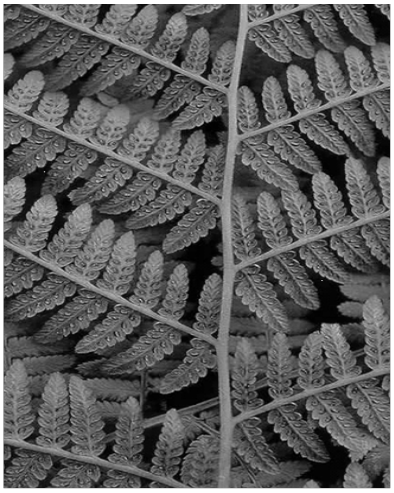


***Woodsia*–basal membranous indusial segments**

***Matonia*–peltate indusium**

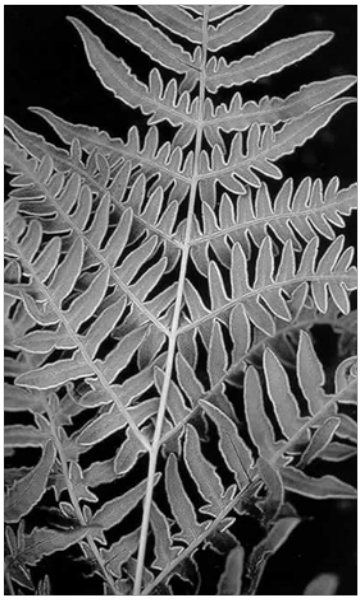
Indusiate Sori

Kidney–shaped near margin of pinnule in *Dryopteris marginalis*

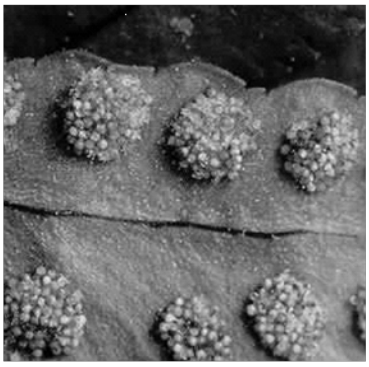


Elongated along pinnule veins in *Athyrium filix-femina*

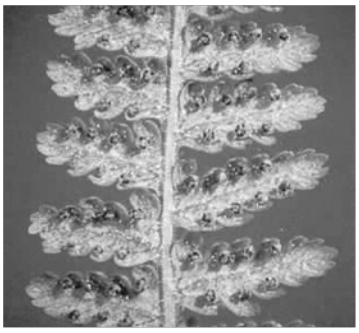
Various Sori, Indusia and False Indusia in Ferns



Linear, marginal, false-indusiate sori of *Pteridium* (Bracken)



Round, naked sori of *Polypodium*

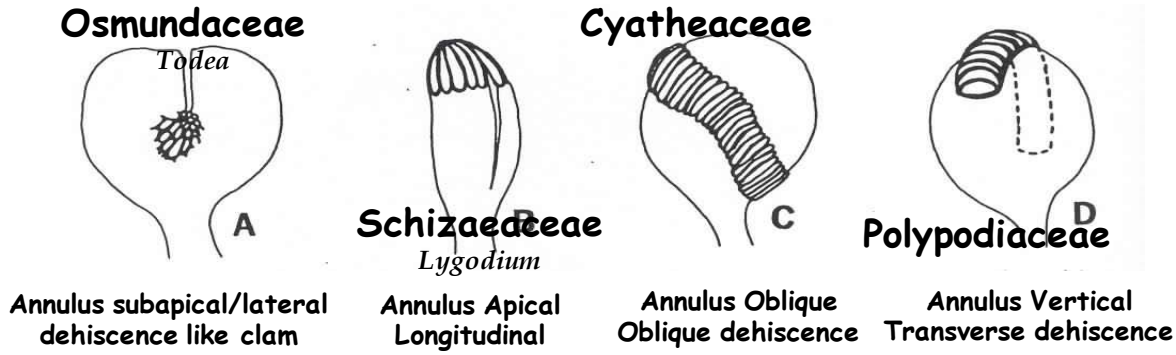


Cup-shaped sori of *Dennstaedtia*

False indusia of *Adiantum capillus-veneris*

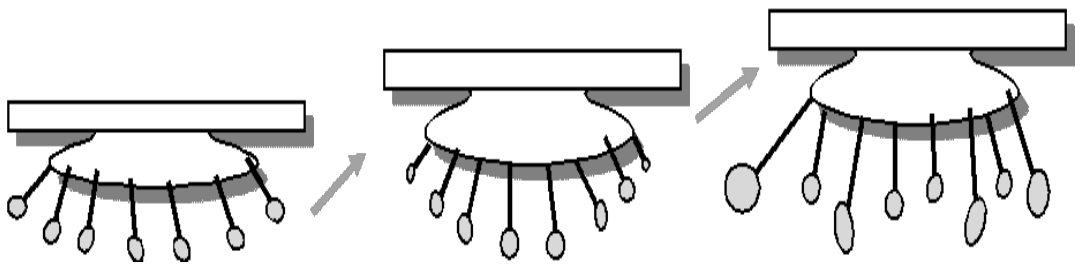
Structure of sporangium

- The two features that are regularly considerable in comparing sporangia are final length of the stalk and the number of cells (or rows of cells) making up the stalk. Short, thick stalks are considered primitive, and long, delicate stalk (frequently consisting of three rows of cells or even one) are derived.
- The sporangial wall is typically one cell in thickness at maturity. The main point of interest, however, is in the means of dehiscence.
- In the Filicales there are various method of dehiscence, depending on the position of the annulus (thick walled cells). In the Osmundaceae the annulus, located to one side, is responsible for the formation of a cleft that runs over the top of the sporangium and down the opposite side. The sporangium opens like a clam. In other ferns the annulus may form a cap at the distal end of the capsule, be obliquely places, or run over the top of the capsule in line with the stalk (in a vertical or longitudinal position). These three positions result in longitudinal, oblique, and transverse dehiscence respectively.



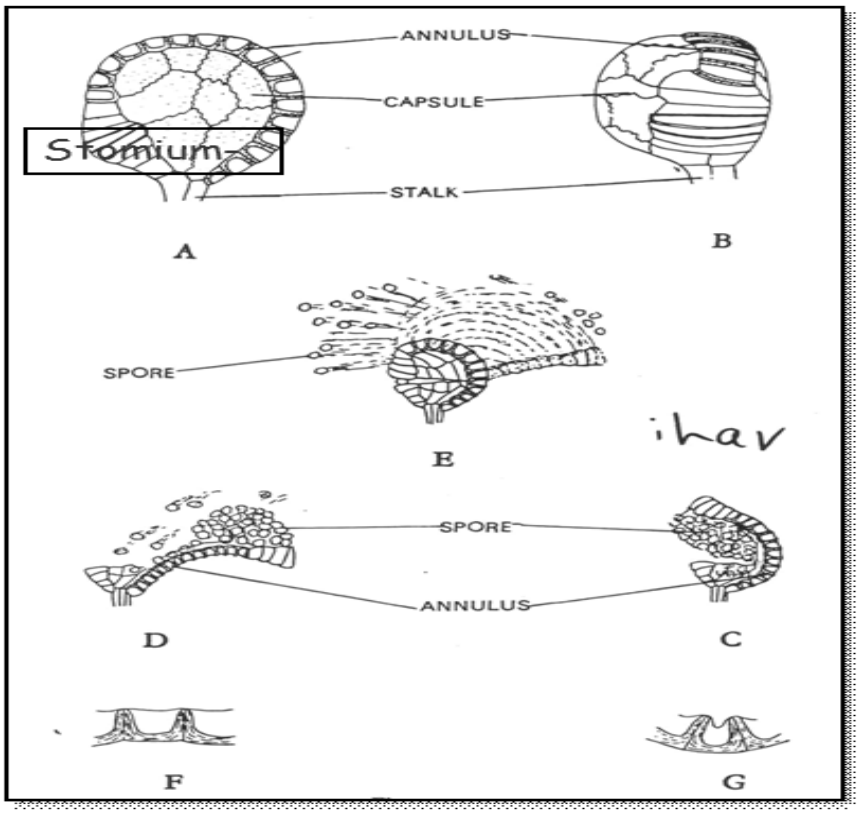
Maturation of sporangia within a sorus

- The simplest way in which fern sporangia are borne is singly along the margins of leaf segments – each with a vascular bundle leading to its base (as in some extant ferns such as *osmunda*). This arrangement is a primitive condition. In the majority of Filicales, sporangia are aggregated to form sori.
- A sorus in which all of the sporangia originate, grow, and mature at the same time is termed a "simple" sorus.
- If sporangia are initiated over a period of time in a definite sequence the "gradate" sorus is produced. The order of sporangial initiation and development is basipetal; the oldest sporangium is near the summit of a receptacle with successively younger sporangia toward the base. When the fossil record is considered and compared with that of living ferns having the simple type of sorus, the gradate maturation is clearly a derived type. It must be emphasized that not all ferns with gradate sori are necessarily closely related. Gradate maturation represents an evolutionary level of specialization that has been achieved by different species.
- The most advanced evolutionary level of development is achieved in the sorus that has intermingles sporangia, all in different stages of growth. This is the 'mixed' sorus. The more highly specialized and evolved families and subfamilies have this mode of soral development.



Sporangial dehiscence

- The dehiscence of the fern sporangium is ingenious. The annulus is the structural feature associated with dehiscence and the forceful ejection of spores.
- As a sporangium matures, water is lost from the cells of the annulus by evaporation. There is a powerful adhesion between the cell walls and water.
- The continuous loss of water from each cell of the annulus results in the thin outer tangential wall of each cell being drawn inward, while the ends of the radial walls are pulled toward each other.
- This results in the tearing open of the sporangium on the weak side and eventually in the complete inversion in the position of the annulus.
- The annulus is now under tremendous tension. Water continues to evaporate.
- Eventually the cohesive force of the water in cells of the annulus is exceeded and the annulus returns suddenly to approximately its original position. In the process the spores are thrown out forcefully for a distance of a centimeter or so.
- The tensions built up prior to dehiscence are equivalent to about 300 or more atmosphere of pressure.



Sporocarp - Marsileaceae

- The sori are marginal structures.
- The presence of protecting body has greatly distorted the normal structure
- It is modified fertile segment of a leaf or equivalent to an entire leaf

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7. Cytogenetics and Speciations: Pteridophytes with low and high chromosome number; polyploidy in microphyllous and megaphyllus forms; intergeneric and interspecific hybridity; obligate interbreeding forms.

Pteridophytes with low and high chromosome number

Much of the work of fern cytologists has concentrated on determining present day basic chromosome numbers and their interpretation in terms of affinities. Unlike in flowering plants, the most important general characteristic of the pteridophytes is the high level of basic numbers. The basic chromosome numbers of homosporous pteridophytes are the highest known in vascular plants, averaging somewhere around x = 35–69. Some genera have much higher basic numbers, such as *Ophioglossum* with x = 120 and *Equisetum* with x = 108. For the most part, heterosporous pteridophytes tend to have low chromosome numbers like those in seed plants, for example *Isoëtes* with x = 11, *Salvinia* with x = 9 or *Selaginella* with x = 7, 8, 9 and 10.

Products of Hybridization

Hybrids are the manifestation of uniting divergent parental genomes in a single nucleus. While this broad definition could apply to organisms derived from crosses between divergent populations of a single species, this review is largely restricted to a discussion of organisms derived from interspecific crosses. At the same time, it is important to acknowledge that distinguishing between hybridization at the intra- and interspecific levels is not clear-cut in some groups of ferns, often because of varying species concepts and evolving species delineations (Haufler, 1996, 2002). The morphological and ecological intermediacy of fern hybrids has contributed greatly to this problem (Barrington et al., 1989).

Homoploid hybrids The ploidy level and the degree of fertility (i.e., the ability to produce functional spores and gametes) of hybrid ferns are directly related to the ploidy level and reproductive mode of their progenitors. For example, homoploid hybrids are derived from hybridization between normal, reduced gametes and have ploidy levels identical to their progenitors. The vast majority of homoploid hybrid ferns are assumed to be sterile, producing

aborted or aberrant spores that often fail to germinate (Wagner & Chen, 1965; Wagner et al., 1986). In reality though, sterility in homoploid hybrids is "relative rather than absolute" (Benedict, 1945), and the production of viable spores can vary between crosses, individuals, and even sporangia on the same individual (Pryer & Britton, 1983; Wagner et al., 1986; Rabe & Haufler, 1992). It is widely hypothesized that the fertility of homoploid hybrids is inversely related to the genetic divergence between their progenitors (Chapman & Burke, 2007; Yatabe et al., 2009a)

Rare, completely fertile homoploid hybrids, such as *Pteris quadriaurita* multiaurita reported by Walker (1958, 1962), result from crosses between sexual species with low genetic divergence and exhibit full pairing of homoeologs (i.e., chromosome copies derived from different progenitor species; Glover et al., 2016) at meiosis. Additional studies have reported homoploid hybrids with spore germination rates as high as 29–40% and gametophytes producing functional sperm and/or eggs.

Recombinant homoploid hybrid speciation has been proposed in *Cyathea* (Conant, 1990) and Ceratopteris (Matsuyama et al., 2002), but may be underrecognized given reports of naturally-formed F2 hybrids in other genera (e.g., *Asplenium*: Vogel et al., 1998a; *Osmunda*: Yatabe et al., 2009b; *Polystichum*: Mayer & Mesler, 1993).

Allopolyploids

In contrast to homoploid hybrids, allopolyploids form through hybridization combined with genome duplication and have chromosome complements that are typically additive of their progenitors (Kihara & Ono, 1926). In even-ploidy allopolyploids (e.g., 2n ¼ 4x), chromosome doubling usually restores bivalent pairing of homologs at meiosis (i.e., chromosome copies inherited from the same progenitor are paired; Fig. 1C; Glover et al., 2016), resulting in fertile individuals that reproduce sexually and are reproductively isolated from their progenitors. Enforced pairing of homologous chromosomes results in disomic inheritance patterns of nuclear loci, allowing allopolyploids to produce offspring that are "fixed" for homoeologous genes (i.e., paralogous gene copies inherited from different progenitors; Glover et al., 2016). Alternatively, odd-ploidy allopolyploids (e.g., 2n ¼ 3x) tend to be sterile or apomictic, a mechanism for circumventing the formation of abortive spores due to univalents and multivalent chromosome pairing during meiosis (DeBenedictus, 1969). Most apomictic ferns undergo an endomitosis immediately preceding meiosis, resulting in the production of unreduced spores with the same ploidy and, presumably, the same genotype as their parent sporophyte.

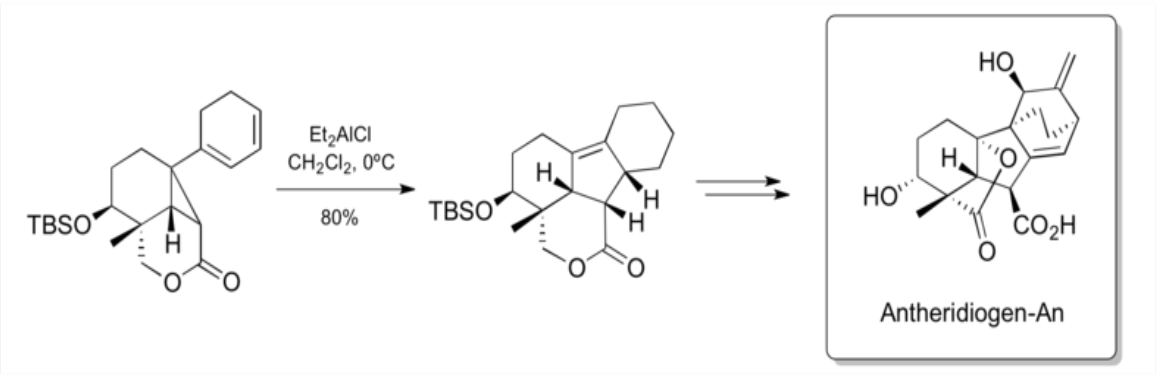
As in homoploid hybrids, the fertility of allopolyploid ferns may be variable. For sexual allopolyploids it has been hypothesized that crosses between progenitors with highly divergent genomes may reinforce bivalent pairing of homolo gous chromosomes and prevents the formation of multivalents and univalents that can cause aberrant spore and gamete formation (Stebbins, 1950, 1971; Chapman & Burke, 2007). This hypothesis is supported by reports of chromosome pairing in the segmental allopolyploids *Adiantum malesianum* (Sinha & Manton, 1970), *Asplenium obovatum* (Herrero et al., 2001), and *Lepisorus thunbergianus* (Shinohara et al., 2010) that formed between weakly diverged progenitor species. In these segmental allopolyploids, the more divergent chromosomes maintain bivalent pairing between homologs at meiosis, whereas the less divergent chromosomes exhibit multivalent pairing.

8. Antheirdiogen in ferns.

In ferns, a chemical substance (pheromone) secreted by gametophytes that causes immature gametophytes to develop as males is called antheridiogen. The hormone (or pheromone) antheridiogen is a potent controller of gametangial initiation in cultures of homosporous fern gametophytes. It has been indicated, as well, as an important force influencing gametophytic mating systems in natural populations. Produced and secreted by mature (meristematic and usually protogynous) gametophytes, antheridiogen induces precocious and abundant formation of antheridia in neighboring, less mature gametophytes.

A careful observation of the antheridial formation in homosporous ferns led to the discovery of specific organ-inducing substances. In cultures of *Pteridium aquilinium*, it was observed by Döpp (1950) that juvenile gametophytes bore antheridia. The induction was suspected to be mediated by a substance elaborated by older gametophytes. The discovery was possible when the medium as well as the extract from mature prothalli of bracken fern accelerated the onset of antheridia on newly formed gametophytes. It has been shown that at least three main classes of antheridiogens occur, antheridiogen A (bracken antheridiogen), antheridiogen B (produced by the members of family Schizaeaceae) and antheridiogen C (produced by species of the genus *Ceratopteris*). Although the chemistry of only antheridiogen B has been studied in detail many similarities among the various types of antheridiogens can be seen from the standpoint of physiology. Chemically, it is a carboxylic acid with some unsaturated linkages. Its activity depended on the presence of free carboxyl groups since the activity disappears on

esterification, and reappears after hydrolysis of ester. Antheridiogens are all water soluble, are active in very low concentration and are formed by large meristic gametophytes. At present, the term pheromone is preferred over the term hormone when speaking of antheridiogens.



The medium or extract from bracken prothalli could also induce the formation of antheridia on gametophytes of *Dryopteris filix-mas*. As compared to controls, the process was accelerated by five to six weeks. Reciprocally, the substance from *Dryopteris filix-mas* was effective in *Pteridium aquilinium*. The media as well as prothallial extracts of these taxa were active at a dilution of 1/1000.

This biologically active factor, a natural metabolite, specifically concerned with the induction of antheridia was rediscovered in an attempt to reverse the filamentous form, by the addition of extract from older prothalli. There was formation of antheridia on filamentous gametophytes. The factor was assayed against *Onoclea sensibilis*. The prothalli of this species are especially suitable, as they do not form antheridia under the prevailing cultural conditions, and the assay can be read in a reasonable time, because the germination and subsequent development in *Onoclea sensibilis* are rapid. Moreover, conditions were defined under which the extract from mature prothalli was active at a dilution of 1:30,000. 'Pteridium factor' (Ap) is specifically active towards *Onoclea* at such a low dilution. Otherwise factor from *Pteris* (Aps) is self-active up to a dilution of 1/1000. The 'Pteridium Factor' (Ap) was designated as "antheridiogen" (Ap). It has been found to be active in members of Pteridaceae, Dryopteridaceae, Gymnogrammaceae, Aspleniaceae, Blechnaceae, Onocleaceae,

Woodsiaceae, Davalliaceae, Dicksoniaceae and Polypodiaceae but inactive in member of Schizaeaceae, Osmundaceae, Parkeriaceae and Cyatheaceae.

Interestingly, the apogamous form *Notholaena sinuata* which does not form antheridia spontaneously but formed antheridia on medium enriched with 'Pteridium factor'.

The 'antheridiogen' either induces the formation of antheridia under condition in which control plants do not form them as in *Onoclea sensibilis*, or accelerates the beginning of antheridium formation. Extremely small amount of the substance is required. Nonetheless, within the wide range of species responsive to 'Pteridium factor' the threshold concentration varies widely. The minimal effective concentration of the substance for *Woodsia obtusa* was 25 times higher than needed in *Onoclea sensibilis*, and it was 125 times higher in *Dennstaedtia punctilobula*.

Investigations on ferns, unresponsive to 'antheridiogen' / 'Pteridium factor' led to the demonstration of a substance that controls antheridium formation in *Anemia phyllitidis*. The substance is named as 'Anemia factor' (Aa), and it is inactive towards species, which respond to 'Pteridium factor'. In *Lygodium japonicum*, antheridium formation seems to be controlled by a second substance (AL). 'Anemia factor' (Aa) was active in *Lygodium japonicum*, but AL was inactive in *Anemia phyllitidis*. *Blechnum gibbum* yielded a substance (Ab), which was active in *Onoclea sensibilis*. *Onoclea sensibilis* yielded another substance (Ao), which was active only after autoclaving, preferably at low pH. Since the unheated medium did not inhibit antheridium formation when added to activate preparation, it is unlikely that heating merely destroys the inhibitor and uncovers the activity of the substance. The response to Pteridium antheridiogen is variable in 3 different species of *Cystopteris*. *Cystopteris protrusa* respond to antheridiogen, *C. bulbifera* remains unaffected whereas an allopolyploid derivative of these two species *C. tennesseensis* had an intermediate level of response.

Antheridiogen from a Parkeriaceous genus *Ceratopteris thallictroides* (Ac) is active within *Ceratopteris*. Antheridiogen from *Pteris vittata* (As) is effective for antheridial induction in many genera, in this way it is similar to Ap. but very distinctive from Ap in being more sensitive towards *Onoclea* than *Pteridium*. It is very likely that there is no common polypodiaceous antheridiogen.

A new note in control of antheridial differentiation in ferns was introduced when it was discovered that gibberellic acid induced antheridium formation in *Anemia phyllitidis*, *Anemia rotundifolia* and *Lygodium japonicum* in exactly the same manner as native 'Anemia factor' (Aa). Later on, it was noticed that GA_3 has been found to be active in all members of

Schizaeaceae. Among non-schizaeaceous species, gibberellin induced antheridia on *Dryopteris filix-mas*, and a weak response occurs in *Onoclea sensibilis*, *Aspidium oreopteris* etc. Gibberellin does not accelerate the onset of antheridium formation in these polypodiaceous species, but merely increases the % of antheridial prothalli. It is very likely that prothalli of polypodiaceous ferns utilize gibberellins as precursors for the synthesis of native antheridiogens.

The antheridia formed in response to GA_3 are quite normal. Seven gibberellins GA_1, GA_3, GA_4, GA_5, GA_6, GA_8 and GA_9 are capable of inducing antheridia in *Anemia phyllitidis*. Besides gibberellic acid, helminthosperol, helminthosporic acid, dihelminthosporic acid and allo-gibberellic acid (a degradation product of gibberellin which serves as a substitute for gibberellin in many higher plants) are active in inducing antheridia in *Anemia phyllitidis*. Halogenated deoxynucleosides also acts as substitute of gibberellin for antheridial induction in *Anemia* but in this case antheridia occur at a low frequency compared to gibberellins.

From these studies it is apparent that different substances in different group of ferns control antheridium formation. The specificity of fern antheridiogens suggests that in different groups different but closely related substances control the induction of antheridium. The gibberellins like steroids meet this specification. It is suggested that schizaeaceous antheridiogens are similar to gibberellin, and the demonstration of gibberellin-induced antheridial induction in *D. filix-mas* indicated that antheridiogens in other families might also belong to this class.

Green antheridia

Sometimes the jacket cells of antheridia of some ferns (*Microsorium*) bear numerous chloroplast and the antheridia are termed green antheridia though they attained the normal size and shape of a typical antheridium. Green antheridia do not release sperm cells and in all likelihood fail to differentiate them. Dopp thought that green antheridia results from the interplay between the antheridiogen and a substance antagonistic to antheridium formation.

9. Habitat diversity of pteridophytes and their conservation; endemic and endangered pteridophytes with special reference to India

Habitat diversity

Well over 8000 species, in at least 300 genera, of living fens are known. These occur throughout an extraordinary range of habitats from high altitude and high latitude Arctic-Alpine situations to the heart of tropical rain forests, and sub desert scrub of continental interiors to rock of the sea coast and mangrove swamps.

Many of the diverse structural adaptations shown by fern sporophytes are associated with their ecological role in particular environments. In different habitats, ferns have adapted to produce sometimes strikingly different sporophyte morphologies. Five main environments are recognized for fern diversity which are Tropical mesic, Tropical alpine, Tropical xeric epiphytic, Tropical xeric terrestrial, and Temperate.

Tropical Mesic Environment: this environment embraces all tropical areas of wet climate, not interrupted by appreciable dry periods. It contains around 65% of living fern species.

a. **Rainforest Floor:** ferns of this habitat are mostly of moderate size, with short, erect, ascending or occasionally creeping rhizomes and erect or arching pinnate or bipinnate fronds. e.g. *Athyrium, Diplazium*

b. **Rainforest Low-Climbers:** long slender rhizomes creep vertically up trunks of trees. *Microsoium, Arthropteris*

c. **Rainforest Low-Epiphytes:** small plants with entire leaf like fronds. These plants are normally restricted to the well-drained habitats offered by the bark of the lower trunks of large forest trees, although several occasionally occur on rocks. e.g. *Asplenium, Vittaria*

d. **Rocks and Cliffs in Rainforest:** rocks and cliffs offer a particularly freely draining habitat in rain forests. The fibrous shallow spreading root systems of ferns are ideally adapted to exploit rocky habitats. e.g. *Nephrolepis, Oleandra*

e. **Rainforest Stream banks:** small streams abound everywhere through rainforests and their banks carry distinctive fern vegetation usually much richer than that of the forest floor. Ferns of streambank communities tend to include many individuals of larger stature than those of the forest floor. e.g. *Adiantum, Blechnum*

f. **Mid-mountain Forests:** Lowland rainforest gradually into mid-mountain forest. Undoubtedly this is floristically the World's richest fern vegetation. Tree ferns especially

Cyathea are typically richly developed in this region, and sometimes carry other pteridophytes upon them. There is also a high frequency of other ferns of large size. Short ascending or erect-rhizomed species predominate, especially in more rocky terrain. However, ferns with long creeping rhizomes become more frequent in more open spots and areas of deeper soils, especially where these have been opened by erosion or landslips. Many of the terrestrial species are large fronded of which the majority have finely cut fronds. e.g. *Gleichenia*, *Polystichum*. Species of the fern ally genus *Selaginella* may be frequent.

g. **Mossy Forest:** mossy forest occurs on tropical mountains flanks wherever cloud and extreme humidity permits luxuriance of epiphytes that they stunt the growth of the trees. In all such moss forests, small mosses like epiphytic ferns are particularly common. This includes many of the filmy fern family Hymenophyllaceae e.g. *Hymenophyllum*, *Trichomanes* etc.

h. **Mountain ridges and mountain fern thickets:** the thin soils on the ridge and in the mountain fern scrub may both be of the acidic peat type. Ferns of lower altitudes and of moss forest are absent, replaced by a few species which are characteristic of this habitat. Many of the ferns of this habitat have long, surface creeping rhizomes and tall, sprawling fronds which usually form thickets 1-2 m high. e.g. *Matonia*, *Cheiropleuria*. Fern ally Lycopodium cernuum may also be present here.

Tropical Alpine Environment: this is the environment of high tropical mountains above the upper most limits of tropical mountain forests. Such areas differ from both tropical conditions at lower altitudes and from more truly alpine environments of temperate latitudes by a combination of low temperatures and lack of thermal or day length seasons. In the tropical alpine environment, plants must contend with alterations of cold might temperatures and warm day temperatures with strong insolation. In the absence of woody vegetation cover, all ferns of the tropical alpine zone occur in terrestrial locations, usually amongst boulders or screes, in rock fissures or occasionally around rocks or hummocks in grassland. There are far fewer fern species in such habitats than at moderate elevation on mountains in the wet tropics and probably no more than 5% of fern species occur in this environment. e.g. *Dryopteris*, *Lepisorus*

Tropical Xeric Epiphytic Environment

a. Rainforest high epiphytes
b. Rainforest high climbers
c. Epiphytes of coastal areas
d. Epiphytes of semi open lowland forests

Tropical Xeric Terrestrial Environment

a. Mangrove swamps
b. Tropical Lowland swamp forest
c. Exposed swampy or marshy ground
d. Exposed inland water
e. Forest margin scrub
f. Seasonally dry open tropical/subtropical forest and savanna
g. Rock outcrops in dry zones
h. Periodically Flooded sub desert scrub
i. Pumicicolous ferns of lava flows
j. Open manmade roadside cuttings

Temperate Environment

a. Temperate rainforest
b. Temperate deciduous and coniferous forest and streamside
c. Open low-altitude rock, scrubland, heathland, moorland and bog conditions
d. Alpine cliffs and screes

Tropical Xeric Epiphytic Environment

Ferns of this environment are chiefly those adapted to life in the high canopy in the tropical rainforests, where conditions are more extreme than those within the forest. They also include epiphytic species growing in communities outside rainforests such as on trees in coastal environments and those of drier forests. About 10% of fern species occur as tropical xeric epiphytes. Of these, the majority occur as high epiphytes of rainforest canopies.

Rainforest High epiphytes

These plants occur on the upper parts of trunks and on the larger branches of tall rainforest trees where conditions may vary rapidly from wet to dry and there is much variation in humidity, light intensity, and presumably leaf temperature. Within the rain forest canopy, these epiphytes often grow to enormous size. e.g. *Platycerium*, *Aspleniumnidus*

Rainforest High Climbers

This type includes all the larger, more vigorous species able to climb to the environment of the high rainforest canopy. The ferns gain the advantages of both establishment if the prothallial and young sporophytic stage in the shelter and assured moisture of the rainforest floor while shedding their spores into the air at the height of the rainforest canopy, thereby presumably achieving potentially more efficient dispersal. e.g. *Teratophyllum*, *Lomagramma*

Some of these ferns undergo considerable change in morphological structure of rhizome and frond. The rhizome first grows horizontally ever the ground on the forest floor during which time it produces an initial type of frond, usually of least divided form. Once the rhizome encounters a tree and begins to climb a second type of frond, typically larger and more finely cut is produced. The third type of frond is generally produced only high in rainforest canopy and bears sporangia. Such fertile fronds seem to be produced only very sporadically, only perhaps in response to a dry season, or when the fern reaches the limit of the tree it is climbing.

Epiphyte of coastal areas

Epiphytic situations such as trunks of palm trees and coastal cliffs may provide habitats with similar degrees of change in environmental conditions to those of the rainforest canopy, while adding the additional hazard of presence of salt-laden air. e.g. *Davallia, Pyrrosia* occasionally members of the fern ally genus *Psilotum* occur epiphytically on low trees in similar situations. In Malay, Holttum records *Humatapumila* as an epiphyte of mangrove plants.

Epiphyte of semi open lowland forest

These include epiphytic ferns of all lowland forests which are drier than rainforest types and are consequently more open and lighter with a usually single layered canopy. Rainfall is much more infrequent and sporadic than in rainforest, and consequently the epiphytes present are species able to tolerate strong light and frequent drought. Almost all have distinctive drought resisting features, frequently including fronds which are fleshy or leathery in texture and have thick cuticles. e.g. *Drynaria*, *Phymatodes*.

Tropical Xeric Terrestrial Environment

A number of unrelated species succeed in tropical climates in which substantial dry periods either seasonally or irregularly. None are adapted to entirely desert conditions, although a few species occur in surprisingly dry areas. Some of these ferns tolerate exposure to full sun; others thrive best in light shade. About 5% of ferns probably occur in this environment.

Mangrove Swamps

Ferns of the genus *Acrostichum* are large clump-forming ferns with short creeping rhizome, large fleshy roots and irregularly pinnate fronds up to 4m high. They are adapted to terrestrial conditions in mangrove swamps in tropical coastal and estuarine conditions. These ferns grow in soft mud towards the drier fringes of mangrove swamps and other brackish conditions, where they tolerate frequent salt water inundation. The roots of these ferns are

sometimes buttress like, giving them further stability in a shifting habitat. The spreading rhizomes are eventually built up extensive colonies fringing muddy river estuaries.

Tropical Lowland Swamp Forest

Various wet tropical lowland conditions support forest of varying density and the fern components of these seem chiefly determined by the soil-water conditions. Swamp forest where the water is still and stagnant have different species from areas in which water is more aerated and moving. Mineral status of the water also influences the species present. e.g. *Stenochlaenapalustris, Lygodium microphyllum*

Exposed Swampy or Marshy Ground

A few species of ferns occur either as widely scattered individuals or sometimes as quite dense single-species stands in open swampy ground and at the margins of lakes. e.g. *Blechnumindicum, Cyclosorusinterruptus*

Exposed Inland Water

Fresh water Lake, pools, and streams in otherwise dry areas provide habitats for particularly distinctive and structurally diverse ferns. Frequently rooted within or beside shallow flowing streams in muddy or sandy conditions are *Ampelopteris prolifera, Ceratopteristhallictroides* .open waterside are also the habitat for several of the amphibious ferns of the genus *Marsilea*. Other completely submerged aquatic pteridophytes are found in the fern ally genus *Isoetes*, while the horsetail *Equisetum ramosissimum* occurs as a marginal species on sandy or rocky ground. Another life form found among ferns in open water conditions is those which have free floating sporophytes. These include the genera *Salvinia*, with two rows of aerial boat shaped floating leaves and two ranks of finely dissected submerges rootlike leaves, and the genus Azolla, a minute fern with reduces leaves of moss like appearance.

In all these aquatic, amphibious and marginal ferns, protallus establishment may be infrequent and all have particularly vigorous vegetative growth and reproduction.

Forest Margin Scrub

Environment conditions at the margin of the rainforests like those of mesic forests with those of much drier savanna forest and possess their own characteristic fern flora. e.g. *Microlepia, Dicranopteris, Gleichenia*

Seasonally-dry Open Tropical/Subtropical; Forest and Savanna (grassland ecosystem characterized by the trees being sufficiently widely spaced so that the canopy does not close)

Vegetation of this type occurs widely in areas marginal to the wet tropics where there is a strong seasonal rhythm of rainfall and dry periods. At dry time of the year habitats within the forest may become considerably desiccated and subjected to natural fires. The ferns of these habitats are adapted to withstand some degree of fire damage. e.g. *Doodiaaspera, Adiantum formosum*.

Rock Outcrops in Dry Zone

It may form refuges for large numbers of small sized ferns which take root in the partial shelter of small fissures. This is particularly so in rocks of a slightly porous nature, which themselves can act as substantial reservoirs of water for at least the beginning of each dry period, and into which the ferns are able to spread their long fibrous roots. e.g. species of *Adiantum, Hemionitis*.

Periodically flooded Sub-Desert Scrub

Poor sandy soil carrying open scrub vegetation flooded at only infrequent intervals provide one of the most extreme habitats in which ferns occur. e.g. *Platyzomamicrophylla*.

Pumicicolous Ferns of Lava Flows

The lava surface itself, although offering no soil, frequently has many vesicles into which some ferns seem well able to establish and spread their roots. e.g. *Nephrolepis*, *Pteris,* fern allies *Selaginella, Lycopodium, Psilotum*

Open Manmade Roadside Cutting

Such habitats, although artificial, closely resemble similar habitats opened by forces of natural erosion, landslips etc. e.g. *Christelladentata, Pterisvittata*. In the field, all act as aggressive pioneers. Most appear to have a wide edaphic tolerance, and in cultivation their ready invasions of pots of other ferns suggests they may be more generally tolerant of other species, chemical inhibitors than most.

Temperate Environment

The temperate environment contains the cooler equivalents of all four tropical fern environments. This environment probably includes 15% of ferns, of which about 1/3rd extend into the cooler regions of the temperate zone.

Temperate Rainforest

Temperature variation in the temperate environment between summer and winter is most pronounced in the interiors of the continents and towards their eastern flanks. Such variation is least pronounced in the island groups of the major ocean areas and along the western margins of continents which border them. Characteristics ferns of these rainforests include

large terrestrial species with finely cut fronds, smaller terrestrial species with more coarsely cut fronds and a variety of small epiphytes on trees and emergent rocks. e.g. Forest Floor Ferns *Asplenium*, *Athyrium*; in the cooler moister forests-*Dicksonia*, *Cyathea*; Larger epiphytes-*Microsorium*, *Polypodium*; smaller epiphytes-Hymenophyllaceae; moist rock-*Adiantum*, *Asplenium*. In Australia, the fern-ally *Tmesipteris* is characteristically epiphytic on tree fern trunks.

Temperate Deciduous and Coniferous Forests and Stream-sides

Temperate deciduous (broadleaf) and coniferous forests occur through a wide range of temperate latitudes which have well distributed, moderate rainfall. All the fern grow in this environment are more abundant though better light availability. In both types of forests, streambanks also provide such lighter locations. e.g. warmer type temperate forest-*Diplazium*, *Gleichenia*; cooler type forest-*Adiantum*, *Polystichum*

Open Low-altitude Rock, Scrubland (plant community with scrub vegetation. Scrub means low shrubs, mixed with grasses, herbs, and geophytes), **Heathland** (shrubland habitat found mainly on free-draining infertile, acidic soils, and is characterised by open, low-growing woody vegetation), Moorland (related to high-ground heaths with - a cooler and damper climate) **and Bog** (peaty soil-deposit of dead plant material) **Conditions**

In all temperate latitudes, open rocky places carry their own characteristic fern vegetation. These are chiefly a small rosette forming species adapted to life in rock fissures. Most show some xeromorphic adaptations, such as frond of rather leathery texture. e.g. *Aspleniummarinum*

Scrubland, heathland, morrland and bog habitats are often developed over edaphically poor soils and rocks, in exposed conditions, in such vegetation, ferns play an increasingly significant role with increasing oceanity of climate. e.g. *Polypodium*, *Dryopteris*

Alpine Cliffs (vertical rock exposure) and Screes (collection of broken rock fragments)

A number of smaller species of ferns are confined to cliff habitats where the ferns are always in close contact with rocks, and the rock type is significant in determining species distributions. Rocks of basic characters are typically much richer than more acidic ones. The ferns of rock faces and cliffs almost always originate in small fissures where they benefit from the increased protection and moisture conditions of the microenvironment. Typical ferns of this habitat have frond usually less than 10cm long, which are pinnately divided and often lie fairly close to the rock surface or remain mainly within sheltered fissures. e.g. *Asplenium*, *Woodsia*

Scree slopes of talus beneath cliffs provide further habitats, often for larger fern species which thrive between the boulders where ferns characteristically form the dominant vascular plants. e.g. *Polystichum, Athyrium* along with several clubmosses and horsetails.

Conservation of Pteridophytes

In evaluating the threat to species survival, the IUCN indicates that the coverage of the pteridophyta is insufficient, and also highlights the lack of information concerning the conservation status of Pteridophytes. Protection measuresare to be implemented for most of the pteridophytic members to counteract the drastic reductionof genetic diversity of natural populations. There is lack ofinformation concerning the conservation status of Pteridophytes and so the need forexperts is one of the challenges for the Global Strategy for Plant Conservation (GSPC) 2002-2010. Today, from the perspective of GSPC 2011-2020,this necessity remains valid.

It is universally accepted that the most effective andefficient mechanism in conservation is habitat protection i.e. *in situ*, but it is also accepted that *exsitu* conservation techniques are critical components in a global conservationprogramme.

In contrast to *in situ, ex situ* conservation aims to preserve the genetic integrity ofpopulations and individuals.These two types of conservation are not mutually exclusive but complementary.

Today, many conservation action plans are developing, but there are largedifferences between geographical areas. Manyareas are well covered but others not at all, and this is not always related to the wealthof biodiversity present in them.

With these circumstances, most fern research today is designed to increaseknowledge of actual biodiversity, species richness and the occurrence of threatenedspecies, soil spore banks, reproductive biology, and population dynamics, to identifythreats and to establish *ex situ* and *in situ* conservation management priorities andprocedures. Finally, studies on spore germination, gametophyte development andcultivation of sporophytes constitute the basis of recovery programmes for damagedpopulations in nature.

***in situ* conservation actions**

In situ conservation is a dynamic conservation, since the evolution of species continuesin the same environment in which plants grows, involving gene pools and also theprocess of co-evolution.

In situ conservation actions could have different approaches oriented to preservegenetic, species and ecosystem diversity. The main objectives of this action are genetic

characterization, re-enforcements, re-introduction, recovery of populations or establishment of natural reserves and protected areas topreserve fern species. Practical actions usually include some study of, or are developedas a result of, previous research on threatened species.

The first step is to know exactly whichcomponents of the biodiversity within the territory under consideration need protectionaction, by determining the conservation status of the species. IUCN Standards andPetitions Subcommittee (2010) established the guidelines to assess the conservationstatus of species.

Strategic plans for fern *in situ* conservation

When an *in-situ* conservation plan must be developed, it is recommended that availableinformation of previous activities is checked and the priorities are discussed withexperts in order to select the best methodology. Afterwards it is useful to share theresults and conclusions through open access publishing which enables futureimprovement of *in situ* techniques.

Natural Soil spore banks have a potentialrole in the conservation of endangered fern species. Tree bark as another kindof *in situ* spore bank that could contribute to fern conservation.

Soil spore banks can be very useful for population reinforcements and to increasethe genetic diversity, especially in threatened species with very small populations andit is the first option for the recovery of spores for the reintroduction of species in placesin which the disappearance of populations is observed.

The persistence of soil spore banks determines the natural capacity for *in situ*regeneration of wild populations. Besides, from soil spore banks additional *ex situ*actions could be developed for propagation and conservation of germplasm.

***ex situ* conservation**

Ex situ conservation could be developed as living collections (sporophytes), usuallymaintained in Botanic Gardens, or through germplasm banks which include conservation of vegetative propagules, gametophytes or spores. Storage of spores preserves large quantities of germplasm with high genetic variation in small spaces withlow financial and technical costs. *Ex situ* conservation activities also include *in vitro* germination from spores and the cultivation of sporophytes from storedgermplasm.

The use of *ex situ* conservation techniques for endangered plants in germplasmBanks iswidely employed for seed-bearing plants but needs further efforts in Pteridophytes.Already

in 1992, Page *et al*. provided a first approach to spore storage in germplasmbanks as an *ex situ* conservation tool.

The study of the longevity of chlorophyllic (green) and non-chlorophyllic (nongreen)spores has evolved during the past 50 years. Methodologies have been improvedand some protocols established to optimize spore preservation in fern spore banks.Green spores show a rapid deterioration which has been attributed to their lack oftolerance to desiccation, their high metabolic rate, and their absence of dormancy.

The study of all the factors influencing spore viability i.e. ploidy level, age, temperatureor spore physiology helps to increase the understanding of spore viability loss under different storageconditions and the optimal conditions to preserve it.

Herbaria are also a valuable source of living germplasm withpotential in the recovery threatened plant species. Magrini *et al*. (2010) propagated *Osmundare galis* L. using spores collected from exsiccate (dried herbarium specimens) stored in herbaria for five and 17 years. Therefore, fern propagation from herbarium spores is proposed as a usefulmethod for preserving rare, threatened or extinct species. However, storage conditions of herbarium specimens are highly variable, especially with respect to the relative humidity, depending on geographical location andavailable facilities. Spore longevity is closely correlated with temperature and humidity and varies between species.

Artificial Spore Banks – spores in storage facilities

There are different approaches to setting up a new artificial bank for spore storage. Short (1-10 years), mid (10-30 years) and long-term storage (>30 years) options are available fordifferent objectives. These options are established under a range of temperatures andrelative humidity values. Wet and dry storage and cryopreservation are widely used for the purpose.

The selection of the particular conditions to use in a spore bank depends basically on the type of material to be preserved, the conservation action to be achieved and theavailable facilities. Lower temperature and lower humidity tend to prolong sporelongevity. But water content must be strictly controlled and the optimal valuedetermined because there seems to be a minimum value below which viability is lost. Cryogenic conditions are aimed at long-term collections.

The main objective of long-term conservation spores of ferns in germplasm banksis to maintain viability and the capability of producing sporophytes that can later beused in restoration of habitats.

Articificial spore bank could be made by dry storage, wet storage, cryopreservation

Wet storage

Lindsay *et al.* (1992) established the effectiveness of wet spore storage, particularlyrecommended for green spores and where low temperature facilities are not available.

Dry storage

Dry storage has been practised under different temperatures; 5°C, -10°C and -20°C arethe usual temperatures in gene banks.

Cryopreservation

Cryopreservation is typically used for the long-term storage, ofgermplasm of plants at -80 °C to -196 °C. It has been widely reported that the spores of diverse species remainviable longer at these temperatures than at higher temperatures (Quintanilla *et al.* 2002; Pence, 2000b; Ballesteros *et al.* 2004; 2006). Cryogenic preservation and *in vitro*culture appear to be the best option for spores with recalcitrant behaviour, but also foroptimal conservation of Pteridophytes in general, due to its simplicity and effectivenessfor a wider range of species and for longer periods.

***In vitro* techniques**

In vitro techniques are mainly needed to rescue plants that produce recalcitrantspores/seeds or propagate only by vegetative means. *In vitro* culture techniques are mainlyrelative to the influence of growth rate, the formation of sexual organs, and theproduction of sporophytes from homogenised gametophytes or sporophytes of severalspecies. Numerous aspects of the regulation of sexual expression of gametophytes werestudied to provide optimal protocols for *In vitro*culture.

Asexual propagation, also known as vegetative techniques, is also recommendedfor conservation purposes in special situations; e.g. for taxa where sexual reproductionthrough spore germination faces difficulties. The most usual protocols use rhizome cuttings, bulbils, stolons and root buds.

The stipule is an optional method efficiently applied to *ex situ* propagation ofmarattioid species as their spores are hard to germinate and gametophytes grow slowly. The only limitation of thismethod is for those species with low production of fronds, in which case the materialfor asexual propagation is strictly limited.

***quasi in situ* conservation: a new concept**

When we apply *ex situ* techniques we preserve the genetic structure of the populationat the moment of sampling. Evolution and co-evolution, the engine driving newdiversity, are interrupted, so that integration between *in situ* and *exsitu* strategies is needed for plant conservation.

In 2010, a new concept for plant conservation was introduced by Volis & Blecher, the "*Quasi in situ*" conservation. This approach basically proposes the maintenance ofliving-plant *ex situ* collections in a natural or semi-natural environment. *Quasi in situ*preservation is considered a novel strategy for biodiversity conservation, with the aimof maintaining interactions between individuals, species and environment, in order toallow the evolution and adaptation of wild genotypes to continue during conservationactions. Detailed guidelines for representative sampling, collection maintenance andutilization for *in situ* actions, as well as advantages of this new strategy over traditional*ex situ* living collections, are compiled by Volis&Blechner (2010). In summary, theadvantages include less restriction on available space, high suitability of environment, natural processes of maintenance of plants and, consequently, low costs.Laguna *et al*. (2012) applied the term "safety neopopulations" to new populations, created in areas distant from the natural populations, which are not exposed by thefactors that reduced or caused the disappearance of the species.

An approach to future fern conservation

As a result of reviewing numerous conservation works published in the last decade Ibars and Estelles (2012) summarized a 10- step approach to plant conservation that is applicable to ferns:

1. Revision of morphological characters and confirmation of the taxonomicidentification.
2. Compilation of complete information of distribution; revision of publishedrecords, herbaria and field data.
3. Characterization of ecological and phenological features of the species in thedifferent locations. Identification of singular ecotypes (especially relevant when thegenetic structure of populations is not well established).
4. Determination of the conservation status of each population (number ofindividuals, mature individuals proportion, actual and potential threats, IUCNcategories, etc…).
5. Research and compilation of data on reproductive biology (vegetative and sexual) of the species. Identification of optimal protocols.
6. Research and compilation of data on genetic diversity of populations in order toestablish conservation priorities.

7. Revision of legal aspects (regional, national or international laws) related to thespecies or their habitats, and those related to natural protected areas when they areincluded in any category.

8. Development of an *ex situ* conservation programme.

9. Revision and research of possible restoration techniques applicable to eachspecies.

10. Proposal of a sustainable management plan for the taxa.

Complementary actions to assure fern conservation are: training of local staff in sporegermination, development of comprehensive research into the biology of the species, creating educational guides and leaflets, and coordination between institutions.

Open access to knowledge will be the key to future sustainability of mankind.Public awareness programmes on the conservation and sustainable utilization of fernsshould be initiated promoting *in situ* and *ex situ* conservation.

Finally, it can be concluded that the fern conservation requires multiple efforts, a compendium ofactions as the result of the application of all knowledge raised through the scientificresearch of different groups from several disciplines all around the world.Coordination between scientists and government departments is necessary to develop and especially to complete, effective conservation or recovery programmes.

A collaborative network of institutions involved in fern biology and biodiversity studies and, a responsible implementation of conservation practices, could helpto improve methodologies by exchange of experiences.

Genetic diversity of populations

Preservation of genetic diversity of a particular population is, finally, the main aim of any conservation strategy. Both *in situ* programmes and *ex situ* collections require the evaluation of genetic diversity to achieve an accurate management and establish priorities. Genetic conservation includes the maintenance of the level of genetic variability of the species and of their natural populations, as well as respect for, and maintenance of, genetic structure within and between existing populations, in order to avoid losing the adaptations already in place. Small populations are more affected by drift and gene flow than large ones; this makes it essential to take into account genetic considerations.

Jimenez (2011) proposes microsatellite technology as a high-quality procedure to determine fern genetic biodiversity.

Peredo*et al.* (2011) recommended that dominant or unspecific markers (RAPD, ISSR, REMAP, IRAP, AFLP, MSAP) are useful tools for assessing genetic diversity, reproductive isolation, orconservation actions in ferns.

RAPD-random amplified polymorphic DNA

ISSR- inter simple sequence repeat

REMAP -retrotransposon-microsatellite amplified polymorphism

IRAP -inter-retrotransposon amplified polymorphism

AFLP amplified fragment length polymorphism

MSAP-methylation sensitive amplified polymorphism

Threatened pteridophytes in India

At present a large number of Pteridophytes in India are threatened mainly due to human activities, such as:

- Environmental degradation of preferred habitat for species, such as uncontrolled tree-felling and destruction for fuel, timber, crop-plantations or grazing, building over sites near to cities or coastal resort areas, or draining of wetlands for agriculture.
- Pollution of the environment, such as stream-banks, lakes and road-sides.
- Unregulated commercial collection, such as of tree-fern trunks for orchid-growing.
- Immoderate collection of species known to be rare by students; however, we consider that constructive small-scale collection by scientific research-botanists generally represents no threat, even though it is often unnecessarily obstructed and prohibited by authorities under the provisions of the International Convention on Biodiversity. Without such collection further advances in botanical research are severely set back and excessive restriction amounts to a misuse of the Convention, for which purpose it was not intended.

The current situation throughout much of India has serious implications for the future. With ever-increasing encroachment upon natural vegetation, huge areas are being denuded annually and continue to be damaged with little or no control. The continued decrease in forest-cover is not conducive (favourable) to achieving a healthy environment for the country's development and resulting climatic change, such as decreased rainfall and increased temperature, is already creating problems for agriculture, increasing storms, floods and droughts *etc*. A crucial factor thought to be causative in the current problem of Global Warming is the destruction of forests and the climatic alteration it causes. The inevitable result is that with increasing destruction of the vegetation there is an accompanying loss of

species of the country's valuable pteridophytic flora from the ever-expanding areas affected.Chanda et al. (2008) lists 29 fern-allies and 385 ferns as threatened taxa of Pteridophytes.

(1) Herbaria. Detailed scrutiny of collections in a large number of herbaria can provide valuable information as to the status of a species. The data on present distribution and on their past distribution along with the trends of shrinkage of population are very important in categorisation of the status of a species. Follow-up work, visiting the locality concerned, which the second author has done in a number of cases, provides very useful information as to the occurrence of the species in the area.

(2) Review of Literature. Floras, checklists, monographs and taxonomic accounts giving reports of species may provide much further information about their distribution-status. Comparison of old floras and recent ones may also give important information about the present status of species, particularly where a species is found by renewed search to be absent from an area where it once occurred. However, an important caveat (caution) is that modern workers must ascertain that the report and identification of the species were genuinely correct for both older and newer reports by taking the trouble to examine the original herbarium-specimens supporting the reports.

(3) Extensive and Specific Field-Surveys. It is necessary a) To find out the present distribution; b) To estimate the past distribution and changes in population-size; c) To measure the threat to the species through accurate field-observation of the population over a period of time.

The Red List recognized nine categories which are:

1) 'Extinct' (EX) - with no reasonable doubt after exhaustive surveys.

2) 'Extinct in the wild' (EW) - only known in cultivation after exhaustive surveys.

3) 'Critically endangered' (CR) - extremely high risk of extinction in the wild.

4) 'Endangered' (EN) - very high risk of extinction in the wild.

5) 'Vulnerable' (VU) - high risk of extinction in the wild.

6) 'Near threatened' (NT) - close or likely to qualify as threatened in the near future.

7) 'Least concern' (LC) - widespread and abundant.

8) 'Data deficient' (DD) - inadequate information on abundance and distribution to assess risk.

9) 'Not evaluated' (NE) - not evaluated against the criteria.

These categories are appropriate to Countries where the local occurrence of species is known in great detail and their former occurrence is also known from reliable data. However, in a number of tropical and developing Countries such precise information is hardly available except in very rudimentary form.

The highly sophisticated and detailed system of local recorders feeding data into a Central data-base simply does not exist in most Countries, including India, where many pteridologists do not know the species well enough and field-work is considerably limited so they inevitably fail to visit the majority of localities, even those well-known from the past. In addition, due to the inaccessibility of many areas the number of localities known in India from herbarium-collections probably represents only a random fraction of the actual extant populations of species. In most cases there is almost no historical information and even today estimates of population-size *etc*. are almost entirely missing. For this reason, the IUCN categories are somewhat impractical outside of advanced, often Western Countries and a handful of others. Therefore, during summarizing the status of pteridophytes in India, partly instead of the IUCN categories mentioned above, the overall term "threatened status" is used by Chanda et al. (2008) in a conservation-context for any indigenous species of taxonomic significance, which falls into one of three categories of threat, defined below, namely:

1. 'At risk',
2. 'Near-threatened',
3. 'Rare'.

(1)'At risk' – These species are recognized by any one of the following criteria: 1) the decline is thought to be rapid and their number and habitat have been drastically reduced to a critical level; 2) the reproductive capacity appears to have fallen considerably below its rate of elimination from the habitat, leading to decline in number and size of population; 3) species with 3 or fewer known localities; 4) known only from one population. Chanda et al. (2008) concluded there are 219 species of pteridophytes which were considered as 'At risk' in India. Of these, 160 come into the IUCN category of '**Critically endangered**'. These species are of particular concern and should be understood as species requiring active focus and efforts for the conservation of the limited localities where they occur in order to preserve both them and the associated species of plants and animals, which are also likely to be of special interest.

(2)'Near-threatened' – This includes species which are declining because of over-exploitation, extensive habitat-destruction or other environmental disturbances. In this

category the decline has started and is apparently slow, but the threat could push the species into the 'At risk' category in the near future. These species are likely to be in danger if modification to their habitat does not stop, or if their reproductive spread is affected.

Gureyeva (2002), however, suggested rather different, more general biological criteria to determine whether a fern species was becoming threatened or not, the main cause of vulnerability being associated with the success-rate of their reproductive biology. According to her, 1) ferns having effective ways of reproduction in their environment, either vegetatively, through buds or rhizome-branching, or through regularly producing viable spores would be the least vulnerable; 2) ferns which do not branch at all but which easily produce sporelings in their environment would be considered slightly vulnerable; 3) species which have sporophytes without effective vegetative reproduction, and also require a combination of many favourable factors for producing gametophytes from the spores, are the most vulnerable and have the least chance for continued survival during environmental change. Her categories key in an environmental factor since if the degradation of the environment, or destruction of plants, causes a drop in effective reproduction the status of the fern will change accordingly.Chanda et al. (2008) considered 82 species as 'Near-threatened' in India.

(3) 'Rare' – These species occur in widely separated small sub-populations (so that interbreeding is seriously reduced or is restricted to a single population) with narrow localised or specialised habitats, often with low climatic tolerance and specialised adaptations; or are thinly scattered over a more extensive range. The rarest are usually ancient species adapted to uncommon habitats, with both hampered spore and vegetative renewal, especially if they inhabit zones of intensive human economic activity. Anthropogenic factors create imbalances in the ecosystem leading to the rarity of many species today. They need not be threatened *per se* (by itself) but are at risk of falling into the 'Near-threatened' category if no attention is paid to their plight. Rarity as such is not mutually exclusive and 'At risk' and 'Near-threatened' species may or may not be rare, though the more threatened species are usually also rare. They estimated 113 species under the category of 'Rare', but not 'At risk' or 'Near-threatened'.

Therefore, the species which are included in the threatened list are mainly those which are:

1. Endemics with restricted distribution. The total number of Indian endemics has been revised by Fraser-Jenkins (2008) to *c*. 45 species of which 27 endemics as threatened in India.

2. Local species with their range restricted to isolated localities, yet they occur in considerable numbers in each locality (many plants but only at one or a few places);
3. Species occurring in small numbers or as few plants but in many separate or distant localities;
4. Species occurring as a few individuals in few localities, or in a single locality;
5. Species overexploited and becoming extirpated in many former areas by man, particularly forcommercial purposes;
6. Rare species, though safe from human expansion;
7. Uncommon species very seldom collected as located in areas extremely difficult to reach;
8. Species suspected to be threatened but for which no or very little information exists.

At present a large number of Pteridophytes in India are threatened mainly due to human activities, such as:

- Environmental degradation of preferred habitat for species, such as uncontrolled tree-felling and destruction for fuel, timber, crop-plantations or grazing, building over sites near to cities or coastal resort areas, or draining of wetlands for agriculture.
- Pollution of the environment, such as stream-banks, lakes and road-sides.
- Unregulated commercial collection, such as of tree-fern trunks for orchid-growing.
- Immoderate collection of species known to be rare by students; however, we consider that constructive small-scale collection by scientific research-botanists generally represents no threat, even though it is often unnecessarily obstructed and prohibited by authorities under the provisions of the International Convention on Biodiversity. Without such collection further advances in botanical research are severely set back and excessive restriction amounts to a misuse of the Convention, for which purpose it was not intended.

The current situation throughout much of India has serious implications for the future. With ever-increasing encroachment upon natural vegetation, huge areas are being denuded annually and continue to be damaged with little or no control. The continued decrease in forest-cover is not conducive (favourable) to achieving a healthy environment for the country's development and resulting climatic change, such as decreased rainfall and increased temperature, is already creating problems for agriculture, increasing storms, floods and droughts *etc*. A crucial factor thought to be causative in the current problem of Global Warming is the destruction of forests and the climatic alteration it causes. The inevitable

result is that with increasing destruction of the vegetation there is an accompanying loss of species of the country's valuable pteridophytic flora from the ever-expanding areas affected.

Endangered and Endemic Pteridophytes

Reasons for being endangered: Both anthropogenic and non anthropogenic

- Environmental degradation of preferred habitat for species- *uncontrolled tree-felling and destruction for fuel, timber, crop-plantations or grazing, building over sites near to cities or coastal resort areas, or draining of wetlands for agriculture*
- Pollution of the environment
- Unregulated commercial collection
- Immoderate collection of species known to be rare by students

Assessment could be done through:

- Herbaria;
- Review of Literature;
- Extensive and Specific Field-Surveys

(1) Herbaria-

Detailed scrutiny of collections in a large number of herbaria can provide valuable information as to the status of a species. The data on present distribution and on their past distribution along with the trends of shrinkage of population are very important in categorisation of the status of a species. Follow-up work, visiting the locality concerned, which the second author has done in a number of cases, provides very useful information as to the occurrence of the species in the area.

(2) Review of Literature

Floras, checklists, monographs and taxonomic accounts giving reports of species may provide much further information about their distribution-status.

Comparison of old floras and recent ones may also give important information about the present status of species, particularly where a species is found by renewed search to be absent from an area where it once occurred.

However an important caveat (caution) is that modern workers must ascertain that the report and identification of the species were genuinely correct for both older and newer reports by taking the trouble to examine the original herbarium-specimens supporting the reports.

(3) Extensive and Specific Field Surveys- It is necessary because

a) To find out the present distribution;

b) To estimate the past distribution and changes in population-size;

c) To measure the threat to the species through accurate field-observation of the population over a period of time.

Indian Scenario

Chanda et al. (2008) lists 29 fern-allies and 385 ferns as threatened taxa of Pteridophytes in India. During summarizing the status of pteridophytes in India, partly instead of the IUCN categories, the overall term "threatened status" is used by Chanda et al. (2008) in a conservation-context for any indigenous species of taxonomic significance, which falls into one of three categories of threat, namely:

1. 'At risk',
2. 'Near-threatened',
3. 'Rare'

If you are interested could visit following website to know the evaluation method for assessing the IUCN RED List

http://cmsdocs.s3.amazonaws.com/keydocuments/summary_sheet_en_web.pdf

- **(1) 'At risk' –** These species are recognized by any one of the following criteria:

1) the decline is thought to be rapid and their number and habitat have been drastically reduced to a critical level;

2) the reproductive capacity appears to have fallen considerably below its rate of elimination from the habitat, leading to decline in number and size of population;

3) species with 3 or fewer in known localities;

4) known only from one population.

Chanda et al. (2008) concluded there are 219 species of pteridophytes which were considered as 'At risk' in India of these, 160 come into the IUCN category of 'Critically endangered'.

- **(2)'Near-threatened' –** This includes species which are declining because of over-exploitation, extensive habitat-destruction or other environmental disturbances. In this category the decline has started and is apparently slow, but the threat could push the species into the 'At risk' category in the near future. These species are likely to be in danger if modification to their habitat does not stop, or if their reproductive spread is affected.

Gureyeva (2002) suggested some biological criteria to determine whether a fern species was becoming threatened or not, the main cause of vulnerability being associated with the success-rate of their reproductive biology. The criteria are-

1) ferns having effective ways of reproduction in their environment, either vegetatively, through buds or rhizome-branching, or through regularly producing viable spores would be the least vulnerable;

2) ferns which do not branch at all but which easily produce sporelings in their environment would be considered slightly vulnerable;

3) ferns which have sporophytes without effective vegetative reproduction, and also require a combination of many favourable factors for producing gametophytes from the spores, are the most vulnerable and have the least chance for continued survival during environmental change.

Chanda et al. (2008) considered 82 species as 'Near-threatened' in India.

- **(3) 'Rare' –** These species occur in widely separated small sub-populations ***(so that interbreeding is seriously reduced or is restricted to a single population)*** either with narrow localized or specialized habitats, often with low climatic tolerance and specialized adaptations; or they are thinly scattered over a more extensive range.

The rarest are usually ancient species adapted to uncommon habitats, with both hampered spore and vegetative renewal, especially if they inhabit zones of intensive human economic activity.

Chanda et al. (2008) estimated 113 species under the category of 'Rare

PRESENT STATUS

Fraser-Jenkins (2012) documented a total of 337 endangered pteridophytes of which 321 species are of ferns but this depiction is not reflected in updated IUCN Red list (www.redlist.org.).

Therefore the species which are included in the threatened list in India are mainly those which are:

- **1.** Endemics with restricted distribution.
- **2.** Local species with their range restricted to isolated localities, yet they occur in considerable numbers in each locality *(many plants but only at one or a few places);*
- **3.** Species occurring in small numbers but in many separate or distant localities;
- **4.** Species occurring as a few individuals in few localities, or in a single locality;

- **5.** Species overexploited and becoming eradicated in many past areas by man, particularly for commercial purposes;
- **6.** Rare species, though safe from human expansion;
- **7.** Uncommon species very seldom collected as because they located in areas extremely difficult to reach;
- **8.** Species suspected to be threatened but for which no or very little information exists.

ENDEMIC FERN SPECIES IN INDIA

Taxa	Endemic to	RET Category
?*Ophioglossum eliminatum* Khand.	C. India (Madhya Pradesh)	VU
Anemia schimperiana C. Presl subsp. *weghtiana* (Gardner) Fraser-Jenk.	S. India (Tamil Nadu)	EN
Oreogrammitisattenuata (Kunze) Parris	S. India (Tamil Nadu)	EN; Globally Threatened
Oreogrammitisaustroindica (Parris) Parris	S. India (Tamil Nadu)	CR or EX; Globally Threatened
Oreogrammitispilifera(Ravi et J. Joseph) Parris	S. India (Kerala, Tamil Nadu)	VU; Globally Threatened
Tomophyllumperplexum (Parris) Parris	S. India (Tamil Nadu)	CR; Globally Threatened
?*Trichomanes agasthianum* (Madhus. Et C. A. Hameed) C. A. Hameed, K. P. Rajesh et Madhus.	S. India (Tamil Nadu; Kerala)	EN
Cyatheaalbosetacea (Scptt. Ex Bedd.) Copel.	Indian Islands (Nicobar)	EN; Globally Threatened
Cyatheacrinita (Hook.) Copel.	S. India (Kerala, Tamil Nadu)	EN
?*Cyatheanicobarica* N. P. Balakr. Et R. D. Dixit	Indian Islands (Nicobar)	CR or EX
Lindsaeamalabarica (Bedd.) Baker	S. and C. India (Karnataka; Kerala; Tamil Nadu; ?Andhra pradesh; Madhya Pradesh)	NT; Globally Threatened
Lindsaeatenera Dryand.	Indian Isldnad (Andaman & Nicobar)	NT; Globally Threatened
Lindsaeavenusta Kaulf. Ex Kuhn	S. India (Kerala; Tamil Nadu)	EN; Globally Threatened
Aleuritopteris(?) *thwaitesii* (Mett. ex Kuhn) Saiki	S. India (Tamil Nadu)	CR; Globally Threatened
Pterisgeminata Wall. Ex J. Agardh	S. India (Tamil Nadu)	EN; Globally Threatened
Pterisquadriaurita Retz.	S. India (Tamil Nadu)	?CR; Globally Threatened
Pterisreptans T. G. Walker	S. India (Kerala)	CR; Globally Threatened

State and Zone wise distribution of endangered and endemic fern species in India

[Total Fern Species: 1100; Total Endangered Fern Species: 321]

States of India	Number of endangered fern species	%age of endangered fern species	Number of endemic fern species
North-West India			
Jammu & Kashmir	010	13	02
Himachal Pradesh	012		
Uttarakhand	019		
Uttar Pradesh	001		
West India			
Maharashtra	002	02	02
Rajasthan	004		
North-East India			
Arunachal Pradesh	103	85	09
Assam	028		
Manipur	044		
Meghalaya	058		
Mizoram	017		
Nagaland	017		
Tripura	005		
North-Central and East India			
Sikkim	038	18	01
West Bengal	020		
Orissa	001		
South India			
Andhra Pradesh	004	41	38
Karnataka	008		
Kerala	048		
Tamil Nadu	071		
Central India		01	02
Madhya Pradesh	004		
Indian Islands		16	04
Andaman & Nicobar	050		

10. **Let's sum up**

- Pteridophytes are considered as first land vascular plants on Earth which appeared in the Late Silurian. Smith et al. (2006) system of classification divides ferns into four Monophyletic classes and 11 monophyletic orders.
- The gametophyte phase of pteridophytes begins with a spore which gives rise to a green, thread-like tissue, called a protonema. The protonema develops into a prothallus, a small, green, multicellular tissue that is rarely seen in nature. The prothallus has numerous subterranean rhizoids to anchor it to the substrate and absorb nutrients.
- Sporophytic plants with leaves, axes and roots and well developed conducting tissue. Stem are rhizomatous, radial or dorsiventral, erect, prostrate, climbing, or subterranean. Leaves either small, simple and bract-like or linear with a simple vein, straight in bud, or a broad frond with branched or divided veins, simple to several times pinnately divided, conduplicate or mostly circinate in bud; bearing sporangia. Sporangia thick or thin walled, homosporous or heterosporous, aggregated in sori.
- The most important general characteristic of the pteridophytes is the high level of basic numbers. The basic chromosome numbers of homosporous pteridophytes are the highest known in vascular plants, averaging x = 35–69.
- It is important to acknowledge that distinguishing between hybridization at the intra- and interspecific levels is not clear-cut in some groups of ferns, often because of varying species concepts and evolving species delineations.
- In ferns, a chemical substance secreted by gametophytes that causes immature gametophytes to develop as males is called antheridiogen. The hormone antheridiogen is a potent controller of gametangial initiation in cultures of homosporous fern gametophytes.
- Well over 8000 species, in at least 300 genera, of living ferns are known. These occur throughout an extraordinary range of habitats from high altitude and high latitude

Arctic-Alpine situations to the heart of tropical rain forests, and sub desert scrub of continental interiors to rock of the sea coast and mangrove swamps.

- In evaluating the threat to species survival, the IUCN indicates that the coverage of the pteridophyta is insufficient, and also highlights the lack of information concerning the conservation status of Pteridophytes.

11. **Suggested Readings**

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11. Christenhusz Maarten J. M., and Chase Mark W. (2014). Trends and concepts in fern classification. Annals of Botany : 1–24, 2014

12. **Assignments**

1. Name two pteridophytes with secondary thickenings.
2. Mention the difference between dictyostelic and solenostelic structures in pteridophytes.
3. What are the differences between fern ally and true fern?
4. Mention the two principal trends in the evolution of fern leaf form.
5. Name one biciliate and one pluriciliate pteridophytes.
6. What type of sporangial development is found in Osmundaceae?
7. What is acrostichoid condition in ferns? Give example.
8. Depict the classification system of fern by Sporne (1962) and Smith *et al.* (2006) by highlighting the differences between two systems.
9. Describe the patterns of gametophyte development in homosporous ferns with suitable diagrams
10. Which fern family gets intermediate position in between eusporangiate and leptosporangiate ferns and why?
11. What is soil spore bank? Mention the utility of soil spore bank.
12. Draw a labelled line diagram of generalized life cycle of a homosporous fern and possible deviations from the complete “sexual cycle”
13. Enumerate the step wise approaches for conservation of pteridophytic species.
14. Specify the morphological changes in early land vascular plants with examples in evolutionary point of view.
15. How do the ferns overcome its sterility attained due to apogamy?

All the materials are self written and collected from ebook, journals and websites.

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - I

Course: BOHCT 1.3

(Biology and Diversity of Gymnosperms and Taxonomy of Angiosperms and Biosystematics)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

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Foreword

Satisfying distance learners' needs of verifying kinds and magnitude as well as minimizing distance and to reach the unreached in Open and Distance Learning (ODL) systems has the novelty in it. Nevertheless, this novelty puts challenges to the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and may other personnel involved in it. A dedicated team of University of Kalyani under leadership of Hon'ble Vice-Chancellor have puts their best efforts, committed professionalism as a Team for promoting Post Graduate Programmes under distance mode under University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 successfully completed with best efforts.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further, suggestions from the learners-end will be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team received continuously positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, made constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either in conceptual level or in the operational level of the DODL of University of Kalyani.

For a comprehensive, learners friendly, adaptable text that meets curriculum requirements of the Post Graduate Programme through distance mode.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 1.3

Biology and Diversity of Gymnosperms and Taxonomy of Angiosperms and Biosystematics

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 1.3	Biology & Diversity of Gymnosperms	**Unit 1. General features of gymnosperm and seed ferns**	1. Introduction : A general account and an outline of recent system of classification of gymnosperms upto order level with characteristic features. 2. Palaeozoic Pteridosperms (Seed Ferns): Major events in evolution of Palaeozoic Pteridosperms; brief account of Lyginopteridaceae, Calamopityaceae, Medullosaceae, Callistophytaceae.	1
		Unit 2. Cycads, Cycadeoids & more diversification among gymnosperm	3. Cycads & Cycadeoids : General traits, circumscriptions of the families of Cycads, early evidence, Cycad and Cyadeoid foliage. 4. More diversification among gymnosperms: Brief account of Caytoniaceae, Corystospermaceae, Peltaspermaceae, Glossopteridceae, Pentoxylaceae.	1
		Unit 3. General accounts of Ginkgo and Gnetophytes	5. Ginkgos : General traits, early evidence, distribution in time and space. 6. Gnetophytes : General traits; characteristics features of the genera of Gnetopsida; comparisons amongst Ephedra, Gnetum and Welwitschia.	1
		Unit 4.General accounts of Conifers	7. Conifers : General traits of conifers; first evidence of conifer organization - Cordaitales, Voltziales; origin of conifer cones and leaves; circumscriptions of the families of extant conifers and their interrelationships; comparative account among conifers on basis of the male gametophyte, pollination mechanisms, female gametophytes, proembryo development.	1
	Taxonomy of Angiosperms and Biosystematics	**Unit 1. General accounts of taxonomy and its classification**	1. Systems of angiosperms classification: Outline of classification of Cronquist (1988) and Takhtajan (1997) up to Subclasses / Super orders. Broad outline of angiosperm phylogeny Group (APG) III, 2009.	1
		Unit 2. General survey of taxa of angiosperms–I	2.1 A general survey of the following taxa of angiosperms with reference to inter-relationship, evolutionary trends, and economic importance: Amborellaceae, Magnoliales, Caryophyllidae, Nepen-thales.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 1.3	Taxonomy of Angiosperms and Biosystematics	**Unit 3. General survey of taxa of angiosperms–II**	2.2. A general survey of the following taxa of angiosperms with reference to inter-relationship, evolutionary trends, and economic importance: Podostemales, Asterales, Alismatales and Poaceae.	1
		Unit 4. ICBN	3. ICBN : Changes, addition and alteration of latest four codes; principles, rank of taxa and names of taxa, nomenclatural types, priority of publication and limitation of the priority of publications. Principle idea about Bio-codes and Phylocodes.	1
		Unit 5. Phytogeography, Botanic Gardens and Herbaria	4. Concepts of phytogeography: Endemism in India; invasion and introduction of plants in India. 5. Botanic Gardens and Herbaria: Importance and examples	1
		Unit 6. Biosystematics and Numerical Taxonomy	6. Biosystematics: Definition, methods, categories, differences with classical taxonomy. 7. Numerical Taxonomy: Definition, principles, logical steps, applications, merits and demerits.	1
		Unit 11. Evolutionary concept and cladistics system of classification	8. Evolutionary concept - Plagiomorphy and Apomorphy; Parallelism and Convergence; Homology and Analogy; Monophyly and Polyphyly including the concept of Heterobathmy, Cline, Polarity, Anagenesis and Cladogenesis, Symplesiomorphy, Synapomorphy, Autopomorphy, Stasigenesis, Cata-genesis, Paraphyly, Holophyly, Homoplasy; Phylogram, Dendrogram and Cladogram. 9. Cladistics system of classifications: Principle, methods, merits and demerits.	1
		Unit 12. Data sources of taxonomy and Taxonomic literatures	10. Data sources of taxonomy: Embryology, photochemistry with brief account of DNA - Taxonomy, DNA - barcoding, e - Taxonomy; nuclear rDNA, chloroplast and mitochondrial DNA; ultrastructure of sieve tube plastids. 11. Taxonomic literatures: classical books, index, flora and manual, revision and monograph, icons, bibliography, catalogue, encyclopedias, glossary and dictionary. Important periodicals of India and abroad.	1

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COURSE – BOHCT1.3

Biology & Diversity of Gymnosperms and Taxonomy of Angiosperms and Biosystematics

Hard Core Theory Paper **Credit: (Groups A+B) = 3**

Group – A (Biology and Diversity of Gymnosperms)

Content Structure

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1. Introduction
2. Course Objectives
3. Introduction: A general account and an outline of recent system of classification of gymnosperms upto order level with characteristic features.
4. Palaeozoic Pteridosperms (Seed Ferns): Major events in evolution of Palaeozoic Pteridosperms; brief account of Lyginopteridaceae, Calamopityaceae, Medullosaceae, Callistophytaceae.
5. Cycads & Cycadeoids: General traits, circumscriptions of the families of Cycads, early evidence, Cycad and Cyadeoid foliage.
6. More diversification among gymnosperms: Brief account of Caytoniaceae, Corystospermaceae, Peltaspermaceae, Glossopteridceae, Pentoxylaceae.
7. Ginkgos: General traits, early evidence, distribution in time and space.
8. Conifers: General traits of conifers; first evidence of conifer organization - Cordaitales, Voltziales; origin of conifer cones and leaves; circumscriptions of the families of extant conifers and their interrelationships; comparative account among conifers on basis of the male gametophyte, pollination mechanisms, female gametophytes, proembryo development.
9. Gnetophytes: General traits; characteristics features of the genera of Gnetopsida; comparisons amongst *Ephedra*, *Gnetum* and *Welwitschia*.
10. Let’s sum up
11. Suggested Reading
12. Assignment

1. Introduction

The first seed producing group that you conventionally study is the gymnosperms. The gymnosperms are a group of seed-producing plants that includes conifers, cycads, Ginkgo, and gnetophytes. The term "gymnosperm" comes from the Greek composite word (gymnos, "naked" and sperma, "seed"), meaning "naked seeds". This module shall help you to understand the recent system of classification and basic idea about present and extict members of gymnosperms. This module shall also guide you to know about evolutionary trends of different gymnosperms group. The module shall introduce you to the general characteristics of some important gymnosperms groups, you will get an overview of their diversity in forms and habitats and conventional resource utilization patterns.

2. Course Objectives

By the end of this section, you will have completed the following objectives:

- To developed a basic idea about gymnosperms classification
- Discuss the type of seeds produced by gymnosperms, as well as other characteristics of gymnosperms
- State which period saw the first appearance of gymnosperms and explain when they were the dominant plant life
- List the four groups of modern-day gymnosperms and provide examples of each

3. Introduction: A general account and an outline of recent system of classification of gymnosperms upto order level with characteristic features.

The **gymnosperms** (spermatophyte) are a group of seed-producing plants that includes conifers, cycads, *Ginkgo*, and gnetophytes. The term "gymnosperm" comes from the Greek word meaning "naked seeds". The name is based on the unenclosed condition of their seeds (called ovules in their unfertilized state). The nonencased condition of their seeds stands in contrast to the seeds and ovules of flowering plants (angiosperms), which are enclosed within an ovary. Gymnosperm seeds develop either on the surface of scales or special leaves, often formed cones, or solitary as in Yew, *Torreya*, *Ginkgo*.

An over view of Gymnosperm:

Diversity in Gymnosperm

The gymnosperms and angiosperms together compose the spermatophytes or seed plants. The gymnosperms are divided into six phyla. Organisms that belong to the Cycadophyta, Ginkgophyta, Gnetophyta, and Coniferophyta (also known as Pinophyta) are living. However, those in the seed ferns Pteridospermales and Cordaitales phyla are now extinct and found in fossil forms. By far the largest group of living gymnosperms are the conifers (pines, cypresses, and relatives), followed by cycads, gnetophytes (*Gnetum*, *Ephedra* and *Welwitschia*), and *Ginkgo biloba* (a single living species). Roots in some genera have fungal association with roots in the form of mycorrhiza (*Pinus*), while in some others (*Cycas*) small specialised roots called coralloid roots nitrogen-fixing cyanobacteria symbiosis.

General classification of Gymnosperm (up to order)

In early classification schemes, the gymnosperms (Gymnospermae) were regarded as a "natural" group. There is conflicting evidence on the question of whether the living gymnosperms form a clade. The fossil record of gymnosperms includes many distinctive taxa that do not belong to the four modern groups, including seed-bearing trees that have a somewhat fern-like vegetative morphology (the so-called "seed ferns"or pteridosperms.) When fossil gymnosperms such as Bennettitales, *Caytonia* and the glossopterids are considered, it is clear that angiosperms are nested within a larger gymnosperm clade, although which group of gymnosperms is their closest relative remains unclear.

The most **recent** system of **classification** for **gymnosperms** is proposed by Christenhusz et al. (2011). They divided the extant **gymnosperms** into four sub-**classes**:
Cycadidae, Ginkgoidae, Gnetidae and Pinidae. Cycadidae includes single order, Cycadales and two families Cycadaceae and Zamiaceae. For the most recent classification on extant gymnosperms see Christenhusz *et al.*(2011). There are 12 families, 83 known genera with a total of ca 1080 known species (Christenhusz & Byng 2016)

Subclass **Cycadidae**

- Order **Cycadales**

  Family **Cycadaceae**: *Cycas*

  Family **Zamiaceae**: *Dioon, Bowenia, Macrozamia, Lepidozamia, Encephalart os, Stangeria, Ceratozamia, Microcycas, Zamia*.

Subclass **Ginkgoidae**

1. Order **Ginkgoales**

   Family **Ginkgoaceae**: *Ginkgo*

Subclass **Gnetidae**

- Order **Welwitschiales**

  Family **Welwitschiaceae**: *Welwitschia*

- Order **Gnetales**

  Family **Gnetaceae**: *Gnetum*

- Order **Ephedrales**

  Family **Ephedraceae**: *Ephedra*

Subclass **Pinidae**

- Order **Pinales**

  Family **Pinaceae**: *Cedrus, Pinus, Cathaya, Picea, Pseudotsuga, Larix, Pseudo larix, Tsuga, Nothotsuga, Keteleeria, Abies*

- Order **Araucariales**

  Family **Araucariaceae**: *Araucaria, Wollemia, Agathis*

  Family **Podocarpaceae**: *Phyllocladus, Lepidothamnus, Prumnopitys, Sundaca rpus, Halocarpus, Parasitaxus, Lagarostrobos, Manoao, Saxegothaea, Microcachrys, Pherosphaera, Acmopyle, Dacrycarpus, Dacrydium, Falcatifolium, Retrophyllum, N ageia, Afrocarpus, Podocarpus*

- Order **Cupressales**

  Family **Sciadopityaceae**: *Sciadopitys*

  Family **Cupressaceae**: *Cunninghamia*, *Taiwania*, *Athrotaxis*, *Metasequoia*, *Se quoia*, *Sequoiadendron*, *Cryptomeria*, *Glyptostrobus*, *Taxodium*, *Papuacedrus*, *Austr ocedrus*, *Libocedrus*, *Pilgerodendron*, *Widdringtonia*, *Diselma*, *Fitzroya*, *Callitris* (in cl. *Actinostrobus* and *Neocallitropsis*), *Thujopsis*, *Thuja*, *Fokienia*, *Chamaecyparis*, *C allitropsis*, *Cupressus*, *Juniperus*, *Xanthocyparis*, *Calocedrus*, *Tetraclinis*, *Platycladus*, *Microbiota*

  Family **Taxaceae**: *Austrotaxus*, *Pseudotaxus*, *Taxus*, *Cephalotaxus*, *Amentotax us*, *Torreya*

Origin of Gymnosperms:

It is widely accepted that the gymnosperms originated in late Carboniferous period, replacing the lycopsid rainforests of the tropical region. This appears to have been the result of a whole genome duplication event around 319 million years ago. Early characteristics of seed plants were evident in fossil progymnosperms of the late Devonian period around 383 million years ago. It has been suggested that during the mid-Mesozoic era, pollination of some extinct groups of gymnosperms was by extinct species of scorpionflies that had specialized proboscis for feeding on pollination drops. The scorpionflies likely engaged in pollination mutualisms with gymnosperms, long before the similar and independent coevolution of nectar-feeding insects on angiosperms. Evidence has also been found that mid-Mesozoic gymnosperms were pollinated by Kalligrammatid lacewings, a now-extinct genus with members which (in an example of convergent evolution) resembled the modern butterflies that arose far later.

Diversity of Gymnosperms :

Conifers are by far the most abundant extant group of gymnosperms with six to eight families, with a total of 65-70 genera and 600-630 species (696 accepted names). Conifers are woody plants and most are evergreens. The leaves of many conifers are long, thin and needle-like, other species, including most Cupressaceae and some Podocarpaceae, have flat, triangular scale-like leaves. *Agathis* in Araucariaceae and *Nageia* in Podocarpaceae have broad, flat strap-shaped leaves. Cycads are the next most abundant group of gymnosperms, with two or three families, 11 genera, and approximately 338 species. A majority of cycads are native to tropical climates and

are most abundantly found in regions near the equator. The other extant groups are the 95-100 species of Gnetales and one species of Ginkgo.

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4. Palaeozoic Pteridosperms (Seed Ferns): Major events in evolution of Palaeozoic Pteridosperms; brief account of Lyginopteridaceae, Calamopityaceae, Medullosaceae, Callistophytaceae.

Pteridosperms or seed ferns:

Pteridosperms or seed ferns are a very heterogeneous group of extinct plants with mostly fern-like foliage but with real seeds. They are mostly reconstructed as small trees but also forms with a climbing growth habit gave been found. They possessed fern-like foliage, bore seeds, and are, therefore, called pteridosperms.

Examples:

Lyginopteris sp., *Heterangium* sp., *Diplopteridium* sp. , *Sphenopteris* sp. , *Glossopteris* sp. , *Medullosa* sp., *Calamopitys* sp. etc.

Geological distribution:

They first appeared on the earth in Upper Devonian times of the Palaeozoic era (Fig. 1.1). They were at their climax in Carboniferous period and became extinct in Jurassic period of Mesozoic era.

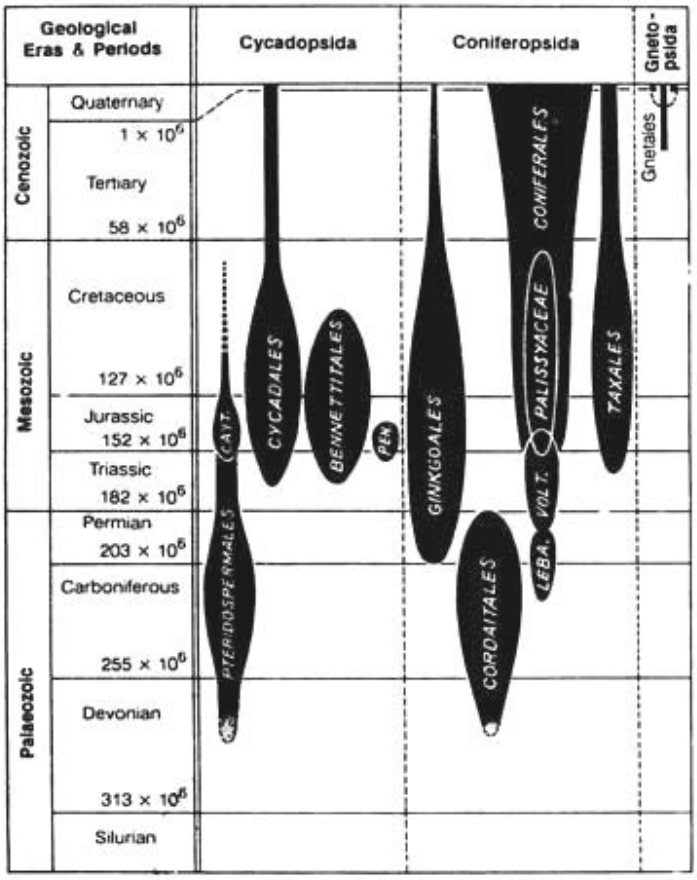

Fig. 1.1. Geological history of gymnosperms (after Melchior and Werdermann, 1954) (*Cayt.*, Caytoniaceae; *Pen*, Pentoxylales; *Leba*, Lebachiaceae; *Volt*., Voltziaceae)

General Characters:

- Extinct Palaeozoic and Mesozoic plants found from Devonian to Jurassic periods.
- Plants possessed slender stems with large frond-like leaves as in *Alethopteris*, *Sphenopteris*, etc.
- Primary xylem was mesarch, represented by solid or medullated protostele. Rarely, the primary xylem was exarch. Polystelic condition was also observed in some members.
- The secondary wood was manoxylic (loose and soft) and limited in amount.
- The radial walls of tracheids had multiseriate pits.
- The cortex was well-developed and had longitudinally aligned fibre strand.
- Leaves were usually fern-like, relatively large, pinnately compound, and often pinnate several times.
- The leaves were covered by a resistant cuticle.
- Ovules borne separately along margins of, or on surface of pinnately compound megasporophylls.
- Ovule-bearing frond or megasporophyll was not part of a cone.
- Megasporophylls were not arranged in strobili.
- Megasporophylls were like foliage leaves, or specialized structures, sometimes peltate.
- Microsporophyll's pinnately compound and not in strobili.
- The microsporangia had no annulus and were sometimes grouped into synangia.
- A well-developed vascular supply was present in the seed.
- The seeds were also provided with a definite pollen chamber, e.g. *Lagenostoma lomaxi*.
- The seeds of Pteridospermales resemble with those of the present day Cycads.

Affinities of Pteridospermales:

Pteridospermales (Cycadofilicales) may be assigned an intermediate position between ferns and Cycadophytes due to their several resemblances with both the groups. But due to the presence of exposed seeds they belong to gymnosperms with certainty.

Some of their possible affinities with ferns and Cycadophytes are mentioned below:

Similarities with Ferns:

- Large and pinnately compound leaves.
- Young leaves are circinately coiled.

- Mesarch or rarely exarch condition. Leaf traces are also mesarch.
- Absence of vessels in the xylem and companion cells in the phloem.
- Polystelic condition of Medullosa resembles with ferns.
- The sporangia were born on the foliage leaves.
- Except the presence of secondary wood, the vascular anatomy of stem is similar with that of ferns.
- Presence of thick wall around the megaspores.

Similarities with Cycadophytes:

- The wood is manoxylic in both Cycadales and Pteridospermales.
- Mucilage canals are present in the cortex and pith of the stem of both cycads and "seed ferns".
- Extensive cortex is present in most of the Cycadales and in some Pteridospermales (e.g. Medullosa).
- Centripetal xylem is present in some Cycadales and also in the vegetative organs of some "seed ferns" or Pteridospermales.
- Male gametes or spermatozoids are multi-ciliate and motile in both the groups.
- In both Pteridospermales and some Cycadales (e.g. *Cycas*), the megasporophylls remain spirally and loosely arranged, and they do not form a compact cone.
- In *Calymmatotheca* the ovules remain attached on the proximal parts of the leaves and their distal or tip regions remain sterile. In this regard, they resemble with the megasporophylls of *Cycas*, in which upper part is dissected, leafy and sterile while the lower part bears ovules.
- The seeds of *Lagenostoma lomaxi* of Pteridospermales remain surrounded by an outer hard stony layer and an inner fleshy layer. They resemble the seeds of cycads to some extent.
- The vascular supply of the ovules of cycads and some Pteridospermales also show some similarity with each other.
- Both Cycadales and Pteridospermales possess pinnately compound leaves.
- Pollen chamber is present in the ovules of both these groups.

Lyginopteridaceae:

Lyginopteridaceae is an extinct family of plants (Pteridospermatophyta) in North America and European Carboniferous coal measures. Lyginopteridaceae were shrubs and vines with radiospermic ovules containing a lagenostome. They consisted of forms with monostelic stem petioles usually with single strand and small seeds. Family members include *Lyginopteris* and *Heterangium*.

Important characters:

- Lyginopteridaceae are relatively large and fern-like.
- Lyginopteridaceaes stems are weak, aerial and well-branched.
- Leaves of lyginopteridaceae are bi-pinnate or tri-pinnate, large; ultimate leaf units dissected.
- In Lyginopteridaceae circinate vernation is seen.
- In Lyginopteridaceae pinnules had free venation.
- Stem stele of discrete vascular bundles or protostelic.

ii) Xylem of lyginopteridaceae is in stems mesarch.

iii) Leaf traces in lyginopteridaceae develop by tangential division of cauline strand.

iv) In Lyginopteridaceae leaf traces is first single and then becoming double.

v) Pith is well-developed in it.

vi) Mucilage canals are absent in lyginopteridaceae .

vii) In lyginopteridaceae secondary xylem is soft- textured and contains a high proportion of ray tissue and long tapering tracheids.

viii) Pericycle is present around the entire vascular region in it.

ix) In lyginopteridaceae the secondary growth is monoxylic.

15. In lyginopteridaceae secondary xylem is exocentric in development.

Kingdom:	Plantae
Division:	Pteridospermatophyta
Class:	Lyginopteridopsida
Order:	Lyginopteridales
Family:	Lyginopteridaceae

Calamopityaceae:

Calamopityaceae is the largest family in Pteriodspermatophyta. This family is composed of gymnosperms, and because of their stem structure discovered through fossil rocks, they are considered to be in this division. However, nothing is known of their reproductive organs,

classified as seed plants based on their similarities to the Lyginopteridaceae and Medullasaceae families within Pteriodspermatophyta.

Important characters:

1.Calamopityaceae resembles Lyginopteridaceae and Medullasaceae in the monoxylic wood structures in their stem; this structure suggests

the stem (diameter less than 1.5 cm) was narrow during the Calamopityaceae plant lifetime.

Kingdom: Plantae

Division: Pteridospermatophya

Order: Calamopityales

Family: Calamopityaceae

Medullosaceae:

The Medullosaceae is a family of pteridospermous seed plants characterised by large ovules with circular cross-section, with a vascularised nucellus, complex pollen-organs, stems and rachides with a dissected stele, and frond-like leaves. Their nearest still-living relatives are the cycads.

Most medullosaleans were small to medium-sized trees. The largest were probably the trees. with *Alethopteris* fronds - these fronds could be at least 7 metres long and the trees were perhaps up to 10 metres tall especially in Moscovian times, many medullosaleans were rather smaller trees with fronds only about 2 metres long, and apparently growing in dense mutually supporting strands. During Kasimovian and Gzhelian time there were also non-arboreal forms with smaller fronds. (e.g. *Odontopteris*) that were probably scrambling or possibly climbing plants.

Kingdom: Plantae

Division: Pteridospermatophyta

Order: †**Medullosales**

Important characters:

- In medullosacceae there are large, fern-like, small trees.
- Stems of medullosacceae are aerial, erect and un-branched.
- Leaves of medullosacceae are bi-pinnate or tri-pinnate, large.
- In medullosacceae vernation is not seen.
- Venation was free, pinnate, reticulate or even dichotomous in it.
- In medullosacceae stems polystelic, advanced types contain a complete cylinder with smaller vascular bundles.

- Xylem in medullosacceae stems are mesarch.
- Leaf traces in medullosacceae is not clearly comparable.
- Leaf traces is multiple In medullosacceae.
- In medullosacceae steles embedded in well-developed ground tissue.
- Mucilage canals are present in stems of medullosacceae.
- In medullosacceae xylem same as in Lyginopteridaceae.
- Pericycle surrounds each stele separately in medullosacceae.
- The secondary growth in medullosacceae monoxylic.
- Secondary xylem in medullosacceae is endocentric in majority of the genera.

Callistophytaceae:

The Callistophytaceae was a family of seed ferns (pteridosperms) from the Carboniferous and Permian periods. They first appeared in late Middle Pennsylvanian (Moscovian) times, 306.5-311.7 million years ago (Ma) in the callistophyte was documented from Late Pennsylvanian coal ball petrifactions in North America.

Kingdom:	Plantae
Division:	Pteridospermatophyta
Order:	Callistophytales
Family:	Callistophytaceae
Order:	Callistophytales

Important characters:

1. The relatively slender stems (fossil genus *Callistophyton*) had a eustele with a well-developed zone of secondary wood, and unlike most (but not all) other Palaeozoic pteridosperms, showed axillary branching.
2. A characteristic feature of the stems is the presence in the cortex of spherical secretory structures.
3. The small ovules (fossil genus *Callospermarion*) with the characteristic secretory structures have an integument that was only fused to the nucellus in the basal part of the ovules and so superficially resemble medullosalean ovules.
4. The ovules were born on the underside of pinnules that did not differ significantly in form from those of the purely vegetative fronds.
5. The pollen-producing organs (fossil genus *Idanothekion*) consisted of small, radially symmetrical synangia, with each pollen-sac having a longitudinal dehiscence structure. Unlike ferns, however, these pollen-organs produced monolete, bisaccate pollen (fossil genus *Vesicaspora*) bearing some similarity to the pollen of many conifers.

6. The foliage, which is the part of these plants most widely-found as macrofossils, consists of fronds with a basal dichotomy of the main rachis, each branch producing pinnately divided segments, but with no pinnae attached below the dichotomy.

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5. Cycads and Cycadeoids: General traits, circumscriptions of the families of Cycads, early evidence, Cycad and cycadeoid foliage.

Cycads & Cycadeoids

Introduction to Cycadeoideales:

The Cycadofilicales, they formed the dominant fossil plants during Palaeozoic age. The Cycadofilicales have of course definite affinities with the cycads on one side and ferns on the other, but they had no cones either in the male or in the female part of the plants, so some workers think that the Cycadofilicales form a separate group quite distinct from gymnosperms. In the Mesozoic times, however, we came across fossils plants which had cones and were definitely related to gymnosperms. So in Mesozoic the Cycadofilicales were replaced by true gymnosperms which formed strobili, and the seeds had a naked dicotyledonous embryo in them. The ovule or the seed was never enclosed in closed carpel.

Broad Classification of Mesozoic gymnosperms:

Cycadeoidale (Bennettitales) and Cycadales. Pant (1957) has placed the cycadeiods in a distinct class, the Cycadeoideopsida of the division Cycadophyta.

Diversity of Cycadeoidales :

The Cycadeoideales (Bennettitales) first appeared in the Permian they reached their highest range during the Jurassic period, after which they disappeared altogether. The second group Cycadales had a world-wide distribution during the Mesozoic period Majority of them had altogether disappeared; only a few types have been left which are confined to special parts of the East. The present day cycads are only the remnants of very large dyeing out group, i.e., they are sometimes described as living fossils, because they are on their way to extinction. The Cycadeoideales (Bennettitales) were very much like the cycads in their general appearance, and as the Mesozoic had these two prominent groups of gymnosperms, so that period sometimes described as age of cycads. These Cycadeoideales are closely related to the Cycadofilicales on one side and to cycads on the other but they have their own characteristic features which

distinguish them from all other gymnosperms except the Gnetales. The important feature which separates the Cycadeoideales from other gymnosperms is the presence of bisporangiate strobili. The plants of this group were diversified in their habit. Some types had short columnar stems like most of the living cycads. The short columnar stem was usually un-branched and at the apex of the plant there was a terminal crown of leaves which in most cases pinnate. Some other forms had branched stems with multiple crown. In present day cycads we know that young leaves and megasporophylls are covered up by unicellular hairy outgrowths known as ramenta. In Cycadeoideales (Bennettitales) these ramenta were not unicellular; they were scale like, flattened and were several cells in breadth. Like cycads the plants had well organized strobili or cones, but in cycads they are monosporangiate whereas in Cycadeoideales they were usually bisporangiate and they were either terminal or axiarlly in position. Majority of Cycadeoideales (Bennettitales) seem to have flowered only once in their life and after flowering the plant died out as we find in some of present day angiosperms.

Sporne classification of the order cycadeodales:

According to Sporne (1965), the order Cycadeoideales (Bennettitales) has been divided into three families. a) Cycadeoideaceae.

b) Williamsoniaceae

c) Wielandiellaceae

Systematic Position of Cycadeoideales:

Gymnosperms

Class. Cycadopsida

Order. Cycadeoideales

Family. Cycadeoideaceae

Genus. Cycadeoidea

(Bennettites, by American workers have been described as Cycadeoidea).

A brief out lines of cycadeoidales :

Cycadeoidea stems were "short and barrel-shaped," with a "crown of pinnate leaves" atop the stem. The majority of *Cycadeoidea* species were bisexual. The genus may have undergone self-pollination, although it is also possible that insects were involved in the process. The size and

shape of the trunk has been used to distinguish species, however forms intermediate between two species suggest the two might be merely different-sized or aged plants can't be excluded.

Features of Cycadeoideales:

(A) Morphological features:

In Cycadeoidea the stem was un-branched with a single crown of pinnate leaves at the tops, but some species had branched stem with a multiple crown. In some the stem was tuberous. In all cases the stem was covered up by persistent leaf bases as we find in *Cycas*.

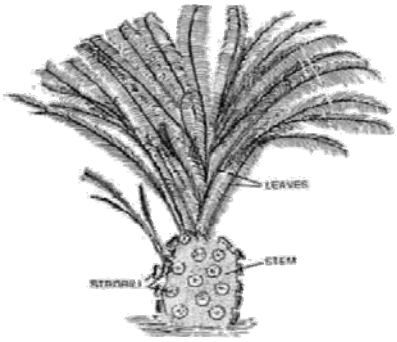


Fig. 2.12. *Cycadeoidea decotensis*. External features.

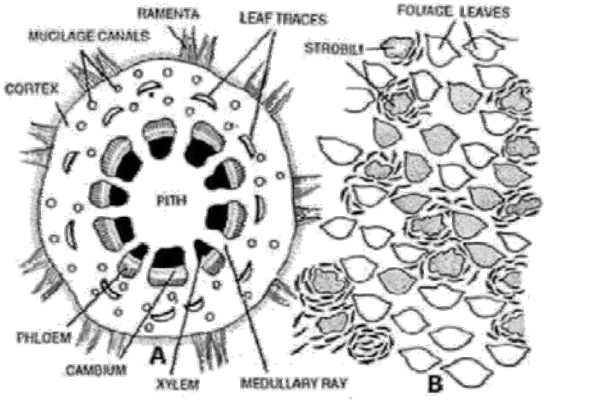


Fig. 2.13. *Cycadeoidea*. A, T.S. of stem showing primary structure; B, tangential section of a trunk.

B) Anatomical Features:

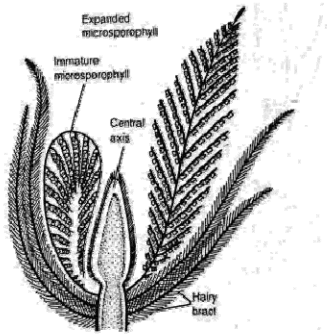


Fig. 2.16. *Cycadeoidea decotensis*. Apical portion with expanded and curved microsporophylls; the conical central axis possesses female reproductive structures.

In structure the stem usually had large pith and thin vascular cylinder in which the protoxylem was endarch, thick cortex with a number of gum canals in it. There was small amount of secondary growth. Growth rings were only in few cases where the cambium persisted and was more active, so on the whole the stem anatomy was like those of present day cycads i.e., with large pith, broad cortex and narrow vascular cylinder. In some few cases, however, the vascular cylinder was sufficiently broad. In the stem there were no traces of mesarch vascular bundles which are a common feature of leaf traces of present day cycads. Another distinction from cycads was that the leaf traces

were direct and no girdles while in present day cycads the girdling of leaf traces is quite common. The xylem had scalariform thickenings; pitted thickenings rather rare. This is an unusual feature because in the xylem of Cycadofilicales pitted thickening was very common and the group is much older than Cycadeoideales (Bennettitales).

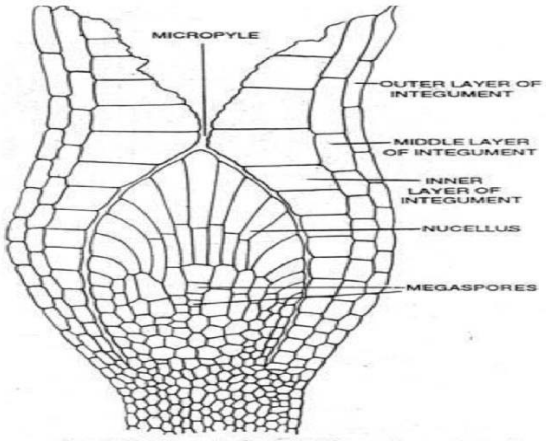


Fig. 2.19. *Cycadeoidea*. V.S. through young ovule.

The feature can only be explained that it was case of reversion.

Fossil specifies of cycadeoidales :

The Isle of Portland was the site of the first specimens recovered, described by Buckland as *C. megalophylla* (the type species) and *C. microphylla*. *Cycadeoidea gibsoniana* is a species collected from Lower Greensand from Luccombe Chine on the Isle of Wight, notable for the remarkable state of preservation of its plant parts. The original specimen was found by Thomas Field Gibson and was extensively broken and sliced to examine its anatomy. Four well preserved cones of a species *C. maccafferyi* were uncovered in Upper Cretaceous beds on Vancouver and Hornby Island in British Columbia.

6. More diversification among gymnosperms: Brief account of Caytoniaceae, Corystospermaceae, Peltaspermaceae, Glossopteridceae, Pentoxylaceae

Caytoniaceae : a brief account

The Caytoniaceae are an extinct family of plants belonging to Pteridospermatophyta, or seed ferns. Different organs attributed to the same original plant can be reconstructed from co-

occurrence at the same locality and from similarities in the stomatal apparatus and other anatomical peculiarities of fossilized cuticles. *Caytonia nathorstii* may have been produced by the same plant as *Caytonanthus arberi* (pollen organs) and *Sagenopteris phillipsii* (leaves).

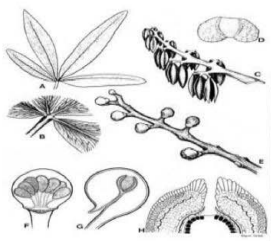
Classification:

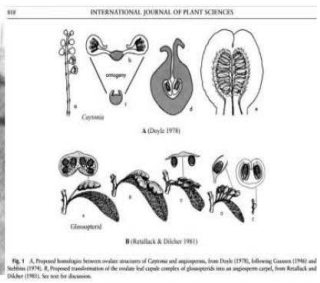
Kingdom:	Plantae
Division:	†Pteridospermatophyta
Order:	†Caytoniales
Family:	†Caytoniaceae
Genus:	†*Caytonia*
Species:	†***C. nathorstii***

The Organ genera: description

The woody nature of associated stalks and preserved short shoots are evidence that Caytoniales were seasonally deciduous, shrubs and trees. Caytoniales had fertile branches with seed-bearing cupules . The ovules were located inside fleshy cupules with tough outer cuticle. Individual ovules had an apical tube called a micropylar canal, that allowed pollen to pass into the pollen chamber. The outer layers of the cupules were fleshy and fruit-like; it is possible this was to aid in animal dispersal. The cupules are 4-5mm in diameter and about 3 mm long (Fig 1-2), and resemble a blueberry. The extra protection of the reproductive organs gave rise to the idea that Caytoniales were predecessors to angiosperms, which have completely enclosed seeds.

The pollen grains were small, between 25 and 30 µm in diameter. The size of the pollen grains supports the idea that they were wind-pollinated, and their bisaccate wings may have enabled entry into the seed by a pollination drop mechanism. In both respects they were like pollen of pine trees. They were produced in pollen sacs in coalesced groups of four, attached to branching structures. The pollen sacs hang off the structure in clusters, and are typically 2 cm in length.





The most common and widespread part found fossilized are leaves of *Sagenopteris* (Figand *Mexiglossa*. These are compound leaves consisting of 3-6 leaflets arrayed in a palmate manner. The individual leaflets are around 6 cm in length. The leaflets have anatomizing veins, like those of some ferns, but lacking orders of venation found in angiosperm leaves.

A) leaf structure B) Venation C) Seed structure D) Pollen grain E) Pollen sacs F) Cupule G) Pollen sac structure H) Ovule

A brief account of Pentoxylales:

This group has been discovered and named as **"Pentoxyleae"** by well-known Indian Palaeobotanist Professor Birbal Sahni (1948). This is a group of some fossil plants described from Rajmahal Hills in Amrapara District (Santhal Parganas) of Eastern Bihar (India) revealing their existence in Jurassic Period.

Distinguishing Features of Pentoxylales:

1. Extinct Mesozoic plants found in Jurassic period.
2. Although the exact habit of these plants is not clearly established, these were probably shrubs or very small trees.
3. Long and short shoots were present on these plants.
4. Short shoots had spirally arranged leaves and terminally located reproductive organs.
5. Leaves were thick, simple, lanceolate, and had diploxylic leaf trace.
6. Stomata were formerly thought to be syndetocheilic, but now they are considered to be haplocheilic.
7. Leaves possessed open venation.
8. Stems were polystelic. Basinger et al. (1974) opined that "it may be more appropriate to call each stele as vascular segment or sympodium".
9. Wood of Pentoxylon was pycnoxylic and resembled Araucaria.
10. Ovules were sessile.
11. Female reproductive organs were like stalked mulberry, consisting of about 20 sessile seeds attached to central receptacle and surrounded by stony layer and then fleshy outer layer of integument uniting them.

12. Male reproductive organs or microsporophyll's form whorl of branched micro-sporangiophores.

13. The micro-sporangiophores were fused basally into a disc-like structure.

Stem Genera of Pentoxyleae:

- *Pentoxylon Sahnii*:

Pentoxylon sahnii and Nipanioxylon guptai are the stem genera of "Pentoxyleae". The stems of Pentoxylon sahnii attained a diameter from 3mm to 2 cm. The stem has always been reported in association with the leaves called Nipaniophyllum. Presence of five steles in a cross- section ofthe stem has been the main reason for giving the name Pentoxylon to the genus. Many short lateral shoots or dwarf shoots were also present on the stem. Five steles occupied greater part of the stem in a cross-section. Each stele had its own cambium. The cambium was uniformly active in the young stems, but at maturity more secondary tissue developed towards the centre, and thus the secondary wood appeared eccentric. Primary phloem and primary xylem were present towards outer and inner sides of the cambium, respectively. Six steles have also been observed by Sahni (1948), although rarely. According to Vishnu-Mittre (1953) the number of steles varied along the length of the stem. There were present five much smaller bundles just alternating with the main bundles of the stem i.e. five steles. Each such bundle had a large amount of secondary wood. These were probably the leaf trace bundles. Medullary rays of the main steles were uniseriate, and they lacked ray tracheids, wood parenchyma and resin canals. The secondary wood resembled greatly with that of Araucaria. Uniseriate or bi-seriate bordered pits were present on the radial wall of tracheids.

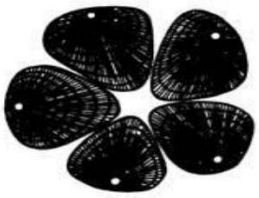


Fig. 7.1. *Pentoxylon sahnii*. T.S. stele. (after Sahni)

(ii) Nipanioxylon:

This stem genus of Pentoxyleae was discovered from the village Nipania and hence named *Nipanioxylon*. Village Nipania is in Rajmahal Hills, near Dumarchir in the Amrapara district (Santhal Parganas) in Bihar (India). Nipanioxylon differed from Pentoxylon in possessing larger number of bundles (steles) and less developed secondary growth in the stem. *Nipanioxylon* resembled *Pentoxylon* in other details.

ii) **Leaves of Pentoxyleae:**

- ***Nipaniophyllum*:**

The leaves have been described under the name *Nipaniophyllum raoi*. They were found attached with the shoots or Pentoxylon sahnii. They were originally described under the name Taeniopteris. They were present on the short lateral shoots. Each leaf was simple, petiolate, strap-shaped, and possessed a well-developed mid rib with many parallel lateral veins. Branching has not been observed in lateral vein.

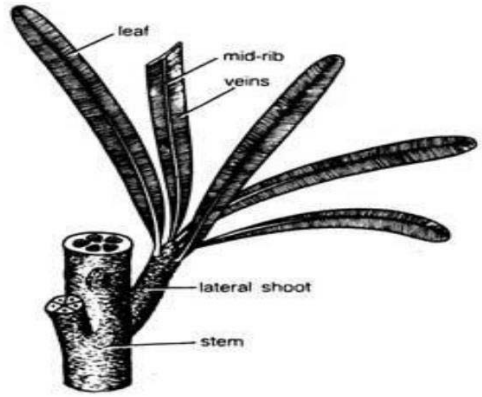


Fig. 7.2. *Pentoxylon sahnii*. Reconstruction of stem and leaves (*Nipaniophyllum raoi*). (after Sahni).

Similar to cycads, the leaf traces had centripetal and centrifugal xylems, thus exhibiting diploxylic condition. Sahni (1948) reported the presence of syndetocheilic stomata in Nipaniophyllum but Vishnu-Mittre (1953) also observed the presence of Cycadalean type ofhaplocheilic stomata.

Later on, Sharma (1969) and Bose et al. (1985) observed that arrangement of stomata was anomocytic as in Cycads and most other gymnosperms. Vascular bundles in Nipaniophyllum were mesarch.

1. Seed-Bearing Organ of Pentoxyleae:

Carnoconites:

The female cones or seed-bearing organs have been described under the name Carnoconites. Two species (*C. compactum* and *C.laxum*) have been described. Both these species have,

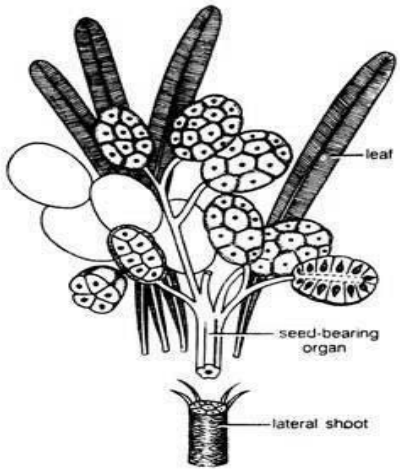


Fig. 7.3. *Carnoconites compactum*. Female cones. (after Sahni).

however, not been reported in organic connection with the stem genus Pentoxylon but from the same rocks. Seed-bearing organs (Fig. 7.3) were forked and found attached terminally on the lateral dwarf shoots. They were mulberry-like, and attained a length of about 1.8 cm in *Carnoconites compactum* and 3 cm in *C. laxum*. They were, however, narrower in *C. laxum*. About 20 sessile ovules were seen attached on the receptacle, and there

were no inter-seminal scales. Any sporophyll-like structure was also not reported. In-spite of these facts Sahni (1948) used the word ‘cone’ for these structures. The ovules were covered by a single integument. The nucellus was free from the integument.

(ii) *Sahnia Nipaniensis*:

The probable microsporangiate or male organs of Pentoxyleae were named as Sahnia nipaniensis by Vishnu-Mittre (1953). They were present terminally on the shoot, and fused basally in a shallow disc (Fig. 7.4). Vishnu-Mittre (1953) reported as many as 24 such pollen-bearing organs. Each microsporophyll possessed many pear-shaped, unilocular sporangia. The terminal position of the sporophyll was also occupied by a sporangium (Fig. 7.5). Several monocolpate and boat-shaped pollen grains were present in each microsporangium. The sexine of the pollen wall is homogeneous while its nexine is present in the form of thin dark zone. In the region of aperture, the sporoderm is highly folded. Taylor & Taylor (1987) observed a few lamellae in the region of aperture. Other details of the male flowers are not yet fully known

Fig. 7.4. *Sahnia nipaniensis*. Reconstruction of male "flower". (after Vishnu-Mittre).

Fig. 7.5. *Sahnia nipaniensis*. A detached microsporophyll. (after Vishnu-Mittre).

Affinities of Pentoxylales: Some of their possible affinities are discussed below: Affinities with Cycadales: The two groups (Pentoxyleae and Cycadophytes) resemble each other in:

i. possession of direct leaf trace in Pentoxyleae and seedlings of some cycads,
ii. leaf traces anatomy in showing diploxylic nature. of their vascular bundles,
iii. haplocheilic stomata,
iv. vestigial polystely in the seedling stages of some modern cycads,
v. nature of wood and pittings,

vi. possessing more or less similar kind of pollen grains, and

vii. structure of their seeds and peduncles.

However, vascular bundles in Pentoxylon are not arranged in the Cycadean manner. There is also no similarity between the polystelic condition of Pentoxylon and mature modern cycads.

Affinities with Conifers: Some of the characteristics, in which Pentoxylales resemble with conifers, include the presence of:

i. Pycnoxylic wood,

ii. Tracheids with circular bordered pits arranged in uniseriate or bi-senate manner,

iii. Uniseriate wood rays, and

iv. dimorphism in the stems of Pentoxylon sahnii and several conifers.

However, the general anatomy of the stem of Pentoxylales is not at all coniferous as mentioned also by Sahni (1948). Pentoxylales are totally stachyospermous (i.e. both the male and female organs were borne on stems, rather than on leaves) while conifer ales are partly phyllospermous and party stachyospermous.

Affinities with Medullosaceae:

Pentoxylales also resemble with members of family Medullosaceae of Palaeozoic Pteridospermales (e.g. Medullosa) in possessing a polystelic primary vasculature in their stems. The secondary wood of Pentoxylon was pycnoxylic, character also encountered in some species of Medullosa.

Affinities with Bennettitales: Under mentioned are some of the resemblances between Pentoxylales and Bennettitales:

- Presence of syndetocheilic stomata, in addition to haplocheihc ones.
- Diploxylic nature of the vascular bundles.
- Whorled micro-sporangiophores.
- Superficial resemblances between male flowers of both the groups.
- The manner in which the ovules were born in Pentoxylales was similar to that of Bennettitales. However, the inter-seminal scales, found in members of Bennettitales, were absent in Pentoxylales.

• *Stachysporous* nature of their male and female organs, i.e. instead of leaves, these organs were born on the stems.

• Both the groups share several common characters in their dwarf shoots.

The two groups also resemble each other in their mode of branching as well as nature of their steles. Coniferous type of pittings was present in the stems of *Pentoxylon* and some species of *Medullosa*.

Affinities with Bennettitales: Under mentioned are some of the resemblances between Pentoxylales and Bennettitales:

1. Presence of syndetocheilic stomata, in addition to haplocheihc ones.
2. Diploxylic nature of the vascular bundles.
3. Whorled micro-sporangiophores.
4. Superficial resemblances between male flowers of both the groups.
5. The manner in which the ovules were born in Pentoxylales was similar to that of Bennettitales. However, the inter-seminal scales, found in members of Bennettitales, were absent in Pentoxylales.
6. *Stachysporous* nature of their male and female organs, i.e. instead of leaves, these organs were born on the stems.
7. Both the groups share several common characters in their dwarf shoots.
8. Presence of direct leaf trace also brings the two groups quite close to each other.

However, the polystelic condition of the stems of *Pentoxylon* and *Nipanioxylon* has no similarity with that found in Bennettitales. In Pentoxyleae, the sporangiophores were erect, radial structures without any sterile part.

They were spirally branched and possessed sac-like unilocular microsporangia. On the other hand, in Bennettitales these structures were completely different. They had circinate dorsiventral pinnate sporophyll with a sterile and synangium-bearing portion.

Affinities with Some Other Groups:

While stem dimorphism of *Pentoxylon sahnii* is a Ginkgoalean feature as also a coniferous one, the diploxylic vascular bundles of Pentoxylales are also seen in Cordaitales as also in Bennettitales. Meeuse (1961) observed several resemblances between Pentoxylales and *Pandanus* (a member of family Pandanaceae of Monocotyledons) and opined that “Pandanaceae and some related monocotyledons” have descended directly “from Pentoxylales”.

Remarks to the Uniqueness to Pentoxylales:

The mulberry-like female cones or infructescences of Pentoxylales (*Carnoconites compactum*) with over twenty sessile ovules attached to a central receptacle is a unique feature of this group. Furthermore, these infructescences had neither any inter-seminal scales, nor anything that could be called a sporophyll, a unique feature again. The sporangiophores of Pentoxylales had spirally arranged branches and the sporangia were unilocular as well as terminal. In view of the above mentioned unique features, (e.g. wood similar to that of a conifer, leaf and pollen grains like that of cycads and cycadeoids, and ovulate cones not reported in any other gymnosperms) as well as resemblances of Pentoxylales with several groups of plant kingdom, Sahni's (1948) remarks that Pentoxyleae **"occupy a unique and rather isolated position",** or Pentoxyleae "is a group of plants that defies classification", still hold good. This group, of course, belongs to gymnosperms, but to establish its phylogenetic relationships a lot more is still to be done.

Glossopteris :

Glossopteris (Ancient Greek: ***meaning*** "tongue", because the leaves were tongue-shaped, and *pteris*, Greek for fern or feathery) is the largest and best-known genus of the extinct order of seed ferns known as Glossopteridales (also known as Arberiales or Ottokariales). The genus *Glossopteris* refers only to leaves, within a framework of form genera used in paleobotany. (For likely reproductive organs see Glossopteridaceae.) These are important because they indicate biological identity of these plants that were critical for recognizing former connections between the varied fragments of Gondwana: South America, Africa, India, Australia, New Zealand, and Antarctica.

Description of Glossopteris

Long considered a fern after its discovery in the 1820s, it was later assigned to the gymnosperms. The genus is placed in the division Pteridospermatophyta. In reality, many of the plant groups included within this division are only distantly related to one another. Glossopterids' relationships with other groups remain obscure. Most recent phylogenetic analyses favour placement of glossopterids as sister to a large group including Corystospermales, Caytoniales, Bennettitales, Pentoxylales, Gnetales (in some analyses), and angiosperms. A few analyses favour alternative links with Ginkgoales, Cordaitales and Pinales.

Glossopteris should strictly be used to refer to the distinctive spathulate fossil leaves with reticulate venation, however, the term has also been used to refer to the parent plant as a whole.

- *G. angustifolia*
- *G. brasiliensis*
- *G. browniana*
- *G. communis*
- *G. indica*
- *G. occidentalis*

7. Ginkgos: General traits, early evidence, distribution in time and space.

General traits:

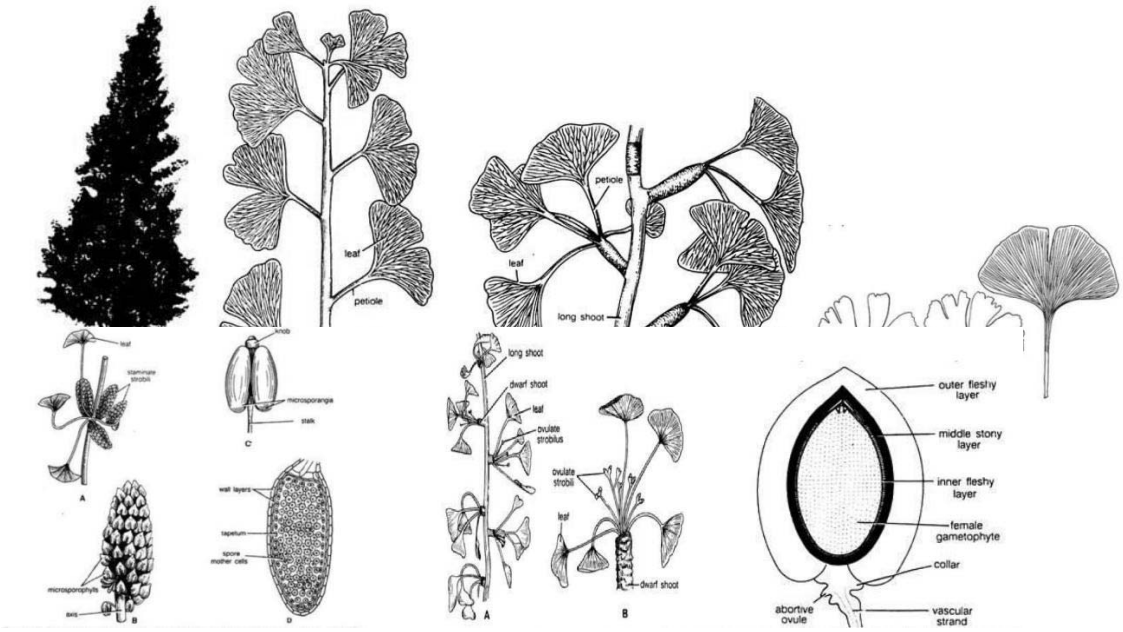


Figure: *Ginkgo biloba* tree (a), a long shoot bearing deeply lobed leaves (b), a long shoot bearing dwarf shoots (c), leaves showing various lobes (d), male strobili (e), female strobili (f), an ovule shortly after pollination (g).

1. The tree is dioecious plant

2. The tree is taller than it is wide, with an average width of up to 60 feet and the females are wider than males.
3. Ginkgos are most commonly grown as a shade or street tree. Despite the showy, drooping thorn-covered branches, the tree is hardy and resistant to breakage, pollution and salty conditions.
4. Ginkgos lose their leaves come winter, after they turn from green to a brilliantly colored yellow in the fall. The color is short-lived because the foliage drops quickly.
5. The leaves grow in an alternate pattern, and are 2 to 4 inches long. The epidermis of leaves is thickly cuticularised and consists of rectangular to polygonal cells. Haplocheilic type of stomata, restricted only to the lower epidermis, are present. The foliage leaves are simple,large, petiolate and wedge-shaped or fan-shaped with expanded apex and narrow base.
6. The branches are dimorphic i.e. bear long shoots which are of unlimited growth with scattered leaves and dwarf shoots which are short branches of limited growth.
7. The young stem (long shoot) is more or less circular in outline and remains surrounded by a single-layered, thickly circularized epidermis made of brick-shaped cells. Epidermis is replaced by periderm in the older stems.
8. The seeds, oval shaped and roughly 1 to 3 inches long, fall to the ground in autumn. The seeds are covered with a fleshy outer coating that splits open when the seeds fall, releasing an unpleasant odor that is said to resemble vomit. The flesh also often splatters, creating a mess. It can take upwards of 20 years or more before a ginkgo tree produces fruit.
9. *Ginkgo biloba* possesses a long tap root system. The roots are extensively branched and penetrate deep into the soil. In transverse section the roots are somewhat circular in outline. Mature roots are surrounded by phellogen or suberized cells of cortex. A large portion of the young root is occupied by multilayered, thin-walled cortex which contains several tannin-filled cells and calcium oxalate crystals. Mucilage canals are also prominently visible.

Early evidence:

The ginkgo (Ginkgoales) is a living fossil, with fossils recognisably related to modern ginkgo from the Permian, dating back 270 million years. The most plausible ancestral group for the

order Ginkgoales is the Pteridospermatophyta, also known as the "seed ferns", specifically the order Peltaspermales. The closest living relatives of the clade are the cycads, which share with the extant *G. biloba* the characteristic of motile sperm. Fossils attributable to the genus *Ginkgo* first appeared in the Early Jurassic, and the genus diversified and spread throughout Laurasia during the middle Jurassic and Early Cretaceous. It declined in diversity as the Cretaceous progressed with the extinction of species such as *Ginkgo huolinhensis*, and by the Palaeocene, only a few *Ginkgo* species, *Ginkgo cranei* and *Ginkgo adiantoides*, remained in the Northern Hemisphere, while a markedly different (and poorly documented) form persisted in the Southern Hemisphere. At the end of the Pliocene, *Ginkgo* fossils disappeared from the fossil record everywhere except in a small area of central China, where the modern species survived. It is doubtful whether the Northern Hemisphere fossil species of *Ginkgo* can be reliably distinguished. Given the slow pace of evolution and morphological similarity between members of the genus, there may have been only one or two species existing in the Northern Hemisphere through the entirety of the Cenozoic: present-day *G. biloba* (including *G. adiantoides*) and *G. gardneri* from the Palaeocene of Scotland.

Distribution in time:

Ginkgo is a genus of highly unusual non-flowering plants. The scientific name is also used as the English name. The order to which it belongs, Ginkgoales, first appeared in the Permian, 270 million years ago, possibly derived from "seed ferns" of the order Peltaspermales, and now only contains this single genus and species. The rate of evolution within the genus has been slow, and almost all its species had become extinct by the end of the Pliocene; the exception is the sole living species, *Ginkgo biloba*, which is only found in the wild in China, but is cultivated across the world.

Distribution in space:

It is the oldest living seed plant. It is cultivated for its edible seeds in some parts of China and Japan. Though, Chamberlain (1935) mentioned that it is doubtful whether *Ginkgo* exits today in the wild state, but Sporne (1965) has stated clearly about *Ginkgo biloba* that **“if it occurs naturally anywhere, is restricted to a small and relatively inaccessible region in South China”.**

Li (1956), however, mentioned certain evidences that *Ginkgo* still exists in the wild state in South-eastern China, along “the north western border of Chekiang and south eastern Anhwei”. In China and Japan it is grown as a sacred tree in temple gardens. It is cultivated in the United States as a shade tree. It is also successfully cultivated in some gardens of Europe, America and India.

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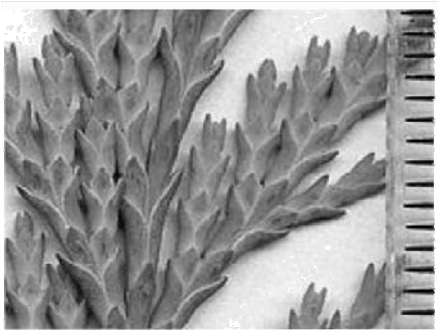
8. Conifers: General traits of conifers, first evidence of conifer organization- Cordaitales, Voltziales; origin of conifer cones and leaves; circumpscription of the families of extant conifers and their interrelationships; comparative account among conifers on basis of the male gametophyte, pollination mechanisms, female gametophytes, proembryo development.

Conifer is a Latin word, a compound of *conus* (cone) and *ferre* (to bear), meaning "the One that bears (a) cone(s) Also known as Pinophyta, **Coniferophyta** or **Coniferae**, or commonly as **conifers**, are a division of vascular land plants containing a single extant class, Pinopsida. They are gymnosperms, cone-bearing seed plants. All extant conifers are perennial woody plants with secondary growth. The great majority are trees, though a few are shrubs. Examples include cedars, Douglas, firs, cypresses, firs, junipers, kauri, larches, pines, hemlocks, redwoods, spruces, and yews. As of 1998, the division Pinophyta was estimated to contain eight families, 68 genera, and 629 living species. Although the total number of species is relatively small, conifers are ecologically important. They are the dominant plants over large areas of land, most notably the taiga of the Northern Hemisphere, but also in similar cool climates in mountains further south. Boreal conifers have many wintertime adaptations. The narrow conical shape of northern conifers, and their downward-drooping limbs, help them shed snow. Many of them seasonally alter their biochemistry to make them more resistant to freezing. While tropical rainforests have more biodiversity and turnover, the immense conifer forests of the world represent the largest terrestrial carbon sink. Conifers are of great economic value for softwood lumber and paper production.

General traits of conifers:

Seed plants all produce woody stems. Conifers are well represented in the fossil record with members dating from the upper Carboniferous. Unlike these other "gymnosperm" phyla, however, the conifers are important today economically and ecologically. The group consists of around 550 species arranged in seven families. All seven families can be dated back to the Mezazoic.



Vegetative Characteristics:

All members produce abundant secondary xylem and grow as either trees or shrubs. Trachiary elements in the xylem include only tracheids, and the sieve elements of the phloem include only sieve cells. Leaves are macrophylls but take the form of needles or scales for most species.

Reproductive Characteristics:

Dioecious or monecious plants. Pollen (microgametophyes) is produced in microsporangiate stobili (pollen cones) made up of sporophylls where the pollen sacs (microsporangia) are borne on the lower surface. All species are wind-pollinated. Sperm are not flagellated and are carried directly to the egg by means of a pollen tube. With the exception of the Taxaceae, ovules are borne in complex or secondarily reduced megasporangiate stobili (seed cones) consisting of seed scales bearing ovules. These are subtended by a sterile bract. Together the seed-scale with its sterile bract is termed a seed-scale complex. These are arranged around the central axis of the ovulate cone.

Foliages of Coniferophyta :

Since most conifers are evergreens, the leaves of many conifers are long, thin and have a needle-like appearance, but others, including most of the Cupressaceae and some of the Podocarpaceae,

have flat, triangular scale-like leaves. Some, notably *Agathis*in Araucariaceae and *Nageia* in Podocarpaceae, have broad, flat strap-shaped leaves. Others such as *Araucaria columnaris* have leaves that are awl-shaped. In the majority of conifers, the leaves are arranged spirally, exceptions being most of Cupressaceae and one genus in Podocarpaceae, where they are arranged in decussate opposite pairs or whorls of 3 (-4). In many species with spirally arranged leaves, such as *Abies grandis* (pictured), the leaf bases are twisted to present the leaves in a very flat plane for maximum light capture. Leaf size varies from 2 mm in many scale-leaved species, up to 400 mm long in the needles of some pines (e.g. Apache Pine, *Pinus engelmannii*). The stomata are in lines or patches on the leaves, and can be closed when it is very dry or cold. The leaves are often dark green in colour, which mat help absorb a maximum of energy from weak sunshine at high latitudes or under forest canopy shade.

Conifers from hotter areas with high sunlight levels (e.g. Turkish Pine *Pinus brutia*) often have yellower-green leaves, while others (e.g. blue spruce, *Picea pungens*) have a very strong glaucous wax bloom to reflect ultraviolet light. In the great majority of genera the leaves are evergreen, usually remaining on the plant for several (2-40) years before falling, but five genera (*Larix*, *Pseudolarix*, *Glyptostrobus*, *Metasequoia* and *Taxodium*) are deciduous, shedding the leaves in autumn and leafless through the winter. The seedlings of many conifers, including most of the Cupressaceae, and *Pinus* in Pinaceae, have a distinct juvenile foliage period where the leaves are different, often markedly so, from the typical adult leaves.

The internal structure of conifer

Tree rings are records of the influence of environmental conditions, their anatomical characteristics record growth rate changes produced by these changing conditions. The microscopic structure of conifer wood consists of two types of cells: **parenchyma**, which have an oval or polyhedral shape with approximately identical dimensions in three directions, and strongly elongated tracheids. **Tracheids** make up more than 90% of timber volume. The tracheids of earlywood formed at the beginning of a growing season have large radial sizes and smaller, thinner cell walls. Then, the first tracheids of the transition zone are formed, where the radial size of cells and thickness of their cell walls changes considerably. Finally, the latewood tracheids are formed, with small radial sizes and greater cell wall thickness. This is the basic pattern of the internal cell structure of conifer tree rings.

Life Cycle pattern of Conifer:

Conifers are heterosporous, generating two different types of spores: male microspores and female megaspores. These spores develop on separate male and female sporophylls on separate male and female cones. In the male cones, microspores are produced from microsporocytes by meiosis. The microspores develop into pollen grains, which are male gametophytes. Large amounts of pollen are released and carried by the wind. Some pollen grains will land on a female cone for pollination. The generative cell in the pollen grain divides into two haploid sperm cells by mitosis leading to the development of the pollen tube. At fertilization, one of the sperm cells unites its haploid nucleus with the haploid nucleus of an egg cell. The female cone develops two ovules, each of which contains haploid haploid megaspores. A megasporocyte is divided by meiosis in each ovule. Each winged pollen grain is a four celled male gametophyte. Three of the four cells break down leaving only a single surviving cell which will develop into a female multicellular gametophyte. The female gametophytes grow to produce two or more archegonia, each of which contains an egg. Upon fertilization, the diploid egg will give rise to the embryo, and a seed is produced. The female cone then opens, releasing the seeds which grow to a young seedling.

1. To fertilize the ovum, the male cone releases pollen that is carried on the wind to the female cone. This is pollination. (Male and female cones usually occur on the same plant.)
2. The pollen fertilizes the female gamete (located in the female cone). Fertilization in some species does not occur until 15 months after pollination.
3. A fertilized female gamete (called a zygote) develops into an embryo.
4. A seed develops which contains the embryo. The seed also contains the integument cells surrounding the embryo. This is an evolutionary characteristic of the Spermatophyta.

- Mature seed drops out of cone onto the ground.
- Seed germinates and seedling grows into a mature plant.
- When the plant is mature, it produces cones and the cycle continues.

Male reproductive organ

Most conifers are monoecious, but some are subdioecious or dioecious; all are wind-pollinated. Conifer seeds develop inside a protective cone called a strobilus. The cones take from four months to three years to reach maturity, and vary in size from 2 mm to 600 mm long.

In Pinaceae, Araucariaceae, Sciadopityaceae and most Cupressaceae, the cones are woody, and when mature the scales usually spread open allowing the seeds to fall out and be dispersed by the wind. In some (e.g. firs and cedars), the cones disintegrate to release the seeds, and in others (e.g. the pines that produce pine nuts) the nut-like seeds are dispersed by birds (mainly nutcrackers, and jays), which break up the specially adapted softer cones. Ripe cones may remain on the plant for a varied amount of time before falling to the ground; in some fire-adapted pines, the seeds may be stored in closed cones for up to 60–80 years, being released only when a fire kills the parent tree.

In the families Podocarpaceae, Cephalotaxaceae, Taxaceae, and one Cupressaceae genus (*Juniperus*), the scales are soft, fleshy, sweet and brightly colored, and are eaten by fruit-eating birds, which then pass the seeds in their droppings. These fleshy scales are (except in *Juniperus*) known as arils. In some of these conifers (e.g. most Podocarpaceae), the cone consists of several fused scales, while in others (e.g. Taxaceae), the cone is reduced to just one seed scale or (e.g. Cephalotaxaceae) the several scales of a cone develop into individual arils, giving the appearance of a cluster of berries.

The male cones have structures called microsporangia that produce yellowish pollen through meiosis. Pollen is released and carried by the wind to female cones. Pollen grains from living pinophyte species produce pollen tubes, much like those of angiosperms. The gymnosperm male gametophytes (pollen grains) are carried by wind to a female cone and are drawn into a tiny opening on the ovule called the micropyle. It is within the ovule that pollen-germination occurs. From here, a pollen tube seeks out the female gametophyte and if successful, fertilization occurs. The resulting zygote develops into an embryo, which along with its surrounding integument, becomes a seed. Eventually the seed may fall to the ground and, if conditions permit, grow into a new plant.

In forestry, the terminology of flowering plants has commonly though inaccurately been applied to cone-bearing trees as well. The male cone and unfertilized female cone are called *male flower* and *female flower*, respectively. After fertilization, the female cone is termed *fruit*, which undergoes *ripening* (maturation).

It was found recently that the pollen of conifers transfers the mitochondrial organelles to the embryo, a sort of meiotic drive that perhaps explains why *Pinus* and other conifers are so productive, and perhaps also has bearing on (observed?) sex-ratio bias.

Evolution of Conifers:

The earliest conifers in the fossil record date to the late Carboniferous (Pennsylvanian) period (about 300 million years ago). possibly arising from *Cordaites*, a genus of seed-bearing Gondwanan plants with cone-like fertile structures. Pinophytes, Cycadophytes, and Ginkgophytes all developed at this time. An important adaptation of these gymnosperms was allowing plants to live without being so dependent on water. Other adaptations are pollen (so fertilisation can occur without water) and the seed, which allows the embryo to be transported and developed elsewhere.

Conifers appear to be one of the taxa that benefited from the Permian–Triassic extinction event, and were the dominant land plants of the Mesozoic. They were overtaken by the flowering plants, which first appeared in the Cretaceous, and became dominant in the Cenozoic era. They were the main food of herbivorous dinosaurs, and their resins and poisons would have given protection against herbivores. Reproductive features of modern conifers had evolved by the end of the Mesozoic era.

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9. Gnetophytes: General traits; characteristic features of the genera of Gnetopsida; comparisons amongst *Ephedra, Gnetum* and *Welwitschia*.

Gnetophytes:

Gnetophytes are the division of plants, grouped within the gymnosperms (which also includes conifers, cycads, and ginkgos), that consists of some 70 species across the three relit genera: *Gnetum*, *Welwitschia* and *Ephedra*.

General traits:

1.The two common characteristics most commonly used are the presence of enveloping bracts around both the ovules and microsporangia as well as a micropylar projection of the outer membrane of the ovule that produces a pollination droplet.

2. *Gnetum* species are mostly woody vines in tropical forests, though the best-known member of this group, *Gnetum gnemon*, is a tree native to western Malesia. The one remaining species of

Welwitschia, *Welwitschia mirabilis*, native only to the dry deserts of Namibia and Angola, is a ground-hugging species with only two large strap-like leaves that grow continuously from the base throughout the plant's life. *Ephedra* species, known as "jointfirs" in the United States, have long slender branches which bear tiny scale-like leaves at their nodes. Infusions from these plants have been traditionally used as a stimulant, but ephedrine is a controlled substance today in many places because of the risk of harmful or even fatal overdosing.

- All extant species are woody.
- Decussate phylotaxis.
- Embryo has two cotyledons.
- Compound structure of both mega and microsporangiate strobili.
- Complex reticulate leaf venetion.
- Meiosis results in a tetrad of four microspores in all genera.

Characteristic features of the genera of Gnetopsida:

Gnetopsida includes three genera. These are *Gnetum*, *Welwitschia* and *Ephedra*.

Characters of *Gnetum*:

1. The general habit of the sporophyte of many species of *Gnetum* resembles with angiosperms.
2. Reticulate venation in the leaves of *Gnetum* is an angiospermic character.
3. Presence of vessels in xylem is again an angiospermic character.
4. Clear tunica and corpus configuration of shoot apices is a character of both *Gnetum* and angiosperms.
5. Strobili of *Gnetum* resemble much more with angiosperms than any of the gymnosperms.
6. Micropylar tube of Gnetales can be compared with the style of the angiosperms because both perform more or less similar functions.
7. Tetrasporic development of the female gametophyte is again a character which brings *Gnetum* close to angiosperms.
8. Absence of archegonia again brings *Gnetum* and angiosperms much closer.
9. Dicotyledonous nature of the embryo of *Gnetum* brings it quite close to the dicotyledons.
10. Most species are climbers.
11. Branches are two types: branches of limited growth and branches of unlimited growth.

12. Mesophyll of the leaves is differentiated into single layered palisade and well developed spongy parenchyma.

Characters of *Welwitschia*:

1. After germination, the seedling produces two cotyledons which grow to 25–35 mm (0.98–1.38 in) in length, and have reticulate venation.
2. The permanent leaves are opposite (at right angles to the cotyledons), amphistomatic (producing stomata on both sides of the leaf), parallel-veined and ribbon-shaped.
3. The two foliage leaves grow continuously from a basal meristem reaching lengths up to 4 m (13 ft). The tips of the leaves split and fray into several well-separated strap-shaped sections by the distortions of the woody portions surrounding the apical slit, and also by wind and adventitious external injuries.
4. *Welwitschia* has an elongated shallow root system consisting of "a tapering taproot with one or more non-tapering extensions, some pronounced lateral roots, and a network of delicate spongy roots" and a woody fibrous unbranched main stem.
5. The main stem consists of an unbranched woody crown roughly shaped like an inverted cone. The only branching in the shoot system occurs in the reproductive branches, which bear strobili.
6. Fertilization is carried out by insects including flies and true bugs.
7. A thick and ridged corky layer covers the upper surface of the stem. A ring of conjoint, collateral, open and endarch vascular bundles is present in the young stem which also contains centrally located distinct pith. Thick-walled spicular cells encrusted with calcium oxalate crystals are present in the cortex and pith both.
8. A layer of thickly cuticularized lower epidermis and an upper epidermis are present. The stomata are syndetocheilic. Palisade tissues alternating with sclerenchyma patches are present below the epidermal layers on both the sides of leaf. Spongy tissue fills the space between the palisade layers of both the sides. Sclereids or spicular cells are also present in the spongy tissue.
9. *Welwitschia* is strictly dioecious, i.e., two sexes are present in separate individuals. The inflorescences develop from a series of several transverse ridges arising parallel to the leaf bases. The branching in inflorescences is dichasial, and each branch ends in an attractive cone.

10. Male or microsporangiate strobilus or male cone is a compound structure bearing a quadrangular cone axis. It contains several bracts or cone scales arranged in opposite decussate manner. In the axil of each subtending bract is present a male flower.

11. The female strobilus, also called ovulate or megasporangiate strobilus or ovuliferous cone is also a compound structure like male cone. The axis of the female strobilus bears many broad decussate bracts or cone scales, in the axil of each of which is present a female flower.

12. The fertilization process is quite unique in *Welwitschia*. At the time of fertilization, the pollen tubes elongate and grow downwards through the nucellus. Simultaneously the apical cells of the female pro-thallus elongate and form prothallial tubes which grow upwards.

Characters of *Ephedra*:

1. Stem and branches are slender and green in colour. It has longitudinal ridges and furrows. Stem becomes woody due to the limited secondary growth. In some cases, underground rhizomes are also produced. The branches arise from azillary buds. Their arrangement gives the plants characteristic bushy and broom-like appearance. Apical meristem produces nodes and internodes. Meristematic tissues are also present at the base of each internode. Their activity further increases the length of stem. Sometimes, meristematic tissue becomes hard at the end of the growing season. Thus many braches fall. These branches are replaced by axillary shoot. It gives bushy appearance to the plants. The ridges and furrows of the successive internodes alternate with each other.

2. The leaves are scale like. They are opposite and arranged in pairs. The leaves of each pair are joined with each other at their bases. It forms a small sheath around the stem.

3. The primary root grows deep in the soil. It develops many secondary roots.

4. Stem is covered by heavily cutinized epidermis. The epidermis has stomata. They are present in pits in the region of furrows. A group of sclerenchymatous cells is present below the epidermis in the region of each ridge.

5. Each male cone arises in the axil of a leaf. The male cones are small. They are 1-2 cm in length. Each cone has a central axis. This axis bears 2-12 pairs of thick bracts. Bracts are arranged in an opposite manner. These bracts are closely set on the axis. A single male microsporophyll is present in the axil of each bract. Two small scales are present at the base of each sporophyll. It has a single stamen. Stamen has short stalk (filament). This stalk bears two to

six microsporangia (anthers) at the top. Each anther has two or three lobes. Each lobe has a pore at its tip for releases of microspore (pollen grain).

6. Female cones are borne on young shoots in the axils of the leaves. The plants are full with these cones during the growing season. Each cone consists of a short axis. This axis bears two or three pairs of opposite bracts. Two or sometimes three megasporangia (ovule) are present at the tip of the axis. Each ovule consists of a central mass of nucellus. It is surrounded by two integuments. Inner integument is supplied by two vascular bundles. Outer integument has four lobes. It is supplied by four vascular bundles. The nucellus is free from the integument in the apical region. Inner integument elongated at the apex to form a long tube called micropylar tube**.** This tube is flattened at the tip and twisted spirally. A mucilaginous drop comes out at this flattened tip. Female Prothallus One of the central nucellus cells enlarges. It functions as the megaspore mother cell. This spore mother cell divides by meiosis to produce four megaspores. Three megaspores disintegrate. Only one remains functional. The functional megaspore increases in size. It divides into 256 or more cells. These cells produce female prothallus. Archegonia are produced in the micropylar region of the pro-thallus. Lower region of the nucellus is known as nutritive region. Each archegonium develops from a superficial cell of the prothallus. Initially the neck of each archegonium consists of three tiers of cells. But later neck becomes very long due to upward growth of the prothalial tissue. It becomes eight cells in height. The venter of the archegonium has no well defined wall. It contains a large oosphere and a ventral canal nucleus. It has no definite ventral canal cell.

- The nucleus of the oospore divides into eight nuclei. The lower nuclei develop cell walls and act as proembryonal cells. Each proembryonal cell produces a tube like outgrowth. This outgrowth swells at the tip. The nucleus migrates into this swollen tip. A septum cut off this tip from the rest of the tube.

Comparision among *Ephedra, Gnetum* and *Welwitschia*:

Ephedra:

- Distribution if *Ephedra* occurs in dry and arid regions of North and South America, and several countries of Old World including India and China.
- Habit of *Ephedra* is most of the species are bushy or shrubby and only a few are lianes.
- Leaves of *Ephedra* are small and scale-like.

- Venation of *Ephedra* is parallel.
- Stomatal Development of *Ephedra* is haplocheilic.
- Shoot Apex of *Ephedra* is same as in *Gnetum*.
- Compound strobili of *Ephedra* are terminal and never cauline.
- **Male Gametophyte of *Ephedra* are:**

(i) A pair of prothallial cells are present in the male gametophyte.

(ii) A sterile cell and a spermatogenous cell are formed in the male gametophyte.

9. Female Gametophyte of *Ephedra* are:

- Normal archegonia are present in the female gametophyte.
- Prior to fertilization, the entire female gametophyte becomes cellular.
- In ephedra endosperm is not formed.
- Fertilization in ephedra takes place in an archegonium.
- Free-nuclear divisions take place in the zygote until about 8 nuclei are formed in *Ephedra*.
- Feeder is absent in *Ephedra*.
- Haploid Chromosome Number of *Ephedra* is 7 or 14.

Gnetum

1. Distribution of *Gnetum* in restricted to tropical or subtropical and humid regions of several countries of the world.
2. Habit of *Gnetum* is most of the species are lianes or trees and only a few are shrubs and trees.
3. Leaves of *Gnetum* are foliaceous, green and angio-sperms-like.
4. Venations of *Gnetum* are reticulate and unicostate.
5. Stomatal Development of *Gnetum* is syndetocheilic
6. Shoot Apex of *Gnetum* follows an open system of growth with a definable tunica layer
7. Compound strobili of *Gnetum* are cauline and only very rarely terminal.

8. Male Gametophyte of *Gnetum* are:

(i) Only a single prothallial cell is present in the male gametophyte.

(ii) No sterile cell and spermatogenous cell are formed in the young male gametophyte.

9. Female Gametophyte of *Gnetum* are:

(i) Normal archegonia are absent in the female gametophyte. A free nucleus organises some cytoplasmic material around itself and functions directly as an egg.

(ii) The upper portion of the female gametophyte remains free-nuclear at the time of fertilization and the lower portion becomes cellular.

(iii) Endosperm of *Gnetum* is like angiosperms, the endosperm in *Gnetum* is formed after fertilization.

(iv) Fertilization of *Gnetum* takes place in an embryo sac.

(v) In *Gnetum* there is no free-nuclear division phase in the embryogenesis.

(vi) Feeder in *Gnetum* is present in the embryo.

(vii) Haploid Chromosome Number of *Gnetum* is 22.

***Welwitschia*:**

1. *Welwitschia* is distributed only in a narrow coastal belt of about 1000 km in south-west Africa.

2. Habit of *Welwitschia* is the only known species of this genus resembles a gigantic turnip.

3. Leaves of *Welwitschia* are only two, large, thick foliaceous, ribbon-like, persistent and attain a length of about two metres. .

4. Venation of *Welwitschia* are parallel veins are joined by transverse or interwoven veinlets

5. Stomatal Development of *Welwitschia* is **s**yndetocheilic.

6. Shoot Apex of *Welwitschia* follows a closed system of growth where the shoot apex stops activity after initiating a pair of foliage-leaf primordia.

7. Compound strobili of *Welwitschia* are cauline and only very rarely terminal.

8. Male Gametophyte of *Welwitschia* are:

(i) Only a single prothallial cell is present in the male gametophyte.

(ii) No sterile cell and spermatogenous cell are formed in the young male gametophyte.

9. Female Gametophyte of *Welwitschia* are:

(i) Only archegonial initials are present. These function directly as eggs.

(ii) Prior to fertilization, the entire female gametophyte becomes cellular.

(iii) Endosperm is not present in of *Welwitschia*.

(iv) Fertilization of of *Welwitschia* takes place in the pollen tube which is not found in any gymnosperms or angiosperms. Same as in *Gnetum*.

(v) There is no free-nuclear division phase in the embryogenesis in *Welwitschia*.

(vi) Feeder present in *Welwitschia*.

(vii) Haploid Chromosome Number of *Welwitschia* is 21.

10. Let's sum up

- The gymnosperms are a seed-producing group of plants that are divided into Cycadophyta, Ginkgophyta, Gnetophyta, Coniferophyta, Pteridospermales and Cordaitales. In Christenhusz & Byng (2016) system of classification, there are 12 families, 83 known genera with a total of ca 1080 known species. Gymnosperms are originated in late Carboniferous period.
- Pteridosperms are an extinct group of plants with mostly fern-like foliage but with real seeds. They first appeared on the earth in Upper Devonian times of the Palaeozoic era.
- The Cycadofilicales are the dominant fossil plants during Palaeozoic age. The Cycadofilicales have of course definite affinities with the cycads on one side and ferns on the other, but they had no cones either in the male or in the female part of the plants.
- The ginkgo is a living fossil, most commonly grown as a shade or street tree of up to 60 feet. Despite the showy, drooping thorn-covered branches, the tree is hardy and resistant to breakage, pollution and salty conditions. The leaves grow in an alternate pattern, and are 2 to 4 inches long.
- Extant conifers are perennial woody plants with secondary growth and grow as either trees or shrubs. The group consists of around 550 species arranged in seven families. All seven families can be dated back to the Mezazoic.
- Gnetophytes are the division of plants that consists of some 70 species across the three relit genera: *Gnetum*, *Welwitschia* and *Ephedra*. The two common characteristics most commonly used are the presence of enveloping bracts around the ovules and microsporangia as well as a micropylar projection of the outer membrane of the ovule that produces a pollination droplet.

11. Suggested Readings

1. Bhatnagar, S.P. & p.Moitra, A.Gymnosperm, 1997, New Age International
2. Gifford, E.M. and Foster, A.S. Morphology & Evolution of Vascular Plants (3rd ed.), 1989, Freeman & Co.
3. Sporne, K.R. The Morphology of Gymnosperms, Latest Ed., Hutchinson &Co. Ltd.
4. Paleo Botany & Evolution of Plants by Wilson N. Stewart and Gar W. Rothwell.

12 Assignment

What are the main divisions and representative species of gymnosperms?

Depict the classification system of gymnosperms by Christenhusz & Byng 2016.

Mention the similarities between Pteridospermae and fern,

Which plant is called living fossil? Why?

Give a general account of Calamopityceae.

Describe with labeled diagram the male and female cone of conifers.

Give a comperative accout of *Gnetum*, *Ephedra* and *Welwitschia.*

COURSE – BOHCT1.3

Biology & Diversity of Gymnosperms and Taxonomy of Angiosperms and Biosystematics

Hard Core Theory Paper **Credit: (Groups A+B) = 3**

Group – B (Taxonomy of Angiosperms and Biosystematics)

Content Structure

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1. Introduction
2. Course Objectives
3. Systems of angiosperms classification: Outline of classification of Cronquist (1988) and Takhtajan (1997) up to Subclasses / Super orders. Broad outline of angiosperm phylogeny Group (APG) III, 2009 with the outline concept of Palaeoherbs and Eudicots.
4. A general survey of the following taxa of angiosperms with reference to their characteristics, inter-relationship, evolutionary trends, changed concepts and economic importance in the light of recent researches: Amborellaceae, Magnoliales, Caryophyllidae, Nepenthales, Podostemales, Asterales, Alismatales and Poaceae.
5. ICBN : Changes, addition and alteration of latest two codes; principles, rank of taxa and names of taxa, nomenclatural types, priority of publication and limitation of the priority of publications, effective and valid publications, author's citation; changes and rejection of names, preliminary concept of appendices. Principle idea about Bio-codes and Phylocodes.
6. Concepts of phytogeography: Endemism in India; invasion and introduction of plants in India.
7. Botanic Gardens and Herbaria: Importance, examples from India and abroad.
8. Biosystematics: Definition, methods, categories, differences with classical taxonomy.
9. Numerical Taxonomy: Definition, principles, logical steps, applications, merits and demerits.
10. Evolutionary concept ; Basic idea about following terms - Plagiomorphy and Apomorphy; Parallelism and Convergence; Homology and Analogy; Monophyly and Polyphyly including the concept of Heterobathmy, Cline, Polarity, Anagenesis and Cladogenesis, Symplesiomorphy, Synapomorphy, Autopomorphy, Stasigenesis, Catagenesis, Paraphyly, Holophyly, Homoplasy; Phylogram, Dendrogram and Cladogram.
11. Cladistics system of classifications of Angiosperms: Principles, methods, merits and demerits.

12. .Data sources of taxonomy: Embryology, photochemistry with brief account of DNA - Taxonomy, DNA - barcoding, e - Taxonomy; nuclear rDNA, chloroplast and mitochondrial DNA; ultrastructure of sieve tube plastids.
13. Taxonomic literatures: Definitions with examples of classical books, index, flora and manual, revision and monograph, icons, bibliography, catalogue, encyclopedias, glossary and dictionary.Important periodicals of India and abroad.
14. Let's sum up
15. Suggested Reading
16. Assignment

1. Introduction

There are slightly more than one third of a million species of plants known to man today, the information having been accumulated through efforts of several millenniums. So taxonomy was made to group plants based on set of characters. For a long time plant taxonomy was considered as 'the science of identifying, naming, and classifying plants'. This module shall give you basic idea about the recent system of classification of angiosperms and evolutionary trends of different angiospermic groups. This module shall also give you an overview about different taxonomic order to identify the angiospermic plant. The module shall introduce you to the numerical, cladistics and molecular taxonomy.

2. Course Objectives

After completation of this module, you will have fullfill the following objectives:

- To classify plant into taxa on the basis of similarities in phenotypic, molecular characteristics.
- To identify and name a plant and fix its rank in a recognised system of classification.
- To study the factors of evolution to find out the origin of species and their interrelationships.
- To know about molecular taxonomy and different taxonomic literature.
- To gather knowledge about Botanical garden and Herbaria

3. Systems of angiosperms classification: Outline of classification of Cronquist (1988) and Takhtajan (1997) up to Subclasses / Super orders. Broad outline of angiosperm phylogeny Group (APG) III, 2009 with the outline concept of Palaeoherbs and Eudicots.

Cronquist System of Plant Classification:

Arthur John Cronquist (1919-1992) was an American botanist (specialist on Compositae), associated with the New York Botanical Garden. He produced a broad classification of Embryobionta along with Takhtajan and Zimmerman (1966). He published his first large scale taxonomic overview in The Evolution and Classification of Flowering Plants in 1968 with a revised and expanded second edition being released in 1988. This work also was a survey of the practices of systematic botany. The Cronquist system is a taxonomic classification system of flowering plants. In 1981 he published his landmark work, An Integrated System of Classification of Flowering Plants.

Cronquist's system places flowering plants into two broad classes, Magnoliopsida (dicotyledons) and Liliopsida (monocotyledons). Within these classes, related orders are grouped into subclasses.

The scheme is still widely used, in either the original form or in adapted versions, but some botanists are adopting the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III.

The system as laid out in An Integrated System of Classification of Flowering Plants (1988) counts 320 families and 64 orders in class Magnoliopsida and with 19 orders and 66 families in class Liliopsida

A broad outline of the classification of Angiosperms by Cronquist (1988)

Division: Magnoliophyta: 2 classess, 11 subclasses, 83 orders, 386 families and 219,300 species.

A. Class: Magnoliopsida (Dicotyledons) 6 subclasses, 64 orders, 320 families and 169,400 species.

A.1- Subclass Magnoliidae (mostly basal dicots) [12 Orders]

A.2- Subclass Hamamelidae [correctly Hamamelididae] [11 Orders]

A.3- Subclass Caryophyllidae [3 Orders]

A.4- Subclass Dilleniidae [13 Oders]

A.5- Subclass Rosidae [18 Orders]

A.6- Subclass Asteridae [11 Orders]

B.Class: Liliopsida 5 subclasses, 29 orders, 66 families and 49,900 species.

B.1- Subclass Alismatidae [4 Oders

B.2- Subclass Arecidae [4 Orders

B.3- Subclass Commelinidae [7 Orders]

B.4- Subclass Zingiberidae [2 Orders]

B.5- Subclass Liliidae [2 Orders]

Merits:

1. This classification is largely based on principles of phylogeny that find acceptance with major contemporary authors. It shows general agreement with major contemporary systems of Takhtajan, Dahlgren and Thorne.

2. This system of classification giving detailed information on phytochemistry, anatomy, ultrastructure and chromosomes besides morphology.

3. As the text is in English, the system has been readily adopted in different books.

4. The system is highly phylogenetic.

5. The placement of Winteraceae family (vessel-less wood) at the beginning of dicotyledons is generally favoured by most authors including Ehrendorfer (1968) and Thorne (up to 1992).

6. Abolition of artificial group names such as Polypetalae, Gamopetalae, Lignosae, Herbaceae etc. has resulted in more natural grouping of taxa.

7. Nomenclature is in accordance with the International Code of Botanical Nomenclature.

8. Compositae in dicotyledons and Orchidaceae in monocotyledons are generally regarded as advanced families.

9. The relationship of various groups has been depicted with diagrams, which provide valuable information on relative advancement, cladistic relationship and size of various subclasses.

Demerits:

1. In spite of being a highly phylogenetic and popular in the USA, the system is not very useful for identification and adoption in herbaria.
2. Dahlgren (1983,1989) and Thorne (1981, 2003) considered angiosperms in the rank of a class and not that of a division.
3. Asteridae represent a loose assemblage of several diverse sympetalous families.
4. Superorder as a rank above the order has not recognized in this system of classification though it is present in other contemporary systems of Takhtajan, Thorne and Dahlgren.
5. Ehrendorfer (1983) pointed out that Hamamelidae do not represent an ancient side-branch of Magnoliidae but are remnants of a transition from Magnoliidae to Dilleniidae-Rosidae-Asteridae.
6. Behnke (1977) and Behnke and Barthlott (1983) advocate that Polygonales and Plumbaginales (S-type plastids) should be removed from Rosidae.
7. Urticales are placed in Hamamelidae, but they are close to Malvales and Euphorbiales (Dahlgren, 1983,1989).
8. Most recent authors do not believe in the aquatic ancestry of monocotyledons.

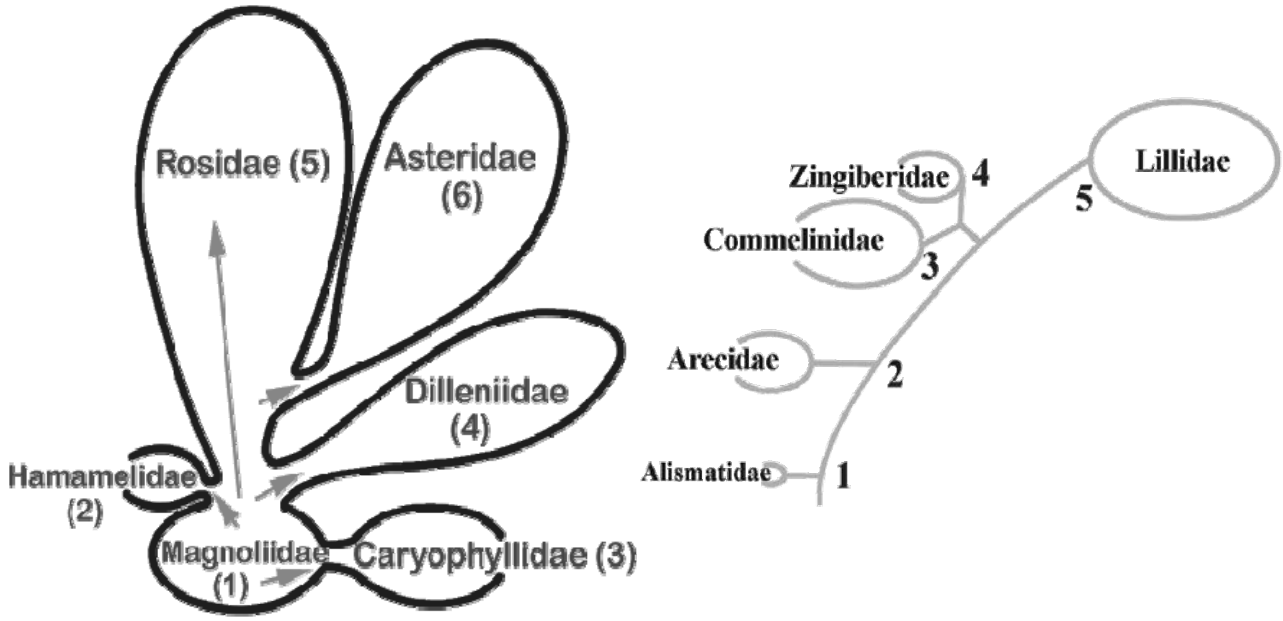

Fig- Phylogram showing the relationship between various subclasses (based on Cronquist 1988). http://botany.csdl.tamu.edu/FLORA/Wilson/tfp/mag/tfplec6s01.htm

Takhtajan system of classification:

Armen Leonovich Takhtajan (1910-2009) was a Soviet-Armenian botanist and one of the most important figures in 20th century plant evolution, systematics and biogeography. He is chiefly famous as the author of works on the origins of flowering plants and palaeobotany.

Takhtajan, who has provided a classification of angiosperms up to the family level, belongs to the Besseyan School and was strongly influenced by Hutchinson, Hallier and the other more progressive German workers. He believes in the monophyletic origin of angiosperms, the group having evolved from seed ferns Lyginopteridophyta.

Takhtajan considered the order Magnoliales as the most primitive and ancient group among the flowering plants. The order Alismatales has been supposed to be most primitive among monocotyledons. The highest evolved order among dicots is Asterales while among monocots, it is Arales.

The suffixes used for designation of taxa in the classification are:

a) Division – 'phyta'
b) Class – 'opsida'
c) Subclass – 'idae'
d) Superorder – 'anae'
e) Order – 'ales'
f) Family – 'aceae'

Takhtajan's system is based on 67 phyletic principles. Some of the important criteria are as follows:

1. Woody plants are primitive than herbaceous plants.
2. Deciduous woody plants are considered evolved from evergreen plants.
3. Xylem fibers evolved from tracheids to libriform fibres, through fibre tracheids.
4. Trilacunar or pentalacunar nodes are primitive to unilacunar nodes.
5. Alternate leaf arrangement is primitive while parallel venation is most advanced.
6. Stomata with subsidiary cells are primitive than those lacking subsidiary cells.
7. Cymose inflorescence is primitive than racemose.
8. Flowers with an indefinite or variable number of floral parts are primitive.
9. Pollen with un-sculptured exine is primitive to sculptured pollen.
10. Apocarpous gynoecium is primitive.

Outline of Takhtajan's system of classification (1997) are as follows:

Division. Magnoliophyta- 2 classes, 17 subclasses, 71 superorders, 232 orders, 589 families (2 classes, 12 subclasses, 53 superorders, 166 orders, 533 families in 1987 classification); estimated genera-13,000, species- 2,50,000.

Class 1. Magnoliopsida (Dicotyledons)- 11 subclasses, 55 superorders, 175 orders, 458 families (8 subclasses, 37 superorders, 128 orders, 429 families in 1987 classification); estimated genera- 10,000, species- 1,90,000

Subclass 1. Magnoliidae

2. Nymphaeidae*
3. Nelumbonidae*
4. Ranunculidae
5. Caryophyllidae
6. Hamamelididae
7. Dilleniidae
8. Rosidae
9. Cornidae*
10. Asteridae
11. Lamiidae

Class 2. Liliopsida (Monocotyledons)-6 subclasses, 16 superorders, 57 orders and 131 families (4 subclasses, 16 superorders, 38 orders, 104 families in 1987 classification); estimated genera-3,000, species- 60,000.

Subclass 1. Liliidae

2. Commelinidae*
3. Arecidae
4. Alismatidae
5. Triurididae
6. Aridae*

Merits:

1. This system is largely accepted mainly due to its clearly defined evolution principles and has the following plus points to its credit:

2. The Dicots (Magnoliopsida) has been discussed prior to Monocots (Liliopsida).

3. The families are small homogeneous units made up of closely related genera.

4. The Dicots begin with Magnoliales; which is highly satisfactory as Magnoliales are universally considered to be the most primitive living Angiosperms.

5. Among Monocots, the Alismatales, which are considered to be the most primitive living Monocots, have been placed at the starting point, which is, satisfactory.

6. Engler and Prantl's division of Dicots into two traditional groups – Archichlamydae and Metachlamydae, has been abolished in this system.

7. Problems such as monophyly or polyphyly, interrelationships of Dicots and Monocots, primitive position of Magnoliales, the secondary nature of anemophilous families with reduced unisexual flowers, etc. have been satisfactorily settled.

8. Depiction of the putative relationships of major subclasses and superorders with the help of a bubble diagram is very useful. It gives some idea about the relative size of different groups.

9. By splitting Asteridae into two subclasses: Lamiidae and Asteridae, a more rational distribution of sympetalous families has been achieved. Separation of Asteridae and Lamiidae has also been followed by Thorne (2000, 2003) and APG II (2003).

Demerits:

1. The main objection to Takhtajan's system is his derivation of Monocotyledons from the stocks ancestral to the Nymphaeales.

2. The extremely narrowly defined taxa in this system has resulted in the unwarranted splitting of related groups.

3. The system, although very sound and highly phylogenetic, is not helpful for identification and for adoption in herbaria, as it provides classification only up to the family level. Also, keys to the identification of taxa are not provided.

4. Dahlgren (1980, 1983) and Thorne (1983,1992, 2003) consider that the angiosperms deserve a class rank equivalent to the main groups of gymnosperms such as Pinopsida, Cycadopsida etc.

5. Dahlgren (1983, 1989) placed Arales next to Liliiflorae (Lilianae). The recent studies have, shown the affinities of Araceae with Alismatales. As such, the family is included under Alismatales in APG II and under Alismatidae—>Aranae—>Arales by Thorne (2003).

6. Although the system is based on data derived from all sources, in final judgment more weightage is given to cladistic information compared to phenetic information.

Angiosperm Phylogeny Group (APG III) system:

The Angiosperm Phylogeny Group, or APG, refers to an informal international group of systematic botanists who came together to try to establish a consensus view of the taxonomy of flowering plants (angiosperms) that would reflect new knowledge about their relationships based upon phylogenetic studies.

In the late 1990s, an informal group of researchers from major institutions worldwide came together under the title of the 'Angiosperm Phylogeny Group' or APG. Their intention was to provide a widely accepted and more stable point of reference for angiosperm classification. Their first attempt at a new system was published in 1998 (the APG system).

As of 2010, two revisions have been published, in 2003 (APG II) and in 2009 (APG III), each superseding the previous system. Eight researchers have been listed as authors to the three papers, and a further 33 as contributors.

The APG III system of flowering plant classification is the third version of a modern, mostly molecular-based, system of plant taxonomy being developed by the Angiosperm Phylogeny Group (APG). Published in 2009, it was superseded in 2016 by a further revision, the APG IV system.

Along with the publication outlining the new system, there were two accompanying publications in the same issue of the Botanical Journal of the Linnean Society. The first, by Chase & Reveal, was a formal phylogenetic classification of all land plants (embryophytes), compatible with the APG III classification. As the APG have chosen to eschew ranks above order, this paper was

meant to fit the system into the existing Linnaean hierarchy for those that prefer such a classification.

The APG III system recognized all of the 45 orders of the previous system, as well as 14 new ones. The order Ceratophyllales was erroneously marked as a new order, as it had been recognized in both of the previous APG systems. The newly recognized orders were:

Amborellales, Nymphaeales, Chloranthales, Petrosaviales, Trochodendrales, Buxales, Vitales, Zygophyllales etc.

The designation of alternative "bracketed families" was abandoned in APG III, because its inclusion in the previous system had been unpopular. APG III recognized 413 families, 43 fewer than in the previous system. Forty-four of the 55 "bracketed families" were discontinued, and 20 other families were discontinued as well.

The discontinued bracketed families were:

Illiciaceae, Alliaceae, Agapanthaceae, Agavaceae, Aphyllanthaceae, Hesperocallidaceae, Hyacinthaceae, Laxmanniaceae, Ruscaceae, Themidaceae, Asphodelaceae,

Main groups in the system (all unranked clades between the ranks of class and order):

clade angiosperms

order Amborellales

order Nymphaeales

order Austrobaileyales

clade magnoliids

order Canellales

order Magnoliales

order Piperales etc.

clade monocots

order Acorales

order Alismatales

order Asparagales

order Liliales etc.

clade commelinids

family Dasypogonaceae—unplaced in an order

order Arecales

order Commelinales

order Poales

order Zingiberales etc.

clade eudicots

family Sabiaceae—unplaced in an order

order Buxales

order Ranunculales etc.

clade core eudicots

family Dilleniaceae—unplaced in an order

order Saxifragales etc.

clade rosids

order Vitales

clade fabids (eurosids I)

order Cucurbitales

order Fabales

order Fagales

order Rosales etc.

clade malvids (eurosids II)

order Brassicales

order Malvales

order Myrtales

order Caryophyllales etc.

clade asterids

order Cornales

order Ericales

clade lamiids (euasterids I)

family Boraginaceae—unplaced in an order

family Oncothecaceae—unplaced in an order

order Lamiales

order Solanales etc.

clade campanulids (euasterids II)

order Apiales

order Asterales

order Escalloniales etc.

Paleoherbs

The paleoherbs are a small group of flowering plants which have traditionally been classified as dicots, but which have many characters in common with monocots. Recent studies have shown that they may actually be closer relatives of the latter group, though there is still considerable debate over this. One alternative view is that the paleoherbs represent relicts descended from some of the earliest flowering plants, a view known as the Paleoherb Hypothesis. The name of the group reflects this viewpoint.The last decade of the twentieth century has seen the strong development of an alternative herbaceous origin hypothesis for angiosperms (Taylor and Hickey, 1996) originally developed as paleoherb hypothesis. The most primitive angiosperms are considered to be rhizomatous or scrambling perennial herbs with simple net-veined leaves, flowers in racemose or cymose inflorescences, with free carpels containing one or two ovules. A number of families are included in the group. Thorne (2000) had placed all of them under Magnoliales, along with Magnoliaceae and Winteraceae. In his later revision (2003), however, placed Amborellaceae and Chloranthaceae (together with Trimeniaceae and Austrobaileyaceae) under Chloranthales, the first order of Magnoliidae (and accordingly angiosperms), the families arranged in that order. Subsequently (2006, 2007) he separated them under distinct subclass Chloranthidae, at the begining of angiosperms. The family Ceratophyllaceae is placed after the monocot families, towards the beginning of Ranunculidae. The placement of Amborellaceae at the beginning of angiosperms is found in the classification schemes of Judd et al. (2003), APG II (2003) and APweb (Stevens, 2003). The position of the other two families is, however, not settled. Judd. et al. and APweb consider both Chloranthaceae (towards the end of basal families before Magnoliid complex) and Cer-atophyllaceae (towards the end of Magnoliid complex) as having uncertain position. APG II, like Thorne places Amborellaceae and Chloranthaceae at the beginning of angiosperms (but as unplaced families), whereas family Ceratophyllaceae is placed before Magnoliids.

Characters:

1. Thery are usually herbaceous plants that may have adaxial prophylls.
2. Leaves are alternate often more or less palmately veined, thin textured and with anomocytic stomata.
3. Flowers has numerous to few parts, those of the perianth and androecium usually are in whorls of three (3).
4. Filaments is well differentiated from the anther, and the connective usually is inconspicuous.
5. Pollen grains usually have a collumellar exine.

Eudicots:

The eudicots are a large, monophyletic assemblage of angiosperms, comprising roughly 190,000 described species, or 75% of all angiosperms. The monophyly of eudicots is well supported from molecular data and delimited by at least one palynological apomorphy: a tricolpate or tricolpatederived pollen grain. Tricolpate pollen grains evolved from a monosulcate type, which is considered to be ancestral in the angiosperms, as well as for many seed plant clades. Many eudicots have pollen grains with more than three apertures, of a great variety of numbers, shapes, and position.

The eudicots can be divided into two groups: the **basal eudicots** and the **core eudicots**. Basal eudicot is an informal name for a paraphyletic group. The core eudicots are a monophyletic group. A 2010 study suggested the core eudicots can be divided into two clades, Gunnerales and a clade called "Pentapetalae", comprising all the remaining core eudicots. The Pentapetalae can be then divided into three clades: Dilleniales superrosids consisting of Saxifragales and rosids (the APG IV system includes the Vitales in the rosids) superasterids consisting of Santalales, Berberidopsidales, Caryophyllales and asterids.

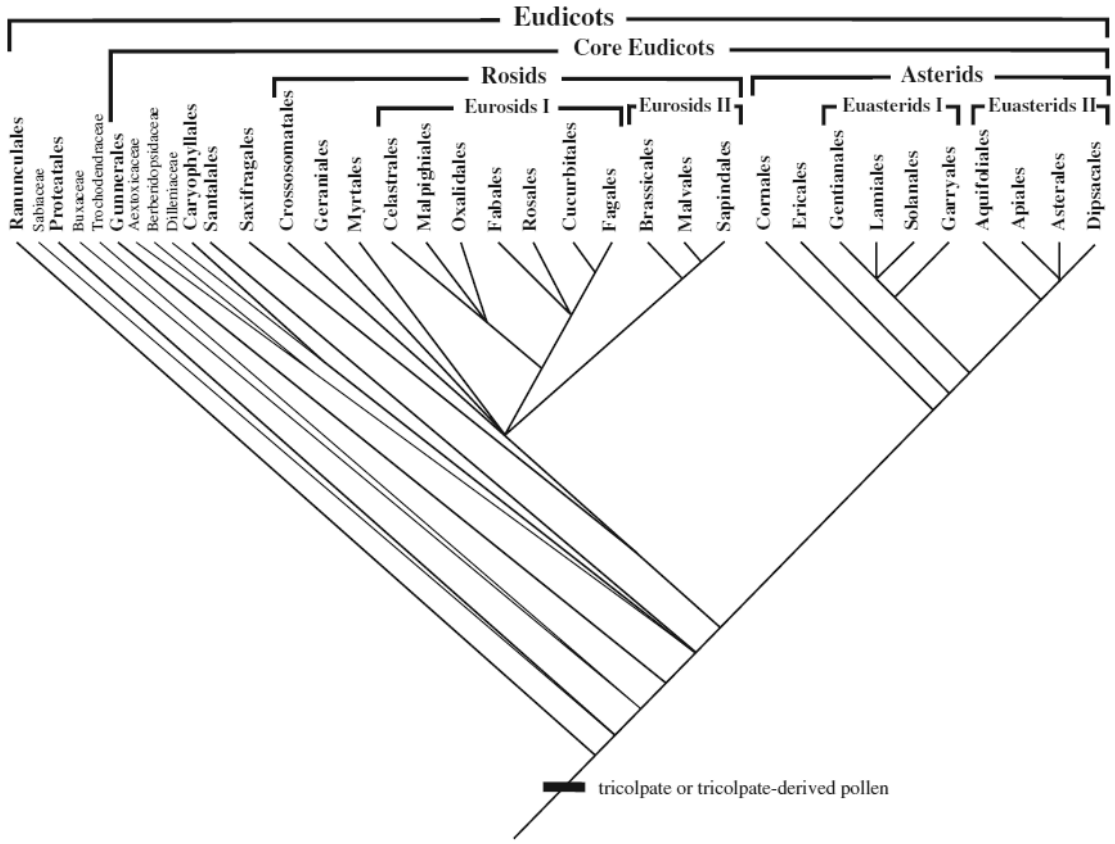

Fig- Eudicots cladogram, Source: Simpson, G. Plant Systematics, 2006.

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4. A general survey of the following taxa of angiosperms (sensu Cronquiust, 1988) with reference to their characteristics, inter-relationship, evolutionary trends, changed concepts and economic importance in the light of recent researches: Amborellaceae, Magnoliales, Caryophyllidae, Nepenthales, Podostemales, Asterales, Alismatales and Poaceae.

Amborellaceae

The Amborellaceae is purported in most molecular studies to be the most basal angiosperm group, although some studies suggest other possibilities (notably that *Amborella* + Nymphaeaceae together are sister to the rest of the angiosperms).

The absence of vessels in the order, which is rare in angiosperms, is possibly an ancestral condition, and the absence of aromatic (ethereal) oil cells is significant in light of other basal groups that have them.

Amborellaceae *Amborella* family. (L. for around a little mouth, perhaps in reference to the flower). 1 genus and species. The Amborellaceae comprises the single species *Amborella trichopoda*.

Diagnostic characteristics:

1. A dioecious, tropical shrub.
2. The leaves are alternate, spiral to distichous, undivided, exstipulate, evergreen, and simple.
3. The inflorescence is an axillary cyme. The flowers are unisexual, actinomorphic, and hypogynous to perigynous.
4. Perianth consists of 5-8, spiral, distinct to basally connate perianth parts (termed sepals by default).
5. The stamens of male flowers are ∞, and somewhat laminar. Anthers are longitudinal in dehiscence.

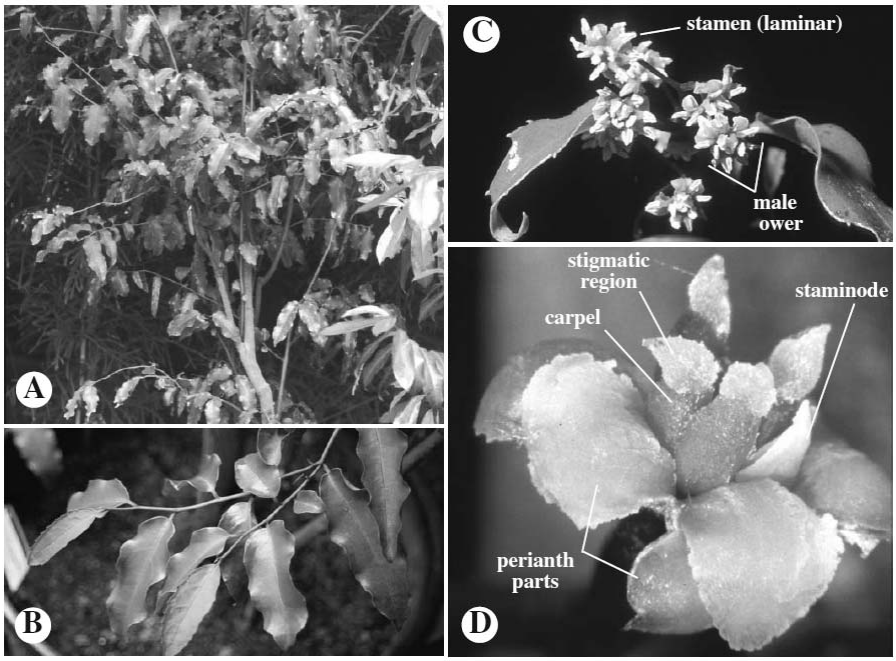


Fig- *Amborella trichopoda*, Source: Simpson, G. Plant Systematics, 2006.

6. The gynoecium of female flowers is apocarpous, comprising 5-6 superior ovaried pistils that are apically open. Placentation is marginal; the ovule is solitary in each pistil.

7. The fruit is a drupecetum. Vessels and ethereal oil cells are lacking.

Fig- *Amborella trichopoda*, Source: http://speciesplantarum.net/sites/default/files/floras/a/amborellaceae.pdf.

Amborella trichopoda, the single species of the Amborellaceae, is native only to New Caledonia. There are no economic uses, other than being a cultivar sought because of its distinctive, basal position in the angiosperms.

Interrelationship, evolutionary trends and recent concept of Amborellaceae:

The group formerly known as magnoliids (Magnoliidae) was long thought to represent the basal most extant angiosperms (e.g., Cronquist 1988). It comprises ca. 35 families (in the circumscription of Takhtajan [1997] and with the inclusion of his Nymphaeidae). However, the magnoliids are extremely diverse in structure, and until recently, it was uncertain which groups of the magnoliids were the basalmost.

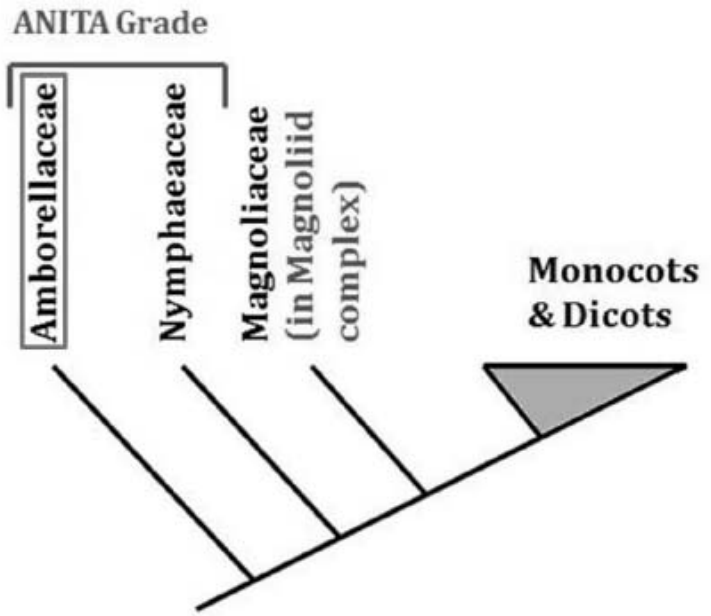
The latest breakthrough came at the Sixteenth International Botanical Congress in St. Louis, at the August 3, 1999, symposium, where four more or less independent groups of authors all reached the same conclusion, that the genus *Amborella* is the basal most clade among extant angiosperms, followed by Nymphaeales and a clade comprising Austrobaileyacae, Trimeniaceae, and Illiciales; this basal grade was termed the ANITA grade (Mathews and Donoghue 1999,

2000; Qiu et al. 2001; Soltis et al. 2000; Graham and Olmstead 2000). Additional support came from Parkinson et al. (1999), Renner (1999) (with Chloranthaceae basal most), Borsch et al. (2000), and Barkman et al. (2000) (with *Amborella* and *Nymphaeales* basal most). These studies also corroborate the view that the magnoliids are highly paraphyletic. This unanimous result is especially remarkable because different approaches were used by these groups of authors. Important in all these studies is the use of multiple genes and a larger sampling of taxa than before. The results seem to be better supported than those of all former studies. In some earlier studies, Nymphaeales (Hamby and Zimmer 1992; Doyle et al. 1994) and the ANITA grade (Soltis et al. 1997) also appeared at the base, but these topologies were less well supported and therefore were not discussed so widely at the time, or members of the ANITA grade came out together as a clade but not as earliest branching angiosperms (Chase et al. 1993; Qiu et al. 1993).

In a combination of the three gene analysis by Soltis et al. (1999, 2000*a*) and a morphological analysis that is more detailed than previous ones, the ANITA grade also comes to the base of the tree, if *Amborella* is chosen as sister to all other angiosperms (Doyle and Endress 2000). In contrast to the gene trees alone, Chloranthaceae immediately follows the ANITA grade. In a tree based on morphology alone, Chloranthaceae goes even more toward the base between *Amborella* and Trimeniaceae.

It is striking that six of the seven families of the ANITA grade have only one or two genera, and two families even have only a single species. Most of these families are geographically very scattered in the Tropics. The two monotypic families (Amborellaceae and Austrobaileyaceae) have very small relic areas and are therefore especially threatened by extinction. They are woody or herbaceous, never large trees; a number of them are scrambling or viny plants (Feild et al. 2000). The latter is true for *Amborella*, *Austrobaileya*, part of Trimeniaceae, and Schisandraceae. Ceratophyllaceae also have a single genus.

These phylogenetic hypotheses on basal angiosperms are supported by ever more fossil finds of these groups from the Lower Cretaceous (leaves reminiscent of taxa of the ANITA grade [Upchurch 1984], Nymphaeaceae-like plants and flowers [Mohr and Friis 2000; Friis et al. 2001], seeds reminiscent of *Illicium* or Nymphaeales [Friis et al. 2000], *Amborella*-like pollen [Hughes and McDougall 1987; Doyle and Endress 2000], and Chloranthaceae-like pollen and flowers [Walker and Walker 1984; Friis et al. 1986, 1999, 2000; Eklund 1999]).



Fig, ANITA Grade and Magnoliid Phylogeny Source: https://www.eeob.iastate.edu/classes/bio366/families/Amborellaceae.pdf

Magnoliales:

Members of Magnoliales include woody shrubs, climbers, and trees. Along with the orders Laurales, Piperales, and Canellales, Magnoliales forms the magnoliid clade, which is an early evolutionary branch in the angiosperm tree; the clade corresponds to part of the subclass Magnoliidae under the old Cronquist botanical classification system. The Magnoliales, sensu APG II (2003), contain six families.

- Annonaceae
- Degeneriaceae
- Eupomatiaceae
- Himantandraceae
- Magnoliaceae
- Myristicaceae

Diagonistic characteristics:

1. Magnoliales are woody plants with showy flowers.
2. 2-ranked simple (seldom lobed) leaves and ethereal oil cells in the parenchymatous tissues of the plant body, paracytic stomates.
3. Perianth generally trimerous.
4. Flowers: on elongated receptacle.
5. laminar stamens
6. The ovary is usually placed above the base of the stamens

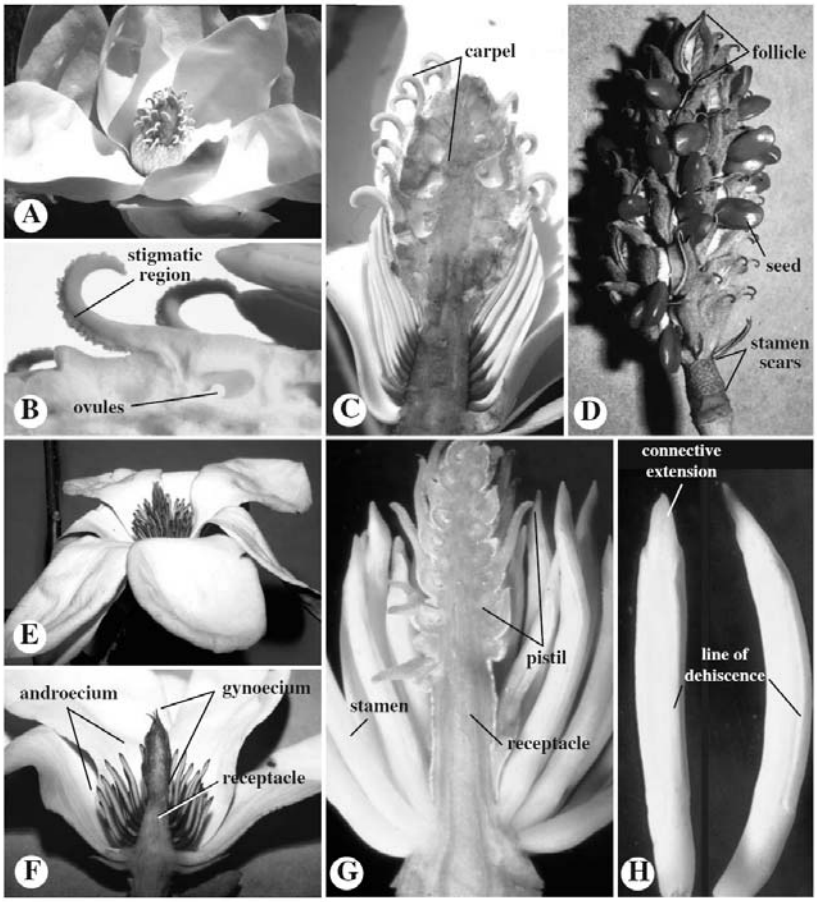


Fig- ***Different floral part of magnoliales,***
Source: Simpson, G. Plant Systematics, 2006.

in the flower (hypogynous), and the perianth is well developed. carpels separate (apocarpous) with superior ovary..

7. - Fruit: aggregate of follicles (in Magnolia) or winged samaras (in Liriodendron).
8. seeds with fleshy seed coat/aril in many; minute embryo, copious endosperm.
9. The pollen is typically uniaperturate (sometimes biaperturate or inaperturate), - Boat-shaped, monosulcate.

General features:

Habit: Usually bisexual (sometimes monoecious or dioecious), evergreen or deciduous trees, shrubs or lianas (rarely dwarf-shrubs or suffrutices). Often aromatic.

Leaves: Alternate (spiral or distichous), simple, usually entire (rarely lobed), with conduplicate or convolute ptyxis. Stipules usually absent (sometimes early caducous, ocreate, open just opposite petiole, enclosing young leaf); leaf sheath absent. Petiole vascular bundle transection arcuate (with an adaxial plate consisting of vascular tissue) or annular (sometimes bicollateral). Palisade parenchyma present. Venation usually pinnate, eucraspedodromous or brochidodromous (rarely palmate). Stomata usually paracytic (rarely anomocytic or actinocytic).

Inflorescence: Terminal, axillary or supra-axillary, cymose, paniculate or fasciculate, or flowers solitary axillary, terminal or pseudo-axillary (terminal on small axillary short shoots). Floral prophylls (bracteoles) usually single (rarely paired).

Flowers: Actinomorphic. Usually hypogyny (rarely half epigyny, with urceolate, campanulate or infundibuliform receptacle and without tepals). **Outer tepals** (two or) three (or four), with valvate or imbricate aestivation, spiral or whorled, sepaloid or petaloid, free or connate at base; inner tepals three to numerous, with valvate or imbricate aestivation, petaloid, spiral or whorled, usually free (rarely connate at base). Nectary usually absent. Disc absent.

Androecium: Stamens 20 to more than 200, laminar (foliaceous), spiral, not differentiated into filament and anther, with separate microsporangia embedded in distal part, or differentiated into filament and anther. Filaments when present usually free from each other (rarely connate at base), free from tepals; filaments often with three vascular bundles. Anthers when present basifixed, non-versatile, tetrasporangiate, sometimes with transversely septate thecae, usually extrorse (rarely latrorse or introrse), longicidal (dehiscing by longitudinal slits) or valvicidal (dehiscing by valves).

Gynoecium: Carpels usually ten to numerous (rarely one to three), spiral or whorled, usually free (sometimes connate or paracarpous); carpel plicate to conduplicate (sometimes basally ascidiate and not differentiated into ovary and style), usually postgenitally partially or entirely occluded by fusion and secretion, with secretory canal often open and filled by secretions (sometimes without canal). Ovary usually superior (rarely semi-inferior), unilocular to 15-locular or more. Stylodium single, simple. Stigma capitate, often decurrent, papillate, Dry or Wet type. Nectar sometimes secreted from exposed carpel surfaces. Pistillodium absent.

Placentation laminar, marginal, parietal, basal, subbasal or lateral. Ovules one to numerous per carpel, usually anatropous (rarely orthotropous or hemiorthotropous), ascending or pendulous, apotropous, usually bitegmic (rarely tritegmic), crassinucellar.

Fruit: A usually fleshy (sometimes leathery or more or less woody), dehiscent or indehiscent, apocarpous follicular fruit or a cluster of follicles (multifolliculus), or a dry or fleshy pseudosyncarp, as ripe usually dehiscing and with sarcotestal seeds pendant in their funiculi, a loculicidal capsule or an assemblage of samaras (rarely berries or dry follicles).

Economic importance:

1. *Liriodendron tulipifera*, the American **tulip tree**, is widely cultivated to yield durable timber, Tulip tree wood is often used as weather board siding for houses, and large logs are suited for the manufacture of rotary-cut veneers for cabinetwork and millwork. The wood also has been used to a lesser extent in making paper.

2. Magnolia, including *Magnolia grandiflora* and *M. champaca* (formerly *Michelia champaca*), has been used for timber. The wood of *Magnolia grandiflora* was once used in the manufacture of venetian blinds because of its uniform texture, hardness, and ability to resist warping.

3. It is in horticulture, *Magnolia* is a well-known genus of cultivated trees and shrubs, and *M. grandiflora* is one of the most popular garden varieties as ornamental trees.

4. *Oxandra lanceolata* (lancewood) is undoubtedly the most important commercial timber source for use in scientific instruments, turnery (objects shaped by lathe), tool handles, and such sporting goods as archery bows and fishing rods. *Guatteria boyacana* (solera, or Colombian lancewood) has most of the same properties and uses.

5. Many species of *Annona* are cultivated for their edible fruits: *A. squamosa* (sweet sop), A. muricata (soursop), *A. reticulata* (custard apple), and *A. cherimola* (cherimoya).

6. Flowers of *Cananga odorata* (ylang-ylang) and *Mkilua fragrans* are used in perfumes.

7. The spicy fruits of West African *Xylopia aethiopica* are the so-called ‘Negro pepper’ used as a condiment, and those of Monodora myristica used as substitutefor nutmeg.

8. Tea made from the roots of *A. squamosa* is highly purgative, while that made from the leaves is a mild laxative and is also considered to have a general tonic effect on the digestive tract. Poultices of the leaves are used in dressing infected wounds.
9. The seeds of *M. fragrans* are the source of nutmeg and mace.
10. Both species of *Degeneria* have been milled for timber, which has been used in building construction and for furniture and veneer. They are too scattered, however, to be deliberately sought for timber.
11. Wood from *Galbulimima* (family Himantandraceae) has been used in Australia for cabinetmaking.
12. The leaves and bark contain piperidine derivatives, which have narcotic and hallucinogenic effects. In Papua New Guinea, *Galbulimima* is used in combination with the leaves of *Homalomena* (family Araceae), which causes violent intoxication followed by sleep with visions and dreams. The wood of *Eupomatia laurina* is used for furniture making in regions where it grows.

Interrelationship, evolutionary trends and recent concept of Magnoliales:

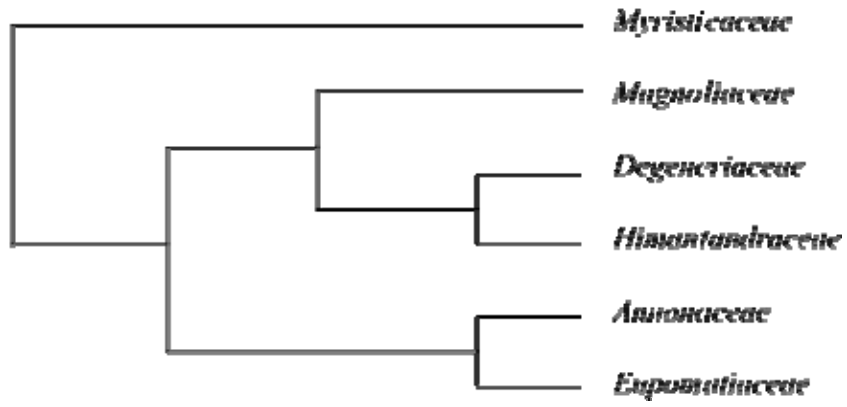


Fig. Phylogeny of Magnoliales based on morphological and DNA sequence data (Qiu & al. 1999; Sauquet & al. 2003; http://angio.bergianska.se/Magnoliales/Magnoliales.html).

The Magnoliales are woody angiosperms that inhabit tropical to warm-temperate climates. At one time this group was thought to represent the most ancient of the flowering plants based on the characters of simple leaves with pinnate venation, laminar stamens producing granular, monosulcate pollen, conduplicate carpels, and absence of vessels in some genera. More recently,

however, some of these traits are now considered as synapomorphies that were-independently derived in other magnoliid angiosperms (Sauquet et al., 2003). Molecular phylogenetic analyses regard the Magnoliales as a monophyletic group of six families (Zanis et al., 2002). All lines of evidence suggest the Magnoliales as being more derived, but still in a basal position relative to the eudicots and monocots (Friis et al., 1997b).

Members of Magnoliales include woody shrubs, climbers, and trees. Along with the orders Laurales, Piperales, and Canellales, Magnoliales forms the magnoliid clade, which is an early evolutionary branch in the angiosperm tree; the clade corresponds to part of the subclass Magnoliidae under the old Cronquist botanical classification system.

Magnoliales were long thought to be 'the most archaic existing order of flowering plants', as stated by Cronquist (1981, 1988), and because of their presumed primitiveness were listed first in most major pre-cladistic systems of angiosperm classification (including Cronquist, 1968, 1981, 1988; Thorne, 1974, 1992; Takhtajan, 1980, 1997). This interpretation was based on a special concentration of characters assumed to be ancestral in angiosperms, including-Simple, entire leaves with pinnate venation and monosulcate pollen with granular exine structure.

Additional presumed plesiomorphic traits included laminar stamens, conduplicate carpels, the spiral arrangement of fertile parts in Magnoliaceae, and the absence of vessels in the wood of Winteraceae.

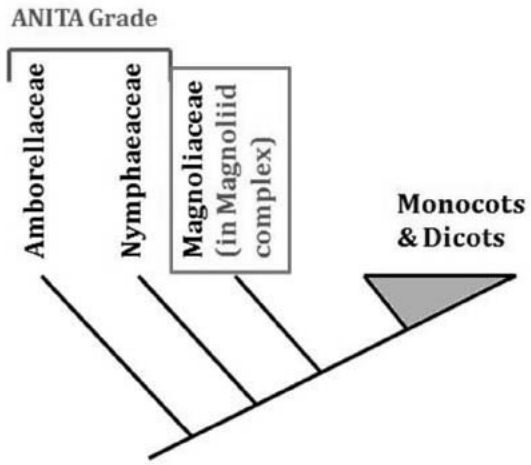
Analyses of rbcL, atpB and 18S rDNA have identified Winteraceae and Canellaceae as the sister-group of Magnoliales. The second outgroup of Magnoliales was Laurales (sensu APG, 1998; Renner, 1999) (Doyle & Endress, 2000), Laurales plus Piperales (sensu APG, 1998) or Chloranthaceae plus the ANITA taxa. The clade consisting of Winteraceae and Canellaceae has been supported by all molecular analyses, but it was not formally named by APG (1998

A larger number of molecular analyses have identified Laurales as the sister group of Magnoliales and a clade consisting of Winterales and Piperales as their next outgroup (Mathews & Donoghue, 1999, 2000; Qiu et al 1999, 2000; Barkmanet al 2000):

Phylogenetic analyses of angiosperms based on morphology gave inconsistent relationships within Magnoliales. In the morphological analysis of Doyle & Endress (2000), Myristicaceae were sister to Magnoliaceae, Annonaceae, Degeneria, Galbulimima and Eupomatia.

In contrast, all recent higher-level phylogenetic analyses based on combined molecular (Qiu et al., 1999, 2000, Zanis et al., 2002) or morphological and molecular (Doyle & Endress, 2000)

data sets have given very similar results. In all of them, Myristicaceae were basal in the order, sister to the five other families.



Fig, ANITA Grade and Magnoliid Phylogeny Source: https://www.eeob.iastate.edu/classes/bio366/families/Amborellaceae.pdf

Caryophyllidae:

Caryophyllidae is a botanical name at the rank of subclass. At the moment there is no complete consensus about what orders it includes, except that it presumably contains the order Caryophyllales. Note that this is only a naming difficulty: what to call various taxa of plants; there is little debate about how the plants in question are related.

A well-known system that used this name is the Cronquist system, and in the original, 1981, version of this system the circumscription was:

subclass Caryophyllidae

order Caryophyllales

order Polygonales

order Plumbaginales

These plants form the order Caryophyllales in the APG II system, 2003.

Diagonastic characteristics:

1. Annual or perennial herbs, rarely shrubs, lianas, or trees.
2. The **stems** often have swollen nodes.
3. The **leaves** are opposite (rarely spiral), simple, usually exstipulate.
4. The **inflorescence** is of dichasial cymes or solitary flowers.
5. The **flowers** are bisexual or unisexual, actinomorphic, hypogynous, rarely perigynous.

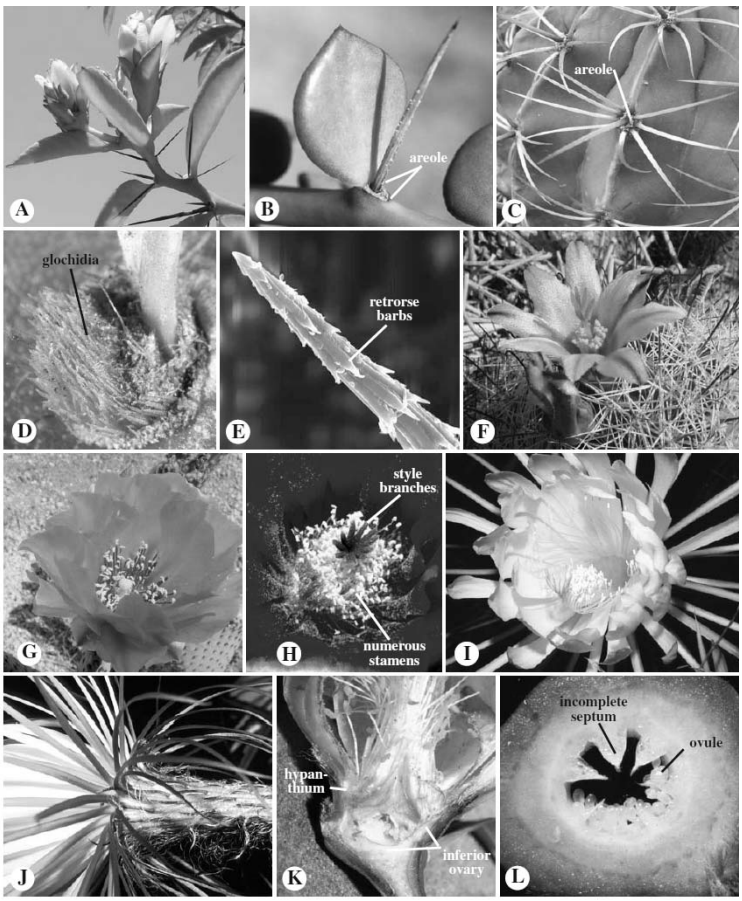


Fig- Different floral part of Caryophyllidae, Source: Simpson, G. Plant Systematics, 2006.

6. The **perianth** is biseriate, dichlamydeous, hypanthium absent [rarely present]. The **calyx** is synsepalous [rarely aposepalous] with 5 [4] sepals.
7. The **corolla** is apopetalous and often unguiculate (clawed), with 5 [4] petals.
8. The **stamens** are 5 10 [1 4], uniseriate or biseriate, apostemonous, epipetalous, or episepalous, basally epipetalous and forming a tube in some species.
9. **Anthers** are longitudinal in dehiscence.
10. The **gynoecium** is syncarpous, with a superior ovary (often with a stipe/gynophore), 2 5+ carpels, and 1 locule, often with basal septa.
11. The **style(s)** are terminal, single below, often branched above.
12. **Placentation** is free-central at least above, often axile below;

13. **ovules** are campylotropous to hemitropous, bitegmic, usually numerous per ovary. **Nectaries** occur as a nectariferous disk in some.
14. The **fruit** is a capsule

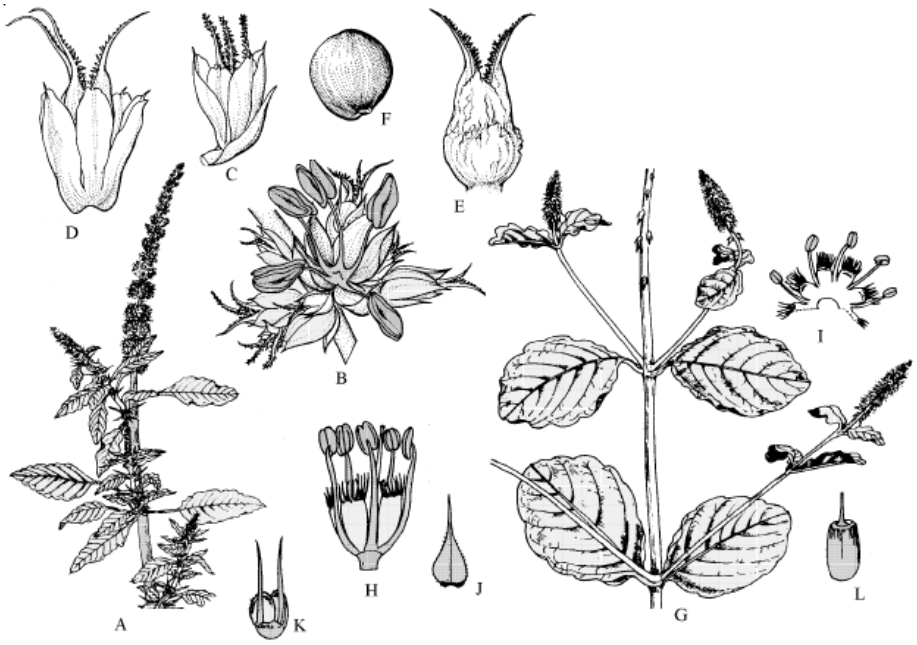


Fig. Different floral part of *Amaranthus spinosus*. Source: Singh, G. Plant Systematics: An Integrated Approach (3rd ed.), 2016.

Economic importance:

1. The family is represented by several ornamentals such as carnation, pinks, sweet william (different species of *Dianthus*), baby's breath (*Gypsophila*) and corn cockle (*Agrostemma*). Species of *Arenaria*, *Cerastium* and *Stellaria* are troublesome weeds.
2. *Portulaca oleracea*) commonly growing wild is frequently cultivated as pot herb. Many species of *Montia* (Miner's lettuce) were used earlier as green salad in America. Root stalks of *Lewisia rediviva* are also eaten in America. Rose moss (*Portulaca grandiflora*), Flame flower (*Talinum spp.*) and Rock purslane (*Calandrinia spp.*) are grown as ornamentals.
3. The family is known for large number of ornamentals (cacti) such as *Opuntia* (prickly pear), *Mammillaria* (pincushion cactus), *Cereus* (hedge cactus), *Echinopsis* (sea-urchin

cactus), *Epiphyllum* (orchid cactus), *Schlumbergera* (Christmas cactus) and *Rhipsalis* (mistletoe cactus).

4. Fruits of several species of *Opuntia* are eaten raw or made into jams or syrups. Spines of cacti are often used as gramophone needles. *Lophophora* contains mescaline alkaloids and is hallucinogenic. Cochineal dye is derived.
5. Species *Bougainvillea* are commonly grown as hedges and for covering walls and fences. Species of *Mirabilis* (Four O'Clock) are grown as garden ornamentals. *Boerhavia repens* is used as medicinal plant as a diuretic. *Fisonia aculeata* is used as hedge plant.
6. *Dorotheanthus* and *Carpobrotus* are grown as garden ornamentals. Some like *Lithops* (stone plant) and *Titanopsis* are grown asmcuriosities. *Tetragonia* is used as vegetable. Some species help stabilize sand dunes and road banks. *Beta vulgaris*: used as leafy vegetable {often confused with spinach}; root vegetable mainly for salad and a source of sugar), spinach (*Spinacea oleracea*) and lambs quarters (*Chenopodium album;* bathoo in Hindi). *Chenopodium ambrosioides* is source of wormseed used as a vermifuge. Seeds and leaves of *C. quinoa* are eaten by Peruvians and Andes.

Interrelationship, evolutionary trends and recent concept of Caryophyllidae:

The concept of Caryophyllales generally corresponded to that of Centrospermae (Harms 1934), a group long recognized as an assemblage of closely related families (e.g., Braun 1864; Eichler 1875–1878). Morphological and embryological characters were used to unite the core families of Centrospermae, but Centrospermae gained particular attention as one of the earliest groups for which circumscription was modified on the basis of chemical characters. The discovery that all but two families of Centrospermae—Caryophyllaceae and Molluginaceae—produced betalain pigments instead of the anthocyanin pigments produced in other angiosperms supported a close relationship among the core group of families. Furthermore, Cactaceae and Didiereaceae, not previously considered closely related to Centrospermae, were also discovered to produce betalains. Revised classifications (e.g., Dahlgren 1975, 1980; Takhtajan 1980; Cronquist 1981; Thorne 1983, 1992a, 1992b), applying the name Caryophyllales and incorporating chemical, morphological, embryological, and anatomical characters, included 12 families. These circumscriptions have remained largely intact for the past 30 years. Cronquist's (1981) Caryophyllidae, comprising Caryophyllales, Polygonaceae, and Plumbaginaceae (the last two

placed in his monofamilial Polygonales and Plumbaginales, respectively), also recognized similarities among these three groups. However, emphasis on certain sets of characters over others has resulted in the inclusion or exclusion of additional families and genera and has altered views on the closest relatives of Caryophyllidae.

A series of molecular phylogenetic analyses has reshaped concepts of Caryophyllales by identifying the closest relatives of the traditional order and resolving patterns of relationship within the clade. Most notable is the discovery that certain carnivorous plants—the sundews and Venus flytrap (Droseraceae) and Old World pitcher plants (Nepenthaceae)—are closely related to Cronquist's Caryophyllidae (Albert et al. 1992; Chase et al. 1993; Williams et al. 1994; Meimberg et al. 2000; Cuénoud et al.2002). In addition, many families previously considered distantly related to Caryophyllales have been included in a large clade with Caryophyllales (e.g., Asteropeiaceae and Physenaceae, Morton et al. 1997; Rhabdodendraceae, Fay et al. 1997a; Simmondsiaceae, e.g., D. Soltis et al. 2000). The strong support for this clade in recent multigene analyses (e.g., D. Soltis et al. 2000; Cuénoud et al. 2002) has led to a revised—and broader—circumscription of Caryophyllales by APG (1998) and APG II (2003). Caryophyllales ***sensu*** APG II (2003) comprise 29 families; two other families have been proposed ("Agdestidaceae" and "Petiveriaceae," discussed below, in Phylogeny of Caryophyllales;

Cronquist (1981) and Takhtajan (1980, 1997), on the basis of floral characters, viewed Caryophyllidae as being derived from Ranunculales- type ancestors. However, phylogenetic analyses using many gene sequences place Caryophyllales firmly within the core eudicots. They have alternatively been considered to be close relatives of rosids, asterids, or Santalales and are best regarded at this time simply as one of the major clades of core eudicots (D. Soltis et al. 2000). The sister group of Caryophyllales may be Dilleniaceae (e.g., D. Soltis et al. 2000).

Although a close relationship between Dilleniaceae and Caryophyllales (sensu APG) was also detected by Chase and Albert (1998; using the "search 2" dataset of Chase et al. 1993), support was less than 50%, and analyses of rbcL and atpB did not find this relationship. A relationship between Dilleniaceae and members of Caryophyllales had not previously been suggested. Cronquist (1981) considered Dilleniaceae, together with Paeoniaceae, as occupying a basal position within subclass Dilleniidae, whereas Takhtajan (1997) viewed Dilleniaceae alone as the "most archaic family in Dilleniidae.

Nepenthales:

Nepenthales is an order of carnivorous flowering plants in the Cronquist system of plant classification.

The order was placed in the subclass Dilleniidae, which in the 1981 version of this system included:

order Nepenthales

family Droseraceae

family Nepenthaceae

family Sarraceniaceae

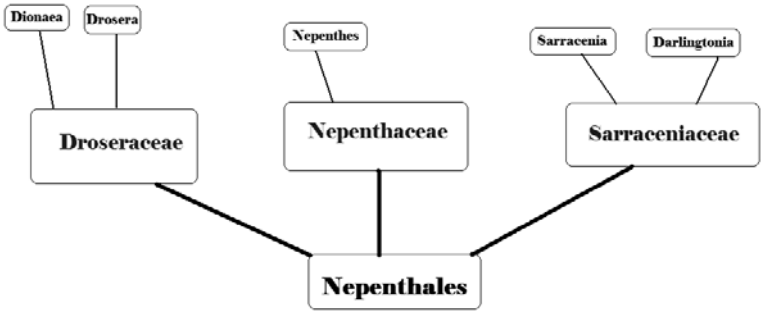


Fig- The phylogenetic tree of order Nepenthales, Source: http://bioweb.uwlax.edu/bio203/s2008/malloy_gera/classificat.htm

All three families are carnivorous plant families. The Droseraceae contains three extant genera: Drosera (sundews), which catch insects with adhesive droplets; and Dionaea (Venus flytrap) and Aldrovanda (waterwheel plant), which capture them in leaves with interlocking teeth. The other two families include pitcher plants, which drown their prey.

Characteristic features:

1. Plants are insectivorous perennial herbs (Droseraseae and Sarraceniaceae), half shrub to shurbs, sometimes clibing or epiphytes (Nepenthaceae).
2. Plants commonly produce proanth cyanins, tanniferos subtances and ellagic acid.
3. Roots are mostly fibrous type.
4. Leaves are stipulate or exstipulate, simple alternate (Sarraceniaceae and Nepenthaceae) or seldom whorled (Droseraseae), modified in one way or another to catch insects, often forming pitchers (Sarraceniaceae and Nepenthaceae).
5. Stomates are usually anomocyatic type.
6. Flowers are actinomorphic, hypgonous,usually bisexual, rarely unisexual (Nepenthaceae).
7. Sepals are 4 to 5, free or connate below, often persistent, imbricate.

8. Petals are 4-5, free, usually as many as sepals or absent.

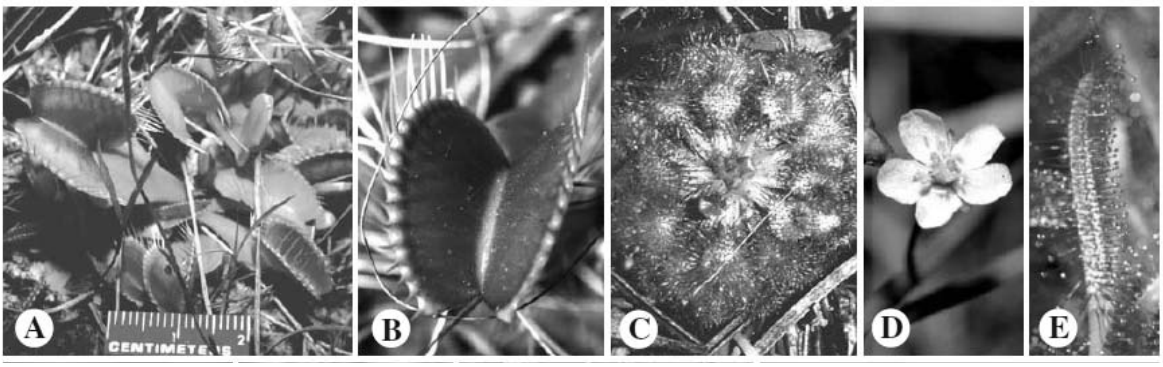


Fig- Different floral part of Droseraceae, Source: Simpson, G. Plant Systematics, 2006.

9. Stamens are 4-numerous, free or united by their filaments (Nepenthaceae).
10. Pollen grains may be monads (Sarraceniaceae and Nepenthaceae) or tetrads (Droseraseae), binucleate or trinucleate.
11. Carpels are 3-5, united in superior ovary, either may be unilocular with parietal or basal placentation (Droseraseae) or may be plurilocular with axile placentation (Sarraceniaceae and Nepenthaceae).
12. Styles are variously distinct or bifid orunited or absent.
13. Ovules vary from 3- numerous, anatrophous, bitegmic or unitegmic (in some Sarraceniaceae), crassinucellar or tenuinucellar.
14. Endosperm development is nuclear or cellular.
15. Fruit is usually loculicidal capsule or rarely indehiscent,
16. Seeds are small, numerous, endospermous with tiny to straight and elongated embryo,

Economic importance:

1. Venus flytrap (*Dionaea muscipula*) and various species of *Drosera* (Sundew) are grown as novelties. Leaves of *Drosera* yield a violet dye, but is no longer of commercial importance.
2. Many species and hybrids of Nepenthes are cultivated domestically in glass houses as novelties.

3. The stems of *Nepenthus distillatoria* (Sri Lanka) and *N. reinwardtiana* (Malaysia) are used as a kind of cordage as well as for making baskets.

Interrelationship, evolutionary trends and recent concept of Nepenthales:

The systematic position or consequent relationships of the three families of Nepenthales are rather controversial and have been differently treated by different taxonomists. This is shown below:

Bentham and Hooker (1886) has placed the 3 familes in different cohorts far apprt from the each others, such as Sarraceniaceae was put underparietales before Papaveraceae. Drosaraceae was included in Rosales under Calyciflorae, Neprnthaceae in multiovulate Terrestres under in complete, consequently affinity of Nepenthaceae was establish with Aristolochiaceae.

Cronquist (1981) considered that the origin of Nepenthales from Theales. Except for their insectivorous habit, the Nepenthales would fit very well into the Theales assuming that the stamens of Sarraceniaceae turn out to be centrifugal at a point not yet fully established). Since as two families have axile placentation, the Viololes do not seem very likely ancestors. The similarities of Ancistocladous and Dioncophyllum, in the viololes, to members of Nepenthaceae are regarded as a common ancestory rather than a direct relationship. According to him none of the 3 families of Nepenthales can be considered ancestral to any of the others.

The insectivorous habit of the Nepenthales may be preseimed to be an evolutionary response to their growth in habitats deficient in available nitrogen. The Sarraceniaceae and Droseraceae commonly grow in water logged soils containing little or no soluble nitrate. The Nepenthaceae occur in nutrient poor soils.

Thorne (1992) has placed the 3 families into 2 differnet orders such as, Nepenthaceae and Sarraceniaceae were put under Theales; Droseraceae under Saxifragales.

The 2009 APG III system assigned the first two families to the order Caryophyllales and the last family to the order Ericales.

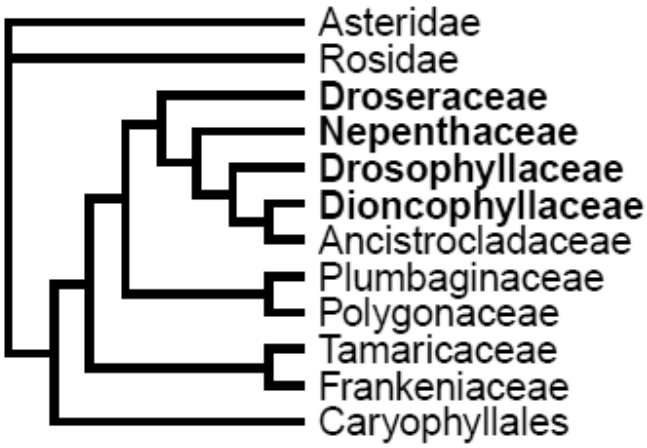


Fig. Phylogenetic tree of Nepenthales, Source:

https://www.researchgate.net/publication/315575998_Carnivorous_Plant_Systematics

Podostemales:

Podostemaceae (riverweed family) is a family in the order Malpighiales. It comprises about 46 genera and ca 300 species of more or less thalloid aquatic herbs.

Riverweeds adhere to hard surfaces (generally rock) in rapids and waterfalls of rivers. They are found mostly in tropical and subtropical areas worldwide. Many species are found in a very small geographic area, often even just a single river or waterfall. Because of their small range, many species are seriously threatened, especially from habitat loss (for example, due to dams flooding their habitat). Riverweeds are submerged when water levels are high, but during the dry season they live a terrestrial existence, flowering at this time. Their root anatomy is specialized for the purpose of clinging to rocks, and in fact details of the root structure are one of the ways of classifying riverweeds.

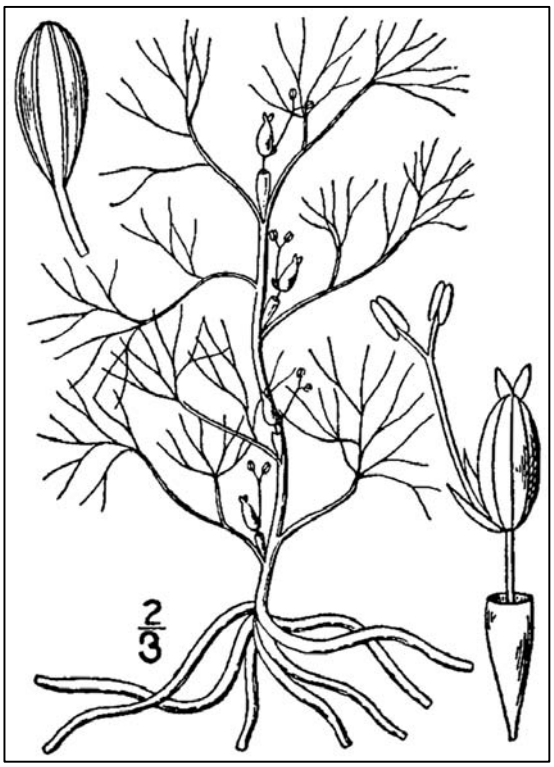


Fig. *Podostemum ceratophyllum* Michx., Source:

https://en.wikipedia.org/wiki/Podostemum

Common genera:

- *Angolaea*
- *Apinagia*
- *Butumia*
- *Castelnavia*
- *Ceratolacis*
- *Cipoia*
- *Cladopus*
- *Dalzellia*
- *Devillea*
- *Podostemum*

Diagnostic characters:

1. Aquatic herbs of fast rivers, annual or perennial. Plant thalloid resembling lichens, bryophytes, seaweeds.
2. They are attached by adhesive hairs or by specialized root like branches called **haptera** (secrete gummy substances) to rock or other hard objects in flowing freshwater
3. Roots usually photosynthetic, creeping or partly floating, thread-like, ribbon-shaped, crustose (foliose), sometimes short-lived or absent.
4. Shoots nearly always arising as endogenous buds from roots; stems reduced or elongate, simple or branched, sometimes dimorphic, occasionally only present when flowering.
5. Photosynthesis takes place under water, flowers or even separate floral shoots develop as the water level drops, the vegetative shoots or leaves often shed as plants become exposed.

6. Leaves are alternate, entire to more or less dissected, without axillary bud, borne on elongate stems or arising from prostrate, often disk-like stems, extremely variable in size and shape, from scale-like to well developed and compound; sheaths single or, in many Podostemoideae, double; sheath lobes sometimes elongated into stipule-like appendages; leaf blades stalked or sessile, entire, lobed or dissected; blade lobes or segments often bearing photosynthetic filaments and/or additional hairs; ultimate leaf segments filiform, linear or spathulate.

7. Flowers bisexual, actinomorphic or zygomorphic, solitary, in clusters or in racemeor cyme-like inflorescences; flower buds naked in Weddellinoideae and some Tristichoideae, surrounded by a **cupula** (a collar-like vascularised cup) in some Tristichoideae, or completely enclosed in a **spathella** (a tubular or sack-like cover) in Podostemoideae; spathellasmostly enclosing a single sessile or pedicellate flower; pedicels often elongatingin fruit.

8. Anthesis takes place in air or flowers cleistogamous under water. Perianth of 1 complete or incomplete whorl of tepals, often confined to one side of the flower; tepals in Tristichoideae and Weddellinoideae large, 5 or rarely 4 or 6, imbricate and sepal-like; tepals in Podostemoideae small, 2–20, linear or subulate, usually alternating with stamens.

9. In flowers with only 2 basally fused stamens occasionally an additional tepal borne at top of andropodium(common stalk); stamens 1–40, in 1 or 2 completewhorls, or in 1 incomplete whorl, or confined to one side of flower and consisting of 1–3 free stamens or a Y-shaped structure consisting of an andropodium carrying 2 stamens; filaments mostly free or, in Tulasneantha,introrsely to latrorsely or rarely extrorsely.

10. Pollen shed in monads, dyads or (rarely) tetrads, tricolporate

11. ovary superior, 2- or 3-locular or 1-locular in some Podostemoideae; ovules, axile, anatropous, bitegmic, tenuinucellate. Fruit a capsule, smooth or ribbed, with 2 or 3, equal or unequal valves, sometimes one or more persisting; stigmas 1–3, variable in shape and size. Seeds 2 to very numerous (over 2,000).

12. seed coat usually mucilaginous and sticky; endosperm 0; **pseudoembryo sac**, embryo straight, with 2 cotyledons and a suspensor.

Economic importance:

Podostemum ceratophyllum is a water plant, an indicator of clean streams. So, it is an indicator of water pollution.

Interrelationship, evolutionary trends and recent concept of Podostemaceae:

The unique combination of characters presented by this family is unparalleled among the angiosperms, The position of podostemaceae is a topic of controversy and the topic is variously treated by different taxonomist.

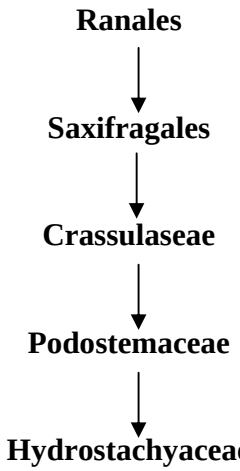
Engler, Rendle and others considered it to be close to **saxifragaceae**. This is due to following reason.

1. Presence of free styles
2. Usually bi-carpellary ovary
3. Numerous anatropous ovule

On the embryological basis, the family is considered that the podostemaceae are much reduced apetalous derivatives of the **crassulaceae** (Maheshwari 1945, Kapil 1970). The presence of well-developed suspensor-haustarium in both this families is one factor for their affinity. Cronquist (1981, 1988) also supports this view i.e. it is related with crassulaceae. However crassulaceae may seem to be an unusual starting point for a group of aquatics; one member of this families , *Tillaea aquatic* is semi-aquatic. This plant may not be the direct ancestor of the podostemaceae, but it shows that the crassulaceae has the potentiality to adapt to aquatic plant.

Hutchinson (1973) placed podostemaceae and **Hydrostachyaceae** in the order podostemales and pointed out that these are highly reduced and apetalous form of **saxifragaceae.** It is now generally considered that the Hydrostachyaceae as a member belong to the subclass Asteridae but not in Rosidae.

According to Mitra (1974), the origin of podostemaceae may be as follows:



Sometimes authors (Dahlgren 1992, Judd et al. 2002) divided family Podostemaceae (s.l.) into 2 distinct families Tristichaceae and Podostemaceae (s.s.).

Asterales:

Asterales is an order of dicotyledonous flowering plants that includes the large family Asteraceae (or Compositae) known for composite flowers made of florets, and ten families related to the Asteraceae. Asterales, daisy order of flowering plants, containing 11 families and some 26,870 species. Asterales is part of the core asterid clade (organisms with a single common ancestor) in the euasterid II group of the Angiosperm Phylogeny Group III (APG III) botanical classification system.

The order is a cosmopolite (plants found throughout most of the world including desert and frigid zones), and includes mostly herbaceous species, although a small number of trees (such as the giant Lobelia and the giant Senecio) and shrubs are also present. Asterales are organisms that seem to have evolved from one common ancestor. Asterales share characteristics on morphological and biochemical levels. Synapomorphies (a character that is shared by two or more groups through evolutionary development) include the presence in the plants of oligosaccharide inulin, a nutrient storage molecule used instead of starch; and unique stamen morphology. The stamens are usually found around the style, either aggregated densely or fused into a tube, probably an adaptation in association with the plunger (brush; or secondary) pollination that is common among the families of the order, where in pollen is collected and stored on the length of the pistil.

Diagnostic characteristics:

1. The flower petals of the order Asterales are joined together by their margins, forming a tubular corolla.
2. The pistil of the Asterales flower is composed of two carpels, united to form a compound ovary with a terminal style.
3. The fruit of the Asterales is tiny with only one seed. Asterales species are distributed by wind.
4. Herbs, shrubs, trees, or vines, with laticifers or resin ducts present in some taxa.
5. The **leaves** are simple or compound, spiral or opposite [rarely whorled], exstipulate.
6. The **inflorescence** consists of one or more heads (capitula) arranged in various secondary inflorescences, each head consisting of a flat to conical compound receptacle that bears one to many flowers (developing centripetally) and is subtended by one or more series of bracts, the phyllaries (collectively termed the involucre); heads of five general types: (1) discoid, with only disk flowers, all bisexual; (2) disciform, with only disk flowers, a mixture of pistillate and sterile with bisexual and staminate, in the same or different heads; (3) radiate, with central (bisexual or male) disk flowers and peripheral (female or sterile) ray flowers; (4) ligulate, with all ray flowers (typically with 5-toothed corolla apices); and (5) bilabiate, with all bilabiate flowers.

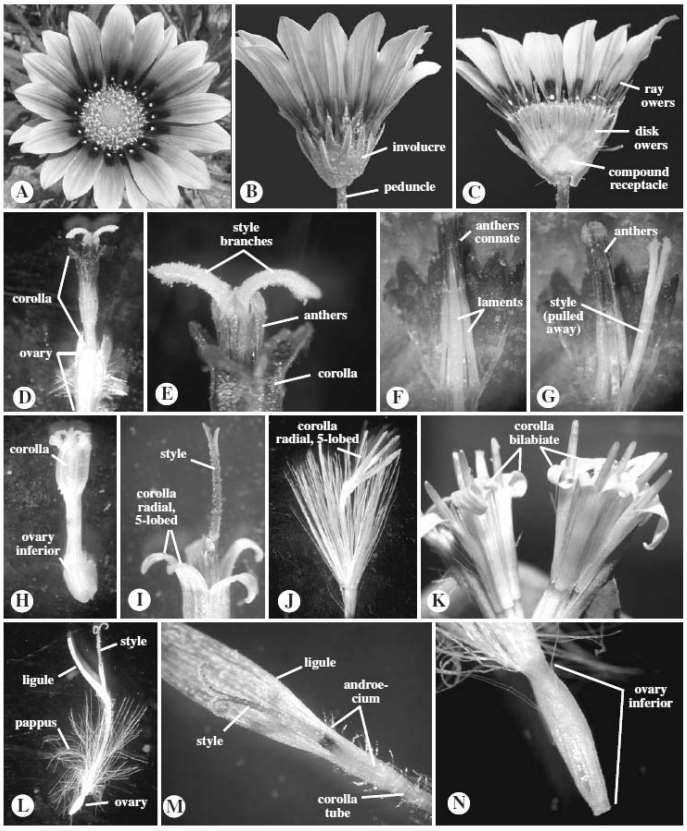


Fig- Different floral part of Asterales,
Source: Simpson, G. Plant Systematics, 2006.

7. The **flowers** are epigynous, bisexual or unisexual, subtended in some taxa by bracts, known as chaff, or bristles (as in the thistles).
8. The **perianth** is biseriate or uniseriate with hypanthium absent. The **calyx**, known as the **pappus**, is modified as 2 ∞ (sometimes connate) awns, scales, or capillary bristles (typically barbed or plumose), pappus absent in some.
9. The **corolla** is sympetalous with 5 [rarely 4] lobes (reduced to 3 marginal teeth in some), of three structural types (also called flower types): (1) **bilabiate**, corolla zygomorphic with a short tube having upper and lower lips; (2) **disk**, corolla actinomorphic with short to elongate tube bearing 5 [4] teethlike or elongate lobes; or (3) **ray** or **ligulate**, corolla zygomorphic

Economic importance:

1. Common valuable ornamentals include species of *Aster*, *Dahlia*, *Chrysanthemum*, *Gerbera*, *Helichrysum*, *Tagetus* and *Zinnia*. A few food plants include *Lactuca* (lettuce), *Cynara* (artichoke), *Helianthus* (sunflower oil), and *Cichorium* (chicory, added to coffee). Safflower a red dye is obtained from *Carthamus tinctorius*.
2. Latter is now more commonly cultivated for its seeds yielding safflower oil, used in cooking. *Chrysanthemum cinerariefolium* is the source of natural insecticide pyrethrum.

Interrelationship, evolutionary trends and recent concept of Asterales:

Asterales (incl. Campanulales of many authors), with Alseuosmiaceae, Argophyllaceae, Compositae (= Asteraceae), Calyceraceae, Campanulaceae (incl. Cyphiaceae, Lobeliaceae, Nemacladaceae), Carpodetaceae (included in Rousseaceae by APG II 2003), Goodeniaceae, Menyanthaceae, Pentaphragmataceae, Phellinaceae, Rousseaceae and Stylidiaceae (incl. Donatiaceae), contain about 26,300 species in c. 1,720 genera. The large majority of species and genera belong to Compositae and Campanulaceae. The order is well supported in all major molecular phylogenetic analyses (APG II 2003), and is part of the Euasterids II or Campanulids sensu Bremer et al. (2002).

Phylogenetic structure within Campanulids (also containing Apiales, Aquifoliales, Dipsacales and several families of uncertain ordinal placement; APG II 2003) is not sufficiently well resolved to identify the sister group of Asterales. It appears to be evident, however, that of all representatives of the ampanulids, Aquifoliales are least closely related to Asterales (Savolainen et al. 2000a, b; Soltis et al. 2000; Albach et al. 2001; Bremer et al. 2001, 2002). Although several of the constituent families of the order had been recognized to be closely related to one another long ago, the recognition of the relationship of others to Asterales (Lundberg and Bremer 2003) is the result mainly (but not only) of recent molecular phylogenetic work. This applies particularly to Alseuosmiaceae (Backlund and Bremer 1997; Cronquist 1981: Rosales; Thorne 1992: Saxifragales; Takhtajan 1997:Hydrangeales), Argophyllaceae (Cronquist 1981: Rosales; Takhtajan 1997: Hydrangeales), Carpodetaceae (Gustafsson and Bremer 1997; Lundberg 2001; Takhtajan 1997: Hydrangeales),Phellinaceae (Cronquist 1981: Celastrales; Thorne 1992: Theales; Takhtajan 1997: Icacinales) and Rousseaceae (Lundberg 2001; Takhtajan 1997: Brexiales), and partly also to Menyanthaceae (Cronquist 1981: Solanales; Thorne 1992: Campanulales; Takhtajan 1997: Menyanthales) and Stylidiaceae (Cronquist 1981: Campanulales; Thorne 1992: Saxifragales; Takhtajan 1997: Stylidiales).

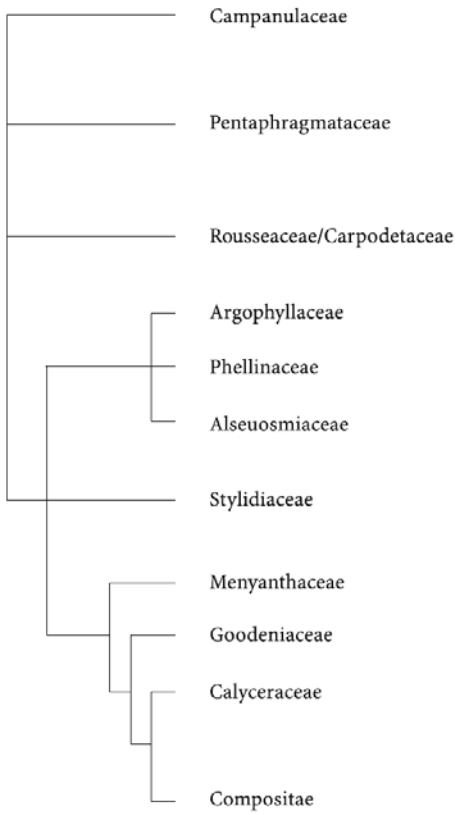


Fig- Phylogenetic hypothesis of Asterales, Source: Kadereit J. W. 2007. Asterales: Introduction and Conspectus.

Members of Asterales are mostly herbaceous and in most cases have alternate leaves without stipules. Flowers are very rarely solitary but mostly aggregated in sometimes axillary but more commonly terminal inflorescences which are capitulate and involucrate in most of the closely related Goodeniaceae, Calyceraceae and Compositae,

Poaceae (Gramineae):

Grass family (from *poa*, Greek namefor a grass)

There are about 9500 species in about 668 genera that are classified into 12 subfamilies. The grass family is one of the most widely distributed and abundant groups of plants on Earth. Grasses are found on every continent, including Antarctica with the presence of Antarctic hair grass on the Antarctic Peninsula.

Diagnostic characters

1. **Habit:** Annual or perennial herbs; rarely tree (*Bambusa*, *Dendrocalamus*)

2. **Roots:** Fibious adventitious, branched, fascicled or stilt (*Zea mays*).

   Stem: Herbaceous; cylindrical; sometimes forms underground rhizome or runner, jointed, hollow at intemodes, culm with conspicuous nodes and internodes, internodes hollow, herbaceous or woody, glabrous or glaucous, vegetative shoots are arising from the base of aerial stem or from underground stems are called tillers.

3. **Leaves:** Alternate, simple, distichous, exstipulate, sessile, ligulate (absent in *Echinochloa*), leaf base forming tubular sheath, sheath open, surrounding internode incompletely, ligule is present at the junction of the lamina and sheath, entire, hairy or rough, linear, parallel venation.

4. **Inflorescence:** Compound spike which may be sessile or stalked. Each unit of inflorescence is spikelet. The spikelets are arranged in various ways on the main axis called rachilla. A compound inflorescence may be spike of spikelets (*Triticum*), panicle of spikelets (*Avena*).

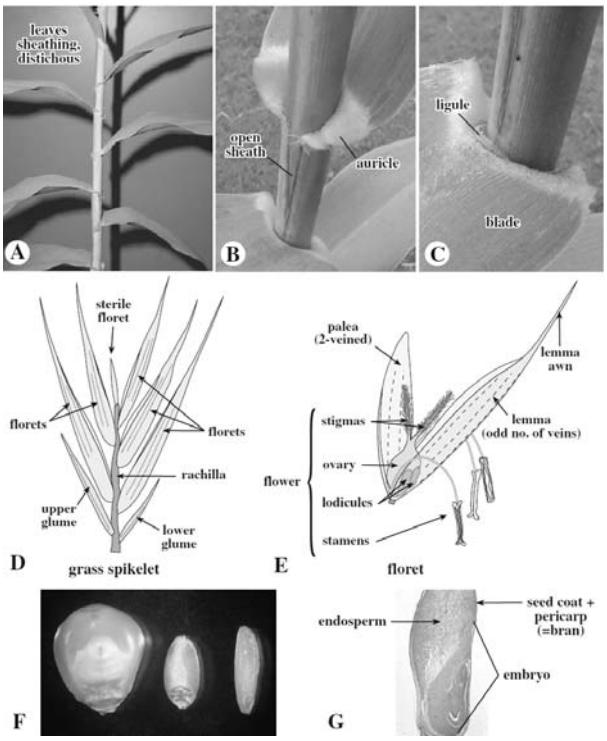


Fig- Different floral part of Poaceae,

Source: Simpson, G. Plant Systematics, 2006.

The spikelet consists of a short axis called **rachilla** on which 1 to many sessile or short stalked flowers are borne. The florets may be arranged in alternate or opposite manner on the central axis. At the base of rachilla two sterile scales, called **glumes**, are present. The glumes are placed one above the other on opposite sides. The lower one is called **first glume** and the upper is called **second glume**. Both the glumes are boat shaped and sterile. Above the glumes a series of florets are present. Each floret has an inferior palea or lemma and above it a superior palea. The lemma frequently bears a long, stiff hair called **awn**.

5. **Flower:** Pedicillate; bracteate, two bracts **palea** and **lemma** enclose flower or floret; lemma contain bristle like **awns** zygomorphic; incomplete; unisexual or hermaphrodite; hypogynous.

6. **Perianth:** Represented by membranous scales called the **lodicules**. The lodicules are situated above and opposite the superior **palea** or may be absent, or many (*Ochlandra*), or 2 or 3.

7. **Androecium:** Usually stamens 3, rarely 6 (*Bambusa*, *Oryza*) and one in various species of *Anrostis, Lepturus*; polyandrous, filaments long, anthers dithecous, versatile, linear, extrorse; pollen grains dry, anther **versatile.**

8. **Gynoecium:** Monocarpellary, according to some authors carpels 3, of which 2 are abortive, ovary superior, unilocular with single ovule, **ovules** are orthotropous to anatropous, usually bitegmic, 1 per ovary. **Nectaries** are absent. **basal placentation**, style short or absent; stigmas two **feathery or papillate** and branched.

1. **Fruits: Caryopsis,** or rarely nut (*Dendrocalamus)* or berry (*Bambusa*).

2. **Seed:** Endospermic and containing a single cotyledon called scutellum, which is shield shaped and pressed against the endosperm.

Economic Importance

This family has greater importance than all other families of the flowering plants. It has importance both for man and animals.

1. **Food**: All the cereals and millets belong to this family. These from the basic food of mankind. These plants are: *Tirticum* sp (Wheat). *Avena saliva, Zea mays* (corn). *Oryza sativa* (rice). *Hordeum vulgare* (barley 2), *Scale cereale* (rye).

2. **Penisetum** typhoideum *Sorghum vulgare* .

3. **Fodders**: Most of the fodders of the animals also belong to this family. The dried stems and leaves of the cereal crops are used as fodder for the cattle.

4. **Sugar**: Sugar is obtained from the juice of *Saccharum officinarum* (sugar cane).

5. **Ornamental plants**: Many grasses are used M lavms, e.g. Agrost is. *Boa, Festuca* etc. So these plants have ornamental significance.

6. **Aromatic oils:** Certain grasses give aromatic oil, e.g. *Cymbopogon throws* (lemon grass). It gives lemon grass oil. This oil **is used** in perfumes and soap industry for making infusions.

7. **Paper industry:** Some species of grasses are used for making papers.

8. **Alcohol and beverages:** Ethyl alcohol and many other beverages are prepared form cereals. For example, wine is prepared from rye, corn and rum molasses form sugar cane.

9. **Ropes:** Fibers are obtained form the leaves of *Saccharin?: munjo.* These fibers are used for making ropes.

10. **Used of Bamboo**: *Bambusa* (bamboo) are used as building material. These are used for thatching huts, making boats, carts pipes etc. Their spilt stems are woven into mars, fans, hats and course umbrella. Their leaves are given to horse for curing cough and cold.

Floral formula: P 2-3 [-6+] lodicules **A** 2-3 [1] **G** (2-3), superior.

Common species

1. ***Triticum vulgare,*** wheat

2. ***Zea mays,*** corn

3. ***Avena sativa,***

4. ***Oryza sativa,*** rice
5. ***Bambusa,*** bamboo
6. ***Saccharam officinarum,*** Sugar cane.

Primitive characters:

1. A few plants arboreal in habit.
2. All florets in a spikelet are fertile.
3. Glumes are persistent.
4. Lemmas are herbaceous and leafy.
5. Stigmas are three.
6. Leaves are simple and alternate.
7. Flowers are hypogynous and hermaphrodite.
8. Seeds are endospermic.

Advanced characters:

1. Plants are mostly herbaceous, annuals and perennials.
2. Leaves are exstipulate.
3. Flowers are arranged in distinct inflorescence.
4. Flowers are small, incospicuous and zygomorphic.
5. Perianth is represented by lodicules.
6. Stamens are reduced to 3.
7. Gynoecium is monocarpellary and unilocular.

8. Basal placentation.

9. Fruit is caryopsis.

10. Seeds are small sized.

Interrelationship, evolutionary trends and recent concept of Poaceae:

Although a very large assemblage Poaceae are easily recognized and form a monophyletic group, as supported by morphology (lodicules, spikelets with glumes, lemma and palea, fruit caryopsis) and DNA characters (*rbcL* and *ndhF* sequences). Cronquist (1988) places Poaceae and Cyperaceae under the same order Cyperales, but similar morphology of two is believed to be due to convergent evolution, Cyperaceae being more closely related to Juncaceae (Judd et al., 1999). The studies of Bremer (2002), using *rbcL* and taq analyses found strong support for Cyperaceae, Juncaceae, and Thurniaceae forming cyperid clade and Poaceae along with other families forming a graminoid clade.

The family is variously classified by different authors. Hutchinson (1973) recognized two subfamilies Pooideae (with 24 tribes) and Panicoideae (with 3 tribes). Studies of Clark et al., (1995) and Soreng and Davis (1998) suggest that Arundinoideae, Chloridoideae and Panicoideae form a well-supported clade (often called PACC clade) based on embryological and DNA data. Arundinoideae as generally defined are not monophyletic, and many of their members such as *Aristida*, *Phragmites*, etc. are spread over in other two subfamilies. Chloridoideae and Panicoideae are generally found to be monophyletic. Stevens (APWeb, 2003) and Thorne (2003) listed 12 subfamilies under Poaceae: Anomochlooideae, Pharoideae, Puelioideae, Panicoideae, Arundinoideae, Centothecoideae, Chloridoideae, Aristidoideae, Danthonioideae (six forming **PACCAD** clade), Bambusoideae, Ehrhartoideae, Pooideae (**BEP** clade). Subsequently (APWeb 2008, Thorne 2006, 2007), however, they have added 13th Micrairoideae, probably sister to the whole clade (Thorne prefers Chondrosoideae to Chloridoideae). There is great diversity in the morphology and biochemistry of C4 photosynthesis in the family (Kellogg, 2000). Studies based on gene expression (Ambrose

et al., 2000) indicate that the palea and perhaps even lemma are calycine in nature and the lodicules are corolline. Clark and Triplett (2006) discuss relationships within Bambusoideae, previously divided into the woody Bambuseae and the herbaceous Olyreae. However, the woody

temperate bamboo group may be sister to the rest of the family. The duplication of AP1/FUL gene, apparently in stem-group Poaceae, may be involved in the evolution of the spikelet (Preston & Kellogg 2006). Malcomber and Kellogg (2005) suggest that there has been duplication of LOFSEP genes within Poaceae, while there has been a duplication of the whole genome in a clade that includes at least *Zea*, *Oryza*, *Hordeum* and *Sorghum* (Schlueter et al. 2004). Developmental gene duplication and subsequent functional divergence seem to have played a very important role in allowing the development of the baroque diversity of inflorescences in the family (Malcomber et al. 2006). Indeed, there has been very extensive duplication of genes - API, AG and SEP families - but not in the AP3 lineage (Zahn et al. 2005a).

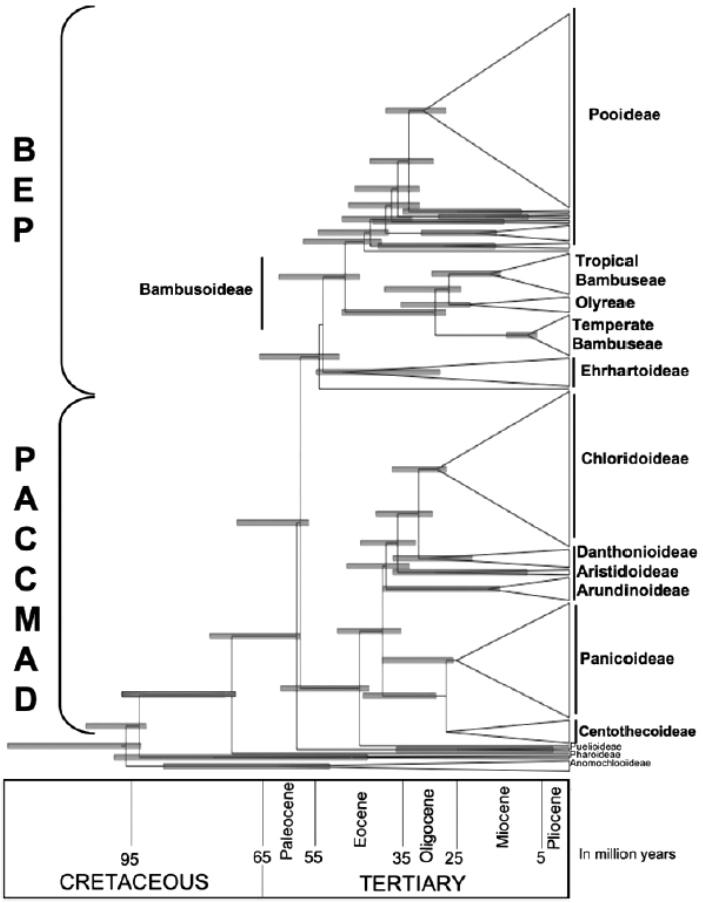


Fig- Phylogenetic tree of Poaceae, Source:https://academic.oup.com/botlinnean/article/162/4/543/2418503.

The origin of Poaceae was retrieved as African and shade adapted. The crown node of the BEP + PACCMAD clade was dated at 57 Mya, in the early Eocene. Grasses dispersed to all continents by approximately 60 million years after their Gondwanan origin in the late Cretaceous. PACCMAD taxa adapted to open habitats as early as the late Eocene, a date consistent with recent phytolith fossil data for North America. C4 photosynthesis first originated in Africa, at least for Chloridoideae in the Eocene at *c.* 30 Mya. The BEP clade members adapted to open habitats later than PACCMAD members; this was inferred to occur in Eurasia in the Oligocene.

Alismatales:

The **Alismatales** (**alismatids**) are an order of flowering plants including about 4500 species. Plants assigned to this order are mostly tropical or aquatic. Some grow in fresh water, some in marine habitats.

The Alismatales comprise herbaceous lowering plants of aquatic and marshy habitats, and the only monocots known to have green embryos other than the Amaryllidaceae. They also include the only marine angiosperms growing completely submerged, the seagrasses. The flowers are usually arranged in inflorescences, and the mature seeds lack endosperm.

Both marine and freshwater forms include those with staminate flowers that detach from the parent plant and float to the surface where they become pollinated. In others, pollination occurs underwater, where pollen may form elongated strands, increasing chance of success. Most aquatic species have a totally submerged juvenile phase, and flowers are either floating or emergent. Vegetation may be totally submersed, have floating leaves, or protrude from the water. Collectively, they are commonly known as "water plantain".

The Cronquist system (1981) places the Alismatales in subclass Alismatidae, class Liliopsida [= monocotyledons] and includes only three families as shown:

- Alismataceae
- Butomaceae
- Limnocharitaceae

Diagnostic Characters:

1. The Alismatales include annuals or perennials.. Some genera (e.g., *Echinodorus, Ottelia, Sagittaria*) contain both annual and perennial species.

2. The perennial species have corms, rhizomes, stolons which usually give rise to an erect leafy stem or a basal rosette of leaves.

3. Erect stem, starchy tubers from which new erect stems can grow.

4. Leaves have sheathing bases and are submersed, floating or emersed. They are phyllodia (petiole-like leaves that are not divided into blade and petiole) sessile, linear blades or divided into a blade and a petiole.The blades are oval to ovate to sagittate.

5. The flowers are bisexual or unisexual. Plants with unisexual flowers are either monoecious (carpellate and staminate flowers on same plant) as in most *Najas* spp., polygamous (perfect, carpellate, and staminate flowers on same plant) or dioecious.

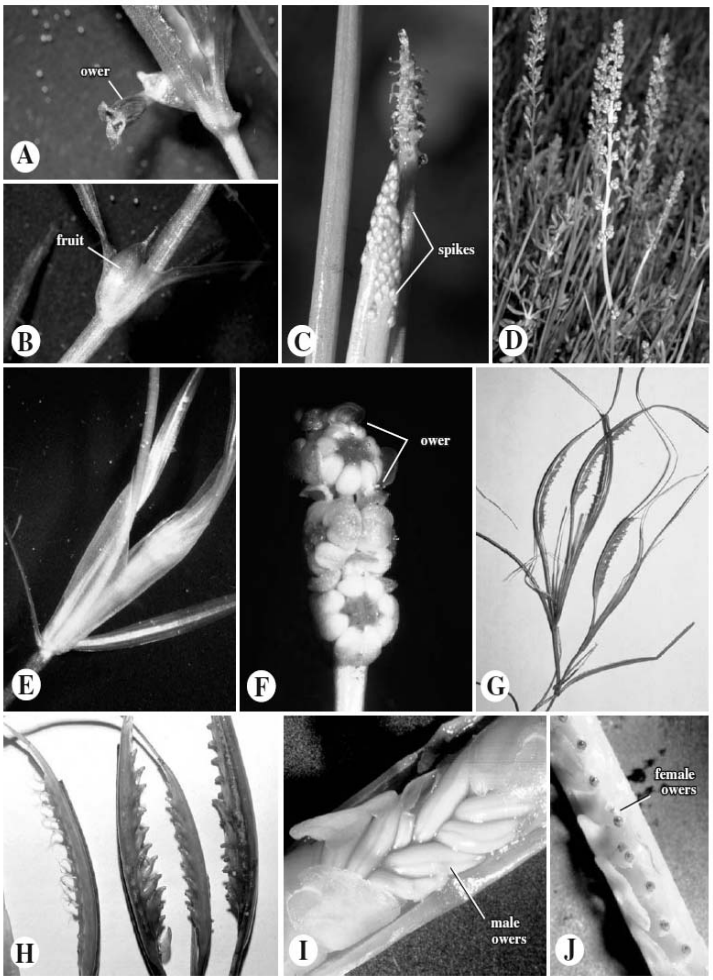


Fig- Different floral part of Alismatales,
Source: Simpson, G. Plant Systematics, 2006.

6. Stamens and carpels are separate and number from one (*Najas*) to fewer than 10 (*Butomus*, *Hydrocleys*, *Elodea*, *Limnobium*) to 20 or 30 (*Echinodorus*, *Sagittaria*).Some species of *Sagittaria* may have several hundred separate carpels.

7. The ovary is inferior in the Hydrocharitaceae and superior in all other families (partly inferior in Butomacae and Damasonium).

8. The fruits are achenes (Alismataceae, Najadaceae), capsules (Hydrocharitaceae) or follicles (Alismataceae, Butomaceae, Limnocharitaceae).

Economic importance:

1. Horticultural ornamentals such as *Pothos*, *Alocasia*, *Arum*, *Dieffenbachia*, *Monstera*, *Philodendron*, *Zantedeschia* and *Syngonium*. *Epipremnum aureum* (money plant) is commonly grown as house plant.

2. The corms of *Colocasia esculenta* (taro or dasheen), *Amorphophallus campanulatus* (Elephant-foot yam), *Cryptosperma* and *Xanthosoma* (tanier, yautia) and fruits of *Monstera* (Mexican breadfruit) are used as food.

3. *Sagittaria sagittifolia* (arrowhead) is cultivated in China and Japan for its edible corms. Several species of *Sagittaria*, *Alisma* (water plantain), and *Echinodorus* (bur-heads) are cultivated as poolside plants and used as aquarium plants.

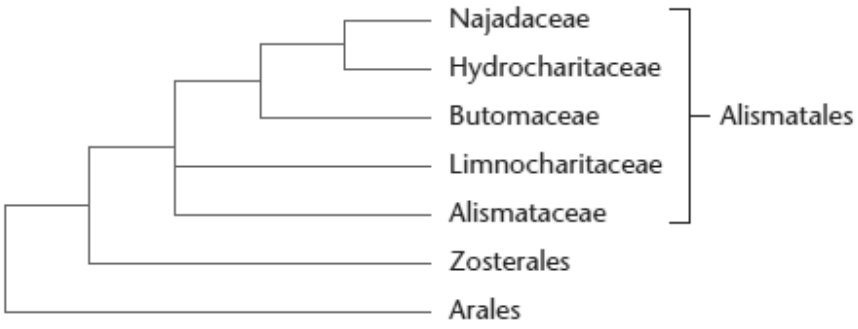
4. Species of many genera (*Hydrilla*, *Vallisneria*, *Elodea*, *Egeria*, etc.) are used as aquarium plants. Some species like *Hydrilla verticillata*, *Elodea Canadensis* have become troublesome weeds in many parts of the world.

5. Many species of *Potamogeton* are troublesome weeds. Fleshy starchy root stocks are sometimes used as food.

Interrelationship, evolutionary trends and recent concept of Alismatales:

The Alismatales are a group of aquatic or semiaquatic plants which have been widely considered as one of the most primitive orders of extant monocotyledons, partly because of their determinate inflorescences. However, their position is complicated because they are more specialised than other apocarpous monocotyledons in the differentiation of their perianth into calyx and corolla, their possession of trinucleate pollen, the anatomy of their vessels and the absence of endosperm in their mature seeds (Lawrence 1951, Eckardt 1964, Cronquist 1981). Many of their characters may represent adaptations to aquatic habitats and may be of limited taxonomic importance (Stant 1964, 1967). In recent treatments by Dahlgren & Clifford (1982), Dahlgren et al. (1985) and Dahlgren & Bremer (1985) the Alismatales are shown to be relatively derived within the monocotyledons. The familial boundaries within the group were redefined on the grounds of macro- and rnicromorphological characters by Pichon (1946) who transferred all genera except Bitrotnits from the Butomaceae to the Alismataceae in which he recognised two tribes, Alismateae and Lirnnochariteae. Lawrence (1951) pointed out that the original spelling had been

Alismaceae but the nomen conseruandim, Alismataceae is now generally used. Later, Takhtajan (1954, 1959) elevated the tribes to familial status, and included Hydrocleys, Litnnocharis, Ostetiia and Tenagocharis in the new family Limnocharitaceae, which has gained widespread acceptance (Kuprianova 1954, Kimura 1956, Stebbins 1974, Cronquist 1981). Hydrocleys bears a striking exomorphological resemblance to Nynzphaea or Lirnnanfhetniot (Willis 1973). Several other genera have been recognised in the Limnocharitaceae including Bitrotnopsis (= Tcnagocharis, Willis 1973), Osfenia (synonymous with Hydrocleys, Eckardt 1964) and Elaftosis (synonymous with Terzagocharis, Eckardt 1964). More recently Butomaceae has been removed from the Alismatales and placed in the closely related order Hydrocharitales (Dahlgren 1975).



Fig, Phylogeny tree of Alismatales Source: http://hydrodictyon.eeb.uconn.edu/people/les/Manuscript_Files/Encyclopedia_Life_Sciences.pdf

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5. ICBN : Changes, addition and alteration of latest four codes; principles, rank of taxa and names of taxa, nomenclatural types, priority of publication and limitation of the priority of publications, effective and valid publications, author's citation; changes and rejection of names, preliminary concept of appendices. Principle idea about Bio-codes and Phylocodes.

ICBN (International Code of Botanical Nomenclature):

The process of naming plants based on international rules proposed by botanists to ensure stable and universal system is called botanical nomenclature. It is an essential process to overcome the problems of common names. The botanists agreed to lay down certain rules and conditions. The main suggestion was that language should be in Latin as it is not a national language of any

country and moreover it is dead language. A lot of previous literature is written in Latin. The ICBN is an agreement between botanists around the world to follow Binomial system of naming. The guiding principle in botanical nomenclature is priority. The ICBN sets the formal starting date of plant nomenclature at **1 May 1753**, the publication of ***Species Plantarum*** **by Linnaeus.**

History of ICBN

Before the middle of 18th century the names of plants were polynomials consisting of several words. Linnaeus proposed elementary rules in his book named 'Philosophia botanica' in 1751. In 1813 A.P. de Candolle proposed details of the rules regarding plant nomenclature in Theorie elementaire de la botanique. In 1867, Alphonse de candolle, son of A.P. de candolle convened a meeting of all botanists to present these rules. This first Botanical congress was held at Paris and the laws framed therein were called as Paris code.

In 1930, the fifth International Botanical Congress (IBC) was held in England to frame rules and regulations for naming plants. In July 1975, twelfth meeting was held at Leningrad, USSR. Based on the resolutions of these meetings, the existing system of International Code of Botanical Nomenclature (ICBN) was adapted from 1978. The IBC meets every 5 to 6 years to decide any additions or changes in the naming and numbering of plants. The 18th IBC congress was held at Melbourne in 2011 and the latest 19th IBC was held in Shenzen, China in July 2017.

Major changes, addition and alteration to the Code of Nomenclature:

Melbourne code:

- The name be changed from *International Code of Botanical Nomenclature* to *International Code of Nomenclature for algae, fungi, and plants*. There was never formal recognition of "*ICBN "* as an abbreviation for the former title and none was proposed for the new title, but the abbreviation "*ICN "* does not compete with the abbreviations of any of the other codes of biological nomenclature.
- Electronic publication online as Portable Document Format (PDF) of all nomenclatural acts permitted from 1 January 2012.
- The Nomenclature Section modified this so that, for names published on or after 1 January 2012, the description and/or diagnosis must be in either English or Latin.

- One fungus, one name is considered. The Code has permitted separate names for asexual and sexual phases of those fungi.
- The Nomenclature Section adopted a set of proposals by which the whole concept of morphotaxa is abandoned, so that when two or more morphotaxa an be shown to belong to the same organism, their names compete for priority in the usual way.
- Registration of fungal names
- As more and more conserved or rejected names have been added to the Appendices of the Code, each successive printed edition has become bulkier

Shenzhen code:

The most extreme change to the Code resulting from the Shenzhen IBC was the Nomenclature Section's decision that future proposals to amend the Code relating solely to names of organisms treated as fungi will be decided exclusively by the Nomenclature Session of an International Mycological Congress (IMC)

- A significant amendment to the proposal of the Special Subcommittee on Governance of the *Code* with respect to Fungi was accepted at the Section, namely to bring together all the provisions of the *Code* that deal solely with names of organisms treated as fungi into a special Chapter, which has been called **Chapter F** (the "F" standing, of course, for fungi), so that the IMC has exclusive authority over this Chapter and the IBC has exclusive authority over the rest
- The second major change to the *Code* accepted at the Shenzhen IBC is the replacement of **Division III**, the Provisions for Governance of the *Code,* with an almost completely new and much-expanded version.
- The third major change to the *Code* in Shenzhen was the acceptance of most of the proposals developed by the Special Committee on Registration of Algal and Plant Names (including fossils)

Division I. Principles

Principle I: Botanical nomenclature is independent of zoological and bacteriological nomenclature. The Code applies equally to names of taxonomic groups treated as plants whether or not these groups were originally so treated.

Principle II: The application of names of taxonomic groups is determined by means of nomenclatural types.

Principle III: The nomenclature of a taxonomic group is based upon priority of publication.

Principle IV: Each taxonomic group with a particular circumscription, position, and rank can bear only one correct name, the earliest that is in accordance with the Rules, except in specified cases.

Principle V: Scientific names of taxonomic groups are treated as Latin regardless of their derivation.

Principle VI: The Rules of nomenclature are retroactive unless expressly limited.

Rank and Name of taxa:

Taxonomic groups of any rank will be referred to as taxa (singular: taxon). Every individual organism is treated as belonging to an indefinite number of taxa of consecutively subordinate rank. Rank is the relative position in a taxonomic hierarchy.

The basic unit in the classification of plants is Species. A species is defined as a single type of living organisms.

The group of closely related species is known as Genus.

For example, the genus *Hibiscus* is made up of closely related species, which differ in their vegetative characters. The group of closely related genera is known as family. Scientific name of a family usually ends in '-ceae'.

For example, *Hibiscus* belongs to family is Malvaceae, it consists of several genera like *Gossypium* (cotton), *Abelmoschus* (lady's finger) etc.

The group of closely related families is known as order. Scientific name of an order ends in '-ales'.

For example, *Hibiscus* order is Malvales which includes family like Malvaceae, Dipterocarpaceae.

The principal ranks of taxa in descending sequence are: kingdom (regnum), division or phylum (divisio or phylum), class (classis), order (ordo), family (familia), genus (genus), and species (species).

Rank	*Ending*	*Example*
Kingdom	-bionta	Chlorobionta
Division	-phyta	Magnoliophyta
	-mycota (Fungi)	Eumycota
Subdivision	-phytina	Pterophytina
	-mycotina (Fungi)	Eumycotina
Class	-opsida	Magnoliopsida
	-phyceae (Algae)	Chlorophyceae
	-mycetes (Fungi)	Basidiomycetes
Subclass	-opsidae	Pteropsidae
	-idae (Seed plants)	Rosidae
	-physidae (Algae)	Cyanophysidae
	-mycetidae (Fungi)	Basidiomycetidae
Order	-ales	Rosales
Suborder	-ineae	Rosineae
Family	-aceae	Rosaceae
Subfamily	-oideae	Rosoideae
Tribe	-eae	Roseae
Subtribe	-inae	Rosinae
Genus	-us, -um, -is, -a, -on	*Pyrus, Allium, Arabis, Rosa, Polypogon*
Subgenus		*Cuscuta* subgenus *Eucuscuta*
Section		*Scrophularia* section *Anastomosanthes*
Subsection		*Scrophularia* subsection *Vernales*
Series		*Scrophularia* series *Lateriflorae*
Species		*Rosa canina*
Subspecies		*Crepis sancta* subsp. *bifida*
Varietas		*Lantana camara* var. *varia*
Forma		*Tectona grandis* f. *punctata*

Some of the families do not end with -aceae, so they are provided with alternative names like,

Cruciferae – Brassicaceae

Compositae – Asteraceae

Graminae – Poaceae

Guttiferae – Clusiaceae

Leguminosae – fabaceae

Palmae – Arecaceae

Umbelliferae – Apiaceae

Nomenclatural type (Art. 7-10):

The application of names of taxa of the rank of family or below is determined by means of nomenclatural types. A **nomenclatural type** (typus) is that element to which the name of a taxon is permanently attached, whether as a correct name or as a synonym. The nomenclatural type is not necessarily the most typical or representative element of a taxon.

The type of a name of a species or infraspecific taxon is either a single specimen conserved in one herbarium or other collection or institution, or an illustration.

1. Holotype: A holotype of a name of a species or infraspecific taxon is the one specimen or illustration used by the author, or designated by the author as the nomenclatural type. As long as a holotype is extant, it fixes the application of the name concerned. A specimen is usually mounted either on a single herbarium sheet or in an equivalent preparation, such as a box, packet, jar or microscope slide. Type specimens of names of taxa must be preserved permanently and may not be living plants or cultures. However, cultures of fungi and algae, if preserved in a metabolically inactive state (e.g. by lyophilization or deep-freezing), are acceptable as types. It is now essential to designate a holotype when publishing a new species.

2. Isotype: An isotype is any duplicate of the holotype, collected from them same place, at the same time and by the same person. Often the collection number is also the same, differentiated as a, b, c, etc.

3. Syntype: Any one of the two or more specimens cited by the author when no holotype was designated, or any one of the two or more specimens simultaneously designated as types. Duplicate of a syntype is an isosyntype.

4. Paratype: A paratype is any specimen cited in the protologue that is neither the holotype nor an isotype, nor one of the syntypes if in the protologue two or more specimens were simultaneously designated as types.

5. Lectotype: A lectotype is a specimen or illustration designated from the original material as the nomenclatural type, if no holotype was indicated at the time of publication, or if the holotype is missing, or if a type is found to belong to more than one taxon.

A lectotype is selected from isotypes, syntypes or paratypes, if such exist. If no cited specimens exist, the lectotype must be chosen from among the uncited specimens and cited and uncited illustrations which comprise the remaining original material, if such exist.

6. Neotype: A specimen or illustration selected to serve as nomenclatural type as long as all of the material on which the name of the taxon was based is missing; a specimen or an illustration selected when no holotype, isotype, paratype or syntype exists.

7. Epitype: An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon. When an epitype is designated, the holotype, lectotype, or neotype that the epitype supports must be explicitly cited.

8. Topotype is often the name given to a specimen collected from the same locality from which the holotype was originally collected.

Principles of Priority:

Principles of Priority are concerned with the selection of a single correct name of taxonomic group. Only legitimate names should be retained while the illegitimate names should be rejected. According to article 11-12 rules for priority are:

(i) Each family or taxon of lower rank with a particular circumscription, position and rank can bear only one correct name.

(ii) For any taxon from family to genus inclusive, the correct name is the earliest legitimate one, validly published with the same rank.

(iii) A name of a taxon has no status under this code unless it is a validly published.

(iv) The application of both conserved and rejected names is determined by nomenclatural type.

(v) “When a name proposed for conservation has been provisionally approved by the general committee, botanists are authorized to retain it pending the decision of a later International Botanical Congress”.

Valid Publication of names is usually considered beginning in May 1753, the date of publication of *Species plantarum* vol. I by Linneaus.

With many names of a taxon, the valid will be the earliest name which is regarded as correct name. Rule of Priority provides stability to his name.

The principle that seniority is fixed by the date of valid publication is known as Principle of Priority.

Example 1:

Nymphea nouchali Burm F. 1768; *N. Pubescence* Willd 1799 and *N.torus* Hook T; 1872 are names of the same species but if rule of Priority is applied the first name is the correct name and other two are synonyms.

Example 2:

Loureiro described a plant and named it *Physkium nataus* in 1790. A.L.de Jussieu transferred it in genus *Vallisneria* in 1828. He instead of *nutans* gave the specific name as *V. physkium*. It is superfluous name. Graebner (1912) described the same plants as *V.gigantee* and Miki (1934) named as *V.asiatica*. Harg while studying Asiatic species confirmed that all these names are synonymous. There is no legitimate combination based on *Physikium natans (*Leru) existed. He made *V.natans* Hara in 1974. The correct name of the specimen is now the recent name, but it is based on earliest basionym, others will be synonym.

Limitations of Principles of Priority:

1. Starting dates:

Principles of Priority starts with the Species Plantarum of Linnaeus published on 1-5-1753.

The starting dates for different groups include:

Seed plants, Pteridophytes, Sphagnaceae

Hepaticae, most Algae, slime moulds

and lichens.....1-5-1753

Mosses (excluding Sphagnaceae)

.....1-1-1801

Fungi 31-12-1801

Fossils 31-12-1820

Algae (Nostocaceae).....1-1-1886

Algae (Oedogoniaceae).....1-1-1900

The publications before these dates for respective groups are ignored while deciding the priority.

2. Limited only upto family ranks:

This principle does not apply over family rank.

3. The corrected name should not be outside the rank. Only when a correct name in the taxon is not available, a combination with other rank is allowed.

4. The application of Principles of Priority resulted in numerous name changes. To avoid it a list of conserved generic and family names has been prepared and Published in the code with some changes. Such Nomina conservanda (non. cons) are to be used as correct name replacing earlier legitimate name, e.g., *Sesbania scop*, 1777 is the conserved genus as against *Sesban adam* 1763 and *Agati adam* 1763.

Effective and Valid publication (Art. 29-50)

Effective publication

1. Publication is effected by distribution of printed matter (through sale, exchange, or gift) to the general public or at least to scientific institutions with generally accessible libraries. Publication is also effected by distribution on or after 1 January 2012 of electronic material in PDF Format (in an online publication with an International Standard Serial Number (ISSN) or an International Standard Book Number (ISBN).

   Ex.1. The paper containing the new combination Anaeromyces polycephalus (Y. C. Chen & al.) Fliegerová & al. (Kirk in Index Fungorum 1: 1. 2012), based on Piromyces polycephalus Y. C. Chen & al. (2002), was effectively published when it was issued online in Portable Document Format with an ISSN on 1 January 2012.
2. Publication is not effected by communication of nomenclatural novelties at a public meeting, by the placing of names in collections or gardens open to the public, by the issue of microfilm made from manuscripts or typescripts or other unpublished material, or by distribution of electronic material other than as described in Art. 29.

   Ex.1. Cusson announced his establishment of the genus *Physospermum* in a memoir read at the Société des Sciences de Montpellier in 1770, and later in 1782 or 1783 at the Société de Médecine de Paris, but its effective publication dates from 1787 (in Hist. Soc. Roy. Méd. 5(1): 279).
3. An electronic publication is not effectively published if there is evidence within or associated with the publication that it is merely a preliminary version that was, or is to be, replaced by a version that the publisher considers final, in which case only that final version is effectively published.

4. Publication by indelible autograph before 1 January 1953 is effective. Indelible autograph produced at a later date is not effectively published. Indelible autograph is handwritten material reproduced by some mechanical or graphic process (such as lithography, offset, or metallic etching).
5. Publication on or after 1 January 1953 in trade catalogues or non-scientific newspapers, and on or after 1 January 1973 in seed-exchange lists, does not constitute effective publication.
6. The distribution on or after 1 January 1953 of printed matter accompanying specimens does not constitute effective publication.
7. Publication on or after 1 January 1953 of an independent non-serial work stated to be a thesis submitted to a university or other institute of education for the purpose of obtaining a degree does not constitute effective publication unless the work includes an explicit statement.
8. The date of effective publication is the date on which the printed matter or electronic material became available
9. When a publication is issued in parallel as electronic material and printed matter, both must be treated as effectively published on the same date unless the dates of the versions are different as determined by Art. 31.1.
10. When separates from periodicals or other works placed on sale are issued in advance, the date on the separate is accepted as the date of effective publication unless there is evidence that it is erroneous.

Valid publication:

1. In order to be validly published, a name of a taxon must: (a) be effectively published on or after the starting-point date of the respective group; (b) be composed only of letters of the Latin alphabet, except as provided in Art.; and (c) have a form that complies with the provisions of Art. 16-27.
2. The date of a name is that of its valid publication. When the various conditions for valid publication are not simultaneously fulfilled, the date is that on which the last is fulfilled.

However, the name must always be explicitly accepted in the place of its valid publication.

3. A name published on or after 1 January 1973 for which the various conditions for valid publication are not simultaneously fulfilled is not validly published unless a full and direct reference is given to the places where these requirements were previously fulfilled.

4. Names in specified ranks included in publications listed as suppressed works are not validly published.

5. A name of a taxon below the rank of genus is not validly published unless the name of the genus or species to which it is assigned is validly published at the same time or was validly published previously.

6. A name is not validly published (a) when it is not accepted by the author in the original publication; (b) when it is merely proposed in anticipation of the future acceptance of the taxon concerned, or of a particular circumscription, position, or rank of the taxon; (c) when it is merely cited as a synonym; or (d) by the mere mention of the subordinate taxa included in the taxon concerned. Art. 36.1(a) does not apply to names published with a question mark or other indication of taxonomic doubt, yet accepted by their author.

7. A name published on or after 1 January 1953 without a clear indication of the rank of the taxon concerned is not validly published.

8. In order to be validly published, a name of a new taxon must be accompanied by a description or diagnosis of the taxon or, if none is provided in the protologue, by a reference to a previously and effectively published description or diagnosis.

9. In order to be validly published, a name of a new taxon (algae and fossils excepted) published between 1 January 1935 and 31 December 2011, inclusive, must be accompanied by a Latin description or diagnosis or by a reference to a previously and effectively published Latin description or diagnosis.

10. Publication on or after 1 January 1958 of the name of a new taxon of the rank of genus or below is valid only when the type of the name is indicated.

11. In order to be validly published, a new combination, name at new rank, or replacement name, must be accompanied by a reference to the basionym or replaced synonym.

12. In order to be validly published, a name of a new fossil-taxon published on or after 1 January 1996 must be accompanied by a Latin or English description or diagnosis or by a reference.

13. In order to be validly published, a name of a new taxon of non-fossil algae published between 1 January 1958 and 31 December 2011, inclusive, must be accompanied by a Latin description or diagnosis or by a reference.

Author citations:

A name of a new taxon is attributed to the author(s) to whom the name was ascribed when the validating description or diagnosis was simultaneously ascribed to or unequivocally associated with the same author(s), who first published the name validly. The names of the authors are commonly abbreviated.

e.g. L. for Carolus Linnaeus, Benth. For G. Bentham, Hook. for William Hooker.

Single author

The name of a single author follows the name of a species (or any other taxon) when a single author proposed a new name, e.g. *Solanum nigrum* L.

Multiple authors

The names of two or more authors may be associatedwith a name for a variety of reasons.

These different situations are exhibited by citing the name of the authors differently:

1. Use of et: When two or more authors publish a new species or propose a new name, their names are linked by et,e.g. *Delphinium viscosum* Hook.f. et Thomson.

2. Use of parentheses: The rules of botanical nomenclature specify that whenever the name of a taxon is changed by the transfer from one genus to another, or by upgrading or downgrading the level of the taxon, the original epithet should be retained. The name of the taxon providing the epithet is termed a basionym. The name of the original author or authors whose epithet is being used in the changed name is placed within parentheses, and the author or authors who made the name change outside the parentheses, e.g. *Cynodon dactylon* (Linn.) Pers., based on the basionym *Panicum dactylon* Linn., the original name for the species.

3. Use of ex: The names of two authors are linked by ex when the first author had proposed a name but was validly published only by the second author, the first author failing to satisfy all or some of the requirements of the Code,

e.g. *Cerasus cornuta* Wall. ex Royle.

4. Use of in: The names of authors are linked using in when the first author published a new species or a name ina publication of another author,

e.g. Carex kashmirensis Clarke in Hook.f. Clarke published this new species in the Flora of British India whose author was Sir J. D. Hooker.

5. Use of emend: The names of two authors are linked using emend. (emendavit: person making the correction) when the second author makes some change in the diagnosis or in circumscription of a taxon without altering the type, e.g. *Phyllanthus* Linn. emend. Mull.

6. Use of square brackets:

Square brackets are used to indicate prestarting point author. The generic name *Lupinus* was effectively published by Tournefort in 1719, but as it happens to be earlier than 1753, the starting date for botanical nomenclature based on *Species plantarum* of Linnaeus, the appropriate citation for the genus is *Lupinus* [Tourne.] L.

Retention and Rejection of name:

When a species is transferred to another genus without change of rank the specific epithet must be retained. If the name of a genus is changed being illegitimate, the binary combinations for all the species under that genus should be changed also and in doing so the new generic name should be used retaining the older specific epithets.

This rule applies equally to infra-specific taxa. A specific epithet is not illegitimate merely because it was originally published under an illegitimate generic name; it is to be taken into consideration for purpose of priority.

A legitimate name must not be rejected merely because it, or its epithet, is inappropriate or disagreeable, or because another is preferable or better known, or because it has lost its original meaning.

Rejection:

The process of selection of correct name for a taxon involves the identification of illegitimate names, those which do not satisfy the rules of botanical nomenclature. A legitimate name must not be rejected merely because it, or its epithet, is inappropriate or disagreeable, or because

another is preferable or better known or because it has lost its original meaning. The name *Scilla peruviana* L. (1753) is not to be rejected merely because the species does not grow in Peru. Any one or more of the following situations leads to the rejection of a name:

1. **Nomen nudum (abbreviated nom. nud.):** A name with no accompanying description. Many names published by Wallich in his Catalogue (abbreviated Wall. Cat.) published in 1812 were **nomen nudum**. These were either validated by another author at a later date.by providing a description (e.g. *Cerasus cornuta* Wall. ex Royle) or if by that time the name has already been used for another species by some other author, the nomen nudum even if validated is rejected

2. Name not effectively published, not properly formulated, lacking typification or without a Latin diagnosis.

3. **Tautonym:** It is the specific epithet repeats exactly the generic name and must be rejected. Whereas the Zoological Code allows binomials with identical generic name and specific epithet (e.g. *Bison bison*), such names in Botanical nomenclature constitute tautonyms (e.g. Malus malus) and are rejected.

4. **Later homonym:** Just as a taxon should have one correct name, the Code similarly does not allow the same name to be used for two different species (or taxa). Such, if existing, constitute **homonyms**. The one published at an earlier date is termed the **earlier homonym** and that at a later date as the **later homonym**. The Code rejects later homonyms even if the earlier homonym is illegitimate.

   Ziziphus jujuba Lam., 1789 had long been used as the correct name for the cultivated fruit jujube. This, however, was ascertained to be a later homonym of a related species *Z. jujuba* Mill., 1768. The binomial *Z. jujuba* Lam., 1789 is thus rejected and *jujube* correctly named as *Z. mauritiana* Lam., 1789.

5. **Later isonym:** When the same name, based on the same type, has been published independently at different times by different authors, then only the earliest of these so-called **'isonyms'** has nomenclatural status. The name is always to be cited from its original place of valid publication, and later 'isonyms' may be disregarded.

e.g.-Baker (1892) and Christensen (1905) independently published the name *Alsophila kalbreyeri* as a substitute for *A. podophylla* Baker (1891) non Hook. (1857). As published by Christensen, *Alsophila kalbreyeri* is a later 'isonym' of *A. kalbreyeri* Baker, without nomenclatural status.

6. **Nomen superfluum (abbreviated as nom. superfl.):** The name which is given to a taxon when already some name is present in existence is called superfluous name.

   e.g.- *Physkium natans* Lour., 1790 thus when transferred to the genus *Vallisneria*, the epithet *natans* should have been retained but de Jussieu used the name *Vallisneria physkium* Juss., 1826 a name which becomes superfluous. The species has accordingly been named correctly as *Vallisneria natans* (Lour.) Hara, 1974

7. **Nomen ambiguum (abbreviated as nom. ambig.):** A name is rejected if it is used in a different sense by different authors and has become a source of persistent error. The name

   Rosa villosa L. is rejected because it has been applied to several different species and has become a source of error.

13. **Nomen confusum (abbreviated as nom. confus.):** A name is rejected if it is based on a type consisting of two or more entirely discordant elements, so that it is difficult to select a satisfactory lectotype.

    The characters of the genus *Actinotinus*, for example, were derived from two genera *Viburnum* and *Aesculus*, owing to the insertion of the inflorescence of *Viburnum* in the terminal bud of an *Aesculus* by a collector. The name *Actinotinus* must, therefore, be abandoned.

14. **Nomen dubium (abbreviated as nom. dub.):** A name is rejected if it is dubious, i.e. it is of uncertain application because it is impossible to establish the taxon to which it should be referred.

    Linnaeus (1753) attributed the name *Rhinanthus crista-galli* to a group of several varieties, which he later described under separate names, rejecting the name *R. crista-galli* L. Several later authors, however, continued to use this name for diverse occasions and the name was finally rejected.

Bio Code:

As information on the world's biota becomes increasingly integrated across different groups of organisms, from bacteria and fungi to animals and plants, there is a concomitant rising need for a consistent and harmonized approach to the regulation of scientific names.

The **BioCode** initiative represents a concerted effort, by biologists intimately involved in the operation of the current system of separate codes, to devise a unified approach to the future naming of organisms of all kinds. This need has become pressing in view of common issues that the separate organismal codes now have to address, consequent on the rapid changes taking place in global informatics, database architecture, molecular systematics and ecology, and electronic publication.

The IUBS/IUMS International Committee on Bionomenclature (ICB) presented the first BioCode Draft in 1997, followed by the BioCode Draft 2011. (DL Hawksworth, IUBS) The Draft BioCode (2011) is most appropriately viewed as a framework over-arching the practices of the current series of codes, but which also addresses ways in which some of the key issues of current concern in systematics could be handled by all codes.

Salient Features

1. General points: No examples are listed, Notes omitted at the present stage, although some will no doubt be needed. A considerable number of articles and paragraphs have been dropped; the Draft BioCode has only 41 Articles, whereas the St. Louis Code has 62.

2. Taxa and Ranks: The present ranks of the Botanical Code are maintained in the Draft BioCode, and a few tentatively added: domain (above kingdom), in use for the pro-/eukaryotes, superfamily (in widespread use in zoology), and the option of adding the prefix super- to rank designations that are not already prefixed.

3. Status: For the purposes of this Code Established names are those that are published in accordance with relevant articles of this Code or that, prior to 1 January 200n, were validly published or became available under the relevant Special Code.

4. **Establishment of names:** In order to be established on or after 1 January 200n, a name of a taxon must be published as provided for by the rules for publication, which are essentially similar to the Effective publication in botany. The new taxon may have a Latin or English description or diagnosis

6. Registration: Registration is affected by submitting the published matter that includes the protologue(s) or nomenclatural acts to a registering office designated by the relevant international body. It is pertinent to mention that this requirement was based on the Botanical Code.

7. Precedence (priority): For purposes of precedence, the date of a name is either the date attributed to it in an adopted List of Protected Names or, for unlisted names, the date on which it was validly published under the botanical or bacteriological Code, or became available under the zoological Code, or was established under the present Code.

8. Homonymy: The major change with respect to the homonymy rule would be that in future, it would operate across the kingdoms.

9. Author citation: The Draft BioCode signals a departure from the botanical tradition of laying great emphasis on the use of author citations, even in contexts where such citations are neither informative nor really appropriate.

10. Hybrids: The Appendix for Hybrids in the Botanical Code is replaced by a single Article in the Draft BioCode.

Principle:

1. The BioCode governs the formation and choice of scientific names of taxa but not the circumscription, position or rank of the taxa themselves. Nothing in this Code may be construed to restrict the freedom of taxonomic action.

2. Scientific nomenclature of organisms builds upon the Linnaean system of binary names (binomina) for species.

3. The application of names of taxa is determined by means of name-bearing types (hereafter referred to as types), although this principle does not apply to certain names at supra-familial ranks.

4. The nomenclature of a taxon is based upon priority (precedence by date) of publication, although application of this principle is not mandatory at all ranks.

5. Each taxon in the family group, genus group or species group with a particular circumscription, position, and rank has only one accepted name, except as may be specified in a Special Code.

6. Scientific names of taxa are treated as Latin, regardless of their derivation.

7. The name as applied to a taxon is not to be changed without sufficient reason, based either on further taxonomic studies or on the necessity of giving up a name that is contrary to the Rules of nomenclature.

8. In the absence of a relevant rule or where the consequences of rules are doubtful, established custom is followed.

9. The rules of nomenclature are retroactive, subject to any specified limitations.

PhyloCode:

The International Code of Phylogenetic Nomenclature, known as the **PhyloCode**, is a developing draft for a formal set of rules governing phylogenetic nomenclature. Its current version is specifically designed to regulate the naming of clades, leaving the governance of species names up to the rank-based Nomenclature codes (ICN, ICZN, ICNB, ICTV).

The PhyloCode is associated with the International Society for Phylogenetic Nomenclature (ISPN). The PhyloCode proposes to regulate phylogenetic nomenclature by providing rules for how to decide which associations of names and definitions will be considered established, which of those will be considered homonyms or synonyms, and which one of a set of synonyms or homonyms will be considered accepted (generally the one registered first; see below). The PhyloCode will only allow the naming of clades, not of paraphyletic or polyphyletic groups, and will only allow the use of specimens, species, and apomorphies as specifiers.

Principle:

1. Reference. The primary purpose of taxon names is to provide a means of referring to taxa, as opposed to indicating their characters, relationships, or membership.

2. Clarity. Taxon names should be unambiguous in their designation of particular taxa. Nomenclatural clarity is achieved through explicit definitions, which describe the concept of the taxon designated by the defined name.

3. Uniqueness. To promote clarity, each taxon should have only one accepted name, and each accepted name should refer to only one taxon.

4. Stability. The names of taxa should not change over time. As a corollary, it must be possible to name newly discovered taxa without changing the names of previously discovered taxa.

5. Phylogenetic context. This code is concerned with the naming of taxa and the application of taxon names in the context of phylogenetic concepts of taxa.

6. Taxonomic freedom. This code permits freedom of taxonomic opinion with regard to hypotheses about relationships; it only concerns how names are to be applied within the context of a given phylogenetic hypothesis.

7. There is no "case law" under this code. Nomenclatural problems are resolved by the Committee on Phylogenetic Nomenclature (CPN) by direct application of the code; previous decisions will be considered, but the CPN is not obligated by precedents set in those decisions.

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6. Concepts of phytogeography: Endemism in India; invasion and introduction of plants in India.

Endemism and Endemic

Endemism is the natural phenomenon or concept of confinement or restriction of any taxonomic unit or taxon which occurs in a restricted geographical areas or any particular country or region or island or mountain.

In Greek *en* means ‘within’ and *demos* means ‘population’. The taxa which occur only in single restricted area, are termed as endemics. Taxon may be a sub-species, species, genera, tribe or even a family. The geographical region where the taxon is restricted, may be termed as endemic area.

The concept of endemism of a family, a genus or species with reference to a particular region is varied.

Endemic Families:

These are restricted in particular floral kingdom or region or a country or even a province. The following families indicate their endemic nature to a particular area:

Madagascar region-

There are 12 endemic families- Didymefaceae, Didiereaceae, Barbe niaceae, Diegolendraceae, Asteropeiaceae, Sarcolaenaceae, Sphaerosepalaceae, Rousseaceae etc.

Cape region-

It has 8 endemic families- Grubbiaceae, Roridulaceae, Bruniaceae, Penaeaceae, Greyiaceae, Geissolomataceae, Retziaceae (Retzia) and Stilbaceae.

Holarctic kingdom- 60 endemic families

Paleotropicol kingdom- 40 endemic families

Neotropicol kingdom- 25 endemic families

Cape kingdom- 8 endemic families

Australian kingdom- 18 endemic families

Holantarctic kingdom- 12 endemic families.

Juan Fernandez islands- 2 endemic families (Lactovidaceae and Thysopteridaceae)

Fiji islands- Degeneriaceae.

Endemic genera:

Unlike families, endemic genera and species occur in isolated or restricted areas. The important islands and geographical regions with high degree of endemic genera are as follows (after Takhtajan 1986)-

Madagascar island- 350 endemic genera

Fiji and adjacent islands- 400 endemic genera

New Caledonia islands- 130 endemic genera

South Africa- 200 endemic genera

India- 142 endemic genera

Australia- 550 endemic genera

Mediterranean region- 150 endemic genera.

Endemic Categories:

Paleoendemism- a species which was once widespread has been reduced to a much smaller range. The restriction of species in a pocket is due to physical barrier like deserts mountain, sea, etc. or change in climate or soil type etc.

The main characteristics are-

a) They are taxonomically isolated components which have no closely related species.
b) Presence of similar life forms in isolated taxa on islands and tops of mountains
c) Low level of polyploidy in endemic plant species of the area
d) Possible fossil evidence
e) Major disjunction in the distribution of many of the endemic plant species.

Neoendemism

On the opposite hand, new species are branching off the evolutionary tree every day. These species are both endemic and indigenous to the location in which they first appeared. They are restricted to a geographical location simply because that is where they started. This is known as neoendemism. There are many species, found on islands, which show this form of endemism.

e.g., *Senecia combrensis.*

A lot of work is going on Neoendemics. On the basis of cytotaxonomic studies Favager and Contandriopoulis (1961) differentiated 3 types of neoendemics.

(a) Schizoendemics:

Derived from or having given rise to a more widespread taxon of same chromosome number.

(b) Patroendemics:

Restricted diploids which have given rise to widespread polyploids.

(c) Apoendemics:

Restricted polyploids which have arisen from widespread diploids.

Holoendemics

According to Richardson (1978) endemics is intermediate between the two extremes i.e., plants which are not of recent origin but have retained a narrow distribution and he called them Holoendemics.

Epibiotics or Relic, endemics:

The plants belong to fossil groups and are restricted to few pockets due to favourable climate, lack of competition e.g., *Ginkgo biloba* which is restricted to China but widely spread in the north temperate zone as a fossil, *Sequoiadendron giganteum* is now restricted to Californian Sierra Nevada.

There is a great confusion in the terms endemic, rare, relicts etc. All endemics are not relicts as there are a larger number of Neoendemics. All endemics are not rare as some are abundantly present in the particular locality. All rare plants are not endemics. Some may occur at several places, with few representatives.

Theories of Endemism:

There are 2 main theories of Endemism. The first theory believes that the last survivors of once flourishing flora which is now declining are the relics or epibiotics which are endemics.

However, second theory believes that these are recent and youthful forms in course of gradual extinction. The theory is also known as Age and Area hypothesis.

The first theory is supported by Geographers e.g., *Sequoia semipenirens* of the central Valley of California and Oregon and *S. gigantea* of Sierra Nevada which are endemic to their respective native homes, were extensively distributed in Cretaceous and Tertiary periods.

The supporters of second theory have the examples of *Primula, Impatiens Rhododendron* etc. According to this theory, Area is directly proportional to its age in the scale of evolution.

So, a small area of distribution shows relatively young in age e.g., Coleus is distributed on the summit of the dry Ritigala mountains in Sri Lanka, with two species *C. elongatus* and *C. barbatus. C. elongatus* is endemic and *C. barbatus* is widely distributed in tropical Asia and Africa. Willis believed *C. elongatus* to be derived from *C. barbatus*.

5. Factors Responsible for Endemism:

Factors responsible for the production of endemics are Natural crossing among the closely related plants growing under favourable conditions and Mutations. If the condition of isolation is developed the effect become more pronounced.

Endemism is found in isolated e.g., islands, isolated areas etc. According the Wulff 80% of Hawaii islands and 72% of New Zealand is endemic. Mountains also have more endemic species as they are isolated e.g., 70% sp. of Himalayas is endemic. Climate also is one of the factors e.g., North of Himalaya is dry plateau of Tibet and South Himalayan range has alluvial fertile soil.

According to Chatterjee the percentage of endemic species of Dicot plants in India is more than 50. Maximum endemic plants are found in the Himalayas and South India. Indo-Gangetic plains have a very small number of endemic species.

Areas of Endemism in India:

The knowledge about the distribution of species, as well as the geographical patterns, constitute crucial information for biodiversity conservation. Because of this, the study of both species distribution and the mechanisms that give them rise have increased since the awareness of biodiversity crisis. In the last few years, endemicity has acquired importance in conservation biology since it is considered an outstanding factor for delimitation of conservation areas. Due to its particular history and its huge biodiversity, India is interesting from a biogeographical point of view. Numerous contributions have been made to address diverse aspects of the distribution of Indian biota

Himalayas:

The eastern and western Himalayan mountain ranges are biologically rich and possess high degree of endemism. This region is considered as one of the mega biodiversity regions of the world (Heywood, 2000). The high altitude ranges of Himalayas in north, Indo-Gangetic regions and Brahmaputra basin on the south, and arid and semi-arid regions of West and North-west acts geographical barriers to check the migration of species from other phytogeograpic regions and vice vera which favour rich and varied biotic potential (Chowdhery & Murti, 2000). The Eastern Himalaya covering the states of Arunachal Pradesh, Sikkim and Darjeeling district of West Bengal is the richest of the phytogeographic regions of India with highest plant diversity (Rao, 1993; Hajra & Mudgal, 1997). 31 Endemic Vascular Plants of India Eastern Himalaya is richer in endemics than the Western Himalaya. No families are endemic to this region, however all the endemics are manifested at species, subspecies or variety level. Western Himalaya contributes 297 endemic taxa while Eastern Himalaya contributes 466 endemic taxa. Western and Eastern Himalaya contributes 7% and 11% of total endemic taxa, respectively. It is also interesting to note that, endemic diversity greatly differ along the altitudinal gradient. Endemics are concentrated in a limited number of taxa, because different taxa respond differently to the process of speciation, hybridization, immigration and extinction (Dhar, 2002). Among the Eastern Himalayan endemic angiosperms, family Orchidaceae with 77 taxa is the most dominant, followed by the family Ericaceae (29 taxa), Lauraceae (27 taxa) and Balsaminaceae (22 taxa).

1. **Western Himalaya:**

Analysis of endemics in Western Himalaya shows that, Central Himalaya comprising the states Uttarakhand and Himachal Pradesh has the maximum number of endemic taxa, while the North-west Himalaya falling within the state of Jammu and Kashmir has about 82 endemic species. The trans-Himalayan region has only 7 exclusive endemic species. Many of the endemic taxa of this region show their eastern extension up to Arunachal Pradesh. In Western Himalaya, maximum endemic taxa are represented by family Asteraceae with 55 taxa, followed by Rosaceae (23 taxa), Apiaceae, Balsaminaceae and Leguminosae - Papilionoideae (16 taxa each) and Poaceae with 15 taxa. A total of 18 families are represented by single species. High concentration of endemic taxa and its peculiar distribution has revealed two areas of endemism in Western Himalaya, i.e., Trans/North-west Himalaya and Central Himalaya.

a) Trans Himalaya including Ladakh:

This includes the drier regions of trans-Himalaya (Leh and Ladakh, Jammu and Kashmir and interior patches of Himachal Pradesh (Lahul, Spiti, Kullu, etc.). The high mountain ranges in the north, semiarid regions on the south, west and warm alluvial Indo-Gangetic plains on the south acts a barrier for migration of species from the north. Some of the important taxa that are found endemic to the Trans Himalaya are *Arnebia bhattacharyyae, Draba alshehbazii, Elymus kuramensis var. stokensis, Senecio ladakhensis* and *Stellaria pinvalliaca*. Some note worthy endemic taxa that are found in Jammu and Kashmir are *Aconitum moschatum, Aralia devendrae, Astragalus gooraiensis, Berberis kashmirana, Dianthus cachemiricus, Draba aubrietoides, Draba tenerrima, Euphorbia thyrsoidea, Impatiens pahalgamensis, Sageretia kashmirensis, Saxifraga chadwellii, Taraxacum banhyhalense*, and *Primula mucronifolia.* Some species that are endemic to Himachal Pradesh are *Artemisia austrohimalayana, Aster molliusculus var. minor, Lactuca lahulensis, Silene kunawurensis, Streptopus chatterjeeanus*, and *Impatiens cothurnoides.*

b) Garhwal and Kumaon:

This is another region of high endemism in Himalaya, which includes the whole state of Uttarakhand, Kumaon and Garhwal. Some of the note-worthy endemic taxa occuring in this region are *Berberis affinis, B. ahrendtii, Carex nandadeviensis, Corydalis boweri, C. magni, Cotoneaster garhwalensis, Cyananthus integer, Delphinium koelzii, Eulophia obtusa, Galium duthiei, Iris duthiei, Ivanjohnstonia jaunsariensis.*

2. Eastern Himalaya:

In the Eastern Himalaya, two areas of endemism, viz., Arunachal Pradesh and Sikkim Himalaya are recognized. Sikkim and Darjeeling Himalaya harbour 208 taxa of endemics while Arunachal Pradesh and adjacent regions harbour 258 endemic taxa.

a) Sikkim Himalaya:

This region comprises the whole state of Sikkim and area under the Darjeeling district of West Bengal. A wide range of climatic variations, high mountain ranges and deep valleys in between provides a number of specialized habitats, where several endemic taxa are found. Dzongri, Green lake, Zema, Muguthang, Tso Lhamo Plateau, Kyngnosla or the Pangola ranges are some of the noteworthy specialized ecosystems of this region.

Nearly 165 species have been named after the state Sikkim, as they were first collected from the state or were known to occur in Sikkim. Some of the representative endemic species of the the region are *Anaphalis hookeri, Acronema nervosum, Actinodaphne sikkimensis, Agrostis sikkimensis, Arenaria thangoensis, Astragalus lachungensis, Berberis umbellata var. branii.*

b) Arunachal Pradesh:

The geographical position, physiography and geological history of Arunachal Pradesh have altogether contributed to high endemism in the relatively younger mountain system. The occurrence of high endemics in a region is suggestive of isolation, centre of speciation, extinction and adaptive evolution of the biota of that region. The genus *Stapletonia* (Poaceae) is exclusively endemic to Arunachal Pradesh. Many of the high mountain ranges such as Vadse hills of Kurung Kumey, Anini and Dalai Valley of Dibang Valley, Lali Ani of Papumpare district, and Zero plateau are some of the important high endemic areas. Some of the important endemic species of Arunachal Pradesh are *Agapetes dalaiensis, Amblyanthus obovatus, Anemone howellii, Begonia scintillans, Berberis dasyclada, Brassaiopsis magnifica, Camellia siangensis, Capparis pachyphylla, Cinnamomum bhaskarii,*

The peninsular India:

The Peninsular India, together with Eastern Ghats along the east, the Western Ghats on the west the Vindhyan range on the north, Indian Ocean on the south and Deccan Plateau in the middle has a characteristic palaeotropic flora derived from the Gondwanaland. Peninsula of Indian region has high degree of endemism. About 2592 taxa of flowering plants are endemic to this region. Most of the endemic plants of Peninsular India are palaeoendemics concentrated in favourable ecological niches on either side of the Western Ghats and Eastern Ghats (Nayar, 1996). On the basis of the analysis of current data on distribution of the endemic plants several centers of endemism in Peninsular India have been identified as below.

a) Western Ghats :

The Western Ghats forms an unbroken chain of mountain ranges along the west coast of Indian peninsula for almost 1600 km (Venu, 2006). The high floristic richness and high concentration of endemic plants in the region has attracted many biogeographers to explore. The region has been dealt under Malabar (Clarke, 1898; Chatterjee, 1939; Puri, 1960; Takhtajan, 1986). Based on the distribution of the endemic species, Western Ghats is divided into two sub-divisions as northern

Western Ghats and southern Western Ghats. An analysis on endemic diversity shows that Western Ghats has 2116 endemic species of which the highest concentration is found in southern Western Ghats with 1278 species while the northern Western Ghats is represented by 354 species. Distribution of 459 taxa is common to both northern and southern Western Ghats

b) The Northern Western Ghats :

The northern Western Ghats forms narrow strips of land mass facing the Arabian Sea lying almost parallel to the coast from mouth of Tapti River to Goa. This is the most homogenous part of the Western Ghats, running almost 750 km parallel to the Arabian sea, while the western boundary is delimited by large peninsula of Kutch and Kathiwar in the north, large plateau by the basaltic formation of the Deccan trap in the west. The altitudes range from 600 to 1500 m with many prominent peaks, Harischandragad (1424 m), Mahabaleshwar (1438 m) and Kalsubai (1646 m). Many isolated conical flat-topped hills with steep sides are the characterstic landscape of the region. Two minor areas of endemism are identified in this region.

c) Southern Western Ghats :

This is a continuous complex hill system comprising Nilgiris, Anaimalai, Palni, Agasthyamalai and Kalakkad hills which provide unique habitats and niches suitable for endemic taxa. On the basis of endemic diversity, southern Western Ghats may be divided in to four subdivisions. The northern most part includes the area between the Coorg to Palakkad gap, secondly the area between the Palakkad gap to Nelliampathy Plateau to Palni hills, thirdly region between Periyar river to Cardamon hills to Kambam valley and the Shencottah pass to Agasthyamalai and Travancore coast (Vajravelu & Vivekananthan, 1996).

d) **Nilgiri-Wayanad-Silent Valley plateau and hills :**

The bizarre geographic location, topography, wide range of altitudinal variation from 700 to 2637 m and marked climatic regimes and significant diurnal range of solar radiation and temperature make this one of the highest rich biodiversity regions of Western Ghats. Almost all important forest types of south India are represented here. This micro area of endemism is a conglomeration of three high-rise plateau, i.e. Nilgiris, Nilgiris-Wayanad, Sigur plateau and outer plains of east and west. Important endemics of Wayanad plateau are *Aglaia canarensis, Arisaema nilamburense, Cynometra beddomei, Desmodium wynaadense, Impatiens nataliae, Justicia wynaadensis, Phyllanthus megacarpa,* and *Tephrosia wynaadensis.*

Deccan:

The Deccan is characterized by its evidences of tertiary flora belonging to Ecocene period. Physiographically, the Deccan plateau consists of two distinctive subdivisions, namely North Deccan and South Deccan. Altogether 312 endemic species belonging to 60 families are distributed in Deccan plateau, which is represented in the Southern and Northern Deccan region in almost equal proportion. Maximum species is represented by the subfamily Papilionoideae with 38 species followed by Cyperaceae (27), Asclepiadaceae (25) and Poaceae (23). As many as 23 families are represented by only single endemic species. The genus *Bhidea, Adenoon, Hardwickia, Indopoa, Lamprachaenium, Leucoblepharis, Lophopogon, Paracaryopsis, Pogonachne, Pseudojacobaea, Trilobachne* and *Triplopogon* are endemic to Deccan.

Eastern ghats:

Unlike Western Ghats, Eastern Ghats does not form a continuous range, thus the species rich zones are isolated by broken hill ranges with plains in between. This is one of the reasons, endemic species of Eastern Ghats show a narrow distribution range as compared to the endemic species of Western Ghats, where a continuous mountain system and uniform humid climatic condition facilitate relatively wider distributional range. A total of 166 endemic taxa, under 117 genera and 43 families are known to occur in Eastern Ghats. These endemic taxa include 120 dicots and 46 monocots. Poaceae have the maximum representation of 22 endemic species followed by Acanthaceae (17 species), Orchidaceae (12 species), Asclepidaceae (9 species); and 18 families are represented by single species. The genus Odisha is exclusively endemic to Eastern Ghats.

a) Vishakhapatnam-Arku-Koraput-Jeypore-Bastar including Sileru Valley:

The northern part of the Eastern Ghats extended over Madgol hills of Visakhapatnam, Arku valley, Koraput and Malkangiri districts of Odisha and culminated near the Jagdalpur of Chattisghar. The hill ranges of this region contain extensive plateau with an altitudinal variation of 300– 650 m. The endemic species, of this region is characterised by very narrow range of distribution. Some of the note-worthy endemic species are *Andrographis ovata, Argyreia arakuensis, Curcuma longa var. vanaharidra, Dimeria orissae, Kalanchoe cherukondensis, Leucas mukerjiana,* and *Strobilanthes jeyporensis.*

b) **Taptapani-Gajapati-Kalinga Hills:**

This region mainly includes southern part of Ganjam, Mohana-Adaba forest divisions of Gajapati district, Pulbani and Kalahandi districts of Odisha. Though this region covers a narrow corridor in the Eastern Ghats, it is rich in plant diversity. *Combretum albidum var. cooperi, Dimeria mooneyi, Eria meghasaniensis, Ficus concinna, Odisha cleistantha, Themeda saxicola, Uvaria eucincta* and *Zeuxine mooneyi* are some of the important endemic angiosperms found in this region.

c) Tirupati-Cuddapah-Nallamalai hills:

This region encompasses southern part from Seshachalam hills downwards up to Madurai with Nallamalais, Cuddapah and Tirupati hill ranges. The southern Eastern Ghats exhibits many important useful endemic plants such as *Shorea tumbuggaia, Cycas beddomei, Pterocarpus santalinus, Argyreia choisyana,* etc. Other interesting endemic plants of Nallamalai hills are *Alysicarpus mahabubnagarensis, Andrographis beddomei, A. glandulosa, Asparagus rottleri, Crotalaria madurensis var. kurnoolica, Croton scabiosus, Euphorbia senguptae,* and *Pimpinella tirupatiensis.*

Islands:

The long geographical isolation and pattern of plant diversity of islands reflects the evolutionary and different ecological processs such as immigration, speciation and extinction. The Andaman and Nicobar group of Islands are another important areas of speciation and endemism in Indian flora. It is a continental Island archipelago lying in north-south direction in Bay of Bengal, stretching from ArakkanYoma in Myanmar to Banda Aceh in Sumatra. Isolation from the neighboring continental land masses and its close proximity to the equator has resulted in the evolution of a rare and distinct flora. The Andaman group consisting of 258 islands and the Nicobar group consisting of 61 islands are separated by a channel of ten degree

a) **Andaman group of Isands:**

The genus Pseudodiplospora with one species is exclusively endemic to Andaman. Some of the important species which are endemic to Andaman group of Islands are *Actinodaphne andamanica, Adenia heterophylla, Aeschynanthus andamanensis, Amomum andamanicum, Ardisia andamanica, Bambusa schizostachyoides, Canthium gracilipes, Codiocarpus andamanicus, Dendrobium tenuicaule, Ficus andamanica, Garcinia andamanica, Garcinia cadelliana, Ginalloa andamanica.*

b) **Nicobar group of Islands:**

The genus Nicobariodendron with one species is endemic to the Nicobar Islands. The other important species that are endemic to the Nicobar group of islands are *Actinodaphne nicobarica, Alseodaphne nicobarica, Artabotrys speciosus, Bentinckia nicobarica, Calamus nicobaricus, C. unifarius, Coptophyllum nicobaricum, Dendrobium shompenii, Eulophia nicobarica, Garcinia calycina.*

Invasion and introduction of plants in India:

An **invasive species** is a species that is not native to a specific location (an introduced species), and that has a tendency to spread to a degree believed to cause damage to the environment, human economy or human health. The criteria for invasive species has been controversial, as widely divergent perceptions exist among researchers as well as concerns with the subjectivity of the term "invasive"

Based on the available databases and some regional reports, nearly 60 invasive plants have been identified from the Indian region, the majority from South and tropical America and Australia, Africa, Europe and even the Asian region. *Arundo donax* - a grass of Indian origin that can pose a major threat to biodiversity owing to its capacity to invade huge areas around water bodies. Most of the invasive plants, irrespective of their origin, belong to the family Asteraceae, while families such as Poaceae, Solanaceae and Fabaceae also predominate. Furthermore, invasive plants belong to a variety of life forms such as herbs, shrubs, trees, climbers/ vines, grasses and aquatic plants. Important invasive plants that have created havoc in a number of habitats include terrestrial herbaceous weeds (e.g. *Ageratum conyzoides* and *Parthenium hysterophorus*), shrubs (e.g. *Lantana camara* and *Chromolaena odorata*), trees (e.g. *Prosopis juliflora* and *Leucaena leucocephala*), vines (e.g. *Mikania micarantha*) and aquatic plants (e.g. *Eichhornia crassipes*). These have entered the alien environment by one of two main pathways - either intentionally to serve some human purpose or accidentally (through import of agricultural/horticultural material, human beings, ballast water, etc.). is perhaps the bestknown example of a serious weed having been intentionally introduced for ornamental value, in this case from tropical America to other parts of the world.

7. Botanic Gardens and Herbaria: Importance, examples from India and abroad.

What is botanical garden?

A **botanical garden** or **botanic garden** is a garden dedicated to the collection, cultivation and display of a wide range of plants labelled with their botanical names. It may contain specialist plant collections such as cacti and other succulent plants, herb gardens, plants from particular parts of the world, and so on; there may be greenhouses, shade houses, again with special collections such as tropical plants, alpine plants, or other exotic plants. Visitor services at a botanical garden might include tours, educational displays, art exhibitions, book rooms, open-air theatrical and musical performances, and other entertainment.

1. **History of botanical garden**

The origin of modern botanical gardens is generally traced to the appointment of professors of botany to the medical faculties of universities in 16th century Renaissance Italy, which also entailed the curation of a medicinal garden. However, the objectives, content, and audience of today's botanic gardens more closely resembles that of the grandiose gardens of antiquity and the educational garden of Theophrastus in the Lyceum of ancient Athens. With the rapid rise of European imperialism in the late 18th century, botanic gardens were established in the tropics, and economic botany became a focus with the hub at the Royal Botanic Gardens, Kew, near London.

Fig- Royal Botanical Garden Kew, London Source: https://www.kew.org/.

Fig- Acharya Jagadish Chandra Bose Indian Botanic Garden Source: http://wahgazab.com/this-old-banyan-tree-looks-like-a-whole-forest-not-a-single-tree/

Over the years, botanical gardens, as cultural and scientific organisations, have responded to the interests of botany and horticulture. Nowadays, most botanical gardens display a mix of the themes mentioned and more; having a strong connection with the general public, there is the opportunity to provide visitors with information relating to the environmental issues being faced at the start of the 21st century, especially those relating to plant conservation and sustainability.

2. Roles of a Botanical Garden

Botanical gardens have been instrumental in motivating several well-known authors to develop their own **systems** of classification while trying to fit the plants grown in the garden, into some previous system of classification, e.g. Linnaeus, while working at Uppsala and Bernard de Jussieu at Versailles. Although the majority of the botanical gardens house plant species which the climate of the area can support, several well-known botanical gardens have controlled enclosures to support specific plants. Tropical gardens often need indoor growing space, **screen houses** for most plants and **glasshouses** for the majority of cacti and succulents in wet tropical and temperate gardens. Glasshouses in temperate gardens often require winter heating. Botanical gardens play the following important roles:

1. **Aesthetic appeal:** Botanical gardens have an aesthetic appeal and attract a large number of visitors for observation of general plant diversity as also the curious plants, as for example, the Great Banyan Tree (*Ficus benghalensis*) in the Indian Botanical Garden at Kolkotta.

2. **Material for botanical research:** Botanical gardens generally have a wide range of species growing together and offer ready material for botanical research, which can go a long way in understanding taxonomic affinities.

3. **On-site teaching:** Collection of plants is often displayed according to families, genera or habitats, and can be used for self-instruction or demonstration purposes.

4. **Integrated research projects:** Botanical gardens with rich living material can support broad-based research projects which can integrate information from such diverse fields as anatomy, embryology, phytochemistry, cytology, physiology and ecology.

5. **Conservation:** Botanical gardens are now gaining increased importance for their role in conserving genetic diversity, as also in conserving rare and endangered species. The Proceedings of the Symposium on Threatened and Endangered species, sponsored by New York Botanical Garden in 1976, published as *Extinction is Forever*, and the conference on practical role of

botanical gardens in conservation of rare and threatened species sponsored by the Royal Botanical Garden, Kew and published as *Survival and Extinction,* are among the major examples of the role of botanical gardens in conservation.

6. **Seed exchange:** More than 500 botanical gardens across the world operate an informal seed exchange scheme, offering annual lists of available species and a free exchange of seeds.

7. **Herbarium and library:** Several major botanical gardens of the world have herbaria and libraries as an integral part of their facilities, and offer taxonomic material for research at a single venue.

8. **Public services:** Botanical gardens provide information to the general public on identification of native and exotic species, methods of propagation and also supply plant material through sale or exchange.

3. Major Botanical Gardens

Thousands of botanical gardens located worldwide are maintained by various institutes.

Of these, nearly 800 important gardens are documented in the *International Directory of Botanical Gardens* published by Henderson (1983). A botanical garden today is an area set aside and maintained by an organization for growing various groups of plants for study, aesthetic, conservation, economic, educational, recreational and scientific purposes. Some of the major botanical gardens are discussed below:

New York Botanical Garden, USA:

This garden was christened the New York Botanical Garden in 1891, when the Torrey Botanical Club adopted its foundation as a corporation chartered by the State. David Hosak founded the garden in 1801 as Algin Botanic Garden. Professor N. L. Britton, the most productive taxonomist of his time, directed the idea of advancement of botanical knowledge through research at this botanical garden. The garden today covers 100 ha. in the heart of New York City along the Bronx River. In addition 778 ha. Mary Flager Cary Arboretum at Millbrook has been added to the jurisdiction of the garden. There are 15,000 species distributed in the demonstration gardens, Montgomery conifer collection, Stout day lily garden, Havemeyer lilac collection, *Rhododendron* and *Azalea* collection, Everett rock garden, herb garden, rose garden, arboretum and conservatory complex. The garden plays a major role in conservation of rare and endangered species. The garden has a well-maintained herbarium of over 5 million specimens from all over the world, but mainly from the New World. The library houses over 200,000 volumes and over

500,000 items (including pamphlets, photographs, letters, etc.). It also maintains a huge botanical database.

Royal Botanic Gardens, Kew:

More popularly known as 'Kew Gardens', this historical garden is undoubtedly the finest botanical garden and botanical research and resource centre in the world. The garden was developed in the 1600s by Kew House owned by Richard Bennet. The widow of the Prince of Wales commissioned the garden in 1759 and William Aiton took over as its superintendent. Sir Joseph Banks introduced large collections from different parts of the world. In 1841, the management of the garden was transferred from the crown to the parliament and Sir William Hooker became its first official director. He was mainly responsible for the advancement of the garden, enlarging it from a mere 6 ha. to more than 100 ha. and building a palm house. Originally the garden covered an area of 120 ha. The Royal Botanic Gardens Kew has directed and financed its development so that **Wakehurst Place** now makes a vital contribution in maintaining the international reputation of the Living Collections Department (LCD). The living collections at Kew are most diverse with 351 families, 5465 genera and over 28,000 species growing successfully. The arboretum covers the greatest area with large mature temperate trees. Tropical plants are maintained indoors, including Aroid House, Palm House, Filmy Fern House etc. Several interesting plants such as *Victoria amazonica* from South America and *Welwitschia mirabilis* from Angola are also growing here. Kew Herbarium, undoubtedly the most famous herbarium of the world, maintains over 6 million specimens of vascular plants and fungi from every country in the world. There are over 275,000 type specimens as well. The library at Kew is very extensive with over 750,000 books and journals a resource for all Kew's research work. *Kew Bulletin* and *Index Kewensis* are its two continuing premier publications. Kew maintains databases on plant names, taxonomic literature, economic botany, plants for arid lands and on plant groups of special economic and conservation value. Kew also makes about 10, 000 identifications a year through its Herbarium service and provides specialist advice on taxonomy and nomenclature in difficult cases.

***Missouri Botanical Garden, USA*:**

Considered one of the top three botanical gardens in the world, the Missouri Botanical Garden is a National Historical Landmark and a centre for botanical research, education and horticultural

display. The garden was founded by an Englishman Henry Shaw and opened to public in 1859 with active help from Asa Gray and Sir William Hooker and Enelmann. Today, the garden covers 79 acres and operates the world's most active tropical botany research programme. The Missouri Botanic Garden is one of the world's leading research centres for botanical exploration and research, with nearly 25 major flora projects. The information is shared via website **TROPICOS,** the world's largest database, containing more than 920,000 scientific plant names and over 1,800,000 specimen records. During the last five years, the herbarium has added an average of 120,000 mounted specimens per year to its collection. In addition to the many gift specimens sent to the specialists, this herbarium loans an average of 34,000 specimens annually, and borrows about 27,000 specimens. The herbarium staff also provides identifications from their area of expertise. The pace of development of the herbarium can be judged from the fact from being number 13th in the world in 1990 (Woodland, 1991), the herbarium today has risen to number six.

Acharya Jagadish Chandra Bose Indian Botanic Garden, India:

The Acharya Jagadish Chandra Bose Indian Botanic Garden (previously known as Indian Botanic Garden) is situated in Shibpur, Howrah near Kolkata. They are commonly known as the Calcutta Botanical Garden, and previously as the Royal Botanic Garden, Calcutta. The gardens exhibit a wide variety of rare plants and a total collection of over 12,000 specimens spread over 109 hectares. It is under Botanical Survey of India (BSI) of Ministry of Environment and Forests, Government of India.

The gardens were founded in 1786 by Colonel Robert Kyd, an army officer of the British East India Company, primarily for the purpose of identifying new plants of commercial value, such as teak, and growing spices for trade. Joseph Dalton Hooker says of this Botanical Garden that "Amongst its greatest triumphs may be considered the introduction of the tea-plant from China ... the establishment of the tea-trade in the Himalaya and Assam is almost entirely the work of the superintendents of the gardens of Calcutta and Seharunpore (Saharanpur).

A major change in policy, however, was introduced by the botanist William Roxburgh after he became superintendent of the garden in 1793. Roxburgh brought in plants from all over India and developed an extensive herbarium. This collection of dried plant specimens eventually became the Central National Herbarium of the Botanical Survey of India, which comprises 2,500,000 items. During the early years of the garden Joseph Dalton Hooker writes:

It contributed more useful and ornamental tropical plants to the public and private gardens of the world than any other establishment before or since. I here allude to the great Indian herbarium, chiefly formed by the staff of the Botanic Gardens under the direction of Dr. Wallich, and distributed in 1829 to the principal museums of Europe.

Over the years attractive display gardens for the public have been developed and many kinds of plants have been cultivated for scientific observation. During the 1970s the garden initiated a program to introduce improved food plants and other varieties of economic benefit to the people of India.

The Garden was designated the *Acharya Jagadish Chandra Bose Indian Botanic Garden* on June 25, 2009 in honor of Jagadish Chandra Bose, the Bengali polymath, and natural scientist.

The best-known landmark of the garden is The Great Banyan, an enormous banyan tree (*Ficus benghalensis*) that is reckoned to be the largest tree in the world, at more than 330 metres in circumference. It partially inspired the novel Hothouse by Brian Aldiss. The gardens are also famous for their enormous collections of orchids, bamboos, palms, and plants of the screw pine genus (*Pandanus*).

Animals seen inside the Botanic Garden include the Jackal (*Canis aureus*), Indian mongoose and the Indian Fox (Vulpes bengalensis). A large numbers of varieties snakes also found in the garden.

What is herbaria?

It was again Luca Ghini who initiated the art of herbarium making by pressing and sewing specimens on sheets of paper. This art was disseminated throughout Europe by his students who mounted sheets and bound them into book volumes.

Although the herbarium technique was a well-known botanical practice at the time of Linnaeus, he departed from the convention of mounting and binding the specimens into volumes. He mounted specimens on single sheets, storing them horizontally, a practice followed even today. From isolated personal collections, herbaria have grown into large institutions of national and international stature with millions of specimens from different parts of the world. *Index Herbariorum,* edited by Thiers, B. [continuously updated] lists the world's important herbaria. Each herbarium is identified by an abbreviation that is valuable in locating the type specimens of

various species. The major herbaria of the world with approximate number of specimens in the order of importance are listed in the table 5.1. In India Central National Herbarium (CAL) of the Indian Botanic Garden, Botanical Survey of India, Kolkotta has over 1.3 million specimens. The herbarium of Forest Research Institute, Dehradun (DD) and National Botanical Research Institute, Lucknow (LUCK) are other major herbaria in India, with collections from all over the world.

Roles of a Herbarium

From a safe place for storing pressed specimens, especially type material, herbaria have gone a long way in becoming major centres of taxonomic research. Additionally, herbaria also form an important link for research in other fields of study. The classification of the world flora is primarily based on herbarium material and associated literature. More recently, the herbaria have gained importance for sources of information on endangered species and are of primary interest to conservation groups. The major roles played by a herbarium include:

1. **Repository of plant specimens:** Primary role of a herbarium is to store dried plant specimens, safeguard these against loss and destruction by insects, and make them available for study.
2. **Safe custody of type specimens:** Type specimens are the principal proof of the existence of a species or an intraspecific taxon. These are kept in safe custody, often in rooms with restricted access, in several major herbaria.
3. **Compilation of Floras, Manuals and Monographs:** Herbarium specimens are the 'original documents' upon which the knowledge of taxonomy, evolution and plant distribution rests. Floras, manuals and monographs are largely based on herbarium resources.
4. **Training in herbarium methods:** Many herbaria carry facilities for training graduates and undergraduates in herbarium practices, organizing field trips and even expeditions to remote areas.
5. **Identification of specimens:** The majority of herbaria have a wide-ranging collection of specimens and offer facilities for on-site identification or having the specimens sent to the herbarium identified by experts. Researchers can personally identify their collection by comparison with the duly identified herbarium specimens.

6. **Information on geographical distribution:** Major herbaria have collections from different parts of the world and, thus, scrutiny of the specimens can provide information on the geographical distribution of a taxon.
7. **Preservation of voucher specimens:** Voucher specimens preserved in various herbaria provide an index of specimens on which a chromosomal, phytochemical, ultrastructural, micromorphological or any specialized study has been undertaken. In the case of a contradictory or doubtful report, the voucher specimens can be critically examined in order to arrive at a more satisfactory conclusion.

Mounting of Specimens

Pressed and dried specimens are finally mounted on herbarium sheets. A standard herbarium sheet is 29 by 41.5 cm (11½ by 16½ inches), made of thick handmade paper or a card sheet. The sheet should be relatively stiff to prevent damage during handling of specimens. Most of the contemporary specimens are fixed using liquid paste or glue in one of the two ways, however:

(i) Paste or glue is applied to the backside (if distinguishable) of the specimen, which is later pressed onto the mounting sheet and allowed to dry in the pressed condition for a few hours. This method is slower but more economical.

(ii) Paste or glue is smeared on a glass or plastic sheet, the specimen placed on the sheet and the glued specimen transferred to a mounting sheet. This method is more efficient but expensive. The use of methylcellulose as adhesive mixed in a solution of 40% alcohol, instead of pure water was suggested by Tillet (1989) for fixing herbarium specimens. It decreases the drying time and also prevents growth of micro-organisms.

Labelling

An herbarium label is an essential part of a permanent plant specimen. It primarily contains the information recorded in the field diary (Field notebook) at the time of collection, as also the results of any subsequent identification process. The label is located on the lower right corner of the herbarium sheet, with the necessary information recorded on the pre-printed proforma, printed directly on the sheet or on the paper slips which are pasted on the sheets. It is ideal to type the information. If handwritten, it should be in permanent ink. Ball pens should never be used, as the ink often spreads after some years. There is no agreement as to the size of a

herbarium label, the recommendations being as diverse as 2¾ by 4¼ inches (Jones and Luchsinger, 1986) and 4 by 6 inches (Woodland, 1991). The information commonly recorded on the herbarium label includes:

a) Name of the institution
b) Scientific name
c) Common or vernacular name
d) Family
e) Locality
f) Date of collection
g) Collection number
h) Name of the collector
i) Habit and habitat including field notes

An expert visiting a herbarium may want to correct an identification or record a name change. Such correction is never done on the original label but on a small annotation label or determination label, usually 2 by 11 cm and appended left of the original label. This label, in addition to the correction, records the name of the person and the date on which the change was recorded. Such information is useful, especially when more than one annotation label is appended to a herbarium sheet. The last label is likely to be the correct one.

Voucher herbarium specimens of a research study often have authentic information about the specimens recorded in the form of a voucher label.

Filing of Specimens

Mounted, labelled and treated (to kill insect pests) specimens are finally incorporated in a herbarium, where they are properly stored and looked after. Small herbaria arrange specimens alphabetically according to family, genus and species. Larger herbaria, however, follow a particular system of classification. Most herbaria usually follow Bentham and Hooker (British herbaria and most commonwealth countries) or Engler and Prantl (Europe and North America).

Virtual Herbarium

Virtual herbarium is a database of consisting of images of Herbarium specimens and the supporting text, available over the internet. It is a huge advancement in herbarium use and design, coupling physical specimens directly with internet and integrating complete specimen

data, with resources or information generation and retrieval. Although a virtual herbarium cannot exist without a physical herbarium,

8. Biosystematics: Definition, methods, categories, differences with classical taxonomy.

Biosystematics:

The term “Biosystematics” was derived from the term “Biosystematy” and was introduced by Camp and Gilly (1943). It is one of the most dynamic aspects of taxonomy , which is concerned with the study of populations of living plants to establish the variation patterns present.

Biosystematics is simply known as “the study of biodiversity and its origins” and it is an art as much as science. In a broader sense, it is a science through which organisms are discovered, identified, named and classified with their diversity, phylogeny, spatial and geographical distributions. It is a science that provides indispensable information to support many fields of research and beneficial applied programmes. Biosystematics is a synthetic branch which uses the characters and data from many disciplines like morphology, anatomy, cytology, genetics, palynology, embryology, ecology, plant geography, phylogeny, physiology, phytochemistry, evalution and paleobotany. Hence, biosystamatics is an integrative and unifying science.

Biosystematics is the taxonomic application of the genecology, is the study of the genotypic and phenotypic variation of species in relation to the environments in which they occur. It is the union of taxonomy and genetics. The biosystematics mainly concerned with genetical, cytological and ecological aspects of taxonomy and it must involve the studies in the experimental gardens.

Objectives of Biosystematics are to:

1. delimit every natural biotic entity composing biodiversity.
2. provide a more meaningful system of nomenclature.
3. resolve microevolution within biodiversity
4. ascertain genetic relationships among members of biota.
5. provide a more scientific basis for recognition of the infraspecific categories.
6. overall improvisation of traditional taxonomy and optimization of services to other disciplines of science.

Principles and Procedures:

The following general principles are proposed by various biosystematists by experimenting different techniques.

1. **Selection of plant groups:** In this stage we have to select the plant groups for study of variations and micro-evolution.

2. **Collection of samples:** Targeted plant groups samples should be collected from its geographical and ecological ranges.

3. **Cultivation of samples:** Collected samples should be cultivated in experimental gardens, where the polymorphy and characters of races can be studied under uniform conditions, and various statistical methods to be followed.

4. **Observation of variations and collection of data:** The samples should be observed on the grounds of their geography and ecology and note down the continuous and discontinuous variations.

5. **Study of fertility relationships and investigation of cytology:** The samples should be studied in the view of their breeding behavior, barriers and cytology.

6. **Synthesis of results:** This also called as synthetic or encyclopaedic phase of principle. This phase aims to synthetize or coordinate the evidences and information derived from the above prinicples in order to express the taxonomic and evolutionary relationships of plants at different levels of hierarchy. The results especially give more details on levels of species, subspecies, varieties, ecotypes, ecophenes, etc.

Modern step:

Micromolecular Characteristics of use in Biosystematic study

Micromolecules (mol mass<1kDa)

1. Primary metabolites:
   a.Universal- Citric acid, aconitic acid etc.
   b.non universal- Non-protein aminoacids
2. Secondary metabolites :
   a. Phenolic compounds : Flavonoids, Phenolic acids, Coumarins, Xanthones, Quinones etc.
   b. Terpenes: Monoterpenes (Geraniol, menthol, pinene, camphor, Carvone etc.)
      Diterpenes (phytol), Sesquiterpenes (Farnesol),
      Triterpene (Squalene),
      Tetraterpene (Carotenoids),
      Polyterpene (Natural rubber)

c. Tannins: Hydrolysable (Ellagitannins), Condensed (Proanthocyanin, Leucocin)

d. Alkaloids

e. Glucosinolates etc.

Micromolecular Characteristics of use in Biosystematic study

Macromolecules (mol mass>1kDa)

Non-Semantides : Large molecules of Starch, cellulose, lipids

Semantides:

Primary: DNA (Nuclear, Chloroplast, mitochondrial)

Secondary: RNAs (m-RNA, r-RNA, t-RNA, Sn-RNA, RNA I, Cp-RNA & Mt-RNA)

Tertiary: Proteins

Procedure and methods in the study of biosystematics:

The experimental methods are supplementary to herbarium and other classical methods of taxonomy. The methods followed in the experimental taxonomy will enrich the existing system of taxonomy and act as complementary, mutually helpful. These methods give all the essential data in separating and solving a number of problems which may develop in the recognition of plants at the level of species and its real position.

The general technical procedure of experimental taxonomy includes the following steps as proposed by W.B. Turrill (1952). They are

i. Planning: Select taxonomic groups with few taxa and collect the available literature.While selecting, annuals, perennial herbs or shrubs are preferable than woody plants.

ii. Collection of material: The material should be collected ideally from wild and make a fair analysis of wild populations. The samples should be planted in the form of seeds or vegetative propagators in the experimental garden.

iii. Labelling: Label all the stocks and samples which are using for experiment.

iv. Attention: Attention should be made in all the stages of experiments

v. **Scoring & Analysis:** Record the qualitative and quantitative characters and abiotic factors with the help of scoring sheets. The obtained scores should be analyzed and preserved.

vi. Harvesting: The dry fruits and seeds of the samples can be collected and stored for future experiments.

Methods:

The following methods are covering all the aspects of experimental taxonomy:

Method 1: It includes careful sampling and analysis of the taxonomic species. The population of taxon, its geographical range, palynology, anatomy, cytology, chromosomal number, phytochemistry, and natural behavior of taxon should be observed and studied for finding genetic variations that may arise between different populations.

Method 2: It involves determination of capability of dissimilar populations to interbreed between one another to form a variant species with vigor and fertility of it. This will reveal the existence or absence of breeding barriers among taxa at several levels.

Method 3: It includes the study of likeness of chromosomes in the hybrids throughout meiosis. The information acquired from the above studies is compared with the data acquired by comparative morphology and geographical distribution resulted in the identification and recognition of a total range of species.

Biosystematics categories:

Biosystematists have developed a classification for experimentally investigated natural taxa, based on the data from various fields of botany such as morphology, cytogenetics, ecology etc. The four most widely accepted biosystematics categories form a hierarchy of ascending units are ecotype, ecospecies, coenospecies and comparium.

The widely accepted categories are as follows:

1. **Ecotype:** The ecotype is the basic unit in biosystematics. It is a phyletic unit “adopted to a particular environment but capable of producing fully fertile hybrids with other ecotypes of the same ecospecies”. Ecotype is considered as equal to subspecies of classical taxonomy.

Ecotype is a genetic variant within a species that is adapted to a particular environment yet remains interfertile with all other members of the species. Edaphic, climatic and biotic ecotypes are generally recognized by the biosystematists.

2. **Ecospecies:** Ecospecies is a group of plants having one or more ecotypes, within the cenospecies, whose members are able to interchange their genes without detriment to the offspring. The term 'ecospecies' coined by Turresson.

When the ecospecies is morphologically distinguishable from others, it corresponds to the taxonomic species. So, ecospecies is also a unit of classification and it is considered as equal to species of classical taxonomy.

3. **Cenospecies:** Cenospecies is a group of plants that representing one or more ecospecies of common evolutionary origin, so far as morphological, cytological and experimental facts indicate. Cenospecies of similar comparium is separated through genetic barriers and all hybrids among them are sterile.

If one or more ecospecies are included in a cenospecies, which is frequently equivalent of subgenus or genus of classical taxonomy.

4. Ecophene: Ecophene is the lower term coined by Turresson, which denotes an ecological variant, purely product of environmental modification of the phenotype. At present the term 'ecad' by F.M. Clements or 'habitat modification' are more often using for such variations.

5. Comparium: Comparium is created of one or more coenospecies which is not capable of intercross. It is biosystematic unit that often is comparable to the genus of classical taxonomy. Distinct comparia are unable to intercross and complete genetic incompatibility prevails between them.

6. Biotype: The biotype consists of all the individuals having the same genotype. Biotypes arise due to mutations, hybridization and isolations. Generally ecotypes are developing from biotypes.

Differences between Classical Taxonomy and Biosystematics:

Classical Taxonomy	**Biosystematics**
It is classical in nature totally based on morphology and to some extent based on anatomy	It is modern systematics primarily based on data from different field like genetics, ecology, morphology, anatomy, chemotaxonomy etc.
Study of variation is based on comparison with type. i.e. the variation is typologically established.	Variation is established on the basis of population sampling in field, it has morphological, genetical and ecological basis.
This is useful mainly in identification of plants for academic.	This is useful delimiting natural biotic units on the basis of experimental findings.

Easier, do not need much money, expertise, sophisticated laboratories. It is quick and less expensive.	Time consuming, sophisticated and expensive.
Basic unit is “Species”	There is no basic unit. Categories are ecotype, ecospecies, coenospecies and comparium.
Studied samples are usually dead	Studied samples are usually living
Process is essentially static.	Process is essentially static.

9. Numerical Taxonomy: Definition, principles, logical steps, applications, merits and demerits.

Numerical taxonomy

Numerical taxonomy received a great impetus with the development and advancement of computers. This field of study is also known as mathematical taxonomy and phenetics. It was Michel Adanson, a French botanist, who for the first time put forward a plan for assigning numerical values to the similarity between organisms and proposed that equal weightage should be given to all the characters while classifying plants.

He used as many characters as possible for the classification, and such classifications came to be known as **Adansonian classifications.** The modern methods of numerical taxonomy had their beginning from the contributions of Sneath (1957), Michener and Sokal (1957), and Sokal

Numerical taxonomy as grouping by numerical methods of taxonomic units into taxa on the basis of their character states.

The principles of modern numerical taxonomy developed by Sneath an Sokal (1973) are based on the modern interpretation of the Adansonian principles and as such are termed neo-Adansonian principles.

These **principles** of numerical taxonomy are enumerated below.

1. The greater the content of information in the taxa of a classification and the more characters it is based upon, the better a given classification will be.
2. A priori, every character is of equal weight in creating natural taxa.

3. Overall similarity between any two entities is a function of their individual similarities in each of the many characters in which they are being compared.

4. Distinct taxa can be recognized because correlations of characters differ in the groups of organisms under study.

5. Phylogenetic inferences can be made from the taxonomic structures of a group and also from character correlations, given certain assumptions about evolutionary pathways and mechanisms.

6. Taxonomy is viewed and practiced as an empirical science.

7. Classifications are based on phonetic similarity.

Logical steps:

Step 1.Taxa-Operational Units:

The first step in data analysis involves the selection of Taxa for data collection, often called Operational Taxonomic Units (OTUs) in Taxometrics. Although it would be ideal to select different individuals of a population, practical considerations make it necessary to select the members of the next lower rank.

Step 2. Unit Characters:

A taxonomic character is a characteristic that distinguishes one taxon from another. Numerical taxonomists defines character (Michener and Sokal, 1957) as a feature, which varies from one organism to another.

e.g.-By this second definition, flower colour (and not white flower or red flower) is a character, and the white flower and red flower are its two **character-states**.

When selecting a character for numerical analysis, it is important to select a **unit character**, which may be defined as a taxonomic character of two or more states, which within the study at hand cannot be subdivided logically, except for the subdivision brought about by the method of **coding.**

Binary and multistate characters:

The characters most suitable for computer handling are two-state (binary or presence/absence) characters (habit woody or herbaceous). However, all characters may not be two-state. They may be qualitative multistate (flowers white, red, blue) or quantitative multistate (leaves two, three, four, five at each node). Such multistate characters can be converted into two-state (flowers white or coloured; leaves four or more vs leaves less than four).

Step 3. Estimation of Resemblances:

Most phenetic methods involve taxon-to-taxon distance, similarity or dissimilarity measures. Distance and dissimilarity are sometimes treated as the same thing, though a distinction can be made between them. As the name implies, distance and dissimilarity measure increase with dissimilarity between taxa, while similarity measures decrease with dissimilarity.

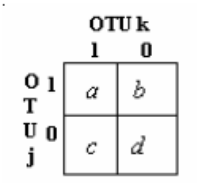
Thus the resemblance between two OTUs is estimated or measured either:

a. In terms of similarity i.e., percentage of characters in which they agree, or

b. In terms of dissimilarity i.e., percentage of characters in which they do not agree.

Character Weighting and Coding:

The coding of character states is done by assigning non-negative integer values. Binary characters are conveniently assigned 0 and 1 for two states. If possible to distinguish, plesiomorphic state is assigned 0 and apomorphic state 1 code.



Number of matches $m = a + d$
Number of positive matches a
Number of mismatches $u = b + c$
Sample size $n = a + b + c + d = m + u$
j and **k** are two OTUs under comparison

Measure of similarity:

Once the data have been codified and entered in the form of a matrix, the next step is to calculate the degree of resemblance between every pair of OTUs. A number of formulae have been proposed by various authors to calculate similarity or dissimilarity (taxonomic distance) between the OTUs.

Some of the common formulae are discussed below:

Simple matching coefficient

This measure of similarity is convenient and highly suitable for data wherein 0 and 1 represent two states of a character, and 0 does not merely represent the absence of a character-state. The coefficient was introduced by Sokal and Michener (1958). The coefficient is represented as:

$$S_{SM} = \frac{\text{Matches}}{\text{Matches} + \text{Mismatches}}$$

$$\frac{m}{m+u}$$

Jaccard Coefficient of association

The coefficient was first developed by Jaccard (1908) and gives weightage to scores of 1 only. This formula is thus suitable for data where absence-presence is coded and 1 represents the presence of a particular character-state, and 0 its absence. The formula is presented as:

$$S_J = \frac{a}{a+u}$$

Where, a stands for number of characters that are present (scored 1) in both OTUs . This can similarly be represented as a percentage similarity.

Taxonomic distance

Taxonomic distance between the OTUs can be easily calculated as a value 1 minus similarity or 100 minus percentage similarity. It can also be directly calculated as Euclidean distance using formula proposed by Sokal (1961):

$$\Delta \mathbf{jk} = \left[\sum_{i=1}^{n} (X_{ij} - X_{ik})^2 \right]^{1/2}$$

The average distance would be represented as:

$$d_{jk} = \sqrt{\frac{\Delta^2_{\mathbf{jk}}}{n}}$$

Step 4. Cluster Analysis:

Cluster analysis or clustering is a type of multivariate statistical analysis. It is used to group organisms into separate clusters based on their statistical behaviour. The main objective of clustering is to find similarities between organisms, and then group similar organisms together to assist in understanding relationships that might exist among them.

Thus, different OTUs are grouped together on the basis of degree of similarity and these groups of OTUs are called clusters.

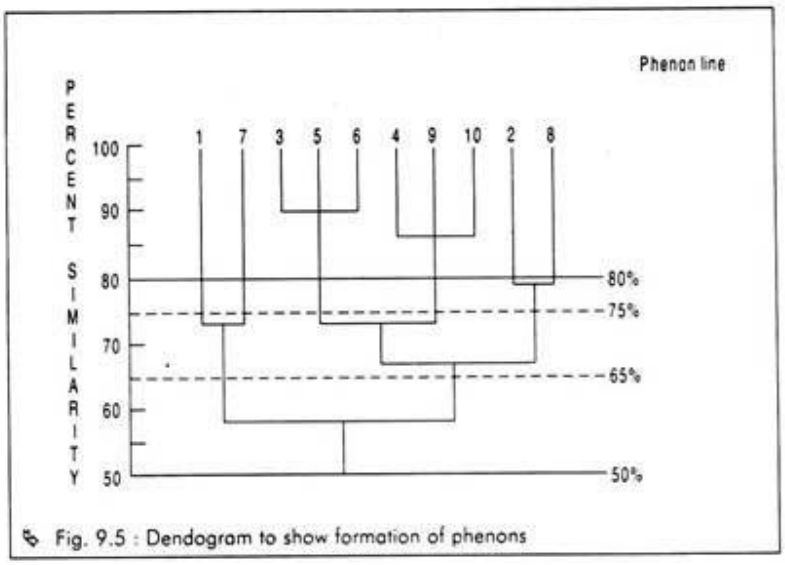


Fig. 9.5 : Dendogram to show formation of phenons

Source: http://www.biologydiscussion.com/plant-taxonomy/construction-of-taxonomic-groups-4-steps/30536

Merits:

1. Numerical taxonomy has the power to integrate data from a variety of sources such as morphology, physiology, phytochemistry, embryology, anatomy, palynology, chromosomes etc. This is very difficult to do by conventional taxonomy.
2. Considerable automation of the data processing promotes efficiency and the work can be handled by even less skilled workers.
3. Data coded in numerical form can be integrated with existing data-processing systems in various institutions and used for the creation of descriptions, keys, catalogues, maps and other documents.
4. The methods, being quantitative, provide greater discrimination along the spectrum of taxonomic differences, and can provide better classifications and keys.
5. The creation of explicit data tables for numerical taxonomy necessitates the use of more and better described characters, which will necessarily improve conventional taxonomy as well.
6. The application of numerical taxonomy has posed some fresh questions concerning classification and initiated efforts for re-examination of classification systems.
7. A number of biological and evolutionary concepts have been reinterpreted, thus introducing renewed interest in biological research.

Demerits:

1. The numerical methods are useful in phenetic classifications and not phylogenetic classifications.

2. The proponents of “biological” species concept, may not accept the specific limits bound by these methods.

3. Character selection is the greatest disadvantage in this approach. If characters chosen for comparison are inadequate, the statistical methods may give less satisfactory solution.

4. According to Steam, different taxonometric procedures may yield different results. A major difficulty is to choose a procedure for the purpose and the number of characters needed in order to obtain satisfactory results by these mechanical aids. It is necessary to ascertain whether a large number of characters would really give satisfactory results than those using a smaller number.

Applications of Numerical Taxonomy:

Numerical taxonomy has been successfully applied in the following studies:

a. Study of similarities and differences in bacteria, other micro-organisms and several animal groups.

b. Delimitation of several angiospermic genera like Oryza, Sarcostemma Solarium, and other groups including Farinosae of Engler and a few others.

c. In the study of several other angiospermic genera including Apocynum, Chenopodium, Crotalaria, Cucurbita, Oenothera, Salix, Zinnia, wheat cultivars, Maize cultivars, etc.

d. Phytochemical data from seed protein and mitochondrial DNA RELP studies has been numerically analyzed by Mondal et al. to study the interspecitic variations among eight species of *cassia* L .Based on the results of electrophoretic patterns, the degree of pairing affinity (PA) or similarity index was calculated Separate dendograms expressing the average linkage were computed using the cluster method UPGMA.

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10. Evolutionary concept ; Basic idea about following terms - Plagiomorphy and Apomorphy; Parallelism and Convergence; Homology and Analogy; Monophyly and Polyphyly including the concept of Heterobathmy, Cline, Polarity, Anagenesis and Cladogenesis, Symplesiomorphy, Synapomorphy, Autopomorphy, Stasigenesis, Catagenesis, Paraphyly, Holophyly, Homoplasy; Phylogram, Dendrogram and Cladogram.

Cladogram:

Phylogeny is commonly represented in the form of a **cladogram**, or phylogenetic tree, a branching diagram that conceptually represents the best estimate of phylogeny. The lines of a cladogram are known as **lineages or clades.** Lineages represent the sequence of ancestral-descendant populations through time, ultimately denoting descent.

Cladograms have an implied, but relative, time scale. Any branching of the cladogram represents lineage divergence or diversification, the formation of two separate lineages from one **common ancestor**. (The two lineages could diverge into what would be designated separate species, the process of forming two species from one termed speciation.) The point of divergence of one

clade into two (where the most common ancestor of the two divergent clades is located) is termed a **node**; the region between two nodes is called an **internode**.

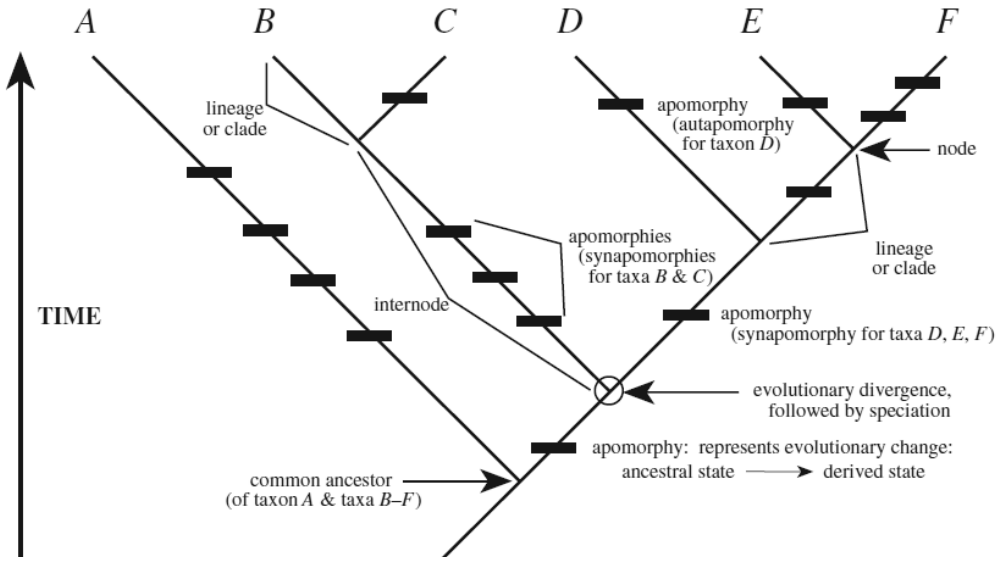
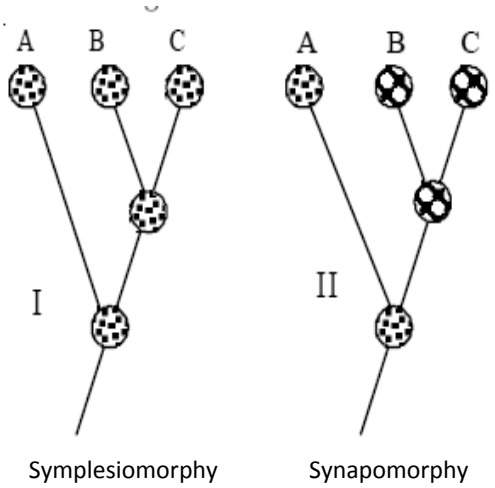


Fig- Eudicots cladogram, Source: Simpson, G. Plant Systematics, 2006.

Evolution may occur within lineages over time and is recognized as a change from a preexisting ancestral (also called **plesiomorphic** or primitive) condition to a new, derived (also called **apomorphic** or advanced) condition. The derived condition, or apomorphy, represents an evolutionary novelty. When two or more taxa that are not nested within each other share a plesiomorphy, it is a **symplesiomorphy**

An **apomorphy** that unites two or more lineages is known as a s**ynapomorphy** (syn, together); one that occurs within a single lineage is called an **autapomorphy** (aut, self). However, either may be referred to simply as an **apomorphy**.



Source: Singh, G. Plant Systematics: An Integrated Approach (3rd ed.), 2016.

Homology and Analogy:

Simpson (1961) defined homology as the resemblance due to inheritance from a common ancestry.

Mayr (1969) similarly defined homology as the occurrence of similar features in two or more organisms, which can be traced to the same feature in the common ancestor of these organisms Analogy represents functional similarity and not due to inheritance from a common ancestry.

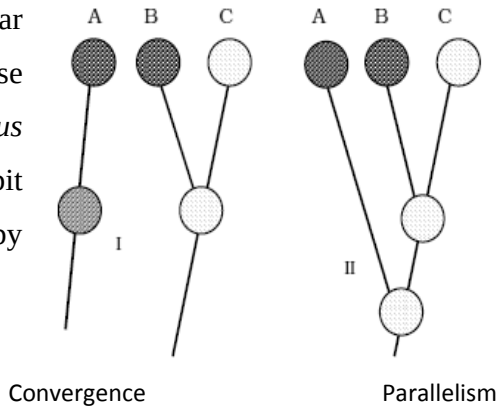
Homoplasy:

Similarity between taxa can arise not only by common ancestry, but also by independent evolutionary origin. Similarity that is not the result of homology is termed homoplasy (also sometimes termed analogy). Homoplasy may arise in two ways: convergence (equivalent to parallelism, here) or reversal.

Parallelism and Convergence:

Parallelism as the independent occurrence of similar changes in groups with a common ancestry, and because they had a common ancestry. The two species *Ranunculus tripartitus* and *R. hederacea* have a similar aquatic habit and dissected leaves and have acquired these characters by parallel evolution.

Convergence is the independent evolution of a similar feature in two or more lineages. Thus, liverwort gametophytic leaves and lycopod sporophytic leaves evolved independently as photosynthetic appendages; their similarity is homoplasious by convergent evolution.



Source: Singh, G. Plant Systematics: An Integrated Approach (3rd ed.), 2016.

Monophyly

A very important concept in phylogenetic systematics is that of monophyly, or monophyletic groups. a **monophyletic group** is one that consists of a common ancestor plus all descendants of that ancestor. The rationale for monophyly is based on the concept of recency of common ancestry. Members of a monophyletic group share one or more unique evolutionary events; otherwise, the group could not generally be identified as monophyletic.

Hennig (1966) defined a monophyletic group as a group of species descended from a single ('stem') species, and which includes all the descendants from this species.

A monophyletic group is separated by a single cut below the group, i.e. it represents one complete branch.

Paraphyly

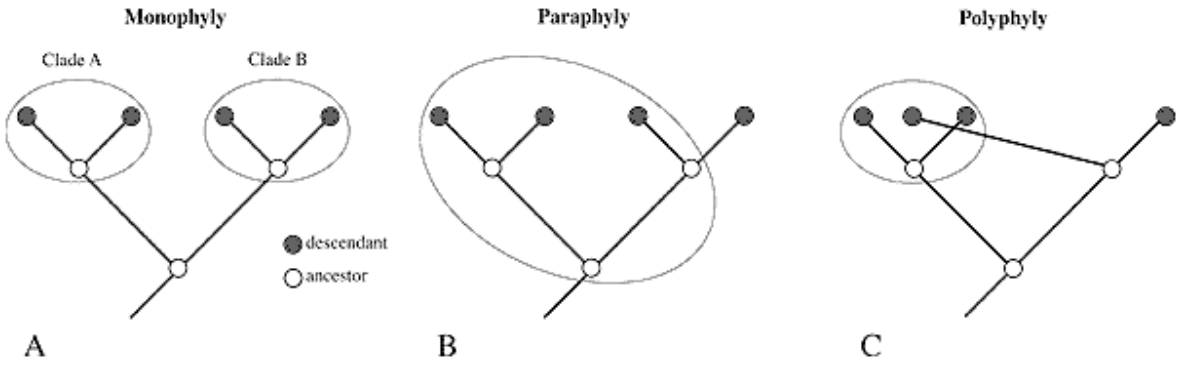
A **paraphyletic group** is one that includes a common ancestor and some, but not all, known descendants of that ancestor. Paraphyletic group do not contain all descendants of the most recent common ancestor of the group.

Paraphyletic group is separated by one cut below the group and one or more cuts higher up, i.e. it represents one piece of a branch.

Polyphyly

A **polyphyletic group** is one containing two or more common ancestors. Polyphyletic group, is one whose most recent ancestor is not cladistically a member of that group.

A polyphyletic group, on the other hand, is separated by more than one cut below the roup, i.e., it represents more than one piece of a branch.



Source: http://training.scicomp.jic.ac.uk/docs/phylogenetics_course_book/glossary.html.

Holophyletic groups are those when all descendants of the most recent common ancestor are contained in the group.

Heterobathmy:

Certain groups of angiosperms have a combination of plesiomorphic and apomorphic characters, a situation known as heterobathmy.

e.g.- *Tetracentron* has primitive vesselless wood but the pollen grains are advanced, being tricolpate.

Polarity

The designation of relative ancestry to the character states of a transformation series/morphocline.

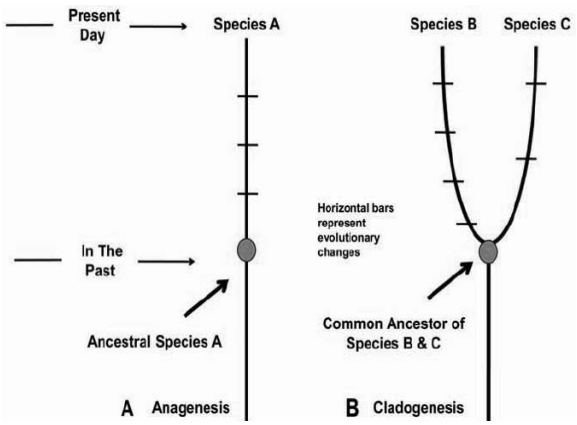
For example, for the character ovary position, with character states superior and inferior , if a superior ovary is hypothesized as ancestral, the resultant polarized morphocline would be superior ⇒inferior.

Morphocline/cline:

A graded series of character states of a homologous character. The hypothesized sequence of evolutionary change from one character state to another. It is also called as transformation series.

Anagenesis and Cladogenesis:

Anagenesis is an progressive evolution defined by a gradual change that occurs in a species without the need for splitting. Anagenesis is not defined by speciation, speciation can assist the process of anagenesis. When speciation occurs and different lineages branch off, if progress is made in one direction without extinction or species selection, then anagenesis occurs.



Source:https://www.researchgate.net/publication/274413892_Why_are_Chimps_Still_Chimps.

Cladogenesis is an evolutionary splitting event where a parent species splits into two distinct species, forming a clade. It is related to the concept of types of speciation.

This event usually occurs when a few organisms end up in new, often distant areas or when environmental changes cause several extinctions, opening up ecological niches for the survivors and causing population bottlenecks and founder effects changing allele frequencies of diverging populations compared to their ancestral population.

Catagenesis and Stasigenesis:

Catagenesis is a regressive evolution towards the loss of independent form or control over the environment.

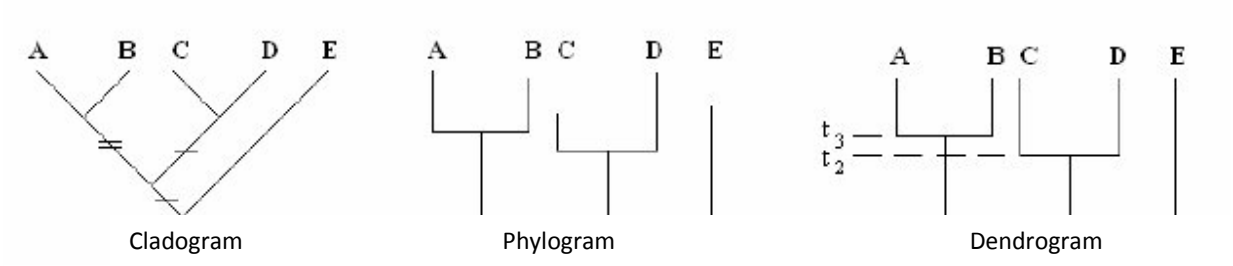
Stasigenesis refers to evolutionary lineage persists through time without splitting or otherwise changing. e.g.-living fossils.

Dendrogram and Phylogram:

A dendrogram (from Greek dendro "tree" and gramma "drawing") is a tree diagram frequently used to illustrate the arrangement of the clusters produced by hierarchical clustering.

Diagrams with vertical axis representing the degree of apomorphy are now more appropriately known as phylograms. The earliest well-known example of such a phylogram is 'Bessey's cactus'.

Phylograms thus not only depict phylogenetic relationships between the groups, they also show the degree of advancement as also the relative number of species in different groups. Such diagrams have been popularly known as **bubble diagrams**.



Source:
https://www.researchgate.net/publication/30072891_Genotype_analysis_and_studies_of_pyrethroid_resistance_of_the_oilseed_rape_Brassica_napus_insect_pest_-_pollen_beetle_Meligethes_aeneus/figures?lo=1

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11. Cladistics system of classifications of Angiosperms: Principles, methods, merits and demerits.

Cladistics:

The cladograms are distinct in the sense that they are developed using a distinct methodology. This method was first proposed by W. Hennig (1950, 1957), a German zoologist who founded the subject of phylogenetic systematics. The term **cladistics** for this methodology was coined by Mayr (1969). An American Botanist, W. H. Wagner, working independently, developed a method of constructing phylogenetic trees, called the groundplan-divergence method, in 1948. Over the years, cladistics has developed into a forceful methodology of developing phylogenetic classifications.

Methodology of Cladistics:

Cladistics is basically a methodology that attempts to analyses phylogenetic data objectively. Cladistic methods are largely based on the **principle of parsimony** (evolutionary route is the shortest hypothetical pathway of changes).

Methodologies of cladistics can be divided into following steps:

Make evolutionary assumptions i.e.

a) **Select OEUs or Operational Evolutionary Units:**

The working units of cladistics are termed as Operational Evolutionary Units (OEUs). It is a unique feature of cladistics. OEUs usually include a **hypothetical ancestor** for comparison with other higher taxa. It is actually a species The entire group of Operational Evolutionary Units is being considered as monophyletic unit. Hypothetical ancestor possess all character in the plesiomorphous state.

b) **Determination of monophyletic groupings** etc, and other terms are as follows:

Clade: A clade is an ancestor and all of its descendents.

Monophyletic: A monophyletic group is a clade.

Paraphyletic: A paraphyletic group is a monophyletic group that excludes some of the descendents, Some cladists discourage the use of this group.

Polyphyletic: A polyphyletic group is a group consisting of memebers from two non-overlapping monophyletic groups. Most Cladists discourage the use of polyphyletic groups.

Outgroup: An outgroup is an organism that is considered not to be part of the group. , but is closely related to the group. The outside taxon is called the outgroups.

Plesiomorphy: A characteristic that is present in both the outgroups and in the ancestors is called a Plesiomorphy (ancestral or primitive state).

Symplesiomorphy: The possession of plesiomorphous character state in common by a group of taxa is termed symplesiomorphy (shared primitive character states).

Apomorphy: A characteristic that occurs only in later descendents is called apomorphy (derived or advanced state).

Synapomorphy: The possession of derived character states in common is termed synapomorphy (shared derived character states).

False synapomorphies: Synapomorphy is usually indicating monophyly, but in some cases it indicate polyphyly due to parallelism and convergence, such cases are called false synapomorphies.

Nested: A clade or species located within another clade is said to be nested within that clade.

Homologous features (or homology): When two species having similar characteristic because it was inherited from a common ancestor, it is called homology.

Analogous features (or homoplasy): When two species have a similar characteristic because of convergent evolution, the feature is called analogous features or homoplasy.

Characters: The observable attributes of organisms which can be examined for similarity or difference are called characters.

Character states: The alternative forms of a character are called character states.

Coding of characters: When presence of one character can be marked as (+ or 1) or absence of that character as (- or 0).

Morphocline: Sometimes any character has more than two character states. Then this series is termed as morphocline and transformation series.

e.g.- 0 ⟷ 1

0 ⟷ 1 ⟷ 2

1 ⟷ 0 ⟷ 2

Sister groups: It is used for outgroup comparison and it a monphyletic group that shares a common direct ancestor.

2. Characters and coding of characters:

This grouping can be subdivided into following categories:

a) Determine or select characters of evolutionary interest. Characters are usually selected for which primitive and derived states can be recognized with confidence.

b) Describe the character states,

c) Select or determine homologous character and character states.

d) Construct character state network.

e) Determine polarity of character states networks. The character state network must be ‘rooted’ to form a character state tree by decoding which state is primitive.

The character states are arranged (ordered) in a sequence of evolutionary transformation.

3. Construct the basic data matrix:

When plesiomorphic and apomorphic character states are accumulated for all EUs, a data matrix t (EUs) × n (characters) is prepared, where '0' represents plesiomorphic character states and '1' is for apomorphic character state of a particular character. The list of EUs in the matrix also includes a hypothetical ancestor as the last row in the matrix, which are all character states of '0'. So, the hypothetical characters possess all characters in plesiomorphic state,

Data matrix table: t (EUs) × n (characters

Characters(n) / EUs (t)	**X**	**Y**	**Z**
Solanum	1	1	0
Physalis	1	1	1
Melongena	0	0	1
Hypothetical ancestor	0	0	0

Divergent index can bemeasured from t × n table. Divergen index for each taxon is the total values of all apomorphic character state of a taxon.

Here divergent index of 4 taxa are as follows:

Taxon	Divergent index
Solanum	2
Physalis	3
Melongena	1
Hypothetical ancestor	0

Measure of Similarity or Measure of Distance from t × n table

A data matrix with coded character-states for each EU can be used for calculating the distance (and consequently, the similarity) between every pair of EUs, including hypothetical ancestor. The distance is calculated as the total number of character state differences between two concerned EUs, the data represented as t × t table.

EUs (t) / EUs (t)	***Solanum***	***Physalis***	***Melongena***	***Hypothetical ancestor***
Solanum	0			
Physalis	1	0		
Melongena	1	2	0	
Hypothetical ancestor	2	3	1	0

Another method of calculating distance involves calculation of the number of apomorphic character states common between the pairs concerned EUs

Data matrix table: t × t matrix presenting distance between EUs expressed as number of apomorphic character-states common between pair of EUs.

EUs (t) / EUs (t)	***Solanum***	***Physalis***	***Melongena***	***Hypothetical ancestor***
Solanum	×			
Physalis	2	×		
Melongena	0	1	×	
Hypothetical ancestor	1	0	0	×

4. Construction of trees or cladograms:

The cladograms can be constructed by various ways or methods. Two methods are very common , such as

i. Henning Argumentation

ii. Wagner Method

There are many other methods in use which are as follows:

i. Ferris parsimony algorithm

ii. Dollo parsimony algorithm

iii. Character compatibility algorithm

iv. Bootstrap analysis

Cladograms may be two types:

1. **Rooted trees:** When the evolutionary polarity is decided in a cladogram, then trees are termed as rooted. Hare ancestral taxon (usually the hypothetical one included among the EUs) is pinpointed. Tree has starting base or point.

2. **Unrooted trees:** When trees are not directional and the evolutionary polarity is not decided, are termed as Unrooted trees.

5. Develop classification or construct classification based on cladograms:

It is the final step of the cladistics analysis as mentioned by Stuessy (1990). There are two basic viewpoints to formation of classification which are:

1. The cladogram should be used directly in constructing the classification and cladograms can be derived from the classification and vice versa.

2. The cladogram should be used as a guide to construct classification and other aspects of phylogeny(such as autapomorphic or phonetic divergence).

Cladograms can not only tell us about relatedness, but also to help us to create a classification. Phylogenetic classification is based on primarily of monophyletic groups and avoids paraphyletic groups or often completely rejects parphyletic groups. Such classificatios are superior over classification based on overall similarity in many aspects which are as follows:

1. It reflects the geneological history more accurately.

2. The classification based on monophyletic groups is predictive and has generates value.

3. It helps understanding distribution patterns, plant interactions, pollen biology etc.

4. It helps considerable in conservation strategies.

Merit:

The impact of cladistics on systematics has been masked that it is superior than both the other two methods in a number of features.

1. The evidence for relationship comes from shared derived characters (synapomorphies). Synapomorphies provide the best evidence for evolution.

2. Organisms should be grouped into monophyletic units.

3. Classifications are hypotheses and they are testable. So cladistics methodology facilitates discussion by a clear presentation of evolutionary assumptions and operational procedures.

4. Cladistic methodology differ from each other primarily in repeatability of the method, Since it is a repeatable method, it is more scientific.

5. Phylogenetic classification is based on assumptions. So two phylogenetic taxonomists independently make the same assumptions; and even if they do, they rarely construct the same classification. Whereas cladist can send his character data to any other cladist and both will construct exactly the same classification.

6. The phonetic classification is not reflecting evolution whereas cladistic classification shows evolution.

7. In a series of papers, Ferris (1977, 79, 80, 83) shows that cladistics methods will produce classification that have more informative and natural than the other system

8. A formal code of phylogenetic nomenclature, the phylocode is currently under development for cladistic taxonomy than others,

Demerits:

1. Cladistic does not include a process for naming of species.

2. It is difficult to understand the essence of a clade.

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12. Data sources of taxonomy: Embryology, photochemistry with brief account of DNA - Taxonomy, DNA - barcoding, e - Taxonomy; nuclear rDNA, chloroplast and mitochondrial DNA; ultrastructure of sieve tube plastids.

Embryology

Embryology has made a relatively lesser contribution in understanding taxonomic affinities. This is primarily because of long preparatory work needed for embryological studies. More often, the study of hundreds of preparations may reveal just a single embryological characteristic of any

significance. It may take many years of laborious and painstaking research to study even a few representatives of a family. The embryological features of major significance include microsporogenesis, development and structure of ovule, embryo sac development, endosperm and embryo development.

Families marked out by distinct

Embryological features

A number of families of angiosperms are characterized by unique embryological features found in all members. These include:

Podostemaceae

Family Podostemaceae includes perennial aquatic herbs, which have a unique embryological feature in the formation of a **pseudoembryo sac** due to the disintegration of the nucellar tissue. The family is also characterized by the occurrence of pollen grains in pairs, bitegmic tenuinucellat ovules, bisporic embryo sac, solanad type of embryogeny, prominent suspensor haustoria, and absence of triple fusion and, consequently, endosperm.

Cyperaceae

Family Cyperaceae is characterized by the formation of only one microspore per microspore mother cell. Following meiosis, of the four microspore nuclei formed, only one gives rise to pollen grain. Besides Cyperaceae, only Epacridaceae in a few members shows the degeneration of three microspore nuclei. Cyperaceae is distinct from these taxa in pollen shedding at the 3-celled stage, as against the 2-celled stage shedding in Epacridaceae.

Onagraceae

Family Onagraceae is characterized by Oenothera type of embryo sac, not found in any other family except as an abnormality. This type of embryo sac is 4-nucleate and is derived from the micropylar megaspore of the tetrad formed.

Specific examples of the role of embryological data

There are a few examples of the embryological data having been very useful in the interpretation of taxonomic affinities:

Trapa

The genus *Trapa* was earlier (Bentham and Hooker, 1883) included under the family Onagraceae. It was subsequently removed to the family Trapaceae (Engler and Diels, 1936;

Hutchinson, 1959, 1973) on the basis of distinct aquatic habit, two types of leaves, swollen petiole, semiepigynous disc and spiny fruit. The following embryological features support this separation:

(i) pyramidal pollen grains with 3 folded crests (bluntly triangular and basin shaped in Onagraceae);

(ii) ovary semi-inferior, bilocular with single ovule in each loculus (not inferior, trilocular, with many ovules);

(iii) *Polygonum* type of embryo sac (not *Oenothera* type)

(iv) endosperm absent (not present and nuclear)

(v) embryo Solanad type (not Onagrad type);

(vi) one cotyledon extremely reduced

Paeonia

The genus Paeonia was earlier included under the family Ranunculaceae (Bentham and Hooker; Engler and Prantl). Worsdell (1908) suggested its removal to a distinct family, Paeoniaceae. This was supported on the basis of centrifugal stamens (Corner, 1946), floral anatomy (Eames, 1961) and chromosomal information (Gregory, 1941). The genus as such has been placed in a distinct monogeneric family, Paeoniaceae, in all modern systems of classification. The separation is supported by the following embryological features:

i. centrifugal stamens (not centripetal);

ii. pollen with reticulately-pittedexine with a large generative cell (not granular, papillate and smooth, small generative cell);

iii. unique embryogeny in which early divisions are free nuclear forming a coenocytic stage, later only the peripheral part becomes cellular (not onagrad or solanad type); and

iv. seed arillate.

Exocarpos

The genus *Exocarpos* (sometimes misspelled *Exocarpus*) is traditionally placed under the family Santalaceae. Gagnepain and Boureau (1947) suggested its removal to a distinct family Exocarpaceae near Taxaceae under Gymnosperms on the basis of articulate pedicel, ‘naked ovule’ and presence of a pollen chamber. Ram (1959) studied the embryology of this genus and

concluded that the flower shows the usual angiospermous character, the anther has a distinct endothecium and glandular tapetum, pollen grains shed at the 2-celled stage, embryo sac of the *Polygonum* type, endosperm cellular, and the division of zygote transverse. This confirms that the genus *Exocarpos* is undoubtedly an angiosperm,

Biochemical systematics or chemosystematics or chemical plant taxonomy or phytochemistry:

What is chemotaxonomy?

This is one of the important and rapidly expanding areas of plant taxonomy. Chemotaxonomy is the application of chemical data or chemical information to systematic problems or to improve the classification of plants. Chemotaxonomy develops as a *hybrid* between the chemistry of natural plant products and systematics.

Purpose of chemotaxonomy:

The information from chemotaxonomy has proved useful at all levels of the hierarchy of classification. There are two main purpose of plant classification.

1. To develop such taxonomic characters which may improve the existing system of plant classification
2. To develop present day knowledge of phylogeny or evolutionary relationships of plants

Reliability of characters

1. All characters of chemotaxonomy are not equally reliable
2. Characters are sporadically present in some taxa
3. Characters of inorganic compounds are less reliable than organic compounds
4. Characters of secondary metabolites are taxonomically significant than primary metabolites.

Causes of rapid growth

The main reasons for rapid expansions are:

1. There are quicker methods of analysis
2. Small amount of plant materials is required for these analysis

Use of chemical criteria in plant taxonomy or chemical characters in plant classification

Chemical characters may be considered under two categories namely,

1. Directly visible characters

a. Starch grains

b. Raphides

c. Silica

d. Lapachol

e. Cystolith

f. Gypsum

2. Non-visible characters

A. Chemical test characters or plant products

I. Primary metabolites: Amino acids, Organic acids, Polysaccharides, Chlorophylls etc.

II. Secondary metabolites: Alkaloids, Phenolic compounds (Flavonoids, Quinones, Coumarins, Phenylpropenes), Terpenoids (Essential oils, Steroids, Saponins, Sesquiterpene lactones), Oils, Fats and waxes.

B. Semantides and Proteins

Semantides are important carrying molecules. DNA is primary semantides, RNA (t-RNA and m-RNA) is secondary semantides and proteins are tertiary semantides.

Sometimes semantides together with larger polysaccharides are known as macromolecules.

DNA ⟶ mRNA ⟶ Protein

(Primary Semantides) (Seondary Semantides) (Tertiary Semantides)

1. Directly visible characters

a. Starch grains:

These are polysaccharide molecules, found in plants, and may be simple or compound, concentric or eccentric and different forms. Grain structure is important in certain tribes of the family Gramineae.

Examples – Eccentric starch grains are found in *Solanum tuberosum*.

Centri starch grains are found in *Pisum sativum*.

Dumbbell shaped in *Opantia*.

b. Raphides:

These are found in certain families like Onagraceae, Rubiaceae, Balsaminaceae. Raphides are crystals of calcium oxalate, usually found within the vacuole of the cell. Shape and size of raphides in different species of Allium are useful for differentiation of species.

Trapa and *Montinia* have been included in earlier classification in the Onagraceae, but lacks raphides. So they should be elsewhere. All the members of Saxifragaceae have raphides,; while, *Hydrangea* which is devoid of them, is better placed in a separate family Hydrageaceae.

c. Silica:

It occurs in large number of dicotyledonous family. Structure of silica is also valuable in Gramineae and Palmae. 20 different forms of silica have been recognized for the classification of grasses.

d. Lapacol:

It is a yellowish powder present in the cells of wood in many plants of Bignomiaceae. Chemically it is known as 2-hydroxy-3-1-4-naphthoquinone. It also exists in *Avicennia* (Verbenaceae) and *Bassia* (Sapotaceae).

e. Cystolith:

It is a crystal of calcium carbonate, usually found in specialized parenchymatous epidermal cells called lithocyst.

f. Gypsum:

Crystals form of $CaSO_4$, $2H_2O$ occurs in some plants. Every member of Tamaricaceae possess these crystals; while, absent in related families like Frankaniaceae and Fonqueriaceae, which have crystals of Ca-oxalate.

2. Non-visible characters

A. Chemical test characters or plant products

These are two types-

I. Primary metabolites:

By products of common metabolic pathways. Most of them are universal in plants and of little taxonomic importance.

a. Distribution of protein, amino acids: Not significant taxonomically but sometimes may have taxonomic significance. Examples – In *Gilgiochloa indurate* (Poaceae), **alanine** is the main amino acid in leaf extracts, **proline** in seed extracts and **asparagine** in seeds extracts.

b. Distribution of polysaccharides: Macleod observed the distribution of water soluble polysaccharides in 22 species of grass and shown that *Festuca* and *Lolium* contain **trisaccharides** and the tribe *Bromeae* have only **fructosans** but lacks **raffinose**.

Storage polysaccharides – Amyloids produced in the genus *Paeonia* among the 30 tested genera from the Ranunculaceae. So the taxonomists have transferred it to a monolytic family Paeoniaceae.

II. Secondary metabolites

These perform non-vital finctions and are less widespread in plants as compared to primary metabolites. These are taxonomically important. Crawford (1989) recognized secondary metabolites as episemantic molecules.

a. **Non-protein amino acids:** There are more than 300 free amino acids found in Angiosperms. Their distribution is not universal but specific to certain groups and is therefore taxonomically significant.

Lathyrine is known from *Lathyrus*.

Canavamine occurs only in the sub-family Lotoideae of Fabaceae. In *Lathyrus*, 7 infrageneric groups and in *Vicia*, 4 infrageneric groups are recognized on the basis of distribution of amino acids. Presence of **cyclopropyl** amino acids in Sapindaceae and Aceraceae show their close relationship.

b. **Alkaloids:** These are organic nitrogen containing bases, often with a heterocyclic ring. These are 5000 alkaloids reported from angiosperms mostly from dicot. Families like

Berberidaceae, Fabaceae, Ranunculaceae and Solanaceae are especially riched in alkaloids species.

The distribution of alkaloids is often specific and thus taxonomically significant.

Morphine is found in poppy (*Papaver somniferum*)

Nicotine is found in *Nicotiana*

Ephedrine is found in *Ephedra*

Benzylisoquinoline alkaloids are found in Apocarpous families of Magnollidae

Isoquinoline alkaloids 'Protopine' are found in Papaveraceae and Fumeriaceae

Papaveraceae and Fumeriaceae are closely related. This affinity is supported by the occurrence of the alkaloid 'protopine; in both.

Nymphaeaceae differs from Nelumbonaceae by the absence of benzylisoquinoline alkaloids.

c. **Phenolic compounds – Betalains:** Betalains are different from the rest of the phenolic compounds due to the presence of nitrogen in them. They are not flavonoids but are functionally equivalent to phenolics. These red (betacyanins) and yellow (betaxanthin) are collectively termed as betalains.

The best example of chemotaxonomy is the classification of Centrospermae or caryophylloles.

Flavonoids: These are phenolic compounds. They function in defense system of plants. More than 500 different flavonoids exist. Flavonoids are found in all plants except for most of the algae. In flowering plants, they are found abundantly in most taxa in vegetative and reproductive organs. Flavonoids are important in systematic work specially valuable at the lower levels of the hierarchy. The common examples of flavonoids are isoflavonoids, anthocyanins, anthoxanthins. Anthocyanins and anthoxanthins are the important pigments in cell sap of petals in many angiospermic families.

d. **Terpenids:** These are used in plant taxonomic studies and use of different types, examples – mono-, di-, tri- sesquiterpenoids. These are ultimately produce steroids. The monoterpenoids are also known as essential oils and are found in many plant families, but they are especially common in the Labiatae, Rutaceae, Umbelliferae and also in the gymnosperms.

Sesquiterpene lactones have been extensively used in studies of some families, specially the compositae (Seaman 1982), where they can occur as much as 2% of the dry weight of the plants. These compounds are principally useful at the lower levels, the taxonomic hierarchy.

Iridoids are another group of terpenoids mostly monoterpene lactones. These are present in more than 50 angiospermic families. Iridoids may occur in several unrelated families eg, Hamamelidaceae and Meliaceae. The occurrence of a distinctive iridoid Aucubin in *Buddleia* has been taken to support its transfer from Logamiaceae to Buddleiaceae. The iridoid compound 'Asperuloside' is the characteristic of the family Rubiaceae; whereas 'ancubin' is found in Orobanchaceae, Scrophulariaceae and Cornaceae.

e. **Lipids:** Lipids are the esters of fatty acids with glycerol. Distribution of some fatty acids is characteristic of some families. For example, petroselinic acid of Umbelliferae, erucic acid of Cruciferae, malvalic acd of Malvaceae and capric acid of Lauraceae and Lythraceae.

f. **Waxes:** These are estersof long chain alcohols with long chain fatty acids. Waxes are chiefly found on the surfaces of leaves and stems. Palmae produce large amount of wax on their leaves. The wax from *Copernicia cerifera* and *Ceroxylon audicola* (stem) is collected and marked in great quantity. Seeds of *Simondsia* have a liquid wax.

Cyanogenic componds:

The aqbility of some plants to release poisonous compounds, such as hydrocyanic acid, amygdalin etc. after injury to their cells, is called cyanogenesis, and these poisonous compounds are called cyanogenic compounds. Over 2000 vascular plants have so far been established as cyanophoric. Some monocottledonous taxa (Gramineae, Araceae, Juncaceae) and dicotyledonous (Asteraceae, Rosidae, Dilleniidae) are cyanogenic.

Proteins:

Electrophoresis of total seed storage proteins yields a series of bands as the proteins separate along the electrical gradient based on the polarities from one taxon to another for an estimate of relationships and the data can be treated phonetically as with other types of information (Crawford and Julian 1976). Protein electrophoresis is helpful for establishing a close relationship between *Vicia* and *Lathyrus*. Symenoidis and Tsekos (1984) suggested that the genus *Taeniantherum*, formerly considered as a part of the genus *Hordeum*, should be treated as an independent genus.

Amino acid sequencing:

Amino acid sequences of cytochrome C, plastocyanin and ribulose bisphosphate carboxylase have been investigated and these are valuable in plants to give a much better view of familial and

ordinal relationships. Data from various fields have pointed to the merger of *Aegilops* with *Triticum*. Autran et al. (1979) on the basis of amino acid sequencing supported this merger.

DNA- taxonomy

DNA taxonomy in the strict sense would refer to the notion that the DNA sequences them serve as the taxonomic reference system. Following this idea, the DNA sequences constitute the catalogue objects, from which the taxonomy objects have to be derived. The latter are groups of sequences which take on a role equivalent to Linnean binomials in the traditional taxonomy (i.e. they serve as the term to which biological information is being associated; see Thiele and Yeates 2002). Given variation between individuals, the grouping procedures are a critical step. Rather than accepting any arbitrary groups, e.g. MOTUs of a particular cut-off, the aim in DNA taxonomy is to identify groups that correspond to entities of reproductively coherent individuals (the species), i.e. to determine a hierarchical level roughly equivalent to the binomials of the traditional system (which are generally considered to represent the true species in nature). While recourse to the Linnean nomenclature provides important evidence for the correct level of the taxonomic hierarchy, it does not follow that the DNA groups are only valid if they correspond precisely to the existing species names; this has been frequently misunderstood in the recent literature (Wheeler 2004; Meyer and Paulay 2005). For some taxa, a growing DNA taxonomy is now available (e.g. Powers 2004; Verbruggen et al. 2005), where sequences serve as the principal means of linking vouchers and collateral information (and hence already represent the primary portrayal of the group for communication). Once in place, the DNA taxonomy also provides a framework for routine identification, and can then serve as the primary database for standard DNA barcoding. Yet, in contrast to DNA barcoding in the strict sense (species identification against a database of mitochondrial coxI sequences), a DNA taxonomy for a particular group of organisms may be based on one or more regions of mtDNA or nuclear DNA, and can be derived from phylogenetic and clustering methods using any gene region (Pons et al. 2006). In addition, the sequences define hierarchical groupings of living organisms into which all species can be included at some level (Tautz et al. 2003; Savolainen et al. 2005). Therefore, the DNA taxonomy can also be useful for fitting unknown or unrepresented species into the database using phylogenetic information.

Procedure

The basic procedures of DNA taxonomy would be straight forward. A tissue sample is taken from a collected individual and DNA is extracted from this. This DNA serves as the reference sample from which one or several gene regions are amplified by PCR and sequenced. The resulting sequences are, as a first approximation, an identification tag for the species from which the respective individual was derived. This sequence is made available via appropriate data bases, together with the species description and other associated information, ideally including its taxonomic status with appropriate references. The sequence now serves as a standard for future reference, together with the type specimen and the respective DNA preparation, which will be deposited in museum collections. Once a significant sequence data base has been built up, new samples can be checked against these existing sequences to assist species re-identification or to assess whether a new species description might be warranted. The data base could also serve to resolve questions about the taxonomic identity of specimens that are derived from larval life stages, or for identification of artefacts from trade with endangered species.

Limitation:

It must be emphasized that the power of DNA sequences for identifying species is limited when species pairs have very recent origins. For some time after the initial split, new sister species will share alleles, either because of ongoing gene flow, or because of recent ancestry. In such cases, sequences from one or few individuals will not be sufficient for an unequivocal assignment to a particular group. There is also a special complication for organelles (mitochondria or chloroplasts), which can occasionally be transferred, at least between closely related species. This could result in different diagnoses, depending on whether one uses a sequence from the nuclear genome or from the organelle genome. The buildup of sequence differences that can serve as unequivocal characters depends on the mutation and fixation rates. The combined rates for neutrally evolving sites are between 0.1% and 2% per million years in nuclear sequences and can reach up to 5% in mitochondria. The random fixation for a new mutation is expected to occur within 4Ne generations for nuclear loci and within 1Ne generations for mitochondria (Ne is the effective population size and measures only the number of reproductively effective individuals, which is usually much lower than the census size). This provides some guideline for assessing how long it will take until one can expect to find a diagnostic difference between newly evolved species after cessation of gene flow.

Application:

1. Naming of species
2. Matching Linnaean names with DNA sequences
3. Role in collection of samples
4. Taxonomy and phylogeny of taxa
5. Need for a new data base

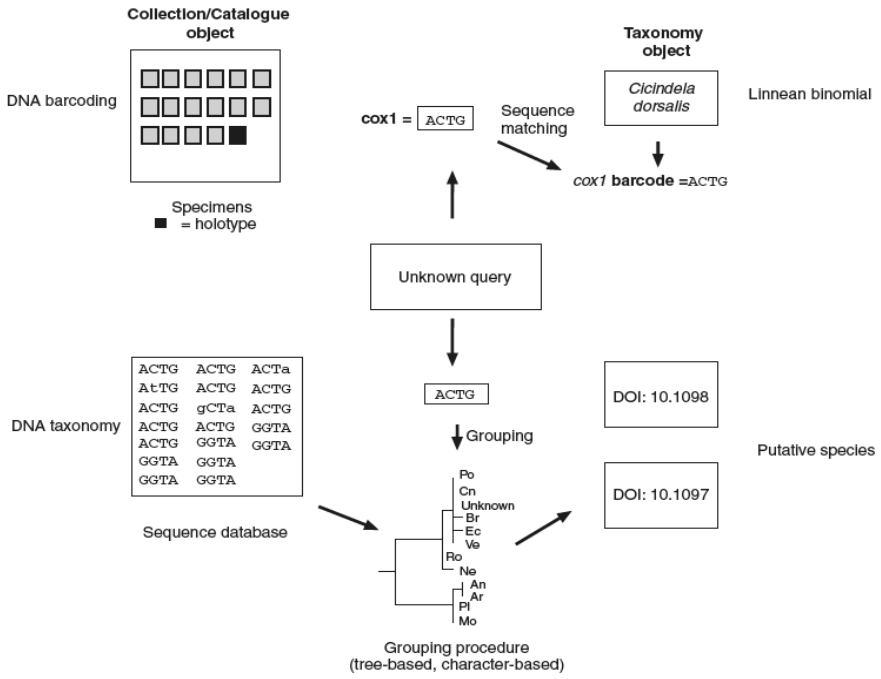

Fig- Schematic illustration of the differences between DNA barcoding and DNA

https://www.researchgate.net/publication/316597985_DNA_Bar-Code_for_Identification_of_Microbial_Communities_A_Mini-Review/figures

DNA Barcoding:

DNA barcoding is a taxonomic method that uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It differs from molecular phylogeny in that the main goal is not to determine patterns of relationship but to identify an unknown sample in terms of a preexisting classification. Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, the utility of DNA barcoding for these purposes is subject to debate. The most commonly used barcode region for

animals and some protists is a segment of approximately 600 base pairs of the mitochondrial gene cytochrome oxidase I (COI or COX1). This differs in the case of fungi, where part of Internal Transcribed Spacer 2 (ITS2) between rRNA genes is used, and again in plants, where multiple regions are used.

Applications include, for example, identifying plant leaves even when flowers or fruit are not available, identifying pollen collected on the bodies of pollinating animals, identifying insect larvae (which may have fewer diagnostic characters than adults and are frequently less well-known), identifying the diet of an animal, based on its stomach contents or faeces and identifying products in commerce (for example, herbal supplements, wood, or skins and other animal parts).

Choice of locus:

A desirable locus for DNA barcoding should be standardized (so that large databases of sequences for that locus can be developed), present in most of the taxa of interest and sequenceable without species-specific PCR primers, short enough to be easily sequenced with current technology, and provide a large variation between species yet a relatively small amount of variation within a species.

Although several loci have been suggested, a common set of standardized regions were selected by the respective committees of COBOL:

- For animals and many other eukaryotes, the mitochondrial COI gene
- For plants, the concatenation of the rbcL and matK chloroplast genes. These provide poor resolution for land plants, and a call was made for regions to be assessed that could complement rbcL and matK.
- For fungi, the internal transcribed spacer (ITS) region

For protists, a final recommendation has not yet been made; a 2012 Working Group report suggests that a 2-stage approach will most likely be required, using a "pre-barcode" based on 18S rDNA followed by a yet to be defined second test according to the result of the first.

Procedure of DNA barcoding:

The process of DNA barcoding involves two basic steps: First step is to build a barcode library of identified species and second is matching the barcode sequence of the unknown sample with the barcode library (known as sequence alignment) for its identification. The first step requires

ecologic expertise in selecting one or several individuals per species as reference samples in the barcode library.

Tissue samples can be collected from live specie in field or from specimen in museum for generating. These specimens go through lab processes that are tissue sampling and DNA processing and sequencing to generate DNA barcode in form of chromatogram. Chromatogram is visual representation of DNA sequence produced by sequencer. This barcode can be stored in database for future use or can be used as query sequence to be compared with sequence already present in database.

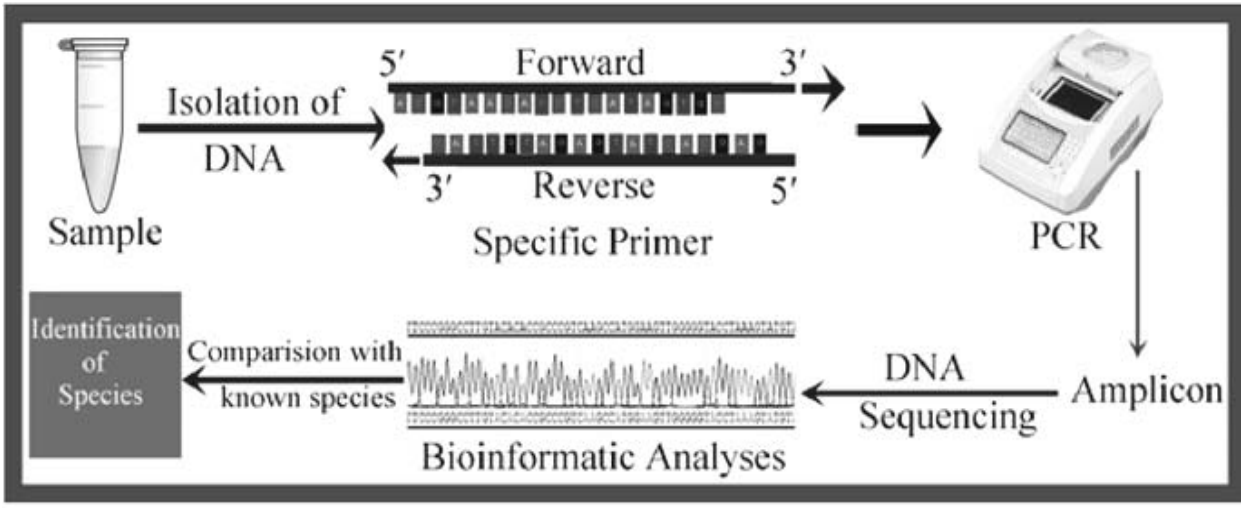

Fig- DNA barcoding procedure

https://www.researchgate.net/publication/316597985_DNA_Bar-Code_for_Identification_of_Microbial_Communities_A_Mini-Review/figures

Applications:

1) Controlling Agricultural Pest

DNA barcoding can help in identifying pests in any stage of life making easier to control them saving farmers from cost of billion dollars from pest damage. The global tephritid barcoding initiative contributes to management of fruit flies by providing tools to identify and stop fruit flies at border.

2) Identifying Disease Vectors

DNA barcoding allows non ecologists to identify the vector species that can cause serious infectious diseases to animals and humans, to understand these diseases and cure them. A global mosquito barcoding initiative in building a reference barcode library that can help public health

officials to control these diseases causing vector species more effectively and with very less use of insecticides.

3) Sustaining Natural Resources

Using DNA barcoding, natural resource managers can monitor illegal trade of products made of natural resources like hardwood trees. Fishbol is reference barcode library for hardwood trees to improve management and conservation of natural resources.

4) Protecting Endangered Species

Primate Population is reduced in Africa by 90% because of bush meat hunting. DNA barcoding can be used by law enforcement to bush meat in local markets which is obtained from bush meat.

5) Monitoring Water Quality

Drinking water is a process resource for living being. By studying organism living in lakes, rivers and streams, their health can be measured or determined. DNA barcoding is used to create a library of these species that can be difficult to identify. Barcoding can be used by environmental agencies to improve determination of quality and to create better policies which can ensure safe supply of drinking water.

6) Routine Authentication of Natural Health Products

Authenticity of natural health products is an important legal, economic, health and conservation issue. Natural health products are often considered as safe because of their natural origin.

7) Identifying of plant leaves even if flowers or fruit are not available

8) Identification of medical plants

E-Taxonomy:

It is an electronic medium to connect users of taxonomy, the taxonomic community with the authors and the taxonomic knowledge base so as to serve the interests as well as get revised and updated by the whole taxonomic community. It uses massive numerical data (metadata) for statistical analysis, modelling and simulation studies.

e.g.- The **EDIT Platform for Cybertaxonomy** is a collection of **open source** tools and services which together cover all aspects of the taxonomic workflow. The workflow is grouped into the following areas: taxonomic editing; publishing of edited data; data storage and exchange; collections and specimens; descriptions; fieldwork; literature; and geography. At the heart of the Cybertaxonomy platform is the Common Data Model (CDM), a repository for every conceivable

type of data produced by taxonomists in the course of their work, and the backend for most EDIT components.

Ultrastructure of sieve tube plastids:

Studies on sieve-tube plastids were first initiated by Behnke (1965) in the family Dioscoreaceae. Since then, nearly all angiosperm families have been investigated for the taxonomic significance of these plastids. All sieve-element plastids contain starch grains differing in number, size and shape. The protein accumulates in specific plastids in the form of crystalloids and filaments. Thus two types of plastids are distinguished: **P-type** which accumulate proteins and **S-type** which do not accumulate proteins. Starch accumulation is of no primary importance in classification, since it may be present or absent in both types of plastids. P-type plastids are further divided into six subtypes (Behnke and Barthlott, 1983).

i. **PI-subtype**. The plastids contain single crystalloids of different sizes and shapes and/or irregularly arranged filaments. This subtype is thought to be the most primitive in flowering plants, mainly Magnoliales, Laurales and Aristolochiales.

ii. **PII-subtype**. This subtype contains several cuneate crystalloids oriented towards the centre of the plastid. All investigated monocots contain this subtype. It is significant to note that only members of dicots with this subtype, *Asarum*, and *Saruma* of Aristolochiaceae are widely regarded among the most primitive members of dicots, a possible link between monocots and dicots.

iii. **PIII-subtype**. This subtype contains a ring-shaped bundle of filaments. PIIIsubtype is confined to Centrospermae (Caryophyllales) and the removal of Bataceae and Gyrostemonaceae has been supported by the absence of this subtype in these families. Further, forms are recognized based on the presence or absence of crystalloids into **PIIIa** (globular crystalloid), **PIIIb** (hexagonal crystalloid) and **PIIIc** (without crystalloid). Based on the distribution of these forms, Behnke (1976) proposed division of the order into three family-groups which exactly correspond to the three subor- ders Caryophyllineae, Chenopodineae and Phytolaccineae, earlier established by Friedrich (1956). Behnke (1997) advocated the removal of genus *Sarcobatus* from the family

Chenopodiaceae on the basis of the presence of **PIIIcf** plastids and absence of **PIIIf**, which are characteristic of family Chenopodiaceae. He places the genus in an independent family, Sarcobataceae.

iv. **PIV-subtype**. The plastid contains a few polygonal crystalloids of variable size. This subtype is restricted to the order Fabales.

v. **PV-subtype**. The plastid contains many crystalloids of different sizes and shapes. This subtype is found in the order Ericales and family Rhizophoraceae.

vi. **PVI-subtype**. The plastid contains a single circular crystalloid. This subtype is found in family Buxaceae.

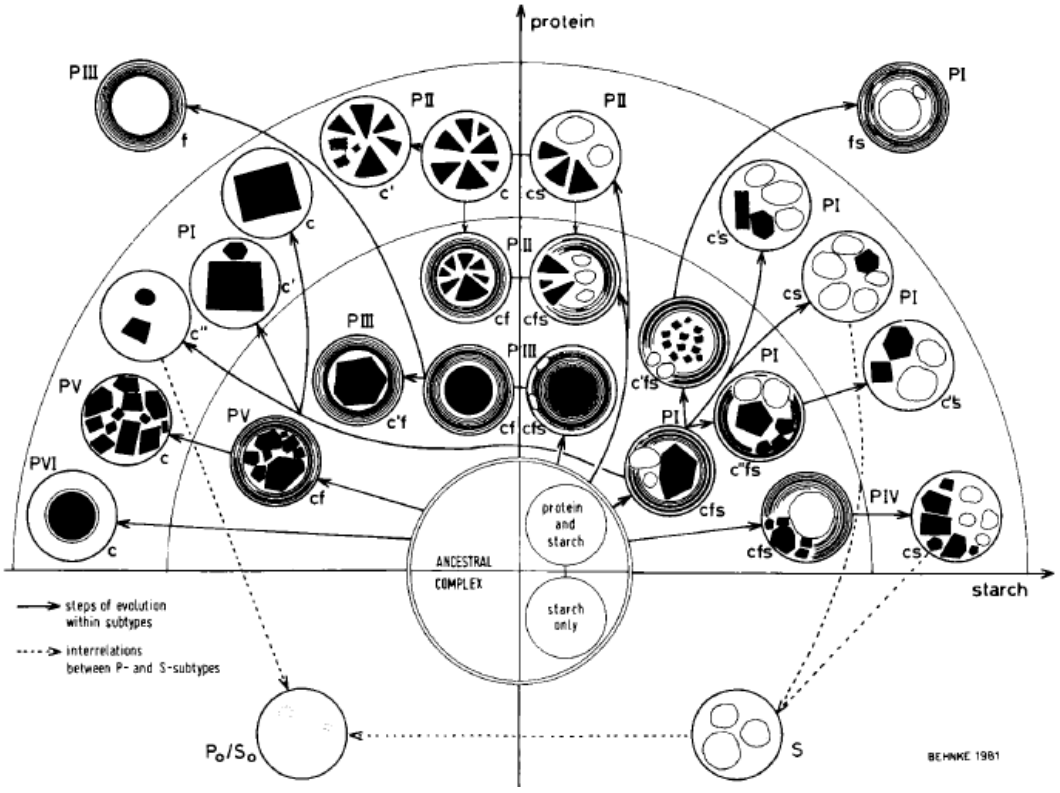

Fig. Various forms of sieve-tube plastids and their possible evolution Source: Singh, G. Plant Systematics: An Integrated Approach (3rd ed.), 2016.

1. ***Nuclear ribosomal DNA (rDNA)*** :

The term rDNA represents the nuclear genes which code for rRNA.

Brief description: The rDNA genes have *three* component genes, viz. 18S, 26S and 5.8S genes. The 18S gene encodes SSU(Smaller subunit) and 26S together with 5.8S genes encodes LSU (Larger subunit) of rRNA. These are separated by two internal transcribed spacers (ITS 1 and ITS 2). The cistron is flanked by the 5' and 3' external transcribed spacers(5'-ETS and 3'-ETS).

The advantages in using this molecular marker are:

(i) These are arranged in tandem repeats in one or a few chromosomal loci.

(ii) They can constitute as much as 10% of the total plant DNA.

(iii) The most remarkable feature of rDNA is the overall sequence homogeneity among members of the gene family. They maintain a kind of intraspecific homogeneity and interspecific homogeneity.

The most widely used molecular markers in plants at interspecific and intergeneric levels:

The nuclear ribosomal ITS region including the 5.8 S gene has been the most widely used in plant taxonomy. The region is relatively short and suitable for sequencing. In flowering plants, the size of these regions are as follows:

ITS 1 – 200-300bp long

ITS 2 – 180-240 bp long

5.8 S – 160bp.

The ITS primers which are recommended for amplifications include N18L18, Nnc18S10, C26A, C5.8S and N5.8S (Wen and Zimmer, 1996).

The most widely used molecular markers in plants at deep level phylogeny:

deep level phylogeny means the evolutionary process concerning the higher.

taxonomic levels from family onwards. Since 18S gene is a slowly evolving molecule it is suitable for interpreting evolution at higher taxonomic levels (Hamby and Zimmer, 1992; Soltis et al., 1997) and reconstructing the land plant phylogeny (Soltis *et al*., 1999).

Why is 26S-gene not widely/less explored as a marker?

(i) The size is very large being about 3.4 Kb long and includes 12 expansion segments (ES) which are more variable than alternating conserved regions (Bult *et al*., 1995).

(ii) The overall-base substitution of 26S is 1.6-2.2 times higher than 18S (Kuzoff *et al*., 1998).

(iii) So far only partial sequences have been used among closely related or in determining phylogenetic position of isolated families.

Chloroplast DNA(cp DNA):

Brief description:

This DNA (genome) is used frequently used in taxonomy. It is a circular molecule ranging in size from 120-217 Kb. Most of the angiosperms score in between 135-160 Kb. There are over 100 functional genes in chloroplast genome.

The chloroplast genome, with a few exceptions, contains two duplicate regions in reverse orientation, known as inverted repeat (IR). IR contains genes encoding rRNA(plastidial). The IR ranges in size from 10 to 76 Kb in land plants and divides the chloroplast DNA into large and small copy regions. The structural organization of chloroplast genome is relatively conserved and free from large deletions, insertions, transpositions and inversions.

The advantages in using this molecular marker in phylogenetic studies are:

(i) Chloroplast DNA is a relatively abundant component of total DNA facilitating extraction and analysis.

(ii) There is extensive molecular information related to evolution since chloroplast genomes have been isolated from different types of plants from algae to angiosperms.

(iii) CpDNA is maternally inherited in angiosperms and thus helpful in determination of maternal origin particularly in cases of hybrid and allopolyploid species.

(iv) Rate of substitution in chloroplast genome is relatively slow and hence conservative and useful in interpreting phylogeny at higher levels (family and order). However some chloroplast DNA regions are there which evolve rather rapidly and hence useful in interpreting phylogeny at lower levels (species and generic levels).

Some commonly used genes of chloroplast genome:

(i) The most commonly used gene is rbcL gene which encodes the enzume Ribulose 1,5-bisphosphate carboxylase oxygenase (RUBISCO).

(ii) trnL(UAA) 5′exon-trnF(GAA) region for interpretation of efolution at lower levels of taxonomic hierarchy.

(iii) matK gene within the trnK intron which encodes a maturase (ORF) and perhaps functions in splicing group II introns.

(iv) ndhF gene encoding a component of chloroplast respiratory chain. This has been found to be useful in interpretations at the family, intergeneric and interspecific levels.

(v) atpB-rbcL intergenic spacer

(vi) rpL 16 intron

Mitochondrial DNA (mtDNA):

Brief description:

This DNA (genome) is used immensely useful in studying phylogeny and population genetics in animals. Plant mtDNAs are rather poorly studied and proved to be not much helpful in resolving evolution in plants.

The demerits of plant mtDNA in taxonomic/phylogenetic interpretations are:

(i) Plant mtDNA molecules encode approximately 5% of the proteins found in mitochondrion.

(ii) Plant mtDNA is greater than 200Kb in size and several times larger than animal mtDNA. In most of the angiosperms the size ranges from 300-600Kb. In Cucurbitaceae and Malvaceae it exceeds 2000Kbs.

(iii) Many foreign sequences can be seen in plant mtDNA, e.g. chloroplast DNA sequences of various kinds, some as large as 12Kb have been found to be integrated with mtDNA of plants. Most of these foreign sequence regions are genetically functionless.

(iv) Plant mtDNA is unstable and intra- and inter- molecular recombinations can occur frequently (Palmer *et al*.,2000). Gene order rearranges frequently in plants.

(v) Rates of nucleotide substitution of plant mtDNA is 3-4 times lower than cpDNA, 12 times lower than plant nuclear DNA, 40-100 times lower than animal mtDNA.

Examples of some useful Plant mtDNA markers:

Only a few mitochondrial markers have shown promise for phylogenetic studies in plants at lower levels (Frendenstein and Chase, 2001), e.g. intron between the *b* and *c* exons of subunit 1 of mitochondrial NADH dehydrogenase gene (nad1 *b/c* exon sequence), mat R sequences.

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13. Taxonomic literatures: Definitions with examples of classical books, index, flora and manual, revision and monograph, icons, bibliography, catalogue, encyclopedias, glossary and dictionary. Important periodicals of India and abroad.

Taxonomic Literature (T. Pullaiah**,** Professor Sri Krishnadevaraya University Anantapur – 515 003A.P**):**

Various forms of literature incorporating description, illustrations and identification keys are useful for proper identification of unknown plants. The library is therefore as important in taxonomic work as a herbarium and knowledge of taxonomic literature is vital to the practising taxonomist. The literature of taxonomy is one of the oldest and most complicated literatures of science. Several bibliographic references, indexes and guides are available to help taxonomists to locate relevant literature concerning a taxonomic group or a geographical region. The major forms of literature helpful in identification are described below-

Floras

A Flora is the systematic enumeration of plants of a given region. It may be a small area, a district, a state, a country or a continent. Floras, especially modern Floras, give complete citation of the species according to ICBN, description, distribution, keys to the genera, keys to species and often illustrations.

e.g.,

Continent: Flora Europaea by T.G.Tutin et al. (1964-80),

Flora Australiensis by G. Bentham (1863-78).

Country: Flora of British India by J.D.Hooker(1872-97),

Flora of India by Botanical Survey of India (1994-),

13

Flora Malesiana by C.G.Steenis (1948),

Flora USSR by V.L.Komarov and B.K.Shiskin (1964-80).

State : Flora of Andhra Pradesh by Pullaiah et al (1997),
Flora of Karnataka by C.J.Saldanha (1986),
Flora of Orissa by Saxena and Brahmam (1994).
District: Flora of Anantapur district by Pullaiah and Yesoda (1989),
Flora of Medak District by Pullaiah et al (1998),
Flora of Hassan Disctrict by Saldanha and Nicolson (1978).

Manuals:

A manual is a more exhaustive treatment than a Flora, always having keys for identification, description and glossary. Manuals do not necessarily indicate place of deposition of plant specimens included. The main emphasis is placed on providing suitable keys and diagnostic descriptions.

e.g., Manual of cultivated plants by L.H. Bailey (1949),
Manual of Aquatic Plants by N.C.Fassett (1957),
Manual of Indian Forest Botany by Bor (1953)

Monographs:

A Monograph is a comprehensive taxonomic treatment of taxonomic group, generally a genus or a family. It gives significant information of a morphological and taxonomic nature concerning taxon. Strictly speaking it should cover the taxon as it exists throughout the world. Monograph gives information on anatomy, cytology, chemistry, genetics, geography, ecology etc.

e.g., The genus Pinus by N.T.Mirov (1967),
The genus Crepis by Babcock (1947),
The genus Datura by Blackslee et al. (1959).
Monograph on Indian Subtribe Cassiinae (Caesalpiniaceae) by
V.Singh 2001.

Revision:

Revision is a taxonomic treatment of a genus or family. It covers only morphological aspects. A revision is less comprehensive than a monograph, incorporating less introductory material and including a synoptic literary review.

e.g.- Fascicles published by BSI in different taxa Cucurbitaceae, Labiales.

Icons (Illustrations)

Illustrations, often with detailed analysis ofthe parts are usually published along with the text in Floras and Monographs, but may sometimes be compiled exclusively and often serve as useful tools for identification. In fact, many species of plants based on published illustrations only, without any accompanying description or diagnosis before 1 January 1908 have been accepted as validly published.

Two principal compilations of Icones are Hooker's Icones and Wight's Icones. Others of interest include Illustrations of plants from Europe (Hegi, 1906-1931), North America (Gleason, 1963), Pacific states (Abrams, 1923-1960), Pacific coast trees (McMinn and Maino, 1946), Germany (Garcke, 1972), Korea (Lee, 1979).

Bibliography:

A bibliography is a list of works (such as books and articles) written on a particular subject or by a particular author. a list of the books referred to in a scholarly work, typically printed as an appendix.

e.g.- Elpel, Thomas (2004). Botany in a Day: The Patterns Method of Plant Identification. Pony, Montana: HOPS Press, LLC.

Baumer-Schleinkofer (1997). Bibliography of the History of Biology/Bibliographie Zur Geschichte De Biologie: Bibliographie Zur Geschichte Der Biologie.

Freeman, R. B. (1977). "On the Origin of Species". The Works of Charles Darwin: An Annotated Bibliographical Handlist (Second ed.). Cannon House Folkestone, Kent, England: Wm Dawson & Sons Ltd. Retrieved 2007-01-14.

Catalogue:

Catalogue are compilation of plant information including name and classifications.

Index:

An Index provides an alphabetic listing of taxa with reference to their publication**.**

General Taxonomic Indexes:

From time to time several in dexes have been prepared for Angiosperms. Indexes serve as an aid to locating quickly the source of original publication of a name.

Index Kewensis Plantarum Phanerogamarum:

This is a comprehensive index of scientific names of seed plants. It was published in 1893-1895 in two volumes under the direction of J.D.Hooker and B.D.Jackson. It consists of an alphabetical list of genera published from the time of Linnaeus to the year 1885. Under each generic name was given, in alphabetical sequence, every species epithet known to have been published followed by the name of the author, the place of publication and an indication of the native country of that plant. Later on supplements were published regularly at the interval of 5 years. So far supplements have been published by Kew Botanical Gardens. The Index Kewensis and supplements are now available on CD-ROM and it includes 968,000 records.

Gray Herbarium Card Index, Cambridge, Mass.

A card index is a data base of 287,225 records of New World Vascular plant taxa at the level of species and below.

Index Holmiensis

Published from Swedish Museum of Natural History it gives Plant distribution maps of vascular plants. 8 Volumes have been published up to 1995.

Index Londonensis, Oxford 1900-1935.

This is an alphabetical index of illustrations of flowering plants, ferns and fern allies appearing from 1753 to 1935. Flowering Plant Index of Illustrations and Information:It was compiled by R. T. Saacson (1979). It is a 2-volume index of all illustrations of flowering plants.

Index Nominum Genericorum:

It is a 3-volume work published in 1979 and gives a list of all generic names. The first supplement appeared in 1986. It is also available on the internet.

Botanical Glossaries:

A glossary is an alphabetical list of different terms with their explanations.

e.g. A glossary of Botanical Terms- B. D. Jackson (1928), 4^{th} ed

Botanical Latin – W. T. Stearn

Dictionaries:

A botanical dictionary may include lists and descriptions of all known genera of certain plant groups.

e.g. - Dictionary of Flowering Plants and Ferns published by J.C.Willis (1973). The 8th edition revised by Airy Shaw.

A dictionary of Economic Products of India- 7 vol –G. Watt (1972), Calcutta.

Dictionary of Economic Plants- J. C. Upholf (1959), New York

Periodicals:

Various herbaria and botanical societies throughout the world publish results of their taxonomic studies in periodicals. These being the authentic and learned treatises on concerned plant taxa, are greatly useful in taxonomic research. Some of the more important ones are:

Acta Phytotaxonomica et Geobotanica, USA

Adansonia, Frace

American Journal of Botany, USA

Annals of Royal Botanical Gardens Calcutta, India

Botanical Journal of the Linnean Society, England

Botanical Gazette, USA

Bullein of Botanical Survey of India

19. Let's sum up

- Cronquist's system places flowering plants into two broad classes, Magnoliopsida (dicotyledons) and Liliopsida (monocotyledons) whereas in Thaktajan's system of classification angiosperms are grouped into division magnoliophyta that are again divided into 2 classes, Magnoliopsida and Liliopsida.
- The APG III system of flowering plant classification is the third version of a modern, mostly molecular-based, system of plant taxonomy being developed by the Angiosperm Phylogeny Group (APG).

- The paleoherbs are a small flowering plants group which has traditionally been classified as dicots, but which have many characters of monocots. The eudicots are a large, monophyletic assemblage of angiosperms with tricolpate or tricolpatederived pollen grain.

- The Amborellaceae is the basal vessel-less angiosperm group. Magnoliales are woody plants with the orders Laurales, Piperales, and Canellales. Caryophyllidae is a botanical name at the rank of subclass. Nepenthales is an order of carnivorous flowering plants. Podostemaceae is a riverweed family in the order Malpighiales. Asterales is an order of dicotyledonous flowering plants that includes the large family Asteraceae known for composite flowers made of florets.

- The grass family is one of the most widely distributed and abundant groups of plants on Earth. The Alismatales are an order of flowering plants including about 4500 species. Plants assigned to this order are mostly tropical or aquatic.

- The ICBN is an agreement between botanists around the world to follow Binomial system of naming. The ICBN sets the formal starting date of plant nomenclature at 1 May 1753, the publication of Species Plantarum by Linnaeus.

- The BioCode initiative represents a concerted effort, by biologists intimately involved in the operation of the current system of separate codes, to devise a unified approach to the future naming of organisms of all kinds. The PhyloCode is a developing draft for a formal set of rules governing phylogenetic nomenclature.

- Endemism is the natural phenomenon or concept of confinement or restriction of any taxonomic unit or taxon which occurs in a restricted geographical areas or any particular country or region or island or mountain.

- A botanical garden or botanic garden is a garden dedicated to the collection, cultivation and display of a wide range of plants labelled with their botanical names. The herbarium technique was a well-known botanical practice at the time of Linnaeus, he departed from the convention of mounting and binding the specimens into volumes.

- Biosystematics is a synthetic branch which uses the characters and data from many disciplines like morphology, anatomy, cytology, genetics etc. Biosystematics is the taxonomic application of the genecology, is the study of the genotypic and phenotypic variation of species in relation to the environments in which they occur.

- Numerical taxonomy as grouping by numerical methods of taxonomic units into taxa on the basis of their character states. Phylogeny is commonly represented in the form of a cladogram, or phylogenetic tree, a branching diagram that conceptually represents the best estimate of phylogeny. Cladistics has developed into a forceful methodology of developing phylogenetic classifications.

- The literature of taxonomy is one of the oldest and most complicated literatures of science. Several bibliographic references, indexes and guides are available to help taxonomists to locate relevant literature concerning a taxonomic group or a geographical region.

20. Suggested Readings

1. Singh, G. Plant Systematics: An Integrated Approach (3rd ed.), 2016, CRC Press
2. Simpson, G. Plant Systematics, 2006, Springer.
3. Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. Plant Systematics, A Phylogenetic Approach (4th ed.), 2016, Sinauer Associaties, Inc.
4. Jones, S.B. and Luchsinger, A.E. Plant Systematics (2nd ed.), 1987, McGraw Hill Book Company
5. Stace, C. A Plant Taxonomy & Biosystematics, Latest Ed., Arnold Publishers
6. Cronquist, A. The Evolution & Classification of Flowering Plant, 1988 (2nd ed.), New York Bot. Garden Bronx. New York.
7. http://www.biologydiscussion.com/plant-taxonomy/
8. https://en.wikipedia.org/

21. Assignment

Give an account of International Code of Botanical Nomenclature. Mention the major changes of the latest code.

Discuss Embryological evidences in relation to Taxonomy.

Give an out line of APG III system of classification. Give an outline idea about Paleoherbs.

Write short notes on any Two of the following:

A) DNA barcoding

B) Monophyly, Paraphyly and Polyphyly

C) Chloroplast DNA

Give a brief accony of interrelationship and evolytionary trends of Amborallaceae.

What is Stasigenesis?

How can homology differs from homoplasy

Define periodicals with example.

Why E-taxonomy is important in modern taxonomy?

Distinguish biosystematics from classical taxonomy

What is invasive species? Give two eample.

Discuss biosystematics categories.

Mention the primitive and advanced features of Poaceae.

What is epitype?

Define haptera.

Name the biggest Herbarium of world.

Mention the principle of Biocode.

Compare between cladogram, dendogram and phylogram.

Discuss the logical steps of Numerical taxonomy? Mention its merits and demerits.

All the materials are self written and collected from ebook, journals and websites.

NOTE

POST GRADUATE DEGREE PROGRAMME (CBCS)

IN

BOTANY

SEMESTER - I

Course: BOHCT 1.4

(Cytology, Cytogenetics and Genetics)

Self-Learning Material

DIRECTORATE OF OPEN AND DISTANCE LEARNING

UNIVERSITY OF KALYANI

Kalyani, Nadia

West Bengal, India

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December, 2018

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Foreword

Satisfying distance learners' needs of verifying kinds and magnitude as well as minimizing distance and to reach the unreached in Open and Distance Learning (ODL) systems has the novelty in it. Nevertheless, this novelty puts challenges to the ODL systems managers, curriculum designers, Self Learning Materials (SLMs) writers, editors, production professionals and may other personnel involved in it. A dedicated team of University of Kalyani under leadership of Hon'ble Vice-Chancellor have puts their best efforts, committed professionalism as a Team for promoting Post Graduate Programmes under distance mode under University of Kalyani. Developing quality printed SLMs for students under DODL within a limited time to cater academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2017 successfully completed with best efforts.

Utmost care has been taken to develop the SLMs useful to the learners and to avoid errors as far as possible. Further, suggestions from the learners-end will be gracefully admitted and to be appreciated.

During the academic productions of the SLMs, the team received continuously positive stimulations and feedback from Professor (Dr.) Sankar Kumar Ghosh, Hon'ble Vice-Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, made constructive criticisms to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Due sincere thanks are being expressed to all the Members of PGBOS (DODL), University of Kalyani, Course Writers- who are serving subject experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have been utilized to develop these SLMs. We humbly acknowledge their valuable academic contributions. I would like to convey thanks to all other University dignitaries and personnel who have been involved either in conceptual level or in the operational level of the DODL of University of Kalyani.

For a comprehensive, learners friendly, adaptable text that meets curriculum requirements of the Post Graduate Programme through distance mode.

Director
Directorate of Open & Distance Learning
University of Kalyani

SYLLABUS

COURSE–BOHCT 1.4

Cytology, Cytogenetics and Genetics

(Full Marks–75)

Course	Group	Details Contents Structure		Study hour
BOHCT 1.4	Cytology, Cytogenetics and Genetics	**Unit 1. Genome organization and Karyotype**	1. Genome organization in Eukaryotes: DNA packing in nucleosome; repetitive, satellite and unique DNA sequences; C-value paradox; structural and functional organization of telomeres and centromeres; spindle organization, polymerization and significance. 2.1. Karyotype concept in relation to evolution; banding techniques	1
		Unit 2. GISH, FISH techniques and sex determination	2.2. GISH and FISH techniques. 3. Sex determination: Sex determination in plants and their interrelationship with human, Drosophila and mice models; dosage compensation; sex linked inheritance.	1
		Unit 3. Special type of chromosome, linkage and crossing over	4. Special type chromosomes: Cytogenetical significance of polytene and B-chromosome; deletion mapping; recombination 5. Linkage and crossing over: chiasma frequency and genetic map distance; Evolutionary significance of recombination; tetrad analysis; centromere mapping with ordered tetrad.	1
		Unit 4. Chromosomal aberration	6. Reciprocal translocation: Cytogenetics of reciprocal translocation in plant species; Gaudens and Velans complex; reciprocal translocation in humans. 7. Polyploidy: Polyploids and aneuploids; Inheritance of autopolyploids and trisomics; significance and limitations of polyploidy; aneuploidy in humans.	1
		Unit 5. Extra-chromosomal inheritance and Microbial genetics	8. Plastids and mitochondrial DNA influenced traits. 9. Microbial genetics: Transformation, conjugation and transduction and their significance in gene mapping.	1

Course	Group	Details Contents Structure		Study hour
BOHCT 1.4	Cytology, Cytogenetics and Genetics	**Unit 6. Gene mutation**	10. Gene mutation: Induction, types, molecular basis, significance; paramutation; DNA repair mechanism; epigenetic changes; genetic imprinting; prion particles; site directed mutagenesis; gene complementation test; rII locus.	1
		Unit 7. Structure of DNA, RNA and DNA replication	11.1 Biology of DNA and RNA: DNA forms; DNA replication; RNA types; characterization of rRNA.	1
		Unit 8. Transcription and translation proceses	11.2 Transcription and translation proceses, pre mRNA processing.	1
		Unit 9. Genetic regulation	12. Genetic regulation: Regulation of prokaryotic gene expression – lac, trp and ara operons; regulation of eukaryotic gene expression – brief account.	1
		Unit 10. Transposable elements and Population genetics	13. Transposable elements: Ac-Ds, IS elements, P-elements and their role in genetics. 14. Population genetics: Hardy-Weinberg principle; gene frequency in a population, genetic equilibrium, factors affecting gene frequency.	1
		Unit 11. Cell cycle and cancer	15. Cell cycle regulation and cancer: Role of proteins in controlling cell cycle; apoptosis; oncogenes and protoon-cogenes; tumour suppressor genes; role of E2F and p53 in controlling cell cycle; cancer therapy.	1
		Unit 12. Recombinant DNA technology	16. Recombinant DNA technology – brief account	1

Content

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Unit 5. Extra-chromosomal inheritance and Microbial genetics	59-70
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Unit 7. Structure of DNA, RNA and DNA replication	98-108
Unit 8. Transcription and translation proceses	108-126
Unit 9. Genetic regulation	127-148
Unit 10. Transposable elements and Population genetics	149-161
Unit 11. Cell cycle and cancer	162-179
Unit 12. Recombinant DNA technology	180-186

COURSE – BOHCT 1.4

Cytology, Cytogenetics & Genetics

Hard Core Theory Paper **Credits: = 3**

Content Structure

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1. Introduction
2. Course Objectives
3. Genome organization in Eukaryotes: DNA packing in nucleosome; repetitive, satellite and unique DNA sequences; C-value paradox; structural and functional organization of telomeres and centromeres; spindle organization, polymerization and significance.
4. Karyotype concept in relation to evolution; banding techniques; GISH and FISH techniques.
5. Sex determination: Sex determination in plants and their interrelationship with human, *Drosophila* and mice models; dosage compensation; sex linked inheritance.
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10. Plastids and mitochondrial DNA influenced traits.
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17. Cell cycle regulation and cancer: Role of proteins in controlling cell cycle; apoptosis; oncogenes and protooncogenes; tumour suppressor genes; role of E2F and p53 in controlling cell cycle; cancer therapy.
18. Recombinant DNA technology – brief account
19. Let’s sum up
20. Suggested Reading
21. Assignment

1. Introduction

Genetics is the study of heredity and variations. Heredity and variations are controlled by genes—what they are, what they do, and how they work. Genes inside the nucleus of a cell are strung together in such a way that the sequence carries information, that information determines how living organisms inherit various features. This course helps the students in gaining sufficient knowledge to pursue academic career or work as an expert at national or international research laboratories.

2. Course Objectives

After completion of the course the learners will be able to:

- To know how the hereditary information in DNA controls what an organism looks like and how it works.
- Relate the structure and function of the DNA molecule to its functional role in encoding genetic material.
- An understanding of the DNA packaging and expression and regulation of gene at transcriptional and translational level.
- Be able to distinguish between maternal effect, sex-linked, and cytoplasmic modes of inheritance.
- Be able to look at a pedigree chart and discern the most likely mode of inheritance.
- To know how inheritance patterns are affected by position on chromosomes
- Gain an appreciation for how genes work together in biological processes.
- Describe variation both in DNA and chromosomal level.
- Explain about the fundamental process of biological systems.
- Apply the Hardy-Weinberg Law in analyzing population genetics for gene frequency, sex linkage, equilibrium, and heterozygote frequency.

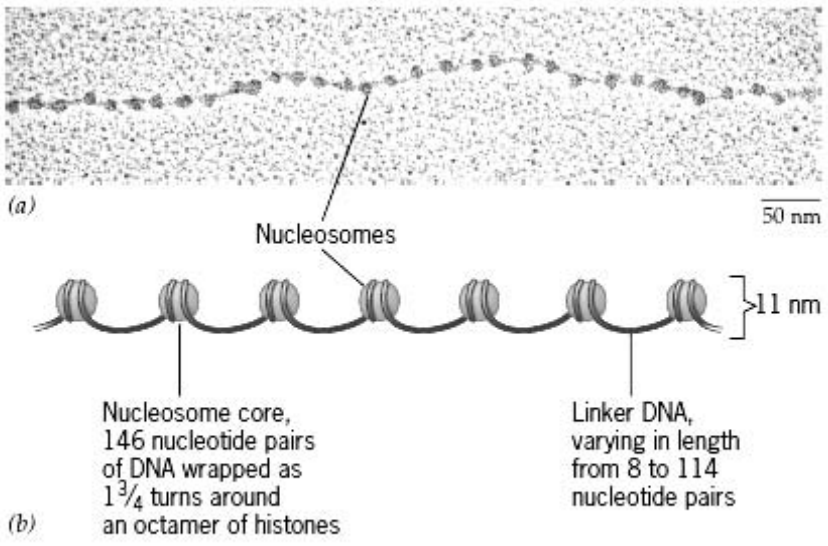
3. Genome organization in Eukaryotes: DNA packing in nucleosome; repetitive, satellite and unique DNA sequences; C-value paradox; structural and functional organization of telomeres and centromeres; spindle organization, polymerization and significance.

Genome organization in Eukaryotes:

DNA packing in nucleosome

The Nucleosome: The unit of chromatin

A basic structural unit of chromatin organization was proposed by Kornberg and Thomas in 1974. Nucleosomes help in DNA packaging at different levels because the bulk of the DNA in eukaryotic cell is wrapped around the nucleosomes and gives a **beads-on-a-string** structure. A complete nucleosome is a total disc of 11 nm diameter and 6 nm in height. It consists of (1) nucleosome core, (2) linker DNA (3) an average one molecule of H1 histone and (4) the other chromosomal protein. A nucleosomes typically have ~200 bp DNA including linker DNA and H1 histone is associated with it. Histones are responsible for the multilevel system of DNA packaging during chromatin/ chromosome organization. Different levels of chromatin organization are discussed below:



■ **FIGURE 9.17** Electron micrograph (*a*) and low-resolution diagram (*b*) of the beads-on-a-string nucleosome substructure of chromatin

(Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

Nucleosome 10nm fiber –

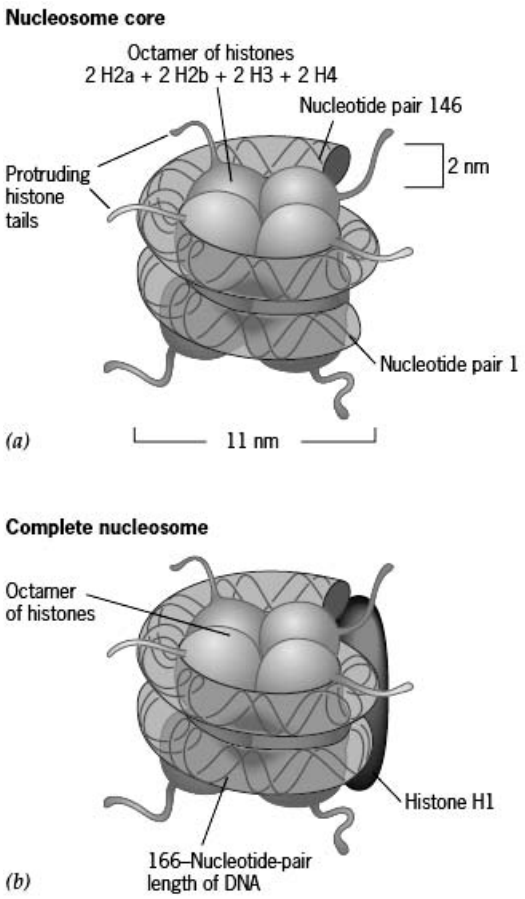
The nucleosomal 10 nm fiber is often called as 'beads on a string' due to its appearance under the electron microscope. It is known as the primary level of chromatin organization.

i) **Nucleosome core:** It consists of a histone octamer composed of two molecules each of histones H2A, H2B, H3 and H4. A 146 bp long DNA molecule is wrapped around this histone octamer in 1¾ turns; this segment of DNA is nuclease resistant. The histones are basic because they contain 20 to 30 percent arginine and lysine, two positively charged amino acids.

ii) **Linker DNA:** The DNA linking two adjacent nucleosomes is called linker DNA. The size of linker DNA varies from 8 bp to 114 bp long DNA and it is nuclease susceptible.

iii) **H1 histone:** Each nucleosome contains one molecule of H1 histone. The H1 histone is located at the point of entry and exit of DNA wrapped around a nucleosome.

On prolonged digestion, the length of the DNA is reduced to 147 bp (DNA is wound about 1.65 times around each nucleosome) and H1 histones are also released.



■ **FIGURE 9.18** Diagrams of the gross structure of (*a*) the nucleosome core and (*b*) the complete nucleosome.

(Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

Solenoid 30 nm fiber – The second level of condensation involves an additional folding or supercoiling in a circular manner of the 11 nm nucleosome fiber, to produce 30 nm chromatin fiber and it is called **solenoid**. It consists of 6 nucleosomes/turn; while, 6 H1 histones are oriented towards the central core of every turn. Non-histone proteins are present within the space enclosed by each turn called **scaffold proteins**.

Loops/ scaffold: Non-histone chromosomal proteins form a **scaffold** that is involved in condensing the 30 nm chromatin fiber into the tightly packed metaphase chromosomes. This third level of condensation appears to involve the separation of segments of the giant DNA molecules present in eukaryotic chromosomes into independently supercoiled domains or loops. In the interphase chromosomes, chromatin fibers appear to be organized into 30,000– 100,000 bp loops or domains anchored in a scaffolding (or supporting matrix) within the nucleus.

Stretches of DNA called scaffold associated regions, or SARs, bind to the non- histone proteins to determine the loops. Further coiling and folding occurs in a metaphase chromosome. Folding of

these longer DNA results a close-packed alignment of nucleosomes and shortens the DNA by another 5 or 10 times into 200-300 Å thick fibers. The overall compression of the DNA has also been described as the packaging ratio, which is the length of DNA divided by the length of the unit that contains it. The ratio of salivary gland chromosome of *Drosophila* at metaphase is 100:1. Each chromatid, therefore, consists of a single unbroken molecule of DNA which is coiled, supercoiled and folded to form the chromatid.

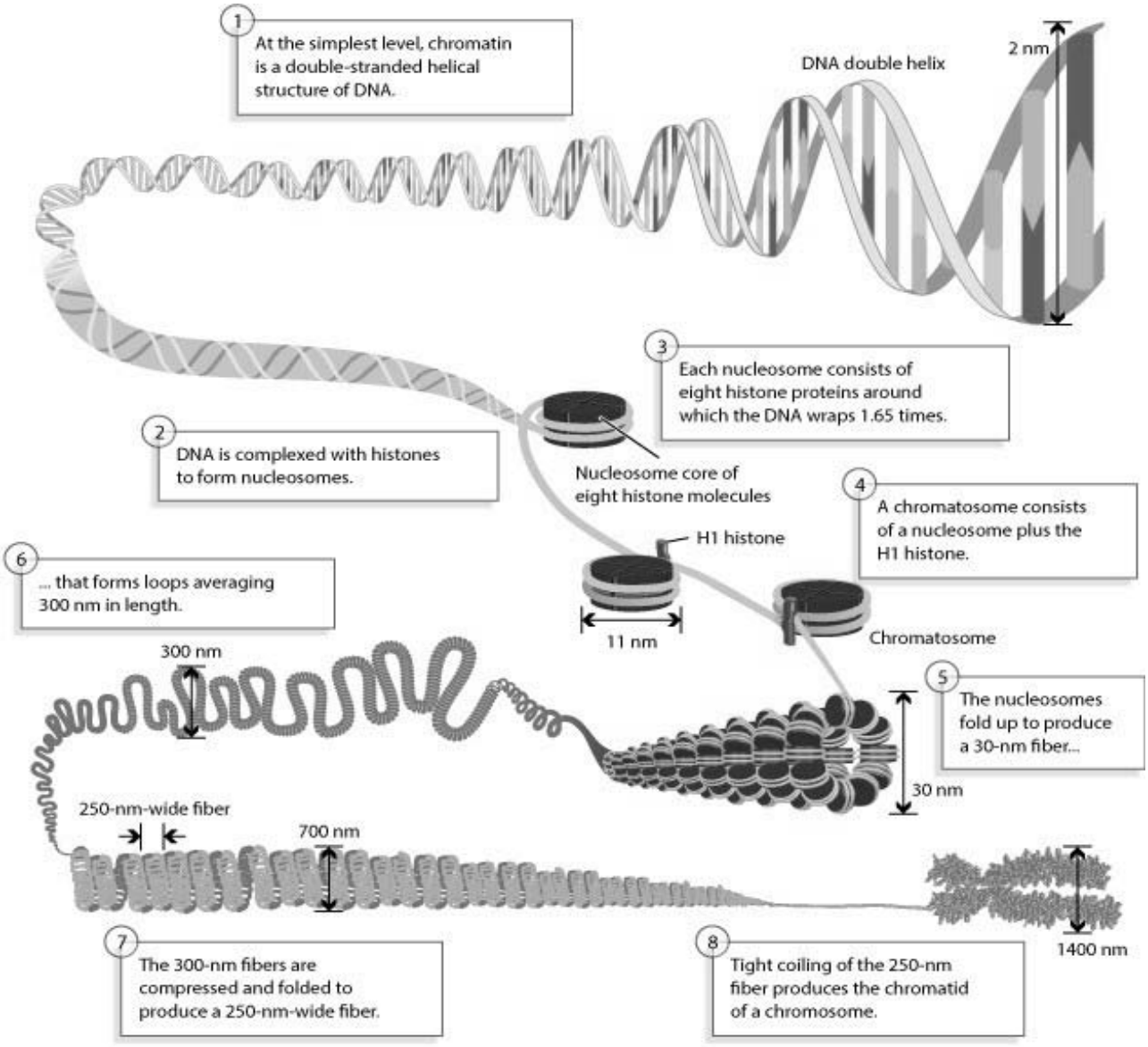


Figure Nucleosome Model (Source-https://brucealberts.ucsf.edu/current-projects/molecular-biology-of-the-cell)

Repetitive, satellite and unique DNA sequences-

Repetitive DNA sequences

The repetitive DNA sequences are present in several to a million copies per genome. Except few genes, majority of repetitive DNA sequences are non-coding sequences. These sequences make eukaryotic genome voluminous. The number of copies of a DNA sequence present in one genome is called **repetition frequency (f)**. Around 45% of the human genome and almost 40% of the mouse genome are composed of repeated DNA sequences. It has been suggested that the event of unequal crossing- over could be the main mechanism for the evolution of repetitive DNAs. Repetitive DNA sequences are grouped into the following two classes: (i) highly repetitive DNA and (ii) moderately repetitive DNA.

(i) Highly repetitive DNA:

It is also called simple sequence DNA. These short sequences, often less than 10 bp in length, are present in hundreds of thousands to millions copies that are repeated in tandem and clustered in certain regions of the chromosome, especially at centromeres and telomeres.

(ii) Moderately repetitive DNA:

Moderately repetitive DNA consists of sequences from 150 to 300 bp in length (although they may be longer) that are repeated many thousands of times.

Much of the moderately repetitive DNA has no known function in the cell while some of these moderately repetitive sequences perform important functions for the cell; for example, the genes for ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) make up a part of the moderately repetitive DNA.

Moderately repetitive DNA is of two types of repeats.

Tandem repeat sequences appear one after another and tend to be clustered at particular locations on the chromosomes.

Interspersed repeat sequences are scattered throughout the genome. An example of an interspersed repeat is the *Alu* sequence, a 200 bp sequence that is present more than a million times and comprises 11% of the human genome.

Short repeats, such as the *Alu* sequences, are called **SINEs (short interspersed elements)**.

Longer interspersed repeats consisting of several thousand base pairs are called **LINEs (long interspersed elements)**. One class of LINE, called LINE1, comprises about 17% of the human genome.

Satellite DNAs-

Satellite DNAs are short sequences ranging from 5 to 200 bp and it consists of very large arrays of tandemly repeating, non-coding DNA. Satellite DNA is the main component of functional centromeres, and forms the main structural constituent of heterochromatin. These highly repetitive sequences are GC-rich DNA due to which they show lower buoyant density than that of bulk of the DNA of the genome and hence appear as a distinct 'satellite' band from the main band of DNA during density gradient centrifugation (hence the name **satellite DNA**). The term satellite DNA is essentially synonymous with simple sequence DNA. There is complex satellite DNA with longer repeat units. For example, alfa and beta satellite DNA found in humans have a repeat unit of 171 bp and ~ 68 bp respectively. Satellite DNAs are species specific and represent less than 10% of the genome.

Depending upon the number of base pairs involved in repeat regions, satellite DNA is of two types, **microsatellite sequences** and **minisatellite sequences**.

Microsatellite DNA:

These include the shortest sequences one to five base pairs long, present in clusters of about 50 to 100 base pairs in length. They are dispersed evenly throughout the DNA. The human genome contains about 30,000 different microsatellite loci.

Minisatellite DNA:

These usually occur in clusters with about 3000 repeats, their size ranging from 12 to 100 bp in length. Minisatellite sequences occupy shorter stretches of the genome than the satellite sequences. Minisatellites are often unstable and the number of copies of minisatellites can increase or decrease from one generation to the next.

Unique DNA Sequences

Unique sequences are non-repeated DNA sequences, sometimes called single-copy sequences, present as single or few copies per haploid genome. These sequences can be coding sequences, gene and gene families or noncoding sequences (introns, regulatory elements, pacer DNA etc.). In prokaryotes, with the exception of few sequences such as ribosomal RNA genes, transfer RNA genes, almost the entire genome consists of unique sequences and the majority of the sequences are coding gene sequences (solitary genes). In eukaryotes, most of the protein coding genes are unique sequences and called solitary genes. A group of genes that encodes similar proteins (not identical) form a gene family. Globins, actins, myosins, collagens, tubulins, integrins, protein kinase and vertebrate immunoglobins are some important examples of such gene families. The multigene families are formed due to gene duplication and mutation in the duplicated genes. The portion of

unique DNA in cells of various species may vary from 70% (man) to only 8% (rye). In human, these unique DNAs contribute ~50% of the total genome and only ~1.5 % DNAs constitutes coding regions within genes that code for proteins. This 26 is because 95% of the gene is made up of the non-protein coding DNAs called introns. Recently discovered miRNA genes, which code small structural RNAs, are also classified under as a unique sequence of DNA. These structural RNAs regulate the expression of other genes.

C-value paradox-

C-value: 'C-value' of an organism is the total amount of DNA present within its haploid genome. It is usually represented in base pairs (bp). The term C-value was given by Hawson Swift in 1950. In C-value, the 'C' stands for the 'constant or characteristic' of DNA content and is measured in picograms. It ranges from 6.6×10^5 for an alga to 10^{11} for some plants and amphibians.

C-value Paradox

The size of the genome (C-value) depends on the organism. It is essentially constant within species, but varies widely among species. There is not a strong correlation between organism complexity and genome size. This is known as the **C-value paradox.**

In general, bacterial genomes are smaller than eukaryotic genomes. Within bacteria, genomes range from 580 kb to 13 Mb, thus there is 20–30-fold size variation within prokaryotes.

A few eukaryotic genomes fall in the size range of bacteria (e.g. yeast), but most are much larger. The size range of eukaryotic genomes is 8.8 Mb to ≈700 Gb. This is 80,000-fold size variation.

The correlation between genome size and organism complexity exists well across the lower eukaryotes. While, a group of higher eukaryotes show the contradictory relationship 21 between C-value and its complexity, for example, genome size of *Xenopus*, (an amphibian) is closer to the genome size of human (most complex organism) and tulips have 40 times larger genome than humans. Similarly even the number of chromosomes in an organism cannot explain the complexity of different organisms. Genome size or C value or DNA content per haploid genome cannot explain the complexity of the organisms which have evolved during evolution over the years due to multiple factors.

Structural and functional organization of telomeres and centromeres-

Centromeres:

The centromere is a constricted region of the chromosome to which spindle fibers is attached and essential for proper chromosome movement in mitosis and meiosis. The centromeric region contains a disk-shaped structure of about 200 nm in diameter which is the site for microtubule attachment; this structure is called kinetochore. The kinetochore functions as the "microtubule organizing

centre" (MTOC) of the chromosome. Kinetochore is the attachment site of spindle fibres and is composed of both DNA and protein. The DNA sequence within these regions is called CEN DNA. Because CEN DNA can be moved from one chromosome to another and still provide the chromosome with the ability to segregate, these sequences must not provide any other function. The yeast centromeric DNA contains three distinguishable sequences-

(i) Conserved element I (CDE I):

It is composed of 9 bp and is located at the left end of the centromere; it shows minor variations.

(ii) Conserved element II (CDE II):

This element is the middle region containing 80-90 bp. A=T rich sequences constitute more than 90% of this region.

(iii) Conserved element III (CDE III):

This element consists of 11 bp and is located at the right end of the centromere. It is a highly conserved sequence.

Mutations in the first two sub-domains have no effect upon segregation, but a point mutation in the CDE-III sub-domain completely eliminates the ability of the centromere to function during chromosome segregation. Therefore CDE-III must be actively involved in the binding of the spindle fibers to the centromere. The protein component of the kinetochore is only now being characterized. A complex of three proteins called CbfIII (Cbf-IIIA, Cbf-IIIB and Cbf-IIIC) binds to normal CDE-III regions. This protein complex has some motor activity due to which the centromeric region of the chromosome becomes attached to microtubules. Mitotic chromosome movement is inhibited when mutation occurs in the genes coding for CBF-III proteins.

Telomeres:

Telomeres are the region of DNA at the end of the linear eukaryotic chromosome that are required for the replication and stability of the chromosome. McClintock recognized their special features when she noticed, that if two chromosomes were broken in a cell, the ends were sticky and end of one could attach to the other and vice versa. Whatever their structure, telomeres must provide at least three important functions. They must (1) prevent deoxyribonucleases from degrading the ends of the linear DNA molecules, (2) prevent fusion of the ends with other DNA molecules, and (3) facilitate replication of the ends of the linear DNA molecules without loss of material.

The telomeres of eukaryotic chromosomes have unique structures that include short nucleotide sequences present as tandem repeats. Although the sequences vary somewhat in different species, the basic repeat unit has the pattern 5′ $T_{1–4}A_{0–1}G_{1–8}$-3′ in all but a few species. For example, the repeat sequence in humans and other vertebrates is TTAGGG, that of the protozoan *Tetrahymena*

thermophila is TTGGGG, and that of the plant *Arabidopsis thaliana* is TTTAGGG. In most species, additional repetitive DNA sequences are present adjacent to telomeres; these are referred to as telomere-associated sequences. In vertebrates, the TTAGGG repeat is highly conserved.

Human telomeres contain the sequence TTAGGG repeated from about 500 to 5000 times. Certain bacteria possess telomeres in their linear genetic material which are of two types; one of the types is called a hairpin telomere. As its name implies, the telomeres bend around from the end of one DNA strand to the end of the complimentary strand. The other type of telomere is known as an invertron telomere. This type acts to allow an overlap between the ends of the complimentary DNA strands.

The telomeres of humans and a few other species have been shown to form structures called t-loops, in which the single strand at the 3′ terminus invades an upstream telomeric repeat (TTAGGG in mammals) and pairs with the complementary strand, displacing the equivalent strand. The DNA in these t-loops is protected from degradation and/or modification by DNA repair processes by a telomere-specific protein complex called **shelterin**. Shelterin is composed of six different proteins, three of which bind specifically to telomere repeat sequences. TRF1 and TRF2 bind to double-stranded repeat sequences, and POT1 (Protection Of Telomeres 1) binds to single-stranded repeat sequences. Subunits TIN2 and TPP1 tether POT1 to DNA-bound TRF1 and TRF2, and the TRF2-associated protein Rap1 helps regulate telomere length. Shelterin is present in sufficient quantities in most cells to coat all the single- and double-stranded telomere repeat sequences in the chromosome complement.

Telomere replication:

Telomerase

Some cells have the ability to reverse telomere shortening by expressing telomerase, an enzyme that extends the telomeres of chromosomes. Telomerase is an RNA dependent DNA polymerase, meaning an enzyme that can make DNA using RNA as a template.

How does telomerase work? The enzyme binds to a special RNA molecule that contains a sequence complementary to the telomeric repeat. It extends (adds nucleotides to) the overhanging strand of the telomere DNA using this complementary RNA as a template. When the overhang is long enough, a matching strand can be made by the normal DNA replication machinery (that is, using an RNA primer and DNA polymerase), producing double-stranded DNA.

The primer may not be positioned right at the chromosome end and cannot be replaced with DNA, so an overhang will still be present. However, the overall length of the telomere will be greater. Telomerase is not usually active most somatic cells (cells of the body), but it is active in germ cells

(the cells that make sperm and eggs) and some adult stem cells. These are cell types that need to undergo many divisions, or, in the case of germ cells, give rise to a new organism with its telomeric "clock" reset.

Interestingly, many cancer cells have shortened telomeres, and telomerase is active in these cells. If telomerase could be inhibited by drugs as part of cancer therapy, their excess division (and thus, the growth of the cancerous tumor) could potentially be stopped.

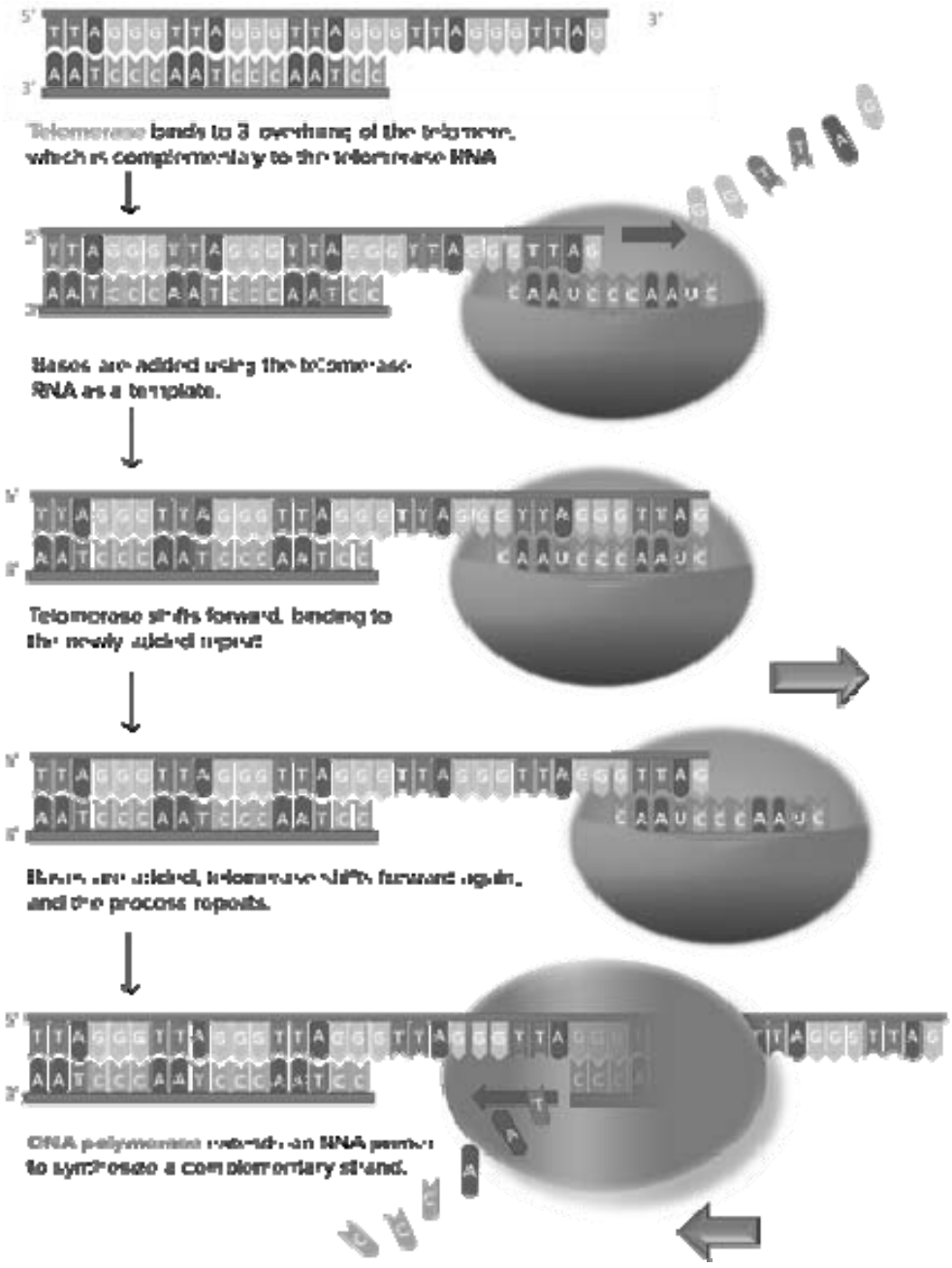


Figure Telomerase activity (Source-https://brucealberts.ucsf.edu/current-projects/molecular-biology-of-the-cell)

Spindle organization:

The term ‘mitotic apparatus’ or ‘spindle apparatus’ has been applied to the asters that surround the centrioles together with the mitotic spindle.

The spindle apparatus has the chromosome fibres, joining the chromosomes to the poles; the continuous fibres, extending pole to pole; the inter-zonal fibres observed between the daughter chromosomes and nuclei in anaphase and telophase; all of which are composed of microtubules.

The continuous fibres, in which birefringence is low in early anaphase, become more conspicuous in late anaphase and telophase. During anaphase in a plant cell, it is possible to differentiate the microtubules attached to the kinetochores of the chromosomes from those forming the continuous and inter-zonal fibres.

Among the so called continuous microtubules which point towards the poles, all of them are not long enough to reach the pole, only a few microtubules may be so long as to span between the poles are called as polar tubules and the rests are called free tubules.

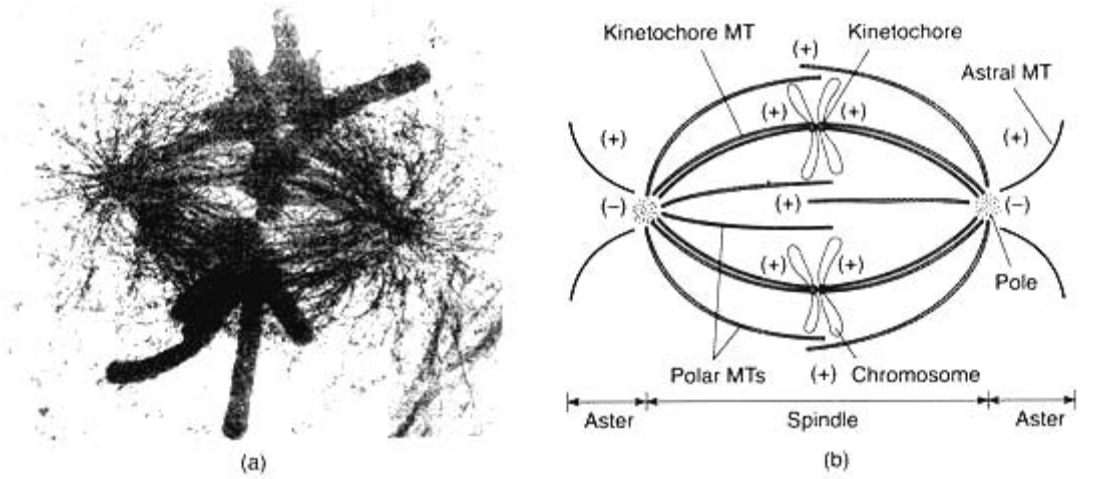


Fig. 5.9A: Mitotic apparatus in a metaphase cell. (a) To visualize the spindle microtubules more clearly, biotin-tagged anti-tubulin antibodies were added to make microtubules more massive. The large cylindrical objects are chromosomes. (b) Diagram showing the three sets of microtubules (MTs) in the mitotic apparatus. Centered around the poles are astral microtubules, kinetochore microtubules, which are connected to chromosomes and polar microtubules. The (+) ends of these microtubules all point away from the centrosome at each pole (after Baltimore)

Source: Kar and Halder 2008 Cell Biology, Genetics and Molecular Biology

Mitotic Apparatus in *S. cerevisiae In vitro* studies have revealed that the assembly of microtubules is controlled by the poles and also by the kinetochores. The lateral interaction between the spindle microtubules may also be involved. When a cell enters prophase, the cytoplasmic microtubules become depolymerized and replaced by the mitotic spindle.

At metaphase, only the spindle microtubules are present; at anaphase with the movement of the chromosomes, the spindle becomes depolymerized; and at telophase the daughter cells are held by the mid-body, and the cytoplasmic microtubules reappear.

The Ca++ ions and the Ca++ binding protein, calmodulin, appear to have a controlling role in the assembly and disassembly of spindle microtubules. The microtubules have distinct polarity with a fast growing or plus end and a slow growing or minus end.

Organizing the spindle apparatus

In a properly formed mitotic spindle, bi-oriented chromosomes are aligned along the equator of the cell with spindle microtubules oriented roughly perpendicular to the chromosomes, their plus-ends embedded in kinetochores and their minus-ends anchored at the cell poles.

Two models predominate the field, which are synergistic and not mutually exclusive.

Centrosome-mediated "search-and-capture" model

In this model, microtubules are nucleated at microtubule organizing centers and undergo rapid growth and catastrophe to 'search' the cytoplasm for kinetochores. Once they bind a kinetochore, they are stabilized and their dynamics are reduced. The newly mono-oriented chromosome oscillates in space near the pole to which it is attached until a microtubule from the opposite pole binds the sister kinetochore. This second attachment further stabilizes kinetochore attachment to the mitotic spindle. Gradually, the bi-oriented chromosome is pulled towards the center of the cell until microtubule tension is balanced on both sides of the centromere; the congressed chromosome then oscillates at the metaphase plate until anaphase onset releases cohesion of the sister chromatids.

In this model, microtubule organizing centers are localized to the cell poles, their separation driven by microtubule polymerization and 'sliding' of antiparallel spindle microtubules with respect to one another at the spindle midzone mediated by bipolar, plus-end-directed kinesins. Such sliding forces may account not only for spindle pole separation early in mitosis, but also spindle elongation during late anaphase.

Chromatin-mediated self-organization of the mitotic spindle

In contrast to the search-and-capture mechanism in which centrosomes largely dictate the organization of the mitotic spindle, this model proposes that microtubules are nucleated acentrosomally near chromosomes and spontaneously assemble into anti-parallel bundles and adopt a spindle-like structure. Classic experiments by Heald and Karsenti show that functional mitotic spindles and nuclei form around DNA-coated beads incubated in *Xenopus* egg extracts and that bipolar arrays of microtubules are formed in the absence of centrosomes and kinetochores. Indeed, it has also been shown that laser ablation of centrosomes in vertebrate cells inhibits neither spindle assembly nor chromosome segregation. Under this scheme, the shape and size of the mitotic spindle are a function of the biophysical properties of the cross-linking motor proteins.

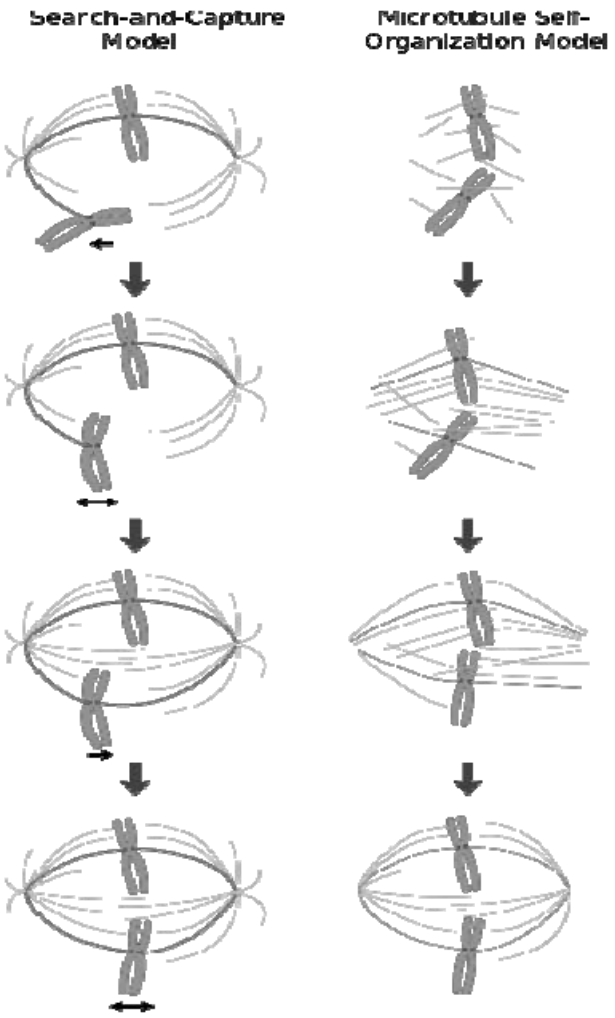


Figure Spindle organization model. source: https://en.wikipedia.org/wiki/Spindle_apparatus

Polymerization and significance:

Microtubules grow and shrink due to polymerization and de-polymerization at the kinetochore. This phenomenon sug-gests that motor activity and microtubule dynamics are coordinated away from and towards the spindle pole during cell division.

Two models have been proposed to explain the de-polymeri-zation of microtubules:

(i) Pac-man model (microtubules are chewed up at the kinetochore end);

(ii) Pole ward flux model (microtubules are depolymerized at the poles)

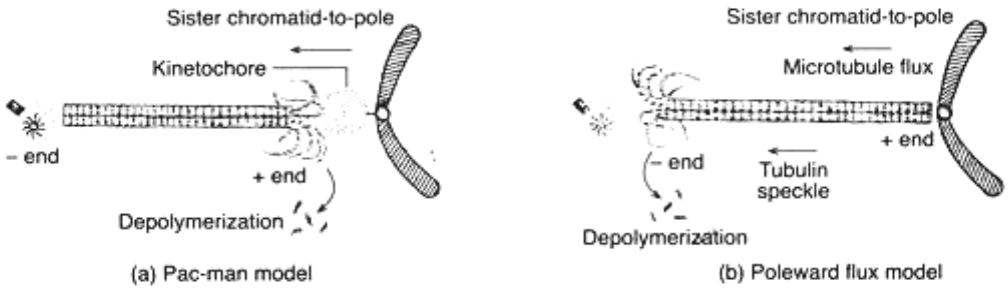


Fig. 5.23: A diagrammatic representation of (a) Pac-man model, suggesting depolymerization of microtubules at plus (+) end and, (b) Poleward flux model, suggesting depolymerization of microtubules at minus (–) end (from P. K.

Source: Kar and Halder 2008 Cell Biology, Genetics and Molecular

2. Karyotype concept in relation to evolution; banding techniques; GISH and FISH techniques.

Karyotype Analysis

The general morphology of the somatic chromosome complement of an individual constitutes its **karyotype**. From karyotype, chromosome number, centromere position, the presence of secondary constriction, size and shape of individual chromosomes and other attributes are known. Karyotypes are presented by arranging the somatic chromosomes complement in a descending order of size keeping their centromeres in a straight line (longest chromosomes is placed on the extreme left and smallest one on the extreme right). Karyotype is usually represented by a diagram called **ideogram** The karyotypes of different groups are sometimes compared and similarities in karyotype are presumed to represent evolutionary relationships. Karyotype also suggest primitive or advanced feature of an organism.

A **symmetric karyotype** has all metacentric chromosomes of the same size; karyotype showing large differences between smallest and largest chromosome of the set and having fewer metacentric chromosomes, is called **asymmetric karyotype** which is considered to be a relatively advanced feature when compared with.

Karyotype Evolution

Two aspects of the process of speciation are of interest in the context of cytogenetics. The first of these is changes in ploidy i.e., changes in the number of the chromosomes, which themselves remain unaltered. Changes in ploidy can have both genetic and phenotypic effects, such as fertility changes, and can be used to great effecting plant breeding to produce new cultivars. Additional sets of chromosomes can be from the individual (autopolyploid) or from an organism of genetically distinct origin (allopolyploids). In the case of humans, changes in ploidy can have very severe consequences, as can the second process of interest: changes in karyotype.

Karyotype changes can be thought of as being due to changes in either DNA content or chromosome structure as well as changes in chromosome numbers. In this context, it is possible to see speciation and karyotype changes that are linked, as in the marsupials, or not linked, as in the hominids. Evolution and speciation are closely related to observable changes in an organism’s chromosomes. It should, however, be clearly borne in mind that karyotype changes are rarely enough for speciation to occur on their own. It is, after all, the phenotype expression of the genome which determines the position of the fine line, sometimes indefinable, between variation and speciation.

Banding techniques

Karyotype analysis is a technique where chromosomes are visualized under a microscope. Cells are collected from an individual, induced to divide, and then arrested at metaphase (a stage of cell division when the chromosome are condensed and therefore visible). The chromosomes are stained with certain dyes that show a pattern of light and dark bands, which are called as the banding patterns. These bands reflect regional differences in the amounts of A and T versus G and C. The banding pattern for each chromosome is specific and consistent allowing identification of each of the chromosomes.

A band is an area of a chromosome which is clearly distinct from its neighboring area, but may be lighter or darker than its neighboring region. The standard methods of banding are the Q, G, R, and C banding techniques. These are defined as follows:

Q- banding- chromosomes are stained with fluorescent dye such as quinacrine. It yields a series of lightly and darkly stained bands — the dark regions tend to be heterochromatic, late-replicating and AT rich. The light regions tend to be euchromatic, early-replicating and GC rich.

G-banding- produced by Giemsa staining after digestion of chromosomes with trypsin. It also stains heterochromatin region.

C-banding- chromosomes are treated with acid and base, the stained with giemsa stain. Constitutive hetrochromatic regions are stained by this technique (centromere staining).

R-Banding- R-bands can be produced either by incubating chromosomes in a hot saline buffer, followed by staining in Giemsa, or by staining heat treated chromosomes directly with acridine orange.

F-banding- Chromosomes are stained by the Feulgen staining method.

Fluorescent in situ hybridization (FISH)

Fluorescent in situ hybridization (FISH) is a molecular cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. It was developed by biomedical researchers in the early 1980s and is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific RNA targets (mRNA, lncRNA and miRNA) in cells, circulating tumor cells, and tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues. FISH is useful, for example, to help a researcher identify where a particular gene falls within an individual's chromosomes. Here's how it works:

How does FISH work?

I. Make a probe complementary to the known sequence. When making the probe, label it with a fluorescent marker, e.g. fluorescein, by incorporating nucleotides that have the marker attached to them.

II. Put the chromosomes on a microscope slide and denature them.

III. Denature the probe and add it to the microscope slide, allowing the probe hybridize to its complementary site.

IV. Wash off the excess probe and observe the chromosomes under a fluorescent microscope. The probe will show as one or more fluorescent signals in the microscope, depending on how many sites it can hybridize to.

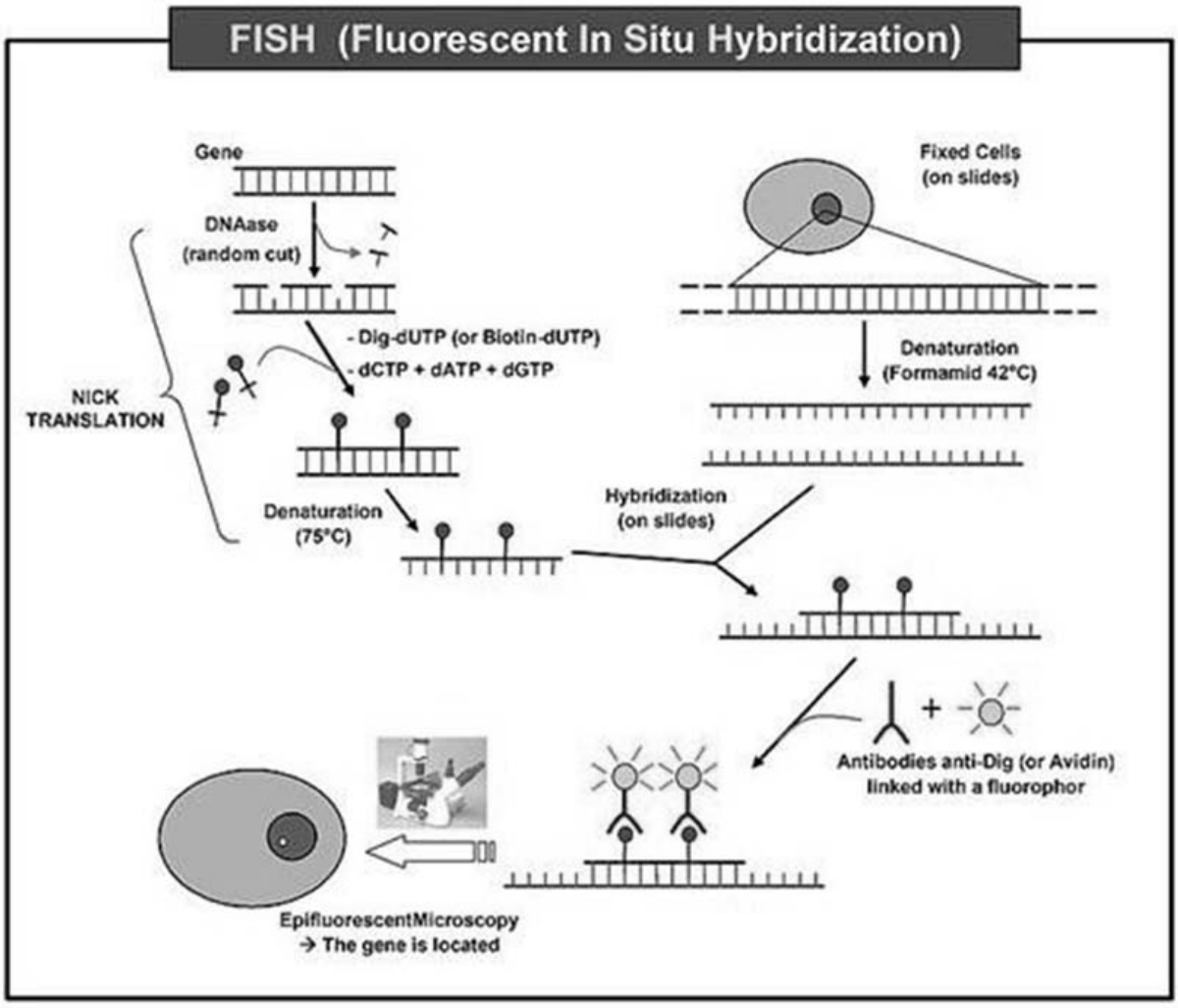


Figure Fluorescent in situ hybridization
Source: https://en.wikipedia.org/wiki/Fluorescence_in_situ_hybridization

What is FISH used for?

FISH is widely used for several diagnostic applications: identification of numerical and structural abnormalities, characterization of marker chromosomes, monitoring the effects of therapy, detection of minimal residual disease, tracking the origin of cells after bone marrow transplantation, identification of regions of deletion or amplification, detection of chromosome abnormalities in non-dividing or terminally differentiated cells, determination of lineage involvement of clonal cells, etc. Moreover, it has many applications in research: identification of non-random chromosome rearrangements, identification of translocation molecular breakpoint, identification of commonly deleted regions, gene mapping, characterization of somatic cells hybrids, identification of amplified genes, study the mechanism of rearrangements. FISH is also used to compare the genomes of two biological species to deduce evolutionary relationships.

Genomic in situ hybridization (GISH)

Genomic in situ hybridization (GISH), which is a modification of fluorescent in situ hybridization, has been widely used in the study of plants. It has become one of the most important techniques for molecular cytogenetics. GISH is a technique that allows distinguishing the genomes in a cell. With this technique, it is possible to differentiate the genomes in a hybrid; consequently, this tool has been applied to the study of hybrid lineages, genetic improvement programs, and studies of the evolution of polyploids. Moreover, GISH can be applied to the analysis of the meiotic behavior in hybrids and polyploids, providing information concerning the relationship between species. This review presents the wide application of this technique in plants when the objective is to distinguish parental chromosomes (or chromosome segments) in an interspecific hybridization or the distinct genomes of an allopolyploid, the entire genome of one parent should be labeled and used as probe. In this case, the technique is called genomic in situ hybridization (GISH). On the other hand, the genome of the second parent (unlabeled) is used as blocking DNA, aiming to avoid non-specific hybridization due to the similarity of the two parental genomes. Thus, both parental genomes (the probe and the blocking DNA) must be used together in the same hybridization mixture.

Main steps of the genomic in situ hybridization (GISH)-

(A) Direct and indirect probe labeling.

(B) Fragmentation of the blocking DNA.

(C) Slide preparation.

(D) Probe and blocking DNA denaturation in a hybridization mixture.

(E) Addition of the hybridization mixture with the probe and the blocking DNA.

(F) Denaturation of the chromosome DNA.

(G) In situ hybridization of probe and blocking DNA in the target sequence of the chromosome.

(H) Detection of the probe in the chromosome DNA of one parent, in an indirect labeling.

(I) Chromosome DNA molecule of the second parent associated to the unlabeled blocking DNA.

(J) Visualization of hybridization signals associated to a probe (green) in a fluorescence microscope. Unmarked chromosomes are visualized with a counter staining (blue). When the probe labeling is direct, the detection step of the GISH can be excluded. The fluorochromes are the signaling molecules and can be directly visualized in a fluorescence microscope with the appropriate filter.

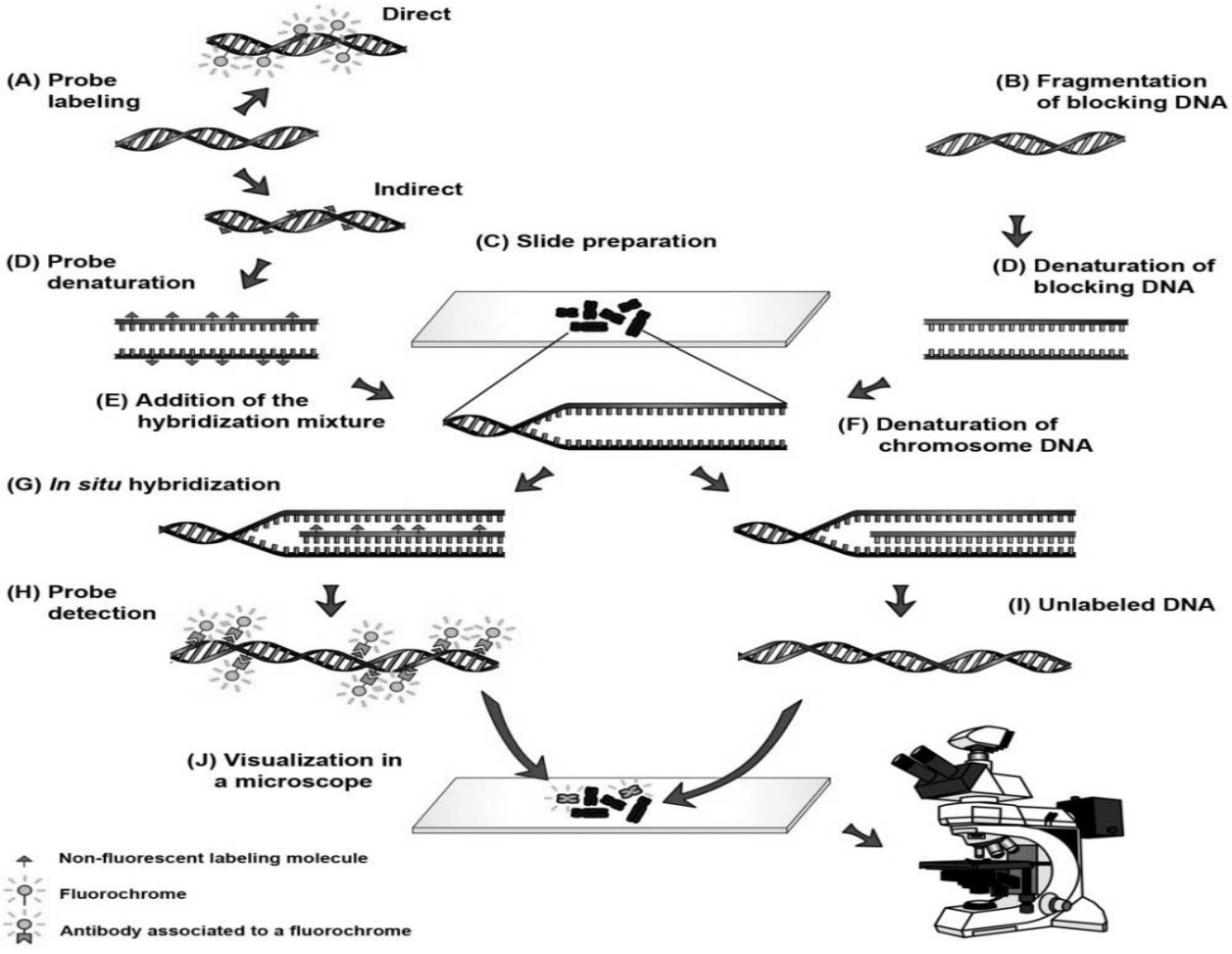


Figure Genomic in situ hybridization Source: Brammer et al. (2013)

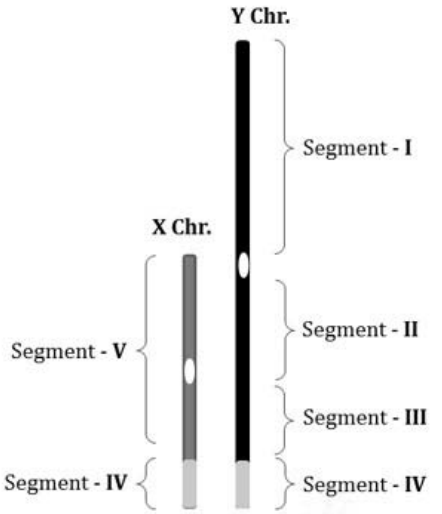
3. Sex determination: Sex determination in plants and their interrelationship with human, *Drosophila* and mice models; dosage compensation; sex linked inheritance.

Sex determination

A sex-determination system is a biological system that determines the development of sexual characteristics in an organism. Most organisms that create their offspring using sexual reproduction have two sexes. Occasionally, there are hermaphrodites in place of one or both sexes. There are also some species that are only one sex due to parthenogenesis, the act of a female reproducing without fertilization.

In many species, sex determination is genetic: males and females have different alleles or even different genes that specify their sexual morphology. In animals, this is often accompanied by chromosomal differences, generally through combinations of XY, ZW, XO, ZO chromosomes, or haplodiploidy. The sexual differentiation is generally triggered by a main gene (a "sex locus"), with a multitude of other genes following in a domino effect.

The **XX/XY sex determination system** is the most familiar, as it is found in humans. The XX/XY system is found in most other mammals, as well as some insects. In this system, most females have two of the same kind of sex chromosome (XX); while, most males have two distinct sex chromosomes (XY). The X and Y sex chromosomes are different in shape and size from each other, unlike the rest of the chromosomes (autosomes), and are sometimes called allosomes. In some species, such as humans, organisms remain sex indifferent for a time after they're created; in others, however, such as fruit flies, sexual differentiation occurs as soon as the egg is fertilized.



Sex Chromosomes (X & Y) of *Melandrium album*

Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Sex determination in plants

The sexual phenotype of individuals is determined by sex chromosomes; males are heterogametic (XY) and females are homogametic (XX). Early cytogenetic studies of sex determining mutants in to conclude that the Y chromosome is divided into three regions relevant to sex expression: one required for the suppression of female development and two required for the promotion of male development. None of these

regions would be necessary for the development of female reproductive organs, because these functions would reside on the X or autosomal chromosomes.

The mechanism of sex determination in *Coccinia indica*, a member of family Cucurbitaceae was studied in some detail by Prof. R.P. Roy and his co-workers at Patna University. They studied the sex in diploid, triploid and tetraploid plants with and without Y chromosome and observed that irrespective of the number of X chromosomes and/or autosomes, presence of a single Y chromosome gave a male individual.

Table 17.7. **The relation between chromosome constitution and sex in *Coccinia indica*.**

Chromosome constitution	X/A Ratio	Sex
2A + XX	1.00	Female
2A + XY	0.50	Male
2A + XYY	0.50	Male
3A + XXY	0.67	Male
3A + XXX	1.00	Female
4A + XXXX	1.00	Female
4A + XXXY	0.75	Male

Zea mays (maize) is an example of a monoecious species that produces only unisexual flowers in separate male and female inflorescences, referred to as the tassel and ear, respectively. Unisexuality in maize occurs through the selective elimination of stamens in ear florets (flowers) and by the elimination of pistils in tassel florets. The anther ear (an1) and dwarf (d1, d2, d3, and d5) mutants of maize are recessive and masculinize ears by preventing stamen abortion in the female florets.

Sexual dimorphism in heterothallic species can be extreme, as exemplified by members of the genus *Micromitrium* (Bryophyte), in which the dwarf male gametophyte grows on the leaves of the markedly larger female plan.

Asparagus is a dioecious form. However, rarely female flowers bear rudimentary anthers and male flowers bear rudimentary pistils. Thus, rare male flowers having poorly developed pistils may set seeds. In one such case, when seeds obtained from a rare male flower, were raised into plants, male and female plants were found to be present in 3:1 ratio. When male plants raised thus were used to pollinate female flowers on female plants, only two-third of them showed segregation indicating that sex is controlled by a single gene. In this case, maleness should be dominant over femaleness and male plants should ordinarily be heterozygous.

Sex determination in Humans

The discovery that human females are XX and that human males are XY suggested that sex might be determined by the number of X chromosomes or by the presence or absence of a Y chromosome. As we now know, the second hypothesis is correct. In humans and other placental mammals, maleness is due to a dominant effect of the Y chromosome.

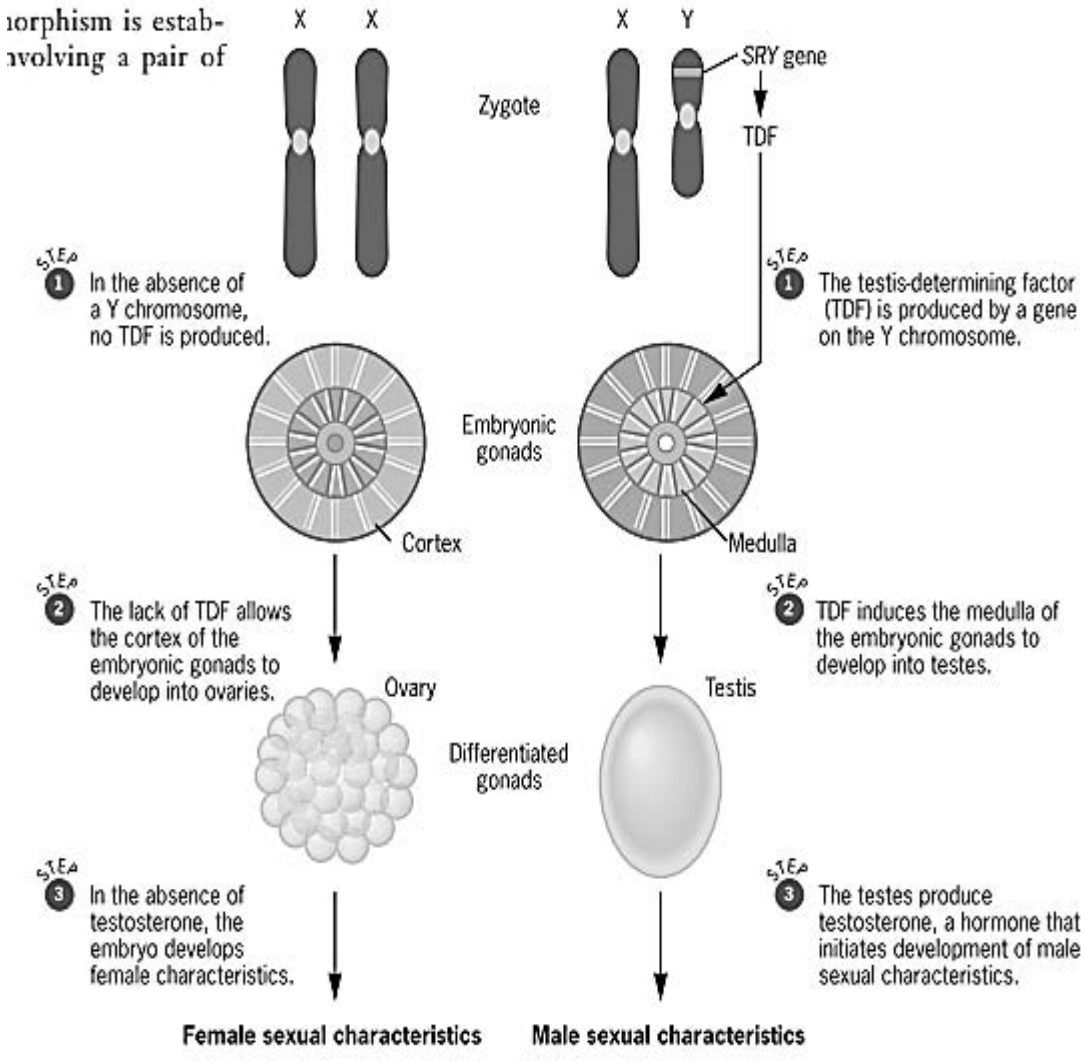


Figure Sexdetermination in human. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

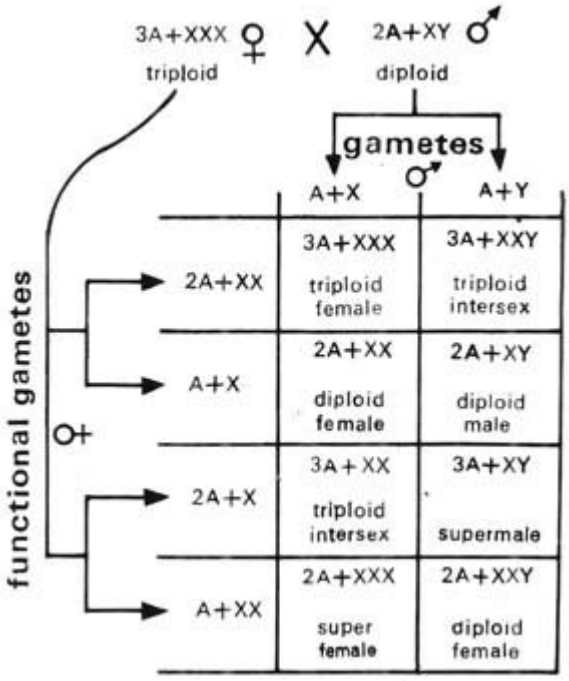
The human Y chromosome plays a key role in determining the sex of a developing embryo. This is mostly due to a gene called SRY ("sex-determining region of Y"). SRY is found on the Y chromosome and encodes a protein that turns on other genes required for male development

XX embryos don't have SRY, so they develop as female.

XY embryos do have SRY, so they develop as male.

Sex determination in Drosophila

The experiment of Bridges mentioned above, though demonstrated that Y-chromosome is not important for determination of sex, it did not indicate whether X-chromosome alone determines the sex or if autosomes also play any role in the mechanism. Individuals could, however, be obtained, which had two X-chromosomes as in the normal female, but were intersexes. Each of these intersexes had an extra set of autosomes (A) indicating that autosomes play a definite role in determination of sex. In this connection, Bridges' experiments on intersexes and supersexes are of special importance. Bridges, as early as 1922, came across certain *Drosophila* individuals which were triploids and thus had three sets of chromosomes (3A + 3X). These triploid individuals were normal females and were crossed with diploid males (2A + XY). The results obtained from such a cross are shown in Figure. As is obvious, from such a cross, normal diploid males, triploid females, intersexes, supermales and superfemales were obtained.



In *Drosophila*, occasionally flies are obtained which have female characters in one part of body and male characters in the remaining parts. Such individuals are known as gynandromorphs and are believed to result due to loss of an X-chromosome in particular cell during development. If this event happens during first mitotic division of zygote, then one of the two cells of two celled proembryo will have 2A + XX with X/A =1.0 and the other cell will have 2A + X with X/A = 0.5.

Dosage compensation

Dosage compensation

In species with XX-XY sex determination, the difference in the number of X chromosomes possessed by males and females presents a special problem in development. Because females have two copies of every X-linked gene and males have only one copy, the amount of gene product (protein) encoded by X-linked genes would differ in the two sexes: females would produce twice as much gene product as that produced by males. This difference could be highly detrimental because protein concentration plays a critical role in development. Animals overcome this potential problem through **dosage compensation,** which equalizes the amount of protein produced by X-linked genes

in the two sexes. In fruit flies, dosage compensation is achieved by a doubling of the activity of the genes on the X chromosome of the male.

Normally, each gene is present in two copies. Departures from this condition, either up or down, can cause abnormal phenotypes, and sometimes even death. It is therefore puzzling

that so many species should have a sex-determination system based on females with two X chromosomes and males with only one. In these species, how is the numerical difference of X-linked genes accommodated? A priori, three mechanisms may compensate for this difference: (1) each X-linked gene could work twice as hard in males as it does in females, or (2) one copy of each X-linked gene could be inactivated in females, or (3) each X-linked gene could work half as hard in females as it does in males. Extensive research has shown that all three mechanisms are utilized, the first in *Drosophila*, the second in mammals, and the third in the nematode *Caenorhabditis elegans*.

These three different mechanisms of **dosage compensation**—inactivation, hyperactivation, and hypoactivation have an important feature in common: many different genes are coordinately regulated because they are on the same chromosome. This chromosome wide regulation is superimposed on all other regulatory mechanisms involved in the spatial and temporal expression of these genes.

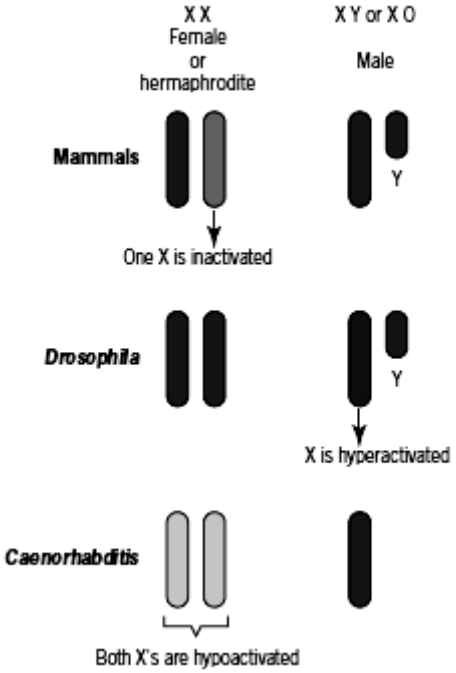


Figure Three different mechanisms of dosage compensation

Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Inactivation of X chromosomes in mammals

In mammals, X chromosome inactivation begins at a particular site called the X inactivation center (XIC) and then spreads in opposite directions toward the ends of the chromosome. Curiously, not all

genes on an inactivated X chromosome are transcriptionally silent. One that remains active is called XIST (for X inactive specific transcript); this gene is located within the XIC. In human beings the XIST gene encodes a 17-kb transcript devoid of any significant open reading frames. It therefore seems unlikely that the XIST gene codes for a protein. Instead, the RNA itself is probably the functional product of the XIST gene. Though polyadenylated, this RNA is restricted to the nucleus and is specifically localized to inactivated X chromosomes; it does not appear to be associated with active X chromosomes in either males or females.

In mice, where fairly detailed experimental analysis has been possible, researchers have found that the homologue of the human XIST gene is transcribed during the early stages of embryonic development at a low level from both of the X chromosomes that are present in females. The transcripts from each of a female mouse's Xist genes are unstable and remain closely associated with their respective genes. As development proceeds, the transcripts from one of the genes stabilize and eventually envelop the entire X chromosome on which that gene is located; the transcripts from the other Xist gene disintegrate, and further transcription from that gene is repressed by methylation of nucleotides in the gene's promoter. Thus, in the female mouse, one X chromosome—the one whose Xist gene continues to be transcribed—becomes coated with Xist RNA and the other does not. The choice of the chromosome that becomes coated is apparently random.

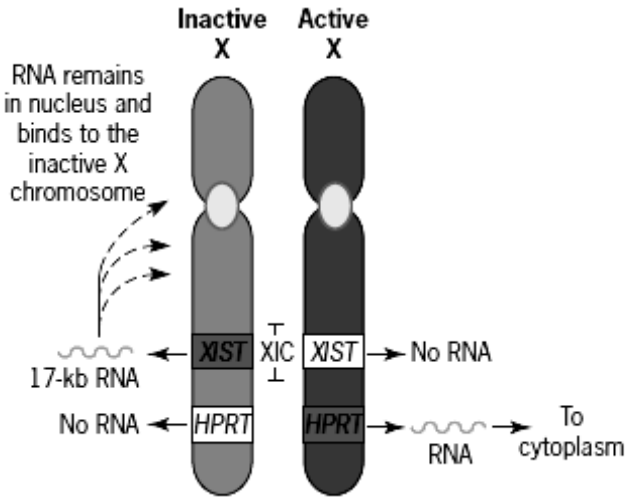
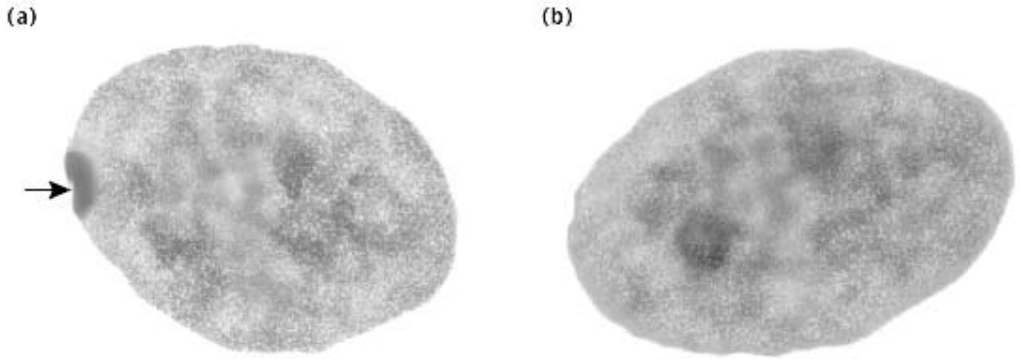


Figure Inactivation of X chromosome. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Lyon hypothesis:

In 1949, Murray Barr observed condensed, darkly staining bodies in the nuclei of cells from female cats; this darkly staining structure became known as a **Barr body.** Mary Lyon proposed in 1961 that the Barr body was an inactive X chromosome; her hypothesis has become known as the **Lyon**

hypothesis. She suggested that, within each female cell, one of the two X chromosomes becomes inactive; which X chromosome is inactivated is random. If a cell contains more than two X chromosomes, all but one of them is inactivated. The number of Barr bodies present in human cells with different complements of sex chromosomes is shown in.



A Barr body is an inactivated X chromosome.

(a) Female cell with a Barr body (indicated by arrow). (b) Male cell without a Barr body

The patchy distribution of color on tortoiseshell cats results from the random inactivation of one X chromosome in females.

Source: Benjamin A. Pierce, 2012, Genetics: A conceptual apporach -4th ed.

Hyperactivation of x chromosomes in *Drosophila*

In *Drosophila*, dosage compensation requires the protein products of at least five different genes. Null mutations in these genes result in male-specific lethality because the single X chromosome in males is not hyperactivated. Mutant males usually die during the late larval or early pupal stages. These dosage compensation genes are therefore called male-specific lethal (msl) loci, and their

products are called the MSL proteins. Antibodies prepared against these proteins have been used as probes to localize the proteins inside cells. The remarkable finding is that each of the MSL proteins binds specifically to the X chromosome in males. These proteins do not bind to the other chromosomes in the male's genome, and they do not bind to any of the chromosomes, including the X's, in a female's genome. The binding of the MSL proteins to the male's X chromosome is facilitated by two types of RNA molecules called roX1 and roX2 (for RNA on the X chromosome) that are transcribed from genes on the X chromosome.

Sex linked inheritance

The inheritance of a trait (phenotype) that is determined by a gene located on one of the sex chromosomes is called sex linked inheritance. The expectations of sex-linked inheritance in any species depend on how the chromosomes determine sex. For example, in humans, males are heterogametic. It has one X chromosome and one Y chromosome. But females are homogametic. They two X chromosomes. In human males, the entire X chromosome is active. But one of a female's X chromosomes is largely inactive. Random inactivation of one X chromosome occurs during the early stages of female embryogenesis. Therefore, every cell that forms from a particular embryonic cell has the same X chromosome inactivated. This pattern of sex determination occurs in most vertebrates, but in birds and many insects and fish the male is the homogametic sex.

In general terms, traits determined by genes on sex chromosomes are not different from traits determined by autosomal genes. Sex-linked traits are distinguishable by their mode of transmission through successive generations of a family. In humans, it is called X-linked or Y-linked inheritance.

Characteristics of Sex Linked Inheritance:

1. It is criss-cross inheritance. Father does not pass the sex-linked allele of a trait to his son. The same is passed to the daughter, from where it reaches the grandson, i.e. diagynic. It is because the males have only one X-chromosome which is transferred to the female offspring. Only Y-chromosome of the father is transferred to the male offspring but this sex chromosome does not carry many alleles.
2. Mother passes the alleles of sex-linked traits to both sons and daughters.
3. Majority of the sex linked traits are recessive.
4. Sex linked traits are more apparent in males than in females.
5. As many sex-linked traits are harmful, males suffer more from sex-linked disorders.
6. Females generally function as carriers of sex-linked disorders because recessive genes can express themselves in females only in the homozygous state.

I. X-linked recessive Traits: These are expressed in all heterogametic and homogametic which are homozygous for the recessive allele. An example is the sex-linked recessive is horns in sheep that

appear only in males. The recessive phenotypes of such genes are more common in males than in females. The examples of X-linked recessive trait in human are Color blindness, Duchenne muscular dystrophy, Hemophilia.

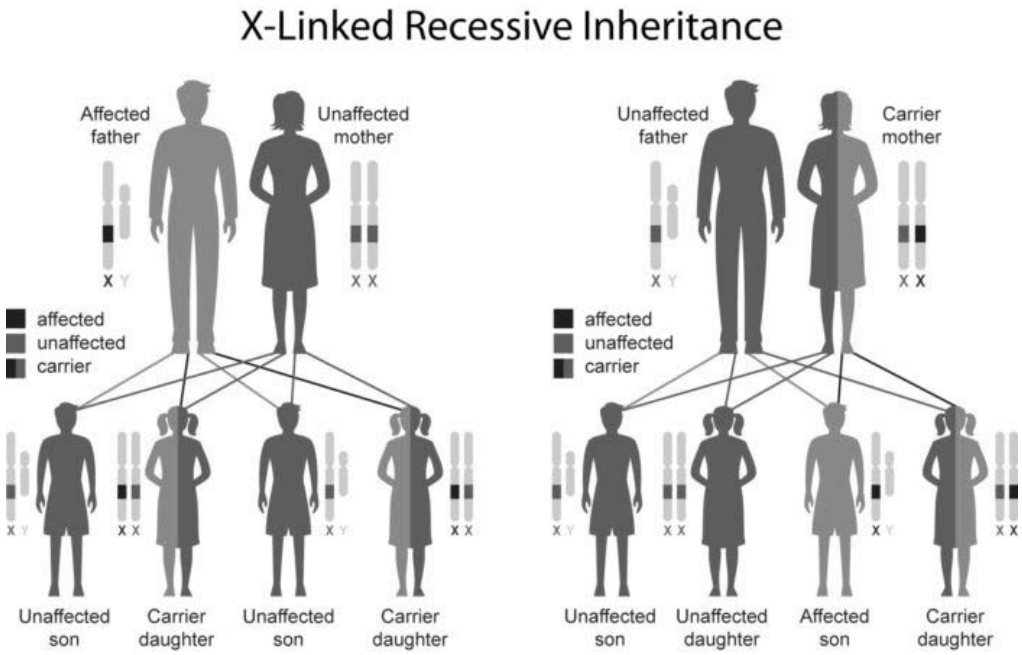


Figure X-linked recessive inheritance. Source: https://www.nfed.org/learn/genetics-inheritance/

4. Special type chromosomes: Cytogenetical significance of polytene and B-chromosome; deletion mapping; recombination.

Polytene Chromosome:

Polytene chromosomes provided the first evidence that eukaryotic gene activity is regulated at the level of RNA synthesis. When dipteran chromosomes become polytenic, the DNA replicates by endomitosis, and the resulting daughter chromatids remain aligned side by side.

There chromatids are visible during interphase and have a characteristic morphology of dark bands and alternating interbands. Within these chromosomes it is possible to observe the genetic activity of specific loci at local enlargements called puffs, which represent DNA undergoing intense gene transcription. Puff distribution varies from one tissue to another and can be induced experimentally, indicating the cell specialization results from variable gene transcription. Polytene chromosomes constitute a valuable material for the study of gene regulation because their gene transcription can be visualized directly in the microscope.

Some cells of dipteran (flies, mosquitoes, midges) larvae become very large and have a high DNA content. The most prominent ones are located in the salivary gland, but other cells from the gut, fat body, and malpighian tubules of the larva also become 'polytenic'. (Polyteny differs from polyploidy, in which there is also excess DNA per nucleus, but in which the new chromosomes are separate from each other).

A polytene chromosome of *Drosophila* salivary glands has about 1000 DNA molecules arranged side by side which arise from 10 rounds of DNA replication (210 = 1024). Other dipteran species have even more DNA molecules per polytene chromosome, for example, *Chironomus* has 16,000.

In polytene cells, the chromosomes are visible during interphase, and the chromomeres (regions in which the chromatin is more tightly coiled) alternate with regions where the DNA fibres are folded more loosely. The alignment of many chromosomes gives polytene chromosomes their characteristic morphology, in which a series of dark bands alternate with clear zones called interbands. There are about 5000 bands in the *Drosophilla* genome. They have characteristic morphology and positions, which permit detailed chromosome mapping.

An additional characteristic of polytene chromosomes is that the maternal and paternal homologue remains associated side by side, in what is called 'somatic pairing'. This permits the identification of deletions, inversions, duplication as regions looped out of the chromosomes. The pericentromeric heterochromatin of all the *Drosophila* chromosomes coalesces in a chromocenter, where the chromosomes are joined together. The satellite DNAs of the chromocenter are underreplicated with

respect to the rest of the chromosome, (i.e., they undergo fewer rounds of replication). Polytene cells are unable to undergo mitosis and are destined to die. Not all the cells in a dipteran larva have polytene chromosomes. Those destined to produce the adult structures after metamorphosis (imaginal discs) remains diploid.

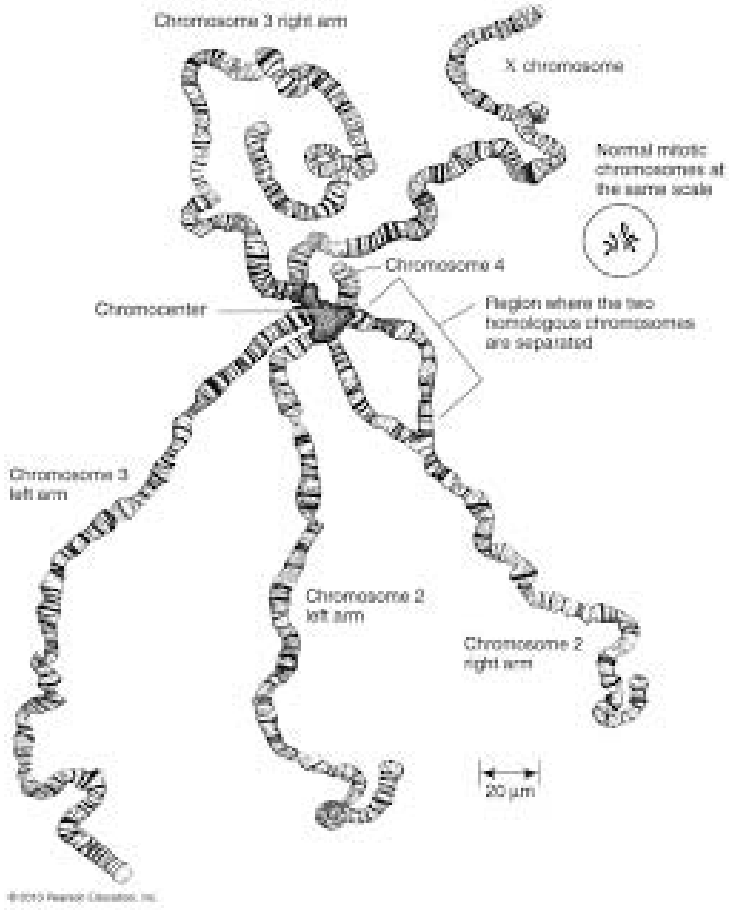


Figure Polytene chromosome. Source: https://www.mun.ca/biology/scarr/Polytene_Chromosomes.html

Polytene chromosomes have become even more important with the advent of recombinant DNA techniques because they make it possible to map any DNA segment to specific chromosomal loci by in sit hybridization. Polytene chromosomes are very suitable for in situ hybridization because their 1000 DNA molecules are aligned side by side, thereby greatly facilitating the detection of single copy genes.

The bands have greatly helped in the mapping of chromosomes in cytogenetics studies. The bands occasionally form reversible "puffs" known as "chromosome puffs" or "Balbiani rings" which are associated with differential gene activation. A puff can be considered a band in which the DNA unfolds into open loops as a consequence of intense gene transcription, i.e., "puffs are sites of intense gene transcription".

In salivary glands, the appearance of some puffs has been correlated with the production of specific proteins which are secreted in large amounts in the larval saliva, e.g. *Chironomou*s has at the base of the salivary glands four specialized cells that contain cytoplasmic granules of a special secretary protein. The gene for this protein is located in a distinct puff that appears only in the four specialized cells. These results show that cell specialization results from variable gene transcription.

In *D. melanogaster* the giant chromosomes are found in the form of five long and one short strands radiating from a single more or less amorphous mass known as chromocentre. One long strand corresponds to the X-chromosome and the remaining four long strands are the arms of IInd and IIIrd chromosomes. The short strand which is small dot like is IVth chromosome. The centromeres of all there chromosomes fuse to form chromocentre. In the male files, the Y chromosome is also found fused within the chromocentre and is therefore not seen as a separate strand.

B chromosomes

B chromosomes are extra chromosomes to the standard complement that occur in many organisms. They can originate in a number of ways including derivation from autosomes and sex chromosomes in intra- and interspecies crosses.

In addition to the normal karyotype, wild populations of many animal, plant, and fungi species contain B chromosomes (also known as **supernumerary, accessory chromosomes**). These chromosomes are not essential for the life of a species, and are lacking in some (usually most) of the individuals. Thus a population would consist of individuals with 0, 1, 2, 3 supernumeraries.

Most B chromosomes are mainly or entirely heterochromatic, but some, such as the B chromosomes of maize, contain sizeable euchromatic segments. In general it seems unlikely that supernumeraries would persist in a species unless there was some positive adaptive advantage, which in a few cases has been identified. For instance, the British grasshopper *Myrmeleotettix maculatus* has two structural types of B chromosomes: metacentrics and submetacentrics. The supernumeraries, which have a satellite DNA, occur in warm, dry environments, and are scarce or absent in humid, cooler localities.

The evolutionary origin of supernumerary chromosomes is obscure, but presumably they must have been derived from heterochromatic segments of normal chromosomes in the remote past.

Function:

B chromosomes may play a positive role on normal A chromosomes in some circumstances. The B chromosomes suppress homologous pairing which reduces multiple pairing between homologous chromosomes in allopolyploids. Bivalent pairing is ensured by a gene on chromosome 5 of the B genome Ph locus. The B chromosomes also have the following effects on A chromosomes:

- increases asymmetry chiasma distribution
- increases crossing over and recombination frequencies: increases variation
- cause increased unpaired chromosomes: infertility
- B chromosomes have tendency to accumulate in meiotic cell products resulting in an increase of B number over generations. However this effect is counterbalanced for selection against infertility.

Deletion mapping

In genetics and especially genetic engineering, deletion mapping is a technique used to find out the location of mutation sites within a gene. The principle of deletion mapping involves crossing a strain which has a point mutation in a gene, with multiple strains who each carry a deletion in a different region of the same gene. Wherever recombination occurs between the two strains to produce a wild-type (+) gene (regardless of frequency), the point mutation cannot lie within the region of the deletion. If recombination cannot produce any wild-type genes, then it is reasonable to conclude that the point mutation and deletion are found within the same stretch of DNA.

This example should demonstrate how the principle works:

Suppose you have a gene X, which in wild-type (+) form can be shown linearly like so:

5'----------

--3' gene X, +

Suppose a strain of organisms has a point mutation in the gene (now called gene X, – to denote that it is no longer wild-type):

5'----------X----------

---3' gene X, –

Now suppose you have two strains of organisms, each with deletions in gene X at different sites, called del-1 and del-2, respectively (the dotted line indicates the site of deletion)

5'----------(.....) ---------- 3' del-1

5'----------(.....)---------3' del-2

Because the point mutation lies within the deletion of del-1, there will be no wild-type (+) recombinants between the point mutant and the del-1 mutant. However, in a cross between the point

mutant and the del-2 mutant, there could be a successful wild-type (+) recombinant produced.
In genetic recombination, if a mutant allele in the donor is within the sequence corresponding to the region deleted in the recipient, then no (+) recombinants will be obtained (as in the cross with del-1). To repair a deletion by recombination, the donor must have wild-type DNA sequence in the region corresponding to the DNA deleted in the recipient (as in the cross against del-2). In other words, there is a feasible recombination possibility between the point mutant and del-2 in which a length of DNA could be made that contained neither the point mutation, nor the deletion, indicating that the mutations in these two strains cannot be in the same region.
Note that not all crossovers between the point mutant and del-2 will yield (+) recombinants; in this case only those crossover events that occur between the point mutant and the 5' end of the deletion would inherit the wild-type sequence.

Recombination

Recombination is the production of new DNA molecule(s) from two parental DNA molecules or different segments of the same DNA molecule; this will be the topic of this chapter.

Types and examples of recombination

At least four types of naturally occurring recombination have been identified in living organisms. **General or homologous recombination** occurs between DNA molecules of very similar sequence, such as homologous chromosomes in diploid organisms. General recombination can occur throughout the genome of diploid organisms, using one or a small number of common enzymatic pathways. This chapter will be concerned almost entirely with general recombination. **Illegitimate or nonhomologous recombination** occurs in regions where no large-scale sequence similarity is apparent, e.g. translocations between different chromosomes or deletions that remove several genes along a chromosome. However, when the DNA sequence at the breakpoints for these events is analyzed, short regions of sequence similarity are found in some cases. For instance, recombination between two similar genes that are several million bp apart can lead to deletion of the intervening genes in somatic cells. **Site-specific recombination** occurs between particular short sequences (about 12 to 24 bp) present on otherwise dissimilar parental molecules. Site-specific recombination requires a special enzymatic machinery, basically one enzyme or enzyme system for each particular site. Good examples are the systems for integration of some bacteriophage, such as l, into a bacterial chromosome and the rearrangement of immunoglobulin genes in vertebrate animals. The third type is **replicative recombination**, which generates a new copy of a segment of DNA. Many transposable elements use a process of replicative recombination to generate a new copy of the transposable element at a new location.

Recombinant DNA technology uses two other types of recombination. The directed cutting and rejoining of different DNA molecules *in vitro* using restriction endonucleases and DNA ligases. Once made, these recombinant DNA molecules are then introduced into a host organism, often a bacterium. If the recombinant DNA is a plasmid, phage or other molecule capable of replicating in the host, it will stay extrachromosomal. However, one can introduce the recombinant DNA molecule into a host in which it cannot replicate, such as a plant, an animal cell in culture, or a fertilized mouse egg. In order for the host to be stably transformed, the introduced DNA has to be taken up into a host chromosome. In bacteria and yeast, this can occur by homologous recombination at a reasonably high frequency. However, this does not occur in plant or animal cells. In contrast, at a low frequency, some of these introduced DNA molecules are incorporated into random locations in the chromosomes of the host cell. Thus random recombination into chromosomes can make stably transfected cells and transgenic plants and animals. The mechanism of this recombination during transformation or transfection is not well understood, although it is commonly used in the laboratory.

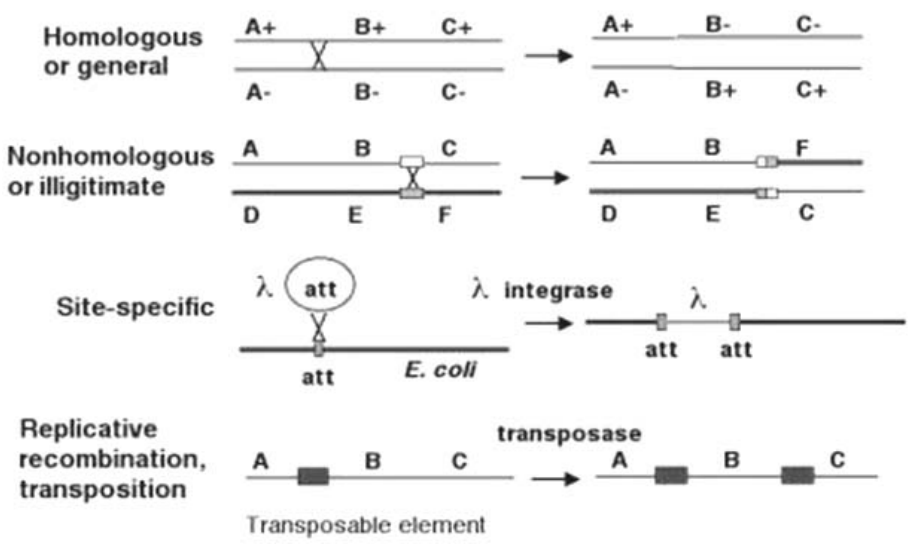


Figure Type of recombination. Source: http://www.bx.psu.edu/~ross/workmg/RecombineDNACh8.htm

Types of natural recombination Each line represents a chromosome or segment of a chromosome; thus a single line represents both strands of duplex DNA. For homologous or general recombination, each homologous chromosome is shown as a different shade of blue and a distinctive thickness, with different alleles for each of the three genes on each. Recombination between genes A and B leads to a reciprocal exchange of genetic information, changing the arrangement of alleles on the chromosomes. For non-homologous (or illegitimate) recombination, two different chromosomes (denoted by the different colors and different genes) recombine, moving, e.g. gene C so that it is now on the same chromosome as genes D and E. Although the sequences of the two chromosomes differ for most of their lengths, the segments at the sites of recombination may be related, denoted by the yellow and orange rectangles. Site-specific recombination leads to the combination of two

different DNA molecules, illustrated here for a bacteriophage l integrating into the *E. coli* chromosome. This reaction is catalyzed by a specific enzyme that recognizes a short sequence present in both the phage DNA and the target site in the bacterial chromosome, called att. Replicative recombination is seen for some transposable elements, shown as red rectangles, again using a specific enzyme, in this case encoded by the transposable element.

5. Linkage and crossing over: chiasma frequency and genetic map distance; Evolutionary significance of recombination; tetrad analysis; centromere mapping with ordered tetrad.

Linkage

Linkage is the phenomenon of certain genes staying together during inheritance through generations without any change or separation due to their being present on the same chromosome.

It was Morgan (1910) who clearly proved and defined linkage on the basis of his breeding experiments in fruitfully *Drosophila melanogaster*. In 1911, Morgan and Castle proposed chromosome theory of linkage. It states that

(i) Linked genes occur in the same chromosome.

(ii) They lie in a linear sequence in the chromosome.

(iii) There is a tendency to maintain the parental combination of genes except for occasional crossovers.

(iv) Strength of the linkage between two genes is inversely proportional to the distance between the two, i.e.,, two linked genes show higher frequency of crossing over if the distance between them is higher and lower frequency if the distance is small.

Linked genes are those genes which occur on the same chromosome while unlinked genes are the ones found on different chromosomes. Linked and unlinked genes can be easily known from breeding experiments. Unlinked genes show independent assortment, a di-hybrid ratio of 9 : 3 : 3 : 1 and the di-hybrid or double test cross ratio of 1 : 1 : 1 : 1 with two parental and two recombinant types.

The linked genes do not show independent assortment but remain together and are inherited en block producing only parental type of progeny. They give a dihybrid ratio of 3 : 1 and a test cross ratio of 1 : 1.

Types of Linkage:

Linkage is of two types, complete and incomplete.

1. Complete Linkage:

The genes located on the same chromosome do not separate and are inherited together over the generations due to the absence of crossing over. Complete linkage allows the combination of parental traits to be inherited as such. It is rare but has been reported in male *Drosophila* and some other heterogametic organisms.

2. **Incomplete Linkage:**

Genes present in the same chromosome have a tendency to separate due to crossing over and hence produce recombinant progeny besides the parental type. The number of recombinant individuals is usually less than the number expected in independent assortment. In independent assortment all the four types (two parental types and two recombinant types) are each 25%. In case of linkage, each of the two parental types is more than 25% while each of the recombinant types is less than 25%.

Linkage Groups:

A linkage group is a linearly arranged group of linked genes which are normally inherited together except for crossing over.

Significance of Linkage:

(i) Linkage plays an important role in determining the nature of scope of hybridization and selection programmes.

(ii) Linkage reduces the chance of recombination of genes and thus helps to hold parental characteristics together. It thus helps organism to maintain its parental, racial and other characters. For this reason plant and animal breeders find it difficult to combine various characters.

Crossing over

Crossing over is the exchange of genetic material between homologous chromosomes that results in recombinant chromosomes during sexual reproduction. It is one of the final phases of genetic recombination, which occurs in the pachytene stage of prophase I of meiosis during a process called synapsis. Synapsis begins before the synaptonemal complex develops and is not completed until near the end of prophase I. Crossover usually occurs when matching regions on matching chromosomes break and then reconnect to the other chromosome.

Crossing over was described, in theory, by Thomas Hunt Morgan.

1. Crossing over takes place during meiotic prophase, i.e.,, during pachytene. Each pair of chromosome has four chromatids at that time.
2. Crossing over occurs between non-sister chromatids. Thus one chromatid from each of the two homologus chromosomes is involved in crossing over.
3. It is universally accepted that crossing over takes place at four strand stage.
4. Each crossing over involves only two of the four chromatids of two homologus chromosomes. However, double or multiple crossing over may involve all four, three or two of the four chromatids, which is very rare.

5. Crossing over leads to re-combinations or new combinations between linked genes. Crossing over generally yields two recombinant types or crossover types and two parental types or non-crossover types.
6. Crossing over generally leads to exchange of equal segments or genes and recombination is always reciprocal. However, unequal crossing over has also been reported.
7. The value of crossover or recombinants may vary from 0-50%.
8. The frequency of recombinants can be worked out from the test cross progeny. It is expressed as the percentage ratio of recombinants to the total population (recombinants + parental types). Thus,

$$\text{Crossing over frequency (\%)} = \frac{\text{No. of recombinants}}{\text{Total progeny}} \times 100$$

Cases of two strand crossing over, somatic crossing over, sister strand crossing over and unequal crossing over is also known. However, frequency of such cases is extremely low, i.e., in fractions. Crossing over differs from linkage in several aspects.

Chiasma Frequency

In genetics during chromosomal crossover chiasma is the point where two homologous non sister chromatids exchange their genetic material which is DNA, as their genetic material is identical there is no noticeable change in daughter cells.

The chiasma becomes visible during prophase I of meiosis, more specifically during the diplotene stage. However, the actual crossover of genetic material occurs only during pachytene stage of prophase I.

Chiasma frequency is considered as the calculation of the level of genetic recombination of a population.

Chiasma frequency is calculated by,

$$f_c = 2 \times f_r$$

Here, f_c is the chiasma frequency, f_r is recombination frequency

Recombination frequency is given by

$$f_r = \frac{(N \times 100)}{N_p}$$

Here, N is the number of recombinants; N_p is the total number of progeny.

According to the given theory on the formations of the chiasmata, it is the cause of genetic crossover. The chiasmata can lead to the division and simultaneous exchange of the chromosomal segments but if crossover takes place, it is the result of the strain acted by the chiasma formation.

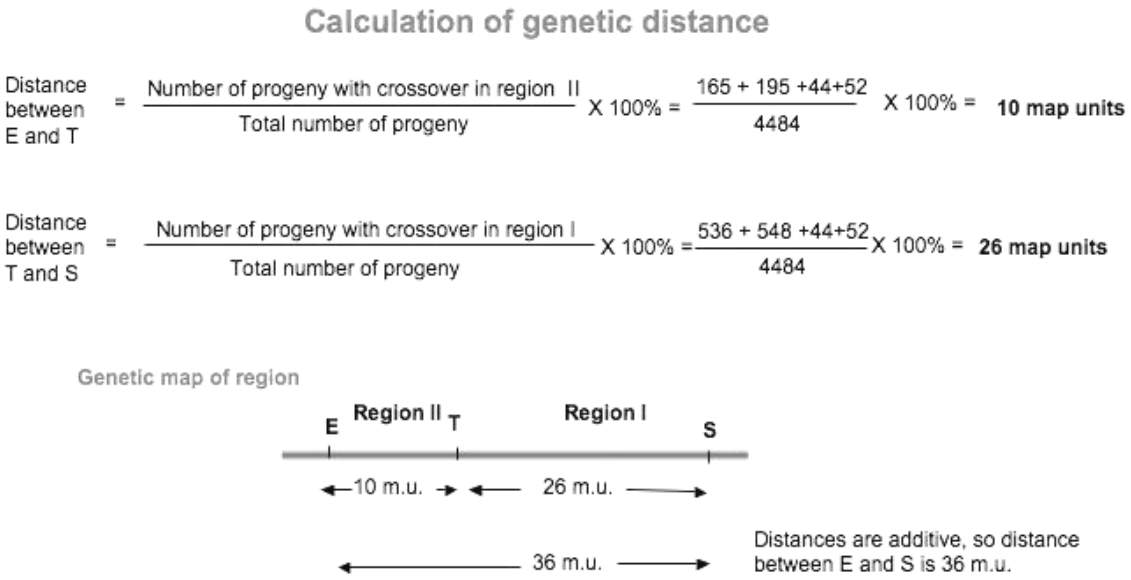
According to the hypothesis, adjacent loops should have some (sister chromatids separating) and reductional (sister chromatids not separating) separation of chromatids.

Chromosome pairs of different sizes are not affected by variation in minimum interchiasma distances. However, the average interchiasma distance increases with the bivalent length. According to the minimum-inter chiasma distance data, chiasma interference is complete over a chromosomal fragment that is about 60 Mb in length.

Genetic map distances

Genetic map distances are the distance between two points on the genetic map of a chromosome is the average number of crossovers between them.

The typical unit of genetic linkage is the centimorgan (cM). A distance of 1 cM between two markers means that the markers are separated to different chromosomes on average once per 100 meioses.



Tetrad analysis

The tetrad is the four spores produced after meiosis of a yeast or other Ascomycota, *Chlamydomonas* or other alga, or a plant. After parent haploids mate, they produce diploids.

Under appropriate environmental conditions, diploids sporulate and undergo meiosis. The meiotic products, spores, remain packaged in the parental cell body to produce the tetrad.

If the two parents have a mutation in two different genes, the tetrad can segregate these genes as the parental ditype (PD), the non-parental ditype (NPD) or as the tetratype (TT).

Parental ditype: a tetrad type containing two different genotypes, both of which are parental. A spore arrangement in Ascomycetes that contains only the two non-recombinant-type ascospores.

Non-parental ditype: a non-parental ditype (NPD) is a spore that contains only the two recombinant-type ascospores (assuming two segregating loci). A tetrad type containing two different genotypes, both of which are recombinant.

Tetratype: a tetrad containing four different genotypes, two parental and two recombinant. A spore arrangement in Ascomycetes that consists of two parental and two recombinant spores indicates a single crossover between two linked loci.

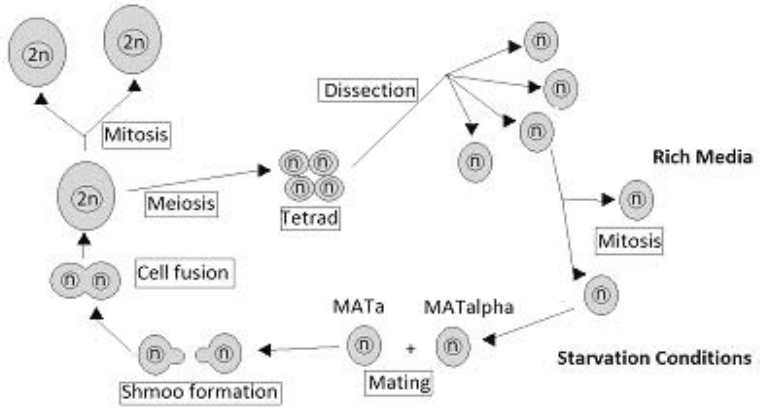
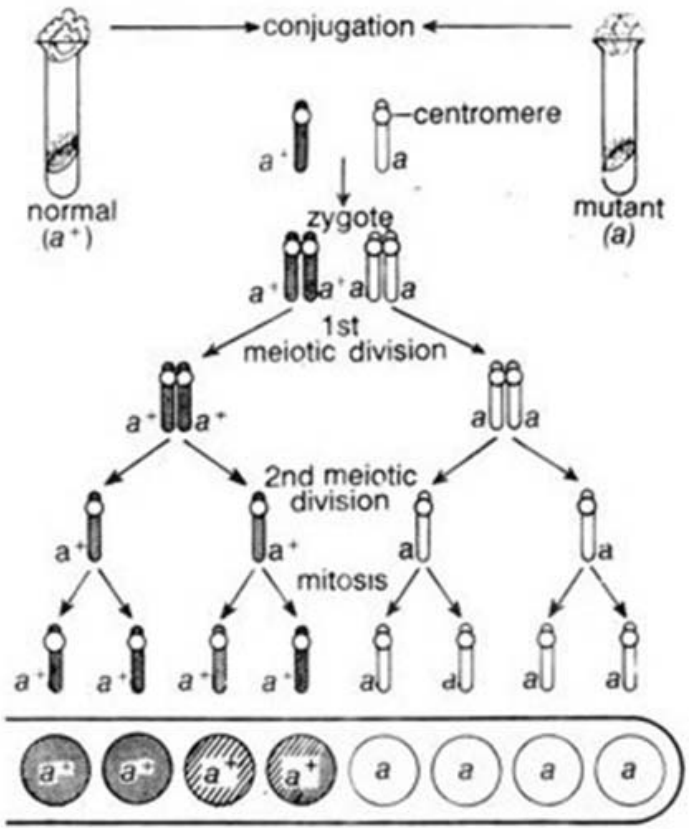


Figure Tedrad analysis. Source: Peter J. Russell IGenetics 3rd ed.

Centromere mapping with ordered tetrad. Analysis of ordered tetrads

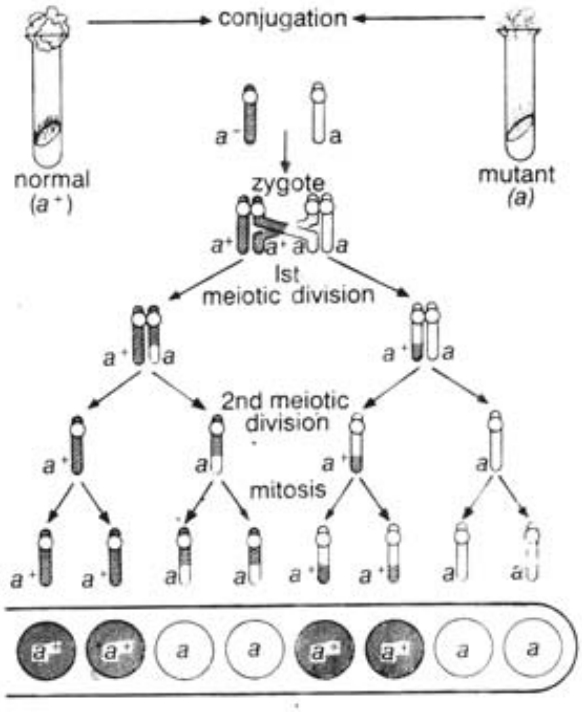
Crossing over between centromere and gene (first and second division segregations).

In *Neurospora*, products of meiosis are present in a linear order, so that ordered tetrads are obtained. Such a situation is not found in *Chlamydomonas* although tetrad analysis can be conducted. Due to ordered tetrads in *Neurospora*, a cross between normal (a+) and mutant (a) strain, will give rise to a linear arrangement of four normal spores (a+) at one end followed by four mutant spores (a) at the other end. Such an arrangement would be disturbed if crossing over occurs between centromere and the gene, because crossing over occurs at four strand and not at two strand stage. If crossing over at two strand stage was possible, it would again lead to four normal spores followed by four mutant spores. Therefore, by analysing linear arrangement, a 4:4 arrangement will suggest absence of crossing over and a paired arrangement (24: 2a : 24 :2a or 2A :4a :2A or 2a :4A :2a) will suggest that crossing over has taken place between the gene and corresponding centromere. When crossing over is absent leading to 4:4 arrangement, this is described as First Division Segregation and when crossing over takes place leading to paired arrangement, it is described as Second Division Segregation.



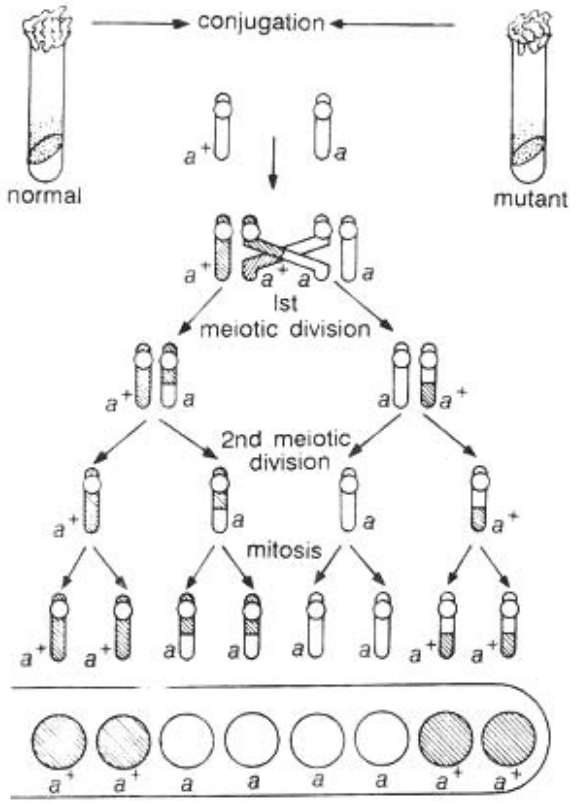
Source: Source: Peter J. Russell IGenetics 3rd ed.

Results showing first division segregation in a cross between normal (a+) and mutant (a) strains of- *Neurospora*, in which crossing over between the gene and centromere does not take place.



Source: Source: Peter J. Russell IGenetics 3rd ed.

Results of a linear order of ascospores (2:2:2:2) showing second division segregation in a cross between normal (a+) and mutant (a) strains of *Neurospora*, in which crossing over takes place between the gene and the centromere.



Source: Source: Peter J. Russell IGenetics 3rd ed.

Results of a linear order of ascospores (2:4:2) which is different than the one shown in Figure. but still showing second division segregation in a cross between normal (a+) and mutant (a) strains of *Neurospora*, in which crossing over takes place between the gene and the centromere. Frequency of recombination between centromere and gene. In *Neurospora*, since crossing over between a gene and centromere leads to second division segregation, the relative proportion of asci exhibiting second division segregation will give an estimate of crossing over. This will not be possible in *Chlamydomonas* due to absence of ordered tetrads. As an illustration, in an experiment in *Neurospora*, cross between albino (al) and wild type (al+) gave first division segregation (al al al+ al+) in 129 asci and second division segregation (al al+ al al+) in 141 tetrads. Since only two of the four strands undergo crossing over in second division segregation, recombination between centromere and the gene is-

$$\frac{1}{2} \times \frac{141}{(129+141)} = 0.26 \text{ or } 26 \text{ per cent.}$$

If a gene is located far away from centromere, crossing over may take place between the gene and centromere in each and every tetrad so that 100% tetrads should exhibit second division segregation. However, since in such cases, double crossovers, and multiple crossovers are also possible, some of the tetrads due to double crossover and other even number of crossover events, will give rise to first division segregation. Thus, second division segregation will never reach 100% level and in practice does not exceed 67% giving rise to only 33% recombination frequency (½ x 67, since only two of the four chromatids are involved in crossing over). This 33% recombination frequency will actually represent 50 units or more. Therefore, recombination frequencies should not be estimated over long distances, but should be estimated over small distances to avoid underestimation due to double and even multiple crossovers.

6. Reciprocal translocation: Cytogenetics of reciprocal translocation in plant species; Gaudens and Velans complex; reciprocal translocation in humans.

Cytogenetics of reciprocal translocation in plant species Gaudens and Velans complex

A translocation occurs when a portion of a chromosome is transferred to another location, either on the same chromosome or on some recipient chromosome not belonging to the same chromosome pair (i.e.,, the same homolog pair) as the donor chromosome. Two chromosomes that belong to the same chromosome pair, that is, that contain the same set of loci in the same order, are called homologous. A **reciprocal translocation**, the most common kind of translocation, is an exact interchange of chromosomal segments between two chromosomes not belonging to the same chromosome pair.

In a translocation heterozygote two distinct pairs of homologous chromosomes have reciprocally exchanged nonhomologous segments between one member of each pair. As a result each of the affected chromosome pairs contain both homologous and nonhomologous segments. Put another way, each such pair has one translocated chromosome, and one normal (untranslocated) chromosome. More than two chromosome pairs can be altered in this way so that some or all of the chromosome pairs are composed of a translocated and an untranslocated member.

Organisms having chromosomes rearranged in this way are known as permanent translocation heterozygotes. Due to the way reciprocal translocations are processed during meiosis, all the translocated chromosomes pass to one gamete and all the normal chromosomes pass to the other. As a result, only two types of gametes are produced. One has all the translocated chromosomes and the other has all the normal ones (here "translocated" and "normal" are relative terms since in naturally occurring organisms it is usually unknown which of the two types was original). These two sets of chromosomes ("normal" and "translocated") are known as Renner complexes. In well- studied organisms of this type, the various Renner complexes have been assigned formal names.

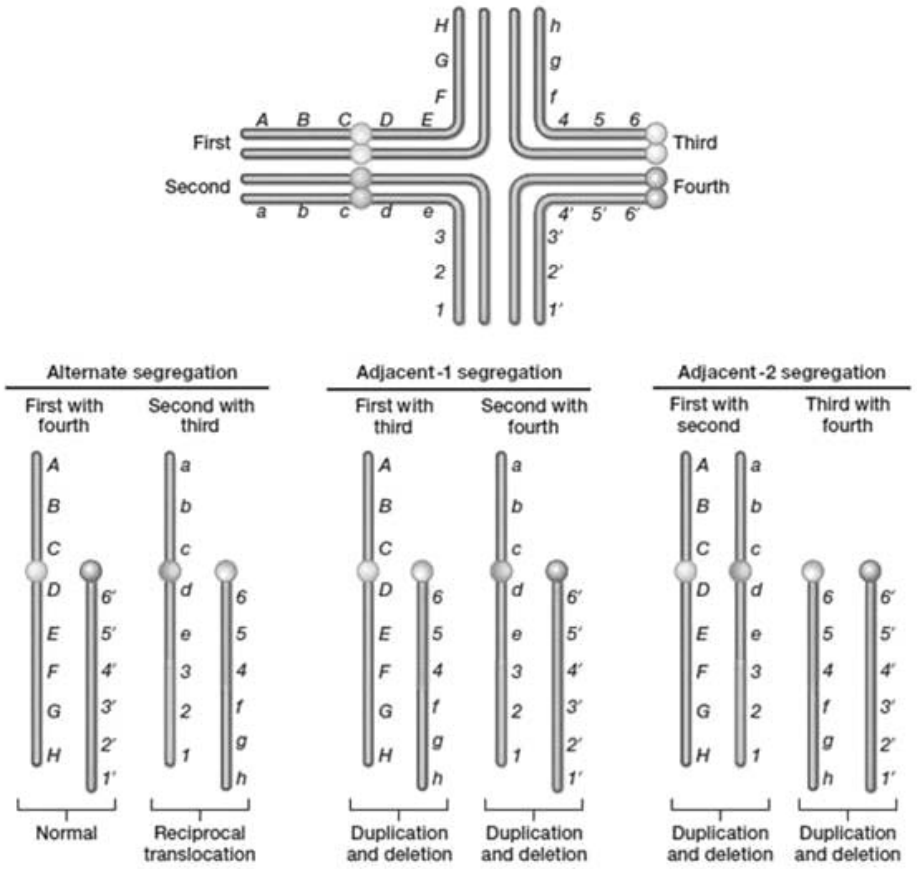


Figure Reciprocal translocation. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Oenothera lamarckiana

In one of the best-known permanent translocation heterozygotes, the evening primrose *Oenothera lamarckiana*, the two Renner complexes are called velans and gaudens. So the karyotype of *O. lamarckiana* is designated as "velans/gaudens." Genetic interchange (crossing-over) between Renner complexes is very rare. So chromosomes in distinct complexes differ genetically in many respects.

Aneuploidization is a common stabilization process among permanent translocation heterozygotes because there is an enhanced tendency to produce aneuploid gametes. The result is the production of numerous trisomic forms. But the most common stabilization process seen in organisms of this type occurs when forms with distinct Renner complexes hybridize to produce new forms. For example, hybridization of *O. lamarckiana* with another evening primrose, *O. strigosa*, which has the Renner complexes deprimens and strigens, yields four different types.

velans/deprimens, velans/strigens, gaudens/deprimens, gaudens/strigens

Some hybrids produced by such recombinations of Renner complexes are reproductively stable and others are not. By this means new stable types can be produced in a single generation. *O. lamarckiana* itself arose by such a process via hybridization between *O. biennis* and *O. hookeri*.

This event occurred within the last two or three centuries in Europe where the two parental forms, both native to North America, were introduced. *O. lamarckiana* spread across Europe and later to North America. In fact, most of the 18 forms of European *Oenothera* that Renner (1942) treated as species arose in this way.

Robertsonian translocation (ROB) is a rare form of chromosomal rearrangement where the participating chromosomes break at their centromeres and the long arms fuse to form a single, large chromosome with a single centromere. In humans, Robertsonian translocations generally occur in the five acrocentric chromosome pairs, namely 13, 14, 15, 21 and 22. Other Robertsonian translocations can occur, but do not lead to a viable fetus. They are named after the American biologist William Rees Brebner Robertson Ph.D. (1881–1941), who first described a Robertsonian translocation in grasshoppers in 1916.They are also called whole-arm translocations or centric-fusion translocations. They are a type of chromosomal translocation.

A Robertsonian translocation is a type of translocation involving two homologous (paired) or non-homologous chromosomes (i.e., two different chromosomes, not belonging to a homologous pair). A feature of chromosomes that are commonly found to undergo such translocations is that they possess an acrocentric centromere, partitioning the chromosome into a large arm containing the vast majority of its genes, and a short arm with a much smaller proportion of genetic content. The short arms also join to form a smaller reciprocal product, which typically contains only nonessential genes also present elsewhere in the genome, and is usually lost within a few cell divisions. This type of translocation is cytologically visible, and can reduce chromosome number

(from 23 to 22 pairs, in humans) if the smaller chromosome that results from a translocation is lost in the process of future cellular divisions. However, the smaller chromosome lost may carry so few genes (which are, in any case, also present elsewhere in the genome) that it can be lost without an ill effect to the individual.

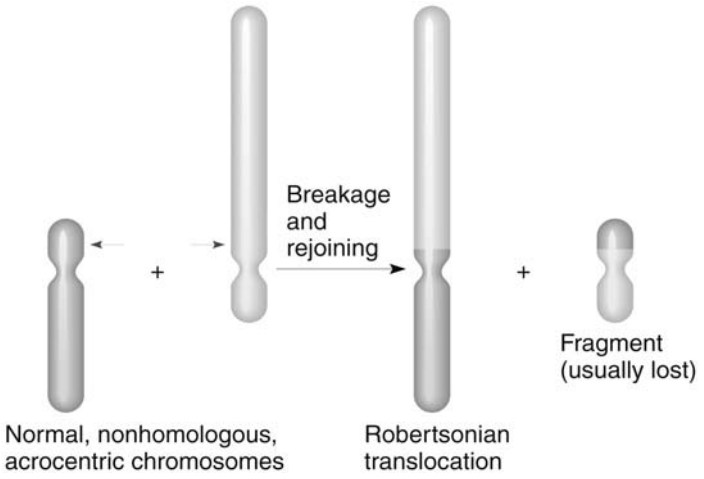


Figure Robertsonian translocation. Source: https://www.mun.ca/biology/scarr/Robertsonian_fusion.html.

7. Polyploidy: Polyploids and aneuploids; Inheritance of autopolyploids and trisomics; significance and limitations of polyploidy; aneuploidy in humans.

Aneuploidy

Aneuploidy is defined as the loss or gain of one or few chromosomes compared to its somatic chromosome number of a species.

a) **Additions to chromosome number:**

i. **Trisomy [2n + 1]:** When somatic cells of an organism contain an extra chromosome (2n+1). The number of possible trisomies in an organism is equal to the haploid chromosome number.

Trisomics are of different types —

primary trisomics where extra chromosome is identical to two homologues.

secondary trisomics where the extra chromosome is an isochromosome with two genetically identical arms.

tertiary trisomies are the products of translocation. Double trisomies (2n + 1 + 1) are also available in nature.

The origin of the trisomics may be from the production of n + 1 types of gametes due to rare non-disjunction of a bivalent in a diploid or may also be produced by triploids through irregular meiosis.

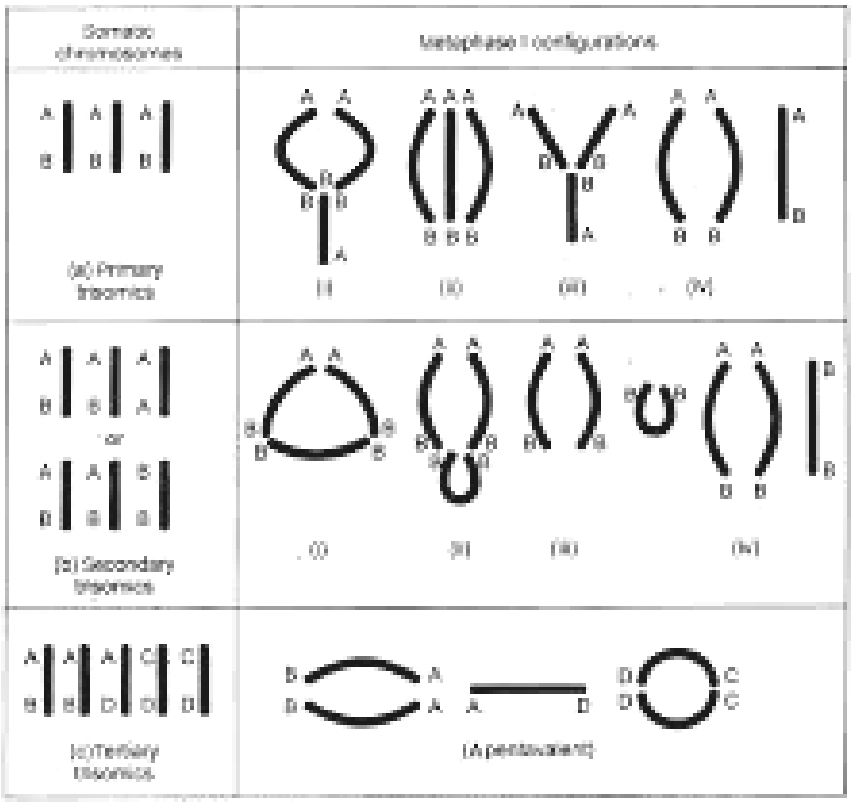


Fig. 11.5 Different types of trisomics and their meiotic configurations at metaphase I

Source: http://www.biologydiscussion.com/

In primary and secondary trisomics, the three homologues of the concerned chromosome may pair with each other to form a trivalent of different configurations at MI, or two of the chromosomes may form a bivalent leaving the additional chromosome as a univalent. In tertiary trisomic, some PMCs would show a dumbbell shaped figure containing five chromosomes at MI.

Gain of a complete chromosome causes a major imbalance, but its effects are not as deleterious as those due to the loss of a full chromosome. Therefore, trisomics are viable in diploid species, e.g. maize, tomato, pea, barley etc.

i. **Tetrasomy [2n + 2]:** When somatic cells contain one pair of a chromosome in excess of the normal somatic compliment, i.e., chromosome compliment is present in four copies. Tetrasomics may be originated by selfing of trisomics. During meiosis, the four homologues of the tetrasomic set tend to form a quadrivalent.

The four homologues of the concerned chromosome may form one quardivalent, a trivalent and a univalent, or two bivalents at MI. Quadrivalents usually disjoin 2:2 at AI, but 3:1 disjunction may also take place.

b) **Reductions in chromosome number:**

(iii) **Monosomy [2n − 1]:** When one chromosome is missing from the somatic complement. Monosomics of diploid species is not tolerated due to genetic imbalance but are fully viable in polyploidy species e.g., wheat, cotton, oats and tobacco. Double monosomics (2n - 1 - 1) as well as triple monosomics (2n - 1 – 1 - 1) may be obtained in polyploidy species.

The one chromosome remains unpaired during meiosis The remaining chromosomes pair normally and produce of n – 1 types of gametes due to rare nondisjunction of a bivalent. In progeny of a monosomic, a mixture of disomic (2n), monosomies (2n – 1) and nuilisomics (2n – 2) is obtained.

(iv) **Nullisomy [2n − 2]:** When one pair of chromosomes is missing from the somatic complement. Nullisomic has a strong deleterious effect on the organism, only viable in the highly polyploidy species e.g. hexaploid or bread wheat (*Triticum aestivum*) and oats (*Avena sativa*).

Cytologically, chromosome pairing is normal and at MI n-1 bivalents can be seen. These bivalents disjoin normally to produce all normal n-1 gametes.

Importance of Aneuploidy in Plants:

Aneuploids have played a role in evolution and have importance in plant breeding in addi-tion to genetic analysis.

(a) Detecting linkage group:

The aneu-ploids have played an important role in locating a linkage group and a gene in a particular chromosome. Particularly nullisomics, mono-somies and trisomies have been used to deter-mine linkage groups in tobacco, wheat, etc.

The study of aneuploidy has shown homoeology between A, B and D genomes of wheat. Identification of the chromosome involved in translocation has also been done with the help of aneuploids.

(b) Chromosome substitution in plant breeding:

The major contribution of aneuploids has been in the field of plant breeding. The substitu-tion of whole chromosome or part of the chro-mosome using aneuploids has been done. These substitutions resulted in significant modification of yield, resistance, lodging, etc.

(c) Speciation:

Aneuploidy can generate variation and source of speciation in vegetatively propagating species. In *Crepis,* aneuploid variations form a series X = 3, 4, 5, 6 and 7 among species. A very extensive aneuploid series has been observed in *Carex* (n = 6 to 56).

Aneuploidy in human:

- **Down syndrome:** A genetic chromosome 21 disorder causing developmental and intellectual delays. Caused by non-disjunction of the 21st chromosome. Typically associated with physical growth delays, characteristic facial features and mild to moderate intellectual disability.
- **Patau syndrome** Trisomy at chromosome 13 causing Intellectual disability and motor disorder, Structural eye defects, polydactyly, abnormal genitalia, kidney defects
- **Edwards syndrome**

  Trisomy at chromosome 18, 47,XX,+18, Multiple malformation of many organs lowest, Malformed ears, small mouth and nose, mental deficiency

- **Klinefelter syndrome:**

47,XXY orXXY, Males with some development of breast tissue normally seen in females. Little body hair is present, and such person are typically tall, have small testes. Infertility results from absent sperm.

- **Turner syndrome**:

45, X, Turner syndrome is associated with underdeveloped ovaries, short stature, webbed, and is only in women. Bull neck, and broad chest. Individuals are sterile, and lack expected secondary sexual characteristics.

Autosomes	Sex-Chromosomes
Numerical abnormalities	Turner syndrome
Trisomy	45, X
8 trisomy	46, Xi (Xq) and mosaics of Xq cell line
9 trisomy	46, Xdel (Xq) and mosaics Xq cell line
13 trisomy (Patau syndrome)	Mosaics 45X/46 XX 45X/47 XXX
18 trisomy (Edwards syndrome)	Mosaics 45, X/46 XY
21 trisomy (Down syndrome)	Other (del, Xp, X mosaics)
22 trisomy	Klinefelters syndrome
Polyploidy	47, XXY
Triploidy	48, XXXY
Tetraploidy	49, XXXXY
Structural abnormalities	Mosaics
Partial trisomy	Others
1 q trisomy	Polysomy X-females
2 q trisomy	47, XXX
3 q trisomy	48, XXXX
5 p trisomy	49, XXXXX
7 q trisomy	Mosaics
9, p, trisomy	Polysomy-Y
10q trisomy	47, XYY
14q trisomy	Others
22q trisomy	

Polyploids (three or more genomes):

The organisms having more than two sets of chromosomes by the addition of another chromosome is called polyploidy. It may arise as a result of abnormal mitosis where chromosomes divide by cytoplasm fails to divide during cytokinesis. The basic set of chromosomes undergoes multiplications. For example, in *Chrysanthemum* basic set is x = 9. Its species and hybrids show multiple of 9, such as 18, 27, 36, 45. In *Nicotiana* and *Solanum* basic set is x = 12 and multiple of somatic chromosome numbers are 24, 48 and 72 and in *Triticum* it is x = 7 and multiples are 14, 21, 42.

Origin of Polyploidy:

i) Polyploids are originated by failure of normal mitotic division in somatic cells.

ii) Polyploids may originate due to abnormal reduction divisions resulting

iii) It may naturally occur due to fertilization of egg by more than one male gamete.

iv) It may originate by artificial induction using colchicines or may be originated by cross hybridization between haploid and diploids.

Types of Polyploidy:

There are mainly four different types of Polyploidy, namely:

i) Auto-Polyploidy,

ii) Allopolyploids,

iii) Segmental allopolyploids and

iv) Auto-allopolyploids.

(i) Auto-Polyploidy:

When more than two genomes developed by the multiplication of chromosome number of some individual is called Autopolyploids. On the other hand autopolyploids are the individuals of which body cells contains more than two identical set of chromosomes derived by self-duplication. It arises due to failure of anaphase.

Examples:

Autotriploids (3n) – *Oenothera*, *Datura*, *Dahlia*, Rose etc. autotriploids are more vigorous, shows more perenation of the organs of vegetative propagation and are highly sterile.

Autotetraploids: (4n) – it contain four identical genomes and arise by fusion of two diploid gametes. It may results from duplication of somatic chromosomes. Autotetraploids show great adaptability, disease resistant, larger seeds, high vitamin-C content and low fertility. These are found in Apples, Grapes, Marigolds.

Meiosis in an Autopolyploid:

Meiotic behaviour in an autopolyploid such as autotetraploid is different than in a diploid. This is due to the presence of four homologous chromosomes of each kind.

Assuming that the primary material is a diploid species with 14 chromosomes (AA), these will form seven pairs (bivalents) at meiosis . In the tetraploid (AAAA) there will be four chromosomes of each type, and at meiosis, these seven groups of four chromosomes may form seven quadrivalents.

A quadrivalent is an association of four homologous chromosomes. Quadrivalents may be of different appearances. Sometimes, the homologous chro-mosomes are represented by an association of three chromosomes, called a trivalent and a univalent or by two bivalents.

As a rule, the average number of quadrivalents per cell is, therefore, lower than the medium possible num-ber. Autotetraploids of different species behave differently in this respect. Some of them have a very high frequency of quadrivalents as in *A. tuberosum*, in some cases bivalents are formed.

The occurrence of trivaients and univalents at meiosis in an autotetraploid leads to distur-bances in chromosome distribution and to the formation of gametes with deviating chromo-some numbers. This is the principal cause for the high degree of sterility in an autotetraploid.

Segregation of Genes in Autopolyploids:

The number of alleles of each gene is represented according to the ploidy level of the Polyploidy individual and gametes containing more than one allele of each gene (homo- or heterozygotic) may be produced.

According to the number of dominant and recessive alleles at a particular locus, the genotype of an autotetraploid may be quadriplex (AAAA or A_4), triplex (AAAa or A_3a), duplex (AAaa or A_2a_2), monoplex or simplex (Aaaa or Aa_3) and nulliplex (aaaa or a_4).

Auto-Polyploidy such as tetraploids show the so called tetrasomic inheritance. The segregation of genes in auto-Polyploidy is affected by factors which play no essential role in diploid.

Among such factors are the number and position of chiasmata in the multivalents, the distance between particular locus and centromere, the behaviour of homologues in multivalent associations during anaphase I and the presence of univalents.

In auto-tetraploids, if it is assumed that the four homologous chromosomes are distributed to poles in 2:2 during anaphase I, theoretical seg-regation ratios for various autotetraploid genotypes of a locus may be calculated.

Table 11.3: Frequencies of the gamete types and zygote types of autotetraploid genotypes

Parent	Gametes				Zygotes					
Genotype	AA	Aa	aa	divisor	A^4	A^3a	A^2a^2	Aa^3	a^4	divisor
Quadriplex (AAAA)	1	–	–	1	1	–	–	–	–	1
Triplex (AAAa)	1	1	–	2	1	2	1	–	–	4
Duplex (AAaa)	1	4	1	6	1	8	18	8	1	36
Monoplex (Aaaa)	–	1	1	2	–	–	1	2	1	4
Nulliplex (aaaa)	–	–	1	1	–	–	–	–	1	1

Applications of Autopolyploidy in Crop Improvement

Autopolyploidy has found some valuable applications in crop improvement. These are briefly summarized below:

Triploids –

Triploids are produced by hybridization between tetraploid and diploid strains. They are generally highly sterile, except in a few cases. This feature is useful in the production of seedless watermelons. In certain species, they may be more vigorous than the normal diploids, e.g., in sugarbeets. These two examples are described in some detail.

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (2x x 4x) is not successful. The triploid plants do not produce true seeds; almost all the seeds are small, white rudimentary structures like cucumber (*Cucumis sativus*) seeds. But a few normal sized seeds may occur, which are empty. For good fruit setting, pollination is essential. For this purpose, diploid lines

are planted in the ratio 1 diploid: 5 triploid plants. There are several, problems, viz., genetic instability of 4x lines, irregular fruit shape, a tendency towards hollowness of fruits, production of empty seeds and the labour involved in triploid seed production (by hand-pollination). Recently, some diploid hybrids of watermelon ('ice-box type') have been developed that produce seedless fruits (all their seeds are like cucumber seeds).

Triploid sugarbeet produce larger roots and more sugar per unit area than do diploids, while tetraploids produce smaller roots and lower yields than diploids. Apparently, 3x is the optimum level of ploidy in sugarbeets.

Tetraploids –

Autotetraploids have been produced in a large number of crop species and have been extensively studied in several cases. Tetraploids may be useful in one of the following ways: (1) in breeding, (2) improving quality, (3) overcoming self-incompatibility, (4) making distant crosses and (5) used directly as varieties.

In banana (*M. sapientum*), autotetraploids are inferior to triploids in that they have weaker leaves and increased fertility. But they offer the only available chance of adding disease resistance to commercially successful varieties. In banana, autoteraploids are produced by chance fertilization of an unreduced triploid egg (AAA) by a haploid pollen from a disease resistant diploid parent. A large number of such tetraploids have been produced, but they have not yet gained any commercial success. This is an unusual case where auto tetraploidy is the only practical approach to breeding an otherwise successful triploid crop species.

Autotetraploidy is able to overcome self-incompatibility in certain cases, e.g., some genotypes of tobacco and white clover (*Trifolium repens*), *Petunia*, etc. Certain distant crosses are not successful at the diploid level, but are relatively successful at the autotetraploid level, e.g., 4x *Brassica oleracea* x *B. chinensis* is successful, but when *B. oleracea* is diploid it is unsuccessful. Similarly, autotetraploids of certain *Solanum* species produce hybrids with *S. tuberosum*, while the diploids do not.

Autotetraploids are larger in size and are more vigorous than diploids. Autotetraploid varieties of forage crops have been considerably successful. The most successful examples are, tetraploid red clover (*Trifolium pratense*) and ryegrass (*Lolium perenne*). Other examples are tetraploids of alsika clover (*Trifolium hybridum*, Variety Tetra) and berseem (*Trifolium alexandrium*, variety Pusa Giant Berseem).

(ii) Allopolyploids:

Polyploidy may also result from doubling of chromosome number in hybrid which is derived from two or more distinctly different species. This brings two (or more) different sets of chromosome in

hybrid. The doubling of chromo-somes in the hybrid, which gives rise to a Polyploidy, is called an allopolyploid.

An allopolyploid in which a sterile hybrid (AB) originating out of the combination of two different species, undergoes duplication of chromosome set, is known as amphidiploid (AABB).

Raphanobrassica is a classical example of amphidiploidy. In 1927, Karpechenko, a Russian scientist, reported a cross between *Raphanus sativus* (2n = 18) and *Brassica oleracea* (2n = 18) to produce F2 hybrids which were completely sterile.

This sterility was due to lack of chromosome pairing, since there is no homology between genomes from *Raphanus sativus* and *Brassica oleracea*. Among these ste-rile hybrids certain fertile plants were found. On cytological examination, these fertile plants were found to have 2n = 36 chromosomes, which showed normal pairing into 18 bivalents.

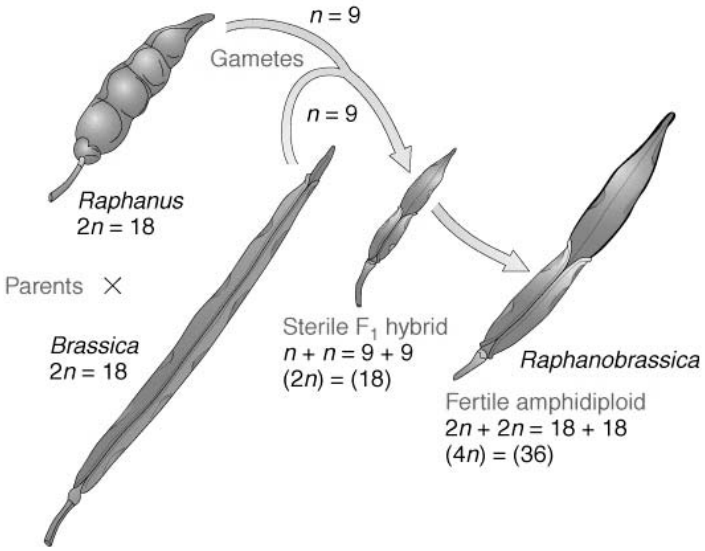


Figure *Raphanobrassica*. Source: http://theelusiveguanaco.blogspot.com/2011/06/joy-of-chromosomes-part-2_16.html

Thus in allopolyploids the paring is of autosyndesis type (paternal-paternal or maternal- maternal pairing) in contrast to allosyndesis (paternal-maternal pairing) in diploids and autopolyploid.

Of the allopolyploids, amphidiploid hybrids containing two sets of each species are of special importance because they are usually fertile, occur rather widely among angiosperms in nature, afford clues to the relationship of certain species, and open a new path to the improvement of cultivated plants.

Triticale is the most successful synthetic allopolyploid produced by crossing wheat (tetraploid or hexaploid) with rye. *Triticales* derived from tetraploid wheats have been the most successful, but those from hexaploid wheats may also become a successful crop species. The breeding strategy involves (l) production of a large number of triticale strains using different combinations (varieties as well as species) of wheat and, rye, (2) hybridization of these triticale strains among themselves, and (3) improvement of the defects of the triticale through selection.

One of the earliest known amphidiploid hybrids was the fertile *Primula kewensis*, with 36 somatic chromosomes. A cross between *P. floribunda* (2n = 18) and *P. verticillata* (2n = 18) had yielded the sterile diploid *P. kewensis* (2n = 18) with one genome from each parent species.

Amphidiploids sometimes arise in ways other than by somatic chromosome doubling. Diploid spores, and, therefore, diploid gametes may appear on failure of meiosis and union of two diploid gametes gives rise to tetraploid. Although the chance of obtaining such plants in this manner seems to be relatively small.

(iii) Segmental Allopolyploids:

In some allopolyploids the different genomes that are present are not quite different from one another, i.e.,, having partial homology with each other (616,8282). Consequently, in these Polyploidy, chromosomes from different genomes do pair together to some extent and multivalents are formed. This means that segments of chromosomes and not the whole chromosome is homologous.

Such allopolyploids are called segmental allopolyploids (Stebbins). These chromosomes which are partially homologous and not completely homologous with each other are sometimes also described as homologous chromosomes. It is also believed that most of the naturally occurring Polyploidy are neither true auto-Polyploidy nor true allopolyploids.

Solanum tuberosum is the best example of segmental allopolyploid.

(iv) Auto-Allopolyploids:

When autopolyploidy is combined with allopolyploidy, autoallopolyploids are produced (AAAA6B). Polyploidy of this type are possible from hexaploid level upward as observed in *Nicotiana tabacum* and *Solanum nigrum*. Autoallopolyploids have importance in the evolution of certain plant species.

Role of Polyploidy:

Some of the important roles played by polyploidy are described below:

i. Role of Polyploidy in Plant Breeding:

When the techniques for artificial chromosome doubling became established, investigations on the origin of many of our economic plants were resumed. Many important crop plants like wheat, oat, sugarcane, cotton, tobacco as well as many fruits and vegetables are the Polyploidy of various degrees.

One of the important effects of polyploidy is the changes in the blooming season of the induced Polyploidy. As such, interspecific hybrids can be obtained of such species which otherwise remain isolated by seasonal isolation and different blooming season.

By artificial polyploidy induction, disease resistance and other desirable characters have been incorporated into some commercial crop plants. For example, *Nicotiana tabacum* is susceptible to TMV whereas *N. glutinosa* appears to be resistant.

The two tobacco species when crossed, the hybrids were found to be resistant but totally sterile. When the chromosomes were doubled it was possible to secure a fertile Polyploidy resistant to the virus. Many Polyploidy are selected and culti-vated because of their larger size, vigour and ornamental values. Several varieties of apples, pears and grapes have produced giant fruits which are of much economic value.

ii. Role of Polyploidy in Evolution:

Polyploidy combined with interspecific hybridization provides a mechanism by which new species may arise in nature and play a role in evolution. Allopolyploidy can produce new species by combining new characters and stable in evolution. It has already been discussed under amphidiploidy how different types of new species may be evolved.

Among the inter-specific hybridization, the most important are *Primula kewensis* (n = 18) obtained by crossing *P. floribunda* (n = 9) and *P. verticillata* (n = 9), *Digitalis mertqnensis* (n = 56) obtained by crossing *D. pur*purea (n = 28) and *D. ambigua* (n = 28) and *Spartina townsendii* (n = 63) obtained from cross of *S. stricta* (n = 28) and *S. alterniflora* (n = 35).

Origin of some of the economically important plants like rice, wheat, cotton, tobacco is important in this aspect. The chromosome number of rice (*Oryza sativa*) is 2n = 24. It is an example of typical secondary allopolyploids with basic chromosome number x = 5.

The present cultivated variety of rice is actually produced by hybridization followed by aneuploidy and euploidy. The origin of wheat, cotton, Mustard, etc. are given below:

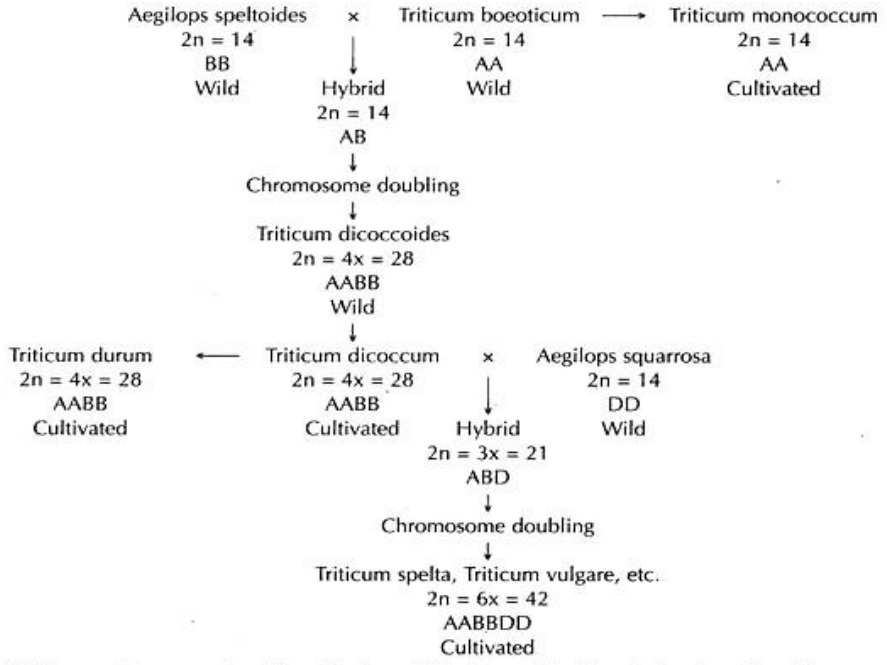


Fig. 11.12: Diagrammatic representation of the origin of tetraploid and hexaploid cultivated wheat from their wild ancestors

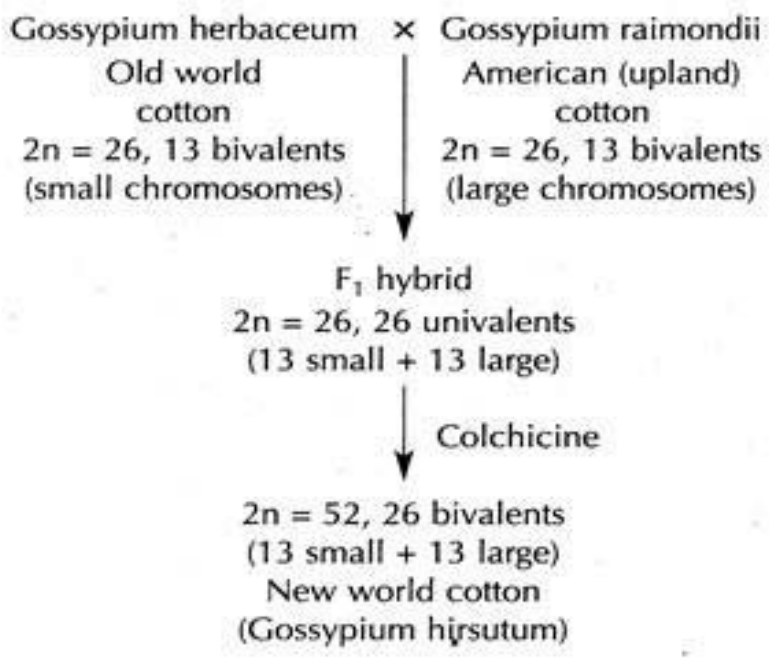


Fig. 11.13: Origin of new world cotton

Source: http://www.biologydiscussion.com/cell/notes-on-polyploidy-cell/38312

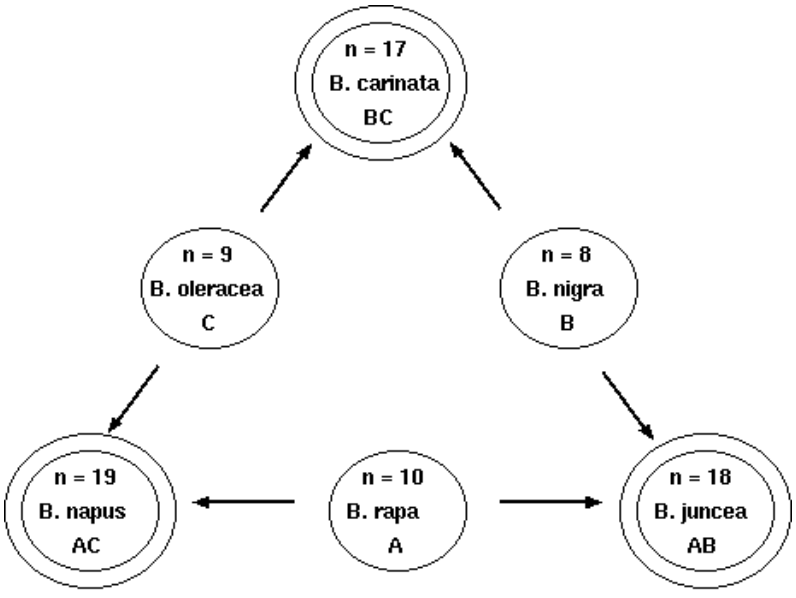


Figure Origin of *Brassica* (triangle)

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

iii. Media of Conservation of Characters:

Polyploidy plays an important role in conserving the characters. A recessive mutation in order to be expressed in an autotetraploid, all four genes must be in recessive condition which is a time requiring process. Thus the characters in a Polyploidy plant could be conserved.

iv. Polyploidy and Geographical Distribution:

The Polyploidy plants can cope with diverse geographical areas than a diploid. Hence, the geographical distributions of Polyploidy plants are greater than diploids. Auto-polyploidy cannot produce new species, but they can colonize a new environment easily. As allopolyploids contain different genomes, they can withstand different environmental condition.

Both these power of colonization and coping with a diverse environment of the Polyploidy plants, help their wide geographical distribution.

Limitation of Polyploidy:

Polyploidy has several limitations. Some important limitations of polyploidy in crop improvement are briefly presented below:

1. Limited use: The single species polyploidy has limited applications. It is generally useful in those crop species which propagate asexually like banana, potato, sugarcane, grapes etc.

2. Difficulty in maintenance: The maintenance of monoploids and triploids is not possible in case of sexually propagating crop species.

3. Undesirable characters: In bispecies or multispecies polyploids characters are contributed by each of the parental species. These characters may be sometimes undesirable as in case of *Raphanobrassica*.

4. Some other defects: Induced polyploids have several defects such as low fertility, genetic instability, low growth rate, late maturity, etc.

5. Chances of developing new species through allopolyploidy are extremely low.

8. Plastids and mitochondrial DNA influenced traits.

Cytoplasmic Inheritance:

The inheritance of most of the characters of an individual is governed by nuclear genes. But in some cases, the inheritance is governed by cytoplasmic factors or genes. When the transmission of characters from parents to offspring is governed by cytoplasmic genes; it is known as cytoplasmic inheritance or **extra nuclear inheritance** or **extra chromosomal inheritance** or **non-mendelian inheritanc**e or **organellar inheritance**.

The first case of cytoplasmic inheritance was reported by Conens in 1909 in four 'o' clock (*Mirabilis jalapa*) for leaf colour. Later on, cytoplasmic inheritance was reported by various workers in various organisms.

TABLE 11.1. Differences between Mendelian inheritance and cytoplasmic inheritance

S.No. Particulars	*Mendelian Inheritance*	*Cytoplasmic Inheritance*
1. Governed by	Nuclear genes	Plasma genes
2. Segregation pattern	Distinct	Not distinct
3. Reciprocal differences	Not observed	Observed
4. Maternal effects	Not observed	Observed
5. Genes mapping	Easy	Difficult
6. Location of genes	Chromosomes	Chloroplasts or mitochondria

Maternal Effects:

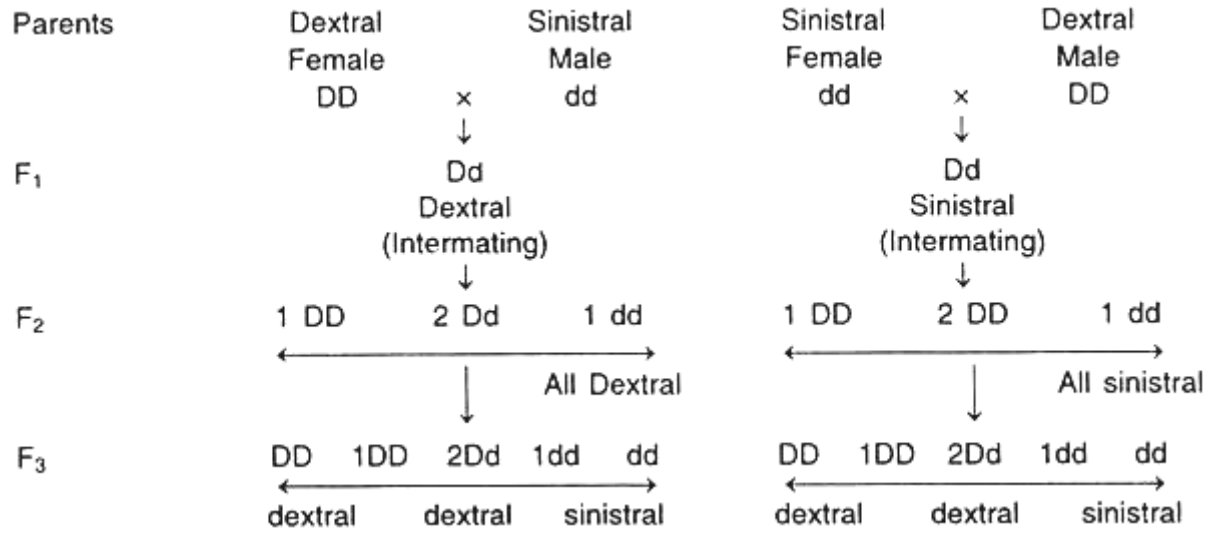
When the expression of a character is influenced by the genotype of female parent, it is referred to as maternal effect. Such characters exhibit clear-cut differences in F_1 for reciprocal crosses. Maternal effects are known both in plants and animals. Some examples of maternal effects are briefly presented below.

(i) Coiling Pattern of Shell in Snail:

The effect of maternal genotype on the coiling behaviour in water snail was studied by Sturtevant. There are two types of coiling pattern of shell in snail (*Limnaeaperegra*), viz., right handed (dextral) and left handed (sinistral).

The coiling behaviour is controlled by a single gene. The dextral coiling behavior is governed by dominant allele D and sinistral by recessive allele d. When a cross is made between dextral female and sinistral male, it produces dextral snails in F_1 as well as in F_2.

However, in F_3 a segregation ratio of 3 dextral and 1 sinistral is observed. Similarly, when a reciprocal cross is made, i.e.,, sinistral as female and dextral as male, all the snails are sinistral in F_1 and dextral in F_2. Again in F_3 a ratio of 3 dextral and 1 sinistral is observed. This indicates that the inheritance of coiling direction in water snail depends on the genotype of female parent and not on its own genotype.



Maternal effect on coiling of water snail

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

Kappa Particles in *Paramecium*:

Kappa particles are found in certain killer strains of *Paramecium* and are responsible for production of substance paramycin, which is toxic to strains not possessing kappa (sensitive strain). The production of kappa particles is dependent on a dominant allele K, so that killer strains are KK or Kk and sensitive strains are ordinarily kk. In absence of dominant allele K, kappa particles cannot multiply and in absence of kappa particles, dominant allele K cannot produce them de novo. Consequently sensitive strains with genotypes KK or kk can be obtained. These will not carry any kappa particles. However, killer strain with genotype kk cannot be obtained, because even if kappa particles are present, these would be lost in absence of dominant allele. If *Paramecium* clones with genotypes KK or Kk are allowed to multiply asexually at such a fast rate, that division of kappa particles cannot keep pace with division of cells, kappa particles will be eventually lost. Consequently sensitive strains with dominant genotype (KK, Kk) having no kappa particles would be obtained.

If the killer (KK) and sensitive (kk)strains are allowed to conjugate, all exconjugants (the cells separating after conjugation) will have same genotype Kk. Phenotypes of these exconjugants will, however, depend upon duration for which conjugation is allowed. If conjugation does not persist long enough for exchange of cytoplasm, heterozygote (Kk) exconjugants will only have parental phenotypes. It means that killers will remain killers and sensitive will remain sensitive even after conjugation. If conjugation persists, sensitive strain will receive kappa particles and will become killer, so that exconjugants will be killers having genotype Kk.

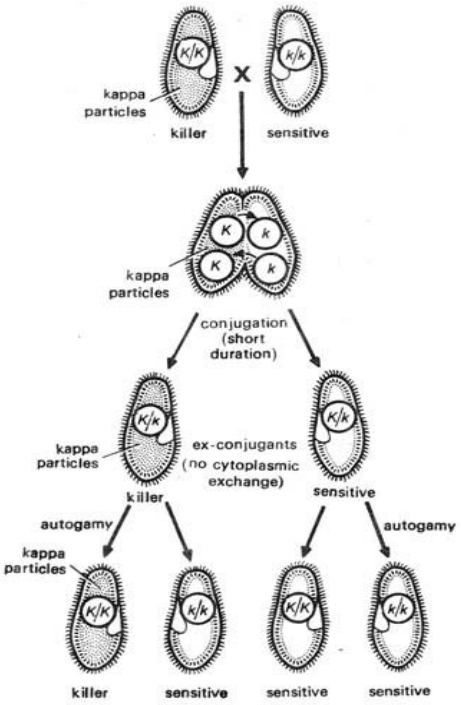


Figure Results of a cross between a killer (KK) and a sensitive (kk) strain of *Paramecium*, when no cytoplasmic exchange is allowed.

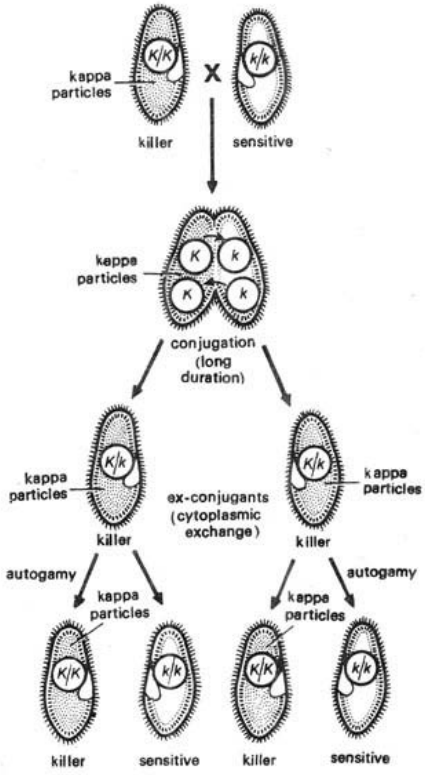


Figure Results of a cross between a killer (KK) and a sensitive (kk) strain of *Paramecium*, when cytoplasmic exchange is allowed.

Source:https://biocyclopedia.com/index/genetics/maternal_effects_and_cytoplasmic_inheritance/kappa_particles_in_paramecium

1. Plastid Inheritance:

Chloroplasts are the important plastids. Plastids have green pigments called chloroplasts. Plastids self-duplicate, have some amount of DNA and play an important role in cytoplasmic inheritance.

Some examples of plastid inheritance are given below:

(i) *Mirabilis jalapa:*

The first conclusive evidence of cytoplasmic inheritance was reported by Correns in 1909 for leaf colour in four 'o' clock plant (*Mirabilis jalapa*). This plant has three types of leaves, viz., green, white and variegated. Three types of results were obtained from crosses between these genotypes as given below.

1. When green was used as female and either green, white or variegated as male, all individuals in F_1 were green.
2. When white was used as female and either green, white or variegated as male, all individuals in F_1 were white.
3. When variegated was used as female and either green, white or variegated as male, various proportions of green, white and variegated individuals were obtained in F_1.

TABLE 11.3. Inheritance of leaf colour in Mirabilis jalapa

Crosses between three leaf colours			*Expression of leaf colour in F_1*
Female		*Male*	
Green	×	Green	Green
	×	White	Green
	×	Variegated	Green
White	×	Green	White
	×	White	White
	×	Variegated	White
Variegated	×	Green	Green, white and variegated in various ratios in each cross
	×	White	
	×	Variegated	

Source: http://www.biologydiscussion.com/genetics/cytoplasmic-inheritance

9. Microbial genetics: Transformation, conjugation and transduction and their significance in gene

Mapping

In molecular biology, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacteria must be in a state of competence, which might occur in nature as a time limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from one bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium). In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram positive and Gram negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection"

Co -Transformation:

It is obvious that if the exogenous DNA that enters a recipient bacterial cell, contains known marker genes, say x, y and z, then these genes appear in the trans-formants, provided the segment or segments containing these genes are successfully integrated into the host chromosome. When x and y or x and z or y and z appear in the same trans-formant, the phenomenon is called co-transformation and the particular transformant is called a co-trans-formant.

Naturally, the probability of co-transformation and hence the frequency of co-trans-formants depend on the relative distance between the pair of marker genes. For detecting co-transformation, the

recipient bacterium must have the corresponding recessive genes, x ,y– and z–, because only then the presence of the x, y and z genes can be detected. For convenience, the x, y and z genes may be represented as x+, y+ and z+.

Co-transformation frequency may be used for preparing gene-maps. Thus, if it is observed that x+ y+ trans-formants appear more frequently than x+z+, it can be concluded that x+ and y+ are closer to each oilier than x+ and z+.

As transforming DNA generally consists of fragments, a particular fragment may or may not contain a marker gene. If the fragment taken up by a cell does not contain any marker gene, there will be no transformation of the marker genes, although other genes not taken into consideration may be present. If the fragment taken up by the recipient contains a marker x+ or y+, or both markers x+ and y+, the trans-formants may have the genotypes, x+x-, y+y- or x+x-y+y–. Only the last genotype represents a co-trans-formant.

Now, if the probability of x+x– and y+y– trans-formants in the population is 10-3 each, then the probability of co-transformation of x+x–y+y– will also be 10-3 provided x+ and y+ are present in the same DNA fragment. But if x+ and y+ are present on different DNA fragments, the probability of taking up the two fragments simultaneously will be 10-3 x 10-3 i.e., 10-6.

The same argument holds good for all the pairs. The probability of x+, y+ and z+ occurring on different fragments being co-transformed is much less, in the order of 10-3 x 10-3 x 10-3 i.e., 10-9. On the other hand, if x+, y+ and z+ occur in the same fragment, the probability of the three genes being co-transformed will be 10-3. Thus, from the probability measurements, it is possible to construct a gene map showing relative distances between the genes, as well as their order in the chromosome.

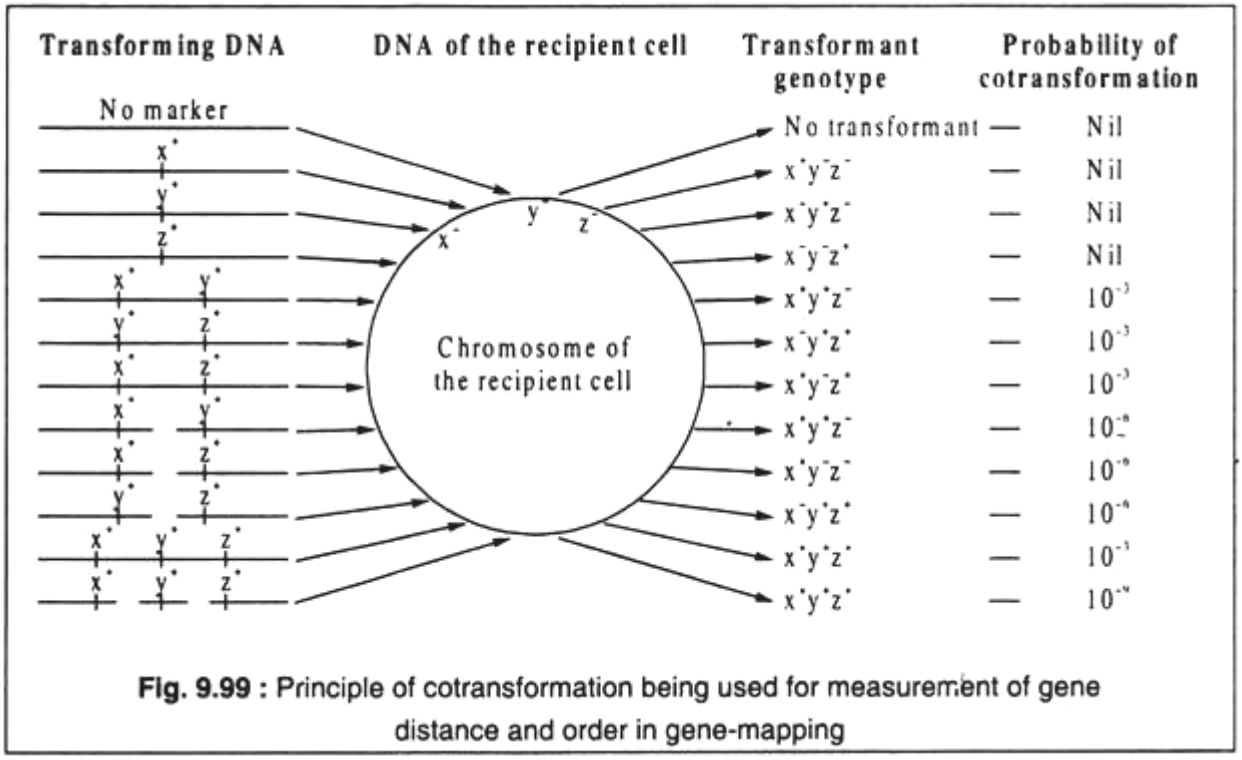


Fig. 9.99 : Principle of cotransformation being used for measurement of gene distance and order in gene-mapping

Source: Peter J. Russell IGenetics 3rd ed.

Transduction:

Transduction is another mode of horizontal gene transfer in bacteria, mediated by transducing bacteriophages which act as vehicles of DNA transfer from one bacterium to another, generally belonging to the same species, because species, because phages are host specific.

Two types of transduction are distinguished:

i. **Generalized transduction** and ii. **Specialized or restrict transduction.**

In the first type, bacteriophages infect a host bacterium and progeny phage particles are assembled. Some of these progeny bacteriophages incorporate host DNA in their heads.

When such bacteriophages infect another host cell after being released by lysis, those carrying the host DNA also inject it into the cell, where it can be incorporated into the bacterial chromosome resulting in genetic recombination. In restricted transduction, temperate bacteriophages take parts which have two alternative types of life cycles, – a lytic cycle and lysogenic cycle. The temperate

phages carry both phage carry both phage DNA and occasionally also some portions of host DNA taken from restricted sites of the host chromosome.

When the released phage particles infect new host cells, they transmit the phage DNA along with the fragments of host DNA. As the bacteriophage enters into a lysogenic state in the infected host, the phage DNA and the bacterial DNA of the previous host are integrated into its chromosome by exchange of parts resulting in genetic recombination.

(i) **Generalized Transduction:**

Transduction was discovered by Zinder and Lederberg in 1952 when they were looking for an E. coli type conjugation system in Salmonella typhimurium. A mixture of two cultures of auxotrophic mutants of this bacterium differing in contrasting characters was found to produce small number of prototrophic recombinants. For example, two populations of auxotroph's having phenotypes, A+B– and A–B+ were mixed in growth medium, and, on incubation, they produced some bacteria having A+B+ phenotype.

To make sure whether the origin of prototrophs was due to conjugation, they took the two cultures in two arms of a U-tube separated by a glass filter which physically prevented cell to cell contact of the two populations which is essential for conjugation. Under this condition also, the prototrophs appeared suggesting that the gene exchange was not due to conjugation.

That it was not due to transformation was also proved by treatment with DNase which destroys free naked DNA. It was finally proved that the gene exchange was mediated by the bacteriophage P22 which infects S. typhimurium and some other species of Salmonella. This new mode of gene exchange leading to genetic recombination was designated as transduction.

In generalized transduction, the transducing phage infects a bacterial host in the usual way by attachment and injection of its DNA. Phage DNA and proteins are synthesized, and the bacterial chromosome is broken down to small fragments. During maturation of progeny phage particles, the host DNA fragments having approximately the same size as that of phage DNA are packaged inadvertently in some of phage heads.

After release by lysis of the infected host cell, such aberrant or defective phage particles may infect new host cells and inject the DNA into the cell. But because the DNA of these phages is of bacterial

origin and does not contain phage genes necessary for replication, its life cycle is not completed. Instead, the DNA can be integrated into the bacterial chromosome by homologous recombination.

In this way, one or more genes can be transferred from one bacterium to another. For example, if a transducing phage carrying a bacterial gene controlling motility infects a non-motile mutant bacterium, the latter may acquire the property of motility.

A characteristic feature of the transducing phages mediating generalized transduction is that any part of the bacterial chromosome can be transferred without any restriction regarding the site. In general, the fragment in a particular phage head is about one-hundredth part of the bacterial chromosome. Also, the frequency of an aberrant phage carrying bacterial DNA in its head in the total phage population is very low, not more than 1 in 105 to 107.

A well-studied example of generalized transduction is by phage PI of *E. coli* K12. PI is a temperate phage which has also a lytic cycle, like other temperate phages. PI DNA has a molecular weight of 5.9 x 107 Daltons. It encodes a DNase which can cleave bacterial chromosome into fragments having molecular weight of 1 x 107 to 1 x 108 Daltons. As the phage particles are assembled following active replication, one of the host DNA fragments may be taken up by mistake and packaged into the head. Such phage particles become defective and they are the transducing phages.

Because the DNase coded by the phage cleaves bacterial DNA at random, the fragment packaged into a defective phage head may originate from any part of the bacterial chromosome. These defective particles are released along with large number of normal PI phages which are not transducing. For reasons, the DNA carried by the transducing phage particles can be injected into new host bacteria, but without replication and production of new progeny phages.

However, as the DNA of the transducing PI phages is of bacterial origin, it can be integrated into the chromosome of *E. coli*. For example, when phage PI is allowed to infect an ampicillin resistant strain of *E. coli* (ampr), some of the transducing phages will package the fragment containing the ampr gene. Such transducing phages on infecting an ampicillin sensitive strain of *E. coli* (ampS) will transfer the amp’ gene, making the sensitive strain resistant.

Interrupted Mating and Gene-Mapping:

The time taken for transfer of the F-integrated Hfr chromosome of E. coli is 100 minutes under optimal conditions, though the transfer of the entire chromosome is of rare occurrence. The mating pair usually breaks apart before 100 minutes. The separation of mating partners can also be effected artificially by vigorous agitation in a blender.

This is called interrupted mating technique. By adopting such technique at different time intervals after the two mating partners i.e., Hfr and F– cells are mixed, it is possible to detect the genes that have been transferred from the donor to the recipient at any particular point of time. The identification of the genes transferred at different intervals in case of a particular Hfr-donox makes it possible to determine the gene order and to prepare a gene map.

In such a map the distance between genes is expressed in terms of time, taking the whole E. coli chromosome to be 100 minutes long. By employing different Hfr-strains in which the F-DNA is integrated at different sites of the circular chromosome, it has become possible to construct the entire gene-map of E. coli containing about 2,000 genes. The relative distances between genes are shown in minutes.

The important features of interrupted mating between Hfr x F– cells of E. coli are briefly mentioned below:

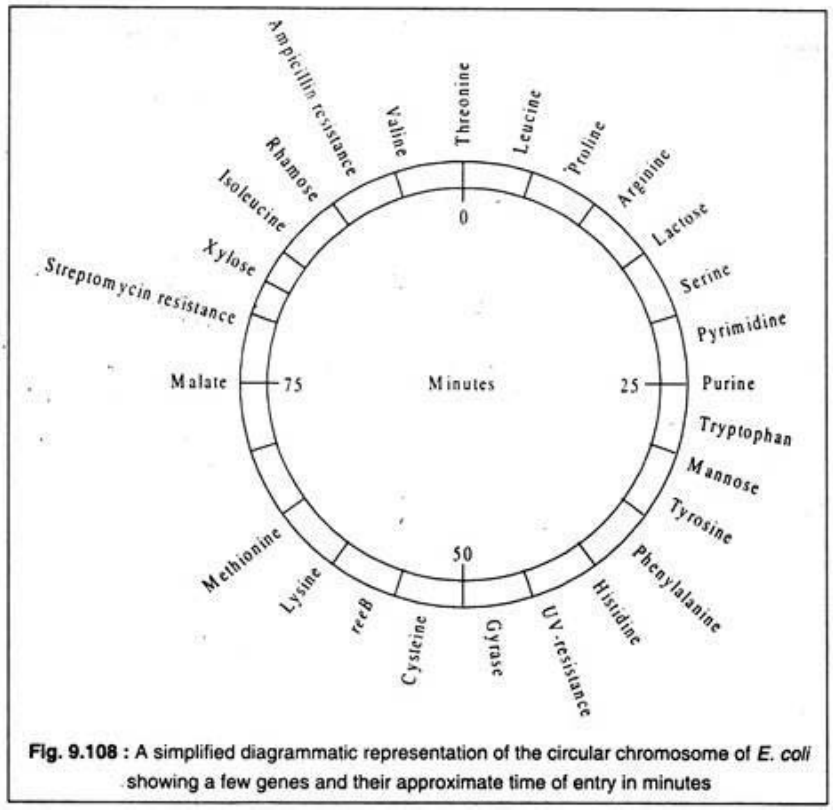


Fig. 9.108 : A simplified diagrammatic representation of the circular chromosome of *E. coli* showing a few genes and their approximate time of entry in minutes

Source: Source: Peter J. Russell IGenetics 3rd ed.

(a) Transfer of Hfr chromosome to P cell begins at a particular point on chromosome determined by the site of integration of the F-plasmid. This means that in different Hfr-strains, transfer begins at different joints of Hfr-chromosome.

(b) The integrated F-DNA nicks at the OriT locus and a part of the F-DNA forms the leading end of the Hfr-chromosome being transferred (see Fig. 9.107). The chromosomal genes enter into the recipient in a linear order. The first gene is the one just behind the part of F-DNA forming the leading end. Other genes follow successively.

(c) The time of entry of any specific gene is the time taken by it to enter the recipient calculated from the time of mixing the Hfr and F– cells. The time of entry, expressed in minutes, is determined by interrupted mating technique.

(d) The number of F– cells showing the presence of a specific gene transferred from Hfr-donor increases with time till a maximum is reached. This happens because all donor cells of an Hfr-strain do not start transferring genes at the same time. The maximum is reached because all do not cells present in the population have transferred the particular gene to the recipients.

(e) Though under appropriate selective conditions, a gene entering into a recipient cell can be expressed as soon as it enters, permanent genetic recombination occurs only after the gene has been incorporated into the F–-chromosome by homologous recombination.

The incoming genes, transferred from Hfr strain, replace homologous genes of F–. The replaced segments of DNA containing these genes, as well as the segments of Hfr-chromosome which have not been incorporated into the F– chromosome, are eventually hydrolyzed by cellular nucleases.

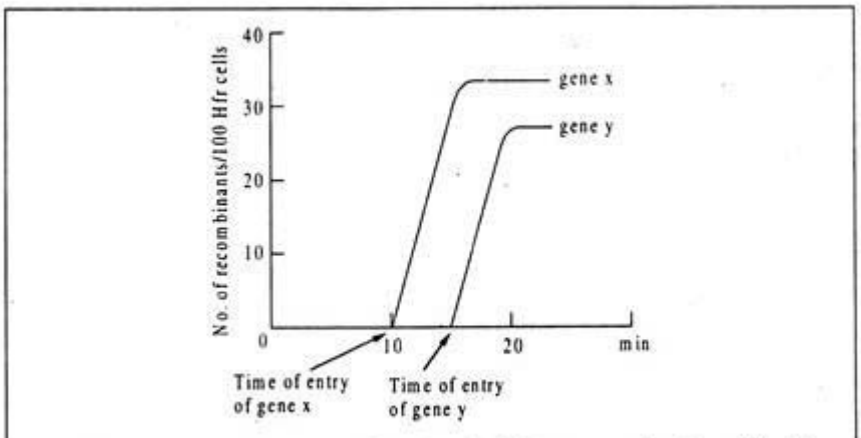


Fig. 9.109 : Time of entry curves of two hypothetical genes x and y of one *Hfr*-strain

Source: Source: Peter J. Russell IGenetics 3rd ed.

(f) In different Hfr-strains of *E. coli*, the F-plasmid is integrated at different loci of the chromosome. There are numerous sites where the F can be integrated. As a result, the linear order of genes during

entry into recipient is different in each strain of Hfr. Moreover, the F-plasmid can be integrated in two different orientations. This also changes the linear order of genes.

(g) In order to detect the entry of a gene in the recipient cell, it becomes necessary to identify such cells in a mixed population of both Hfr cells and F– cells. Such identification needs the use of marker genes present hi both the donor and the recipient. Auxotrophy for various amino acids or other metabolites and antibiotic resistance are often used as useful markers. For example, an F– strain resistant to streptomycin (Strr) can grow in a medium containing an inhibitory concentration of the antibiotic, while a streptomycin-sensitive Hfr-strain (Strs) fails to grow in such a medium.

Thus, in a mixed population of P and Hfr cells, the latter can be eliminated. Again, if the P strain is an auxotrophic mutant, requiring an essential metabolite, like leucine (leu–), it fails to grow in a minimal medium unless it receives a gene for leucine synthesis (leu+) from the Hfr-strain. Thus, when a mixture of Strr leu– F– cells and Strs leu+ Hfr cells is plated on a minimal agar containing streptomycin, neither the leu– F– cells nor Strs Hfr cells can grow. Only the Strr leu+ F– cells will form colonies on such plates.

Thus, by adopting the interrupted mating technique and using different selected markers, it is possible to determine the time of entry of specific genes into the recipients. Many markers had to be used and large number of interrupted mating experiments had to be done for determining the gene map of *E. coli*.

10.Gene mutation: Induction, types, molecular basis, significance; paramutation; DNA repair mechanism; epigenetic changes; genetic imprinting; prion particles; site directed mutagenesis; gene complementation test; rII locus.

Features of Molecular Mutations:

Main features of molecular mutations are given below:

1. It is a change in the number or arrangement of nucleotide sequence of a gene.
2. It is a heritable change in DNA sequence.
3. It is permanent structural change in hereditary material [DNA].
4. Mutations can be harmful, beneficial, or have no effect.
5. Mostly mutations are harmful and very rarely beneficial.
6. Mutations may be caused by mistakes during cell division, or they may be caused by exposure to DNA damaging agents in the environment such as radiation and mutagenic chemicals.
7. It is an alteration in a gene from its natural state. In other words, one allele of a gene changes into a different allele.
8. It may be a spontaneous or induced change in the DNA of a cell.
9. A mutation results in the appearance of a new heritable characteristic in an individual.
10. Mutations are sometimes attributed to random chance events.

Causes of Molecular Mutation:

Mutations in molecular terms are caused by two types of changes at the DNA level, viz:

(i) Base substitution, and Base additions or deletions.

1. Base Substitution:

The replacement of one base pair by another is called base substitution. Some mutations affect only a part of a nucleotide, resulting in replacement of base pair. The replacement of base pair may take place during replication of DNA without breakage. These base pair replacements are of two types, viz. transitions and transversions.

(i) Transition:

Replacement of one purine by another purine or one pyrimidine by another pyrimidine is known as transition. In other words, it is the replacement of a base by other base of the same chemical group

[purine replaced by purine: either A to G or G to A; pyrimidine replaced by pyrimidine: either C to T or T to C].

It means that both way change between purines [A and G] and pyrimidines [C and T] can occur. Such type of change yields a normal base.

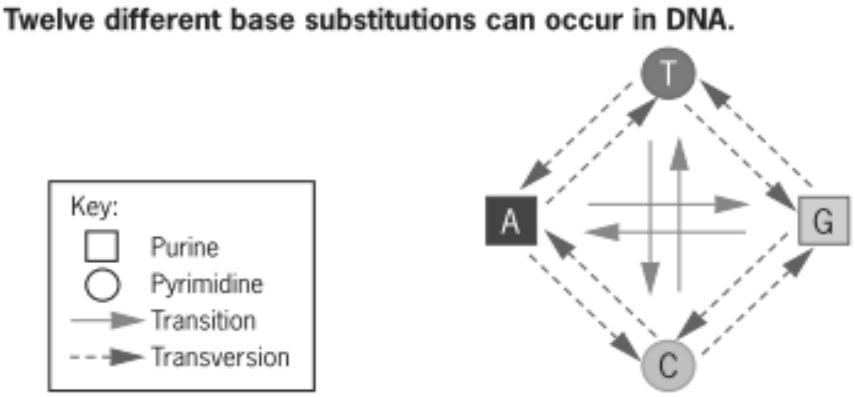
(ii) Transversion:

Replacement of a purine by a pyrimidine and vice versa is called transversion. In other words, it is the replacement of a base of one chemical category by a base of the other [pyrimidine replaced by purine: C to A, C to G, T to A, T to G; purine replaced by pyrimidine: A to C, A to T, G to C, G to T].

Such mutations are the result of breaking the backbone of genetic material [DNA] at two or more places. Such alterations include addition, deletion, replacement, transposition and inversion. All these mutations except inversions are possible for single stranded DNA or RNA.

Inversion requires double stranded nucleic acid. The simplest forms of such mutations are single base pair additions or single-base-pair deletions. There are examples in which mutations arise through simultaneous addition or deletion of multiple base pairs.

Like nonsense mutations, single base additions or deletions have consequences on polypeptide sequence that extend far beyond the site of the mutation itself. Because the sequence of mRNA is "read" by the translational apparatus in groups of three base pairs (codons), the addition or deletion of a single base pair of DNA will change the reading frame starting from the location of the addition or deletion and extending to the carboxy terminal of the protein.



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Types of Molecular Mutation:

Non-Sense Mutations:

Mutations in which the codon for one amino acid is replaced by a translation termination (stop) codon are referred to as non-sense mutations. In non-sense mutation a stop codon replaces an amino acid codon, resulting in premature termination of nucleotide chain.

(i) **Codon Involved:**

Non-sense mutations have non-sense codons which do not code for any amino acid.

(ii) **Frequency:**

The frequency of non-sense mutations is much lower than missense mutations.

(iii) **Effects:**

Non-sense mutations lead to the premature termination of polypeptide chain and hence are also called chain terminating mutations. They have a considerable effect on protein function.

Generally, nonsense mutations will produce completely inactive protein products. When they occur very close to the 3′ end of the open reading frame, only a partly functional truncated polypeptide is produced.

(iv) **Origin:**

Non-sense mutations result due to formation of non-sense codons after origin of frame shift mutations.

2. **Missense Mutations:**

Mutations in which the codon for one amino acid is replaced by a codon for another amino acid are called missense mutations. Missense mutations result in a protein in which one amino acid is substituted for another.

(i) **Codon Involved:**

Missense mutations have missense codons which code for different amino acid. Missense mutations usually result in replacement of a single amino acid in the polypeptide chain.

(ii) **Frequency:**

The frequency of missense mutations is more than non-sense mutations.

(iii) **Effects:**

The effects of such mutations vary. For example, if a missense mutation causes the substitution of a chemically similar amino acid, referred to as a synonymous substitution, then it is likely that the alteration will have a less severe effect on the protein's structure and function.

If a missense mutation causes the substitution of a chemically different amino acid, called non-synonymous substitutions, then it is more likely to produce severe changes in protein structure and function.

(iv) **Origin:**

Missense mutations result due to formation of missense codons after origin of frame shift mutations.

Silent Mutations:

Mutations that code for the same or similar amino acid are known as silent mutations. Such mutations change one codon for an amino acid into another codon for that same amino acid. Hence such mutations never alter the amino acid sequence of the polypeptide chain. In other words, they do not have any effect.

4. **Frame Shift Mutations:**

There is another category of point mutation in which the normal reading frame of the base triplet [codon] is changed. Such mutations are known as frame shift mutations. In these mutations, the normal reading frame of base triplets [codons] is altered due to addition or deletion of single base pair or nucleotides in mRNA. These are generally followed by a stop codon.

(i) **Origin:**

The frame shift mutations arise due to addition or deletion of single base pair. They arise in two ways, viz:

(a) By error during DNA repair or replication, and

(b) By acridine dyes.

The addition or deletion of nucleotides occurs in numbers other than three or multiple of three. The reading frame in such case is shifted from the point of addition or deletion onwards.

(ii) **Position:**

The addition or deletion of base pairs takes place in interstitial or intercalary position. Sometimes, addition and deletions take place at the same position, they are known as double frame shifts. Such changes may restore the normal reading frame in mRNA.

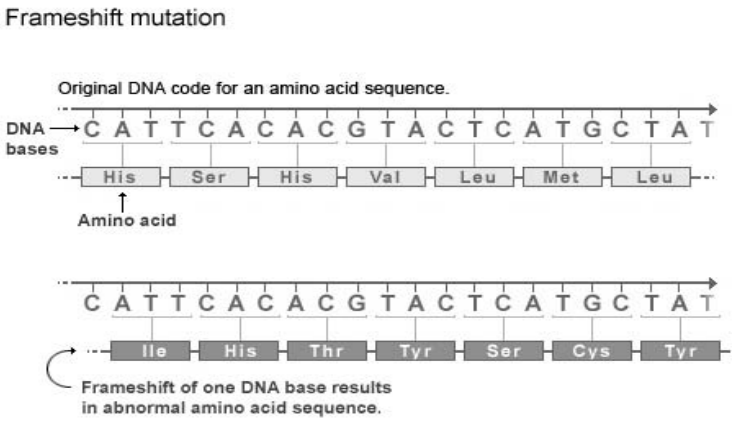
(iii) Effects: Frame shift mutations typically exhibit complete loss of normal protein structure and function. After frame shift mutations, three types of codons are produced, viz:

(a) Sense codons,

(b) Missense codons, and

(c) Non-sense codons.

Sense codons are normal codons which are read in the same way as before frame shift mutations. Mutations also effect the gene regulation both in eukaryotes and prokaryotes.



Source: https://www.youtube.com/watch?v=JaW42lROslE

5. **Induced and Spontaneous Mutation:**

Generally mutations are classified as induced and spontaneous. Induced mutations are defined as those that arise after purposeful treatment with mutagens. Spontaneous mutations are those that arise in the absence of known mutagen treatment. The frequency at which spontaneous mutations occur is low, generally in the range of one cell in 105 to 108.

Therefore, if a large number of mutants are required for genetic analysis, mutations must be induced. The induction of mutations is accomplished by treating cells with mutagens. The mutagens most commonly used are high energy radiation or specific chemicals. Induced and spontaneous mutations arise by generally different mechanisms.

Mechanisms of Induction Mutation:

Induced mutations are developed through the application of mutagenic agents called mutagens. There are three different mechanisms of mutation induction through use of mutagens, viz. by:

(i) **Base replacement,**

(ii) **Base alteration**, and

(iii)**Base damage in the DNA.**

These are briefly discussed as follows:

1. **Base Replacement:**

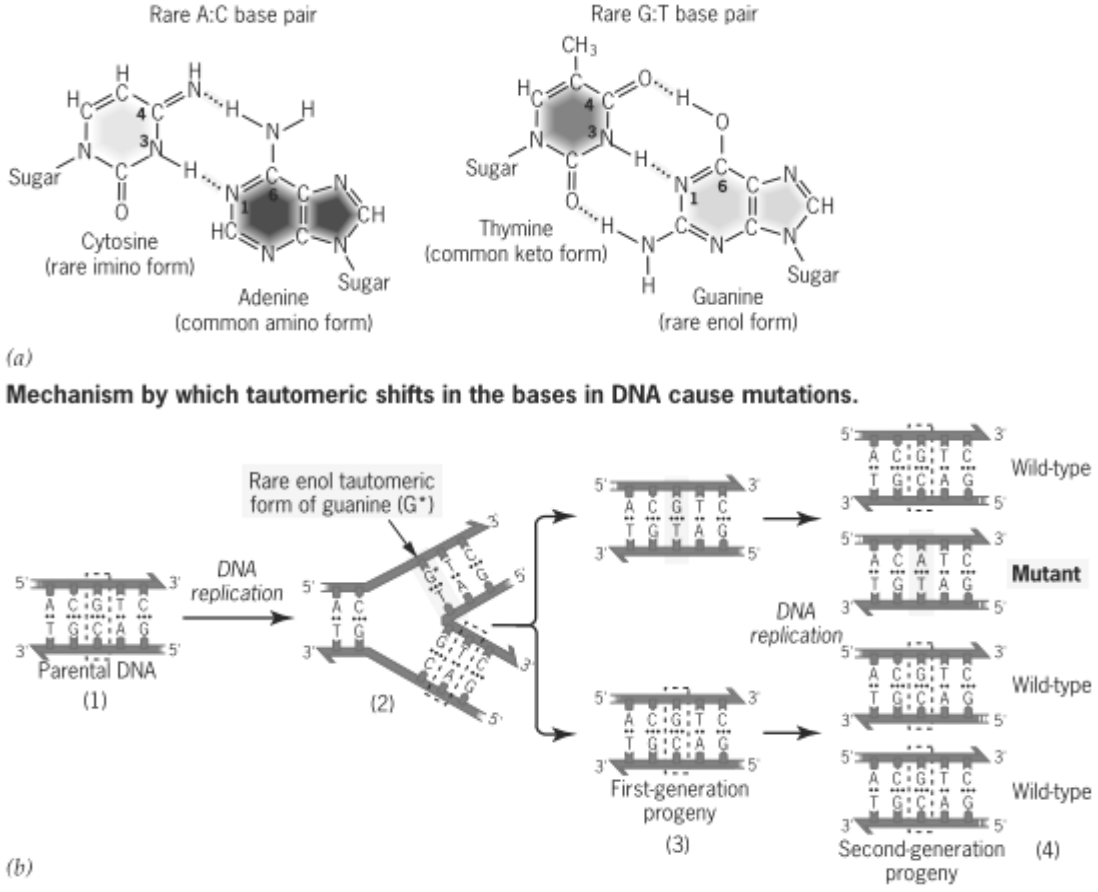
Some chemical compounds replace a base in the DNA because they are very similar to DNA bases. Such chemical compounds are called base analogues. They are sometimes incorporated into DNA in place of normal bases.

Thus they can produce mutations by wrong base pairing. An incorrect base pairing results in transitions or transversions after DNA replication. The most commonly used base analogues are 5 bromo uracil [5BU] and 2 amino purine [2AP].

The 5 bromouracil is similar to thymine, but it has bromide at the C5 position, 'whereas thymine has C3 group at C5″position. The presence of bromine in 5BU enhances its tautomeric shift from keto form to enol form. The keto form is usual and more stable form, while enol form is rare and less stable or short lived. Tautomeric change takes place in all the four DNA bases, but at a very low frequency. The change or shift of hydrogen atoms from one position to another either in a pyridine or in a pyridine base is known as tautomeric shift and such process is known as tautomerization. The base which is produced as a result of tautomerization is known as tautomeric form or tautomer.

As a result of tautomerization, the amino group [-NH2] of cytosine is converted into imno group [-NH]. Similarly, keto group [C=O] of thymine is changed to enol group [-OH].

5BU is similar to thymine, therefore, it pairs with adenine [in place of thymine], A tautomer of 5BU will pair with quanine rather than with adenine. Since the tautomeric form is short lived, it will change to keto form at the time of DNA replication which will pair with adenine in place of guanine.



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

In this way, it results in A to G or G to A; and C to T or T to C transitions. The mutagen 2AP acts in a similar way and causes A to G or G to A and T to C or C to T transitions. This is an analogue of adenine that can pair with thymine but can also mispair with cytosine and cause transitions.

1. **Base Alteration:**

Some chemical compounds alter a DNA base so that it specifically mispairs with another base. Such mutagens are not incorporated into the DNA but instead alter a base, causing specific mispairing.
They induce mutations especially transitions and transversions by adding an alkyl group [either ethyl or methyl] at various positions in DNA. Alkylation induces mutation by changing hydrogen bonding in various ways.
Alkylating agents can cause various large and small deformations of base structure resulting in base pair transitions and transversions. Transversions can occur either because a purine has been so reduced in size that it can accept another purine for its complement, or because a pyridine has been so increased in size that it can accept another pyrimidine for its pairing.
In both cases, diameter of the mutant base pair is close to that of a normal base pair.
Base Damage: Some mutagens damage a DNA base so that it can no longer pair with any base under normal conditions. A large number of mutagens damage one or more DNA bases, as a result no specific base pairing is possible. The result is a replication block, because DNA synthesis will not proceed beyond a base that cannot specify its complementary partner by hydrogen bonding.
In bacterial cells, such replication blocks can be bypassed by inserting nonspecific bases. The process requires the activation of a special system, the SOS system the name SOS comes from the idea that this system is induced as an emergency response to prevent cell death in the presence of significant DNA damage.
SOS induction is a last resort, allowing the cell to trade death for a certain level of mutagenesis. In nature DNA can be damaged by two main sources, viz. Ultraviolet light and Aflatoxin found in fungal infected peanuts.
Ultraviolet (UV) light generates a number of photoproducts in DNA. Two different lesions that unite adjacent pyrimidines in the same strand have been most strongly correlated with mutagenesis. These lesions are the cyclobutane pyrimidine photodimer and the 6-4 photoproduct.
These lesions interfere with normal base pairing; hence, induction of the SOS system is required for mutagenesis. The insertion of incorrect bases across from UV photoproducts is at the 3′ position of the dimer, and more frequently for 5′-CC-3′ and 5′- TC-3′ dimers.
The C -> T transition is the most frequent mutation, but other base substitutions (transversions) and frame shifts also are induced by UV light, as are larger duplications and deletions.

Aflatoxin B1 (AFB1) is a powerful carcinogen originally isolated from fungal-infected peanuts. Aflatoxin forms an addition product at the N-7 position of guanine. This product leads to the breakage of the bond between the base and the sugar, thereby liberating the base and resulting in an apurinic site.

Studies with apurinic sites generated in vitro have demonstrated that the SOS bypass of these sites leads to the preferential insertion of an adenine across from an apurinic site. This predicts that agents that cause depurination at guanine residues should preferentially induce G C à TA transversions.

Role of Induced Mutation in Crop Improvement:

Induced mutations are useful in crop improvement in the following five principal ways:

1. **In the development of improved varieties:**

More than 2000 improved varieties of field and horticultural crops with high yield, improved quality, earliness, resistance to biotic and abiotic stresses have been developed through induced mutations.

2. **Induction of Male Sterility:**

The male sterility has been induced in many crops such as pearl millet which is used in hybrid seed production.

3. **Production of Haploids:**

X-ray induced haploids have been developed in many crops which are used for development of pure lines after chromosomal doubling.

4. **Creation of Genetic Variability:**

Induced mutations lead to creation of vast genetic variability in a population which provides basis for selection.

5. In some cases, induced mutations have been used for overcoming problem of self-incompatibility.

Mechanisms of Spontaneous Mutation:

It is known now that spontaneous mutations arise from a variety of sources. There are three important mechanisms of spontaneous mutations, viz:

(i) Errors in DNA replication,

(ii) Spontaneous lesions, and

(iii) Transposable genetic elements.

These are briefly discussed below:

1. **Errors in DNA Replication:**

Mispairing in the course of replication is a source of spontaneous base substitution. (Mispairing was covered earlier in the discussion of 5BU.) Most mispairing mutations are transitions.
This is likely to be because an A-C or G-T mispair does not deform the DNA double helix as much as A-G or C-T base pairs do. However, transversions also can occur through mispairing. Replication errors can also lead to frame shift mutations.

2. **Spontaneous Lesions:**

Naturally occurring damage to the DNA, called spontaneous lesions, also can generate mutations. The spontaneous lesions are of three types, viz:

(i) Depurination,

(ii) Deamination, and

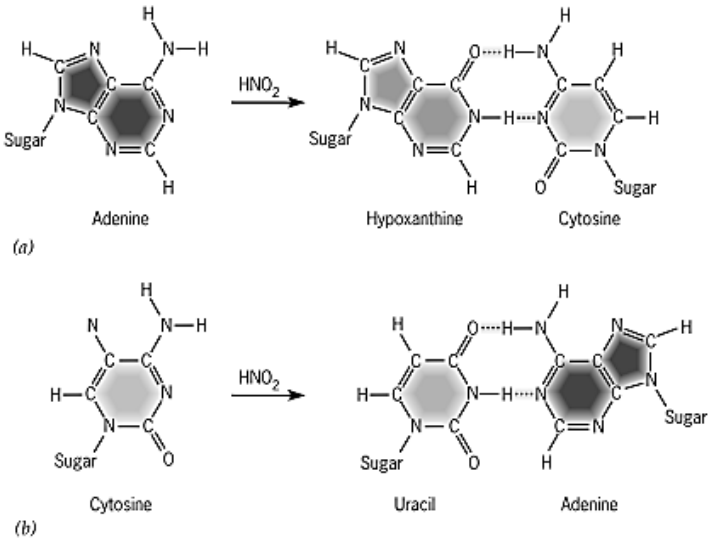
(iii) Oxidatively damaged bases.

The first one is more common.

(i) **Depurination:**

As stated above, aflatoxin induces depurination. However, depurination also occurs spontaneously. A mammalian cell spontaneously loses about 10,000 purines from its DNA during a 20-hour cell generation period at 37°C. The deamination of cytosine yields uracil. Unrepaired uracil residues will pair with adenine in the course of replication.
(ii) **Deamination:** Conversion of a G-C pair into an A-T pair (a G-C —> A-T transition). Deamination's at certain cytosine positions have been found to be one type of mutational hot spot. DNA sequence analysis of hot spots for G-C to A-T transitions in the lacl gene has shown that 5-methylcytosine residues are present at the position of each hot spot.

Enzyme uracil-DNA glycosylase [one of the repair enzymes in the cell], recognizes the uracil residues in the DNA that arise from deamination's and excises them, leaving a gap which is subsequently filled in.
However, the deamination of 5-methylcytosine generates thymine (5-methyluracil), which is not recognized by the enzyme uracil-DNA glycosylase and is thus not repaired. Therefore, C —> T transitions generated by deamination are seen more frequently at 5-methylcytosine sites, because they escape this repair system.



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

(iii) **Oxidatively damaged bases:**

This type of lesion by active oxygen species, such as superoxide radicals (O_2D), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OHD), which are produced as by-products of normal aerobic metabolism.

These oxygen species can cause oxidative damage to DNA, as well as to precursors of DNA (such as GTP), resulting in mutation. Such mutations have been implicated in a number of human diseases. Such product frequently mispairs with A, resulting in a high level of G —> T transversions.

3. Transposable Genetic Elements:

Transposable elements are also reported to play important role in the induction of spontaneous mutations in various organisms.

If these lesions persist, they will result in significant DNA damage because, during replication, the apurinic sites cannot specify any kind of base. However, under certain conditions, a base can be inserted across from an apurinic site, frequently resulting in a mutation.

Paramutation

In epigenetics, a paramutation is an interaction between two alleles at a single locus, whereby one allele induces a heritable change in the other allele. The change may be in the pattern of DNA methylation or histone modifications. The allele inducing the change is said to be paramutagenic, while the allele that has been epigenetically altered is termed paramutable. A paramutable allele may

have altered levels of gene expression, which may continue in offspring which inherit that allele, even though the paramutagenic allele may no longer be present. Through proper breeding, paramutation can result in sibling plants that have the same genetic sequence, but with drastically different phenotypes.

Mechanism

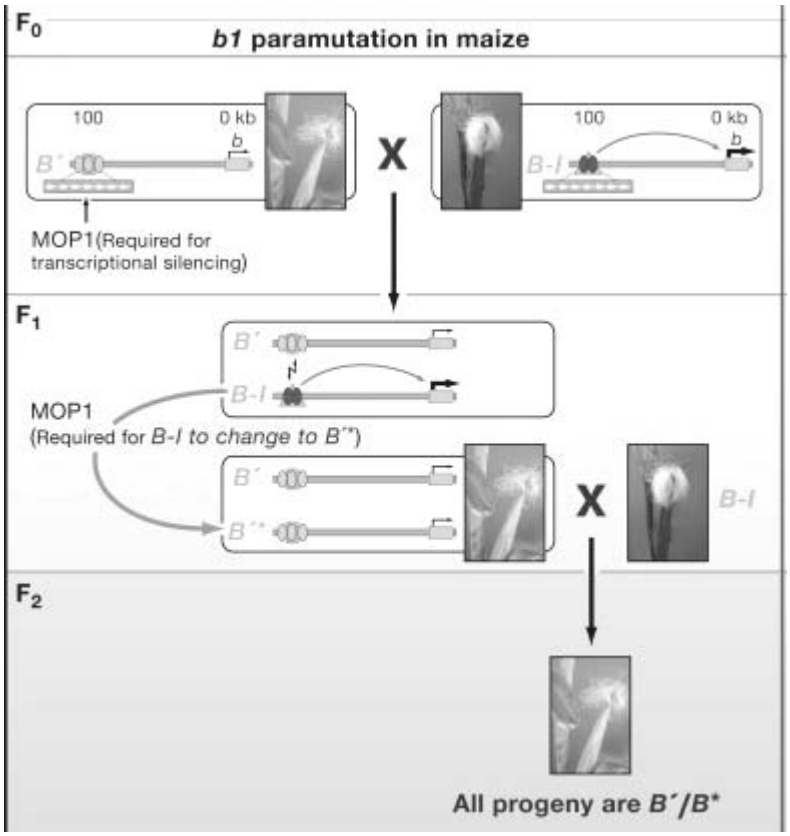
Though the specific mechanisms by which paramutation acts vary from organism to organism, all well documented cases point towards epigenetic modification and RNA silencing as the underlying mechanism for paramutation.

In the case of the r1 locus in maize, DNA methylation of a region of tandem repeats near the coding region of the gene is characteristic of the paramutagenic B' allele, and when the paramutable B-I allele becomes paramutagenic, it too takes on the same DNA methylation pattern. In order for this methylation to be successfully transferred, a number of genes coding for RNA-dependent RNA polymerases and other components of RNA-silencing pathways are required, suggesting that paramutation is mediated via endogenous RNA-silencing pathways. The transcription of short interfering RNAs from the tandem repeat regions corroborates this. In animal systems such as *Drosophila*, piRNAs have also been implicated in mediating paramutation. In addition to the characteristic DNA methylation state changes, changes in histone modification patterns in the methylated DNA regions, and/or the requirement of histone modifying proteins to mediate paramutation have also been noted in multiple systems. It has been suggested that these histone modifications play a role in maintaining the paramutated state. The previously mentioned tandem repeat region in the r1 locus is also typical of other loci showing paramutation or paramutation like phenomena.

However, it has been noted that it is not possible to explain all occurrences and features of paramutation with what is known about RNAi-mediated transcriptional silencing, suggesting that other pathways and/or mechanisms are also at play.

Implications

It has been speculated that in any particular population, relatively few genes would show observable paramutation since the high penetrance of paramutagenic alleles (like B' at the r1 locus in maize) would drive either the paramutagenic or paramutable allele to fixation. Paramutation at other loci with paramutagenic alleles with lower penetrance may persist; however, which may need to be taken into account by plant breeders.



Source: http://mmg-233-2013-genetics-genomics.wikia.com/wiki/Paramutations

Since there are examples of paramutation, or paramutation like phenomena, in animals such as fruit flies and mice, it has been suggested that paramutation may explain the occurrence of some human diseases that exhibit non-Mendelian inheritance patterns.

Repair Mechanisms:

Most kinds of damage create impediments to replication or transcription. Altered bases cause mispairing and can cause permanent alteration to DNA sequence after replication.

In order to maintain the integrity of information contained in it, the DNA has various repair mechanisms.

I. reversal of damage,

II. nucleotide excision repair,

III. base excision repair,

IV. mismatch repair,

V. recombinational repair, and error-prone repair.

Reversal of damage

Some kinds of covalent alteration to bases in DNA can be directly reversed. This occurs by specific enzyme systems recognizing the altered base and breaking bonds to remove the adduct or change the base back to its normal structure.

1. Photo reactivation

Ultraviolet light is a physical mutagen and can induce mutation. Ultra violet radiation (254 nm) causes formation of pyrimidine dimers (cyclobutane ring), when two pyrimidine bases occurs together in single strand of DNA.

Thymine dimer is most common one but cytosine dimer as well as thymine-cytosine may also occur. Thymine dimer is a state in which two adjacent thymine molecules are chemically joined distorting the structure of DNA, so that impeding transcription and replication process.

This pyrimidine dimer formation is lethal to the cell unless it is corrected. A repair mechanism known as photo reactivation can repair this mutation.

When UV radiated population of bacteria is subsequently exposed to visible light of wave length of 300-450nm, the survival rate increases and frequency of mutation decreases. This is due to activation of photo reactivating enzyme **photolyase**, which splits thymine dimer.

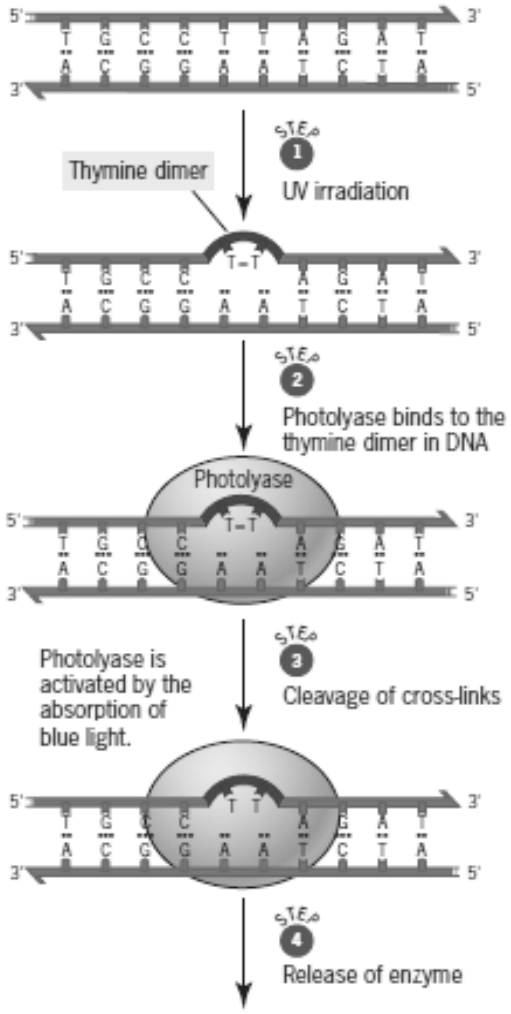


Figure Photo reactivation, Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Excision Repair

The repair system removes and replaces the altered bases from damaged DNA. Excision repair system involves nucleotide repair and base excision repair.

i. Base excision repair:

In this mechanism modified bases are recognized and cut out. Mutation causes alkylation and deamination of bases which are recognized by special DNA glycosylase enzyme.

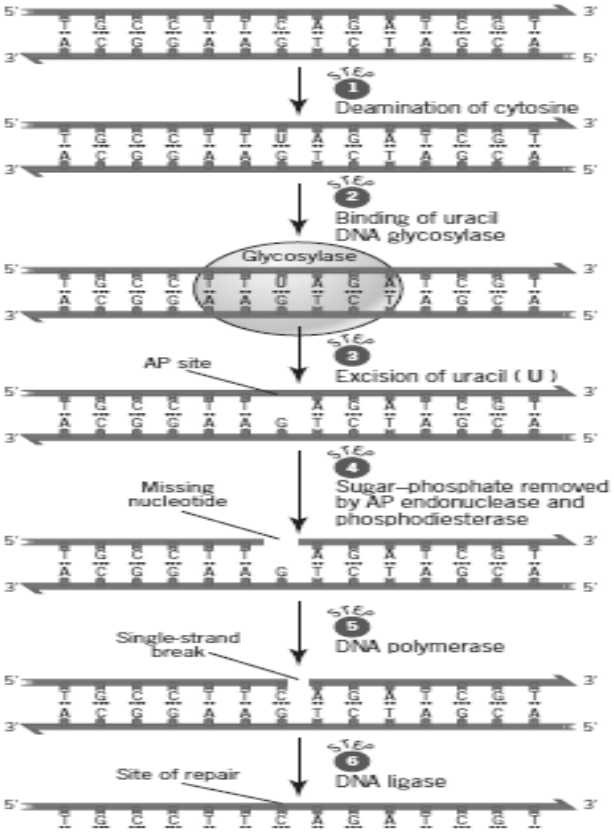


Figure Base excision repair pathway. Snustad and Simmons, 2012, Principles of Genetics -6th ed.

After the damaged nucleotide is removed, the gap is repair by DNA polymerase I and ligated by DNA ligase.

Nucleotide excision repair:

In nucleotide excision repair mechanism, the defective nucleotide are cut out and replaced.

Unlike base excision repair, the enzymes in nucleotide excision repair recognize the distortion in shape of double stranded DNA structure caused by thymine dimers or intercalating agents.

The multi-subunit enzyme excinucleases (endonuclease and exonuclease activity) hydrolyses two phosphodiester bond one on either side of distortion caused by lesion creating 3'-OH group and 5'-P group.

Glycosylase recognizes and remove the damaged bases by hydrolyzing the glycosidic bond and cut out the damaged base creating AP site (a purinic or pyrimidinic site).

The AP site is recognized by AP endonucleases which split the phosphodiester bond on DNA strand at AP site and removes the AP sugar.

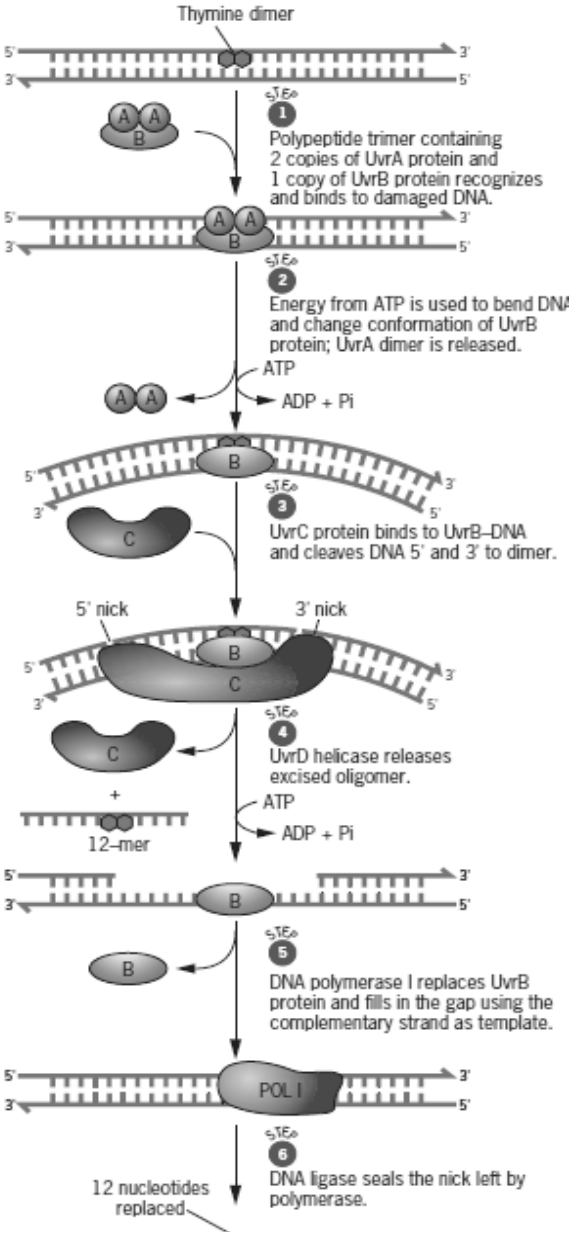


Figure Nucleotide excision repair pathway. Snustad and Simmons, 2012, Principles of Genetics -6th ed.

The excised nucleotide is removed and the resulting gap is filed by DNA polymerase-I in E. coli and DNA polymerase E in Human and finally joined by DNA ligase.

Mismatch Repair

The third type of excision repair we will consider is mismatch repair, which is used to repair errors that occur during DNA synthesis. In contrast to nucleotide excision repair, mismatch repair does not operate on bulky adducts or major distortions to the DNA helix. Most of the mismatches are substitutes within a chemical class, e.g. a C incorporated instead of a T. This causes only subtle helical distortions in the DNA, and the mis-incorporated nucleotide is a normal component of DNA. The ability of a cell to recognize a mismatch reflects the exquisite specificity of MutS, which can distinguish normal base pairs from those resulting from mis-incorporation.

The enzyme complex MutH-MutL-MutS or MutHLS, catalyzes mismatch repair in *E. coli*. The genes that encode these enzymes, mutH, mutL and mutS, were discovered because strains carrying mutations in them have a high frequency of new mutations. This is called a mutator phenotype, and hence the name mut was given to these genes.

MutS will recognize seven of the eight possible mismatched base pairs (except for C:C) and bind at that site in the duplex DNA. MutH and MutL (with ATP bound) then join the complex, which then moves along the DNA in either direction until it finds a hemi-methylated

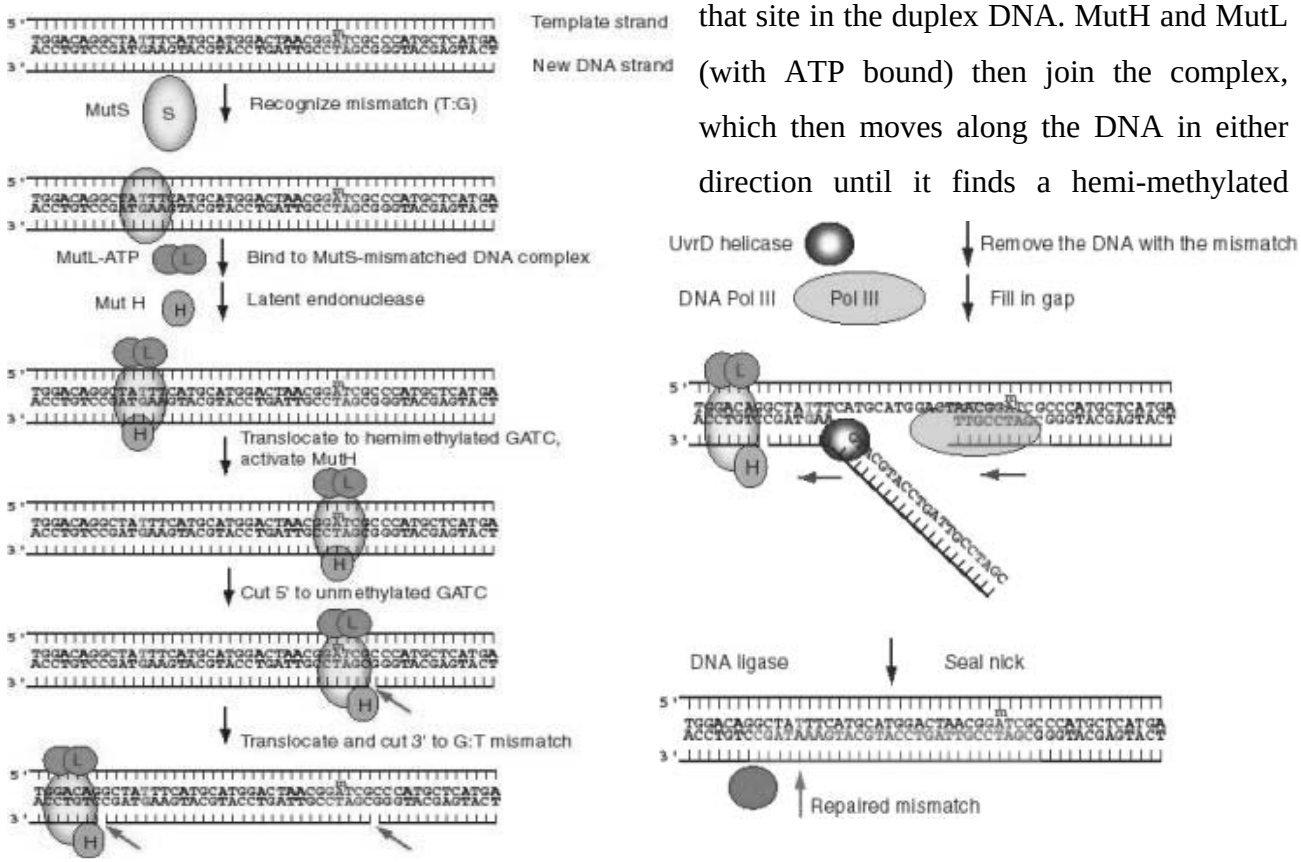


Figure Mismatch Repair of DNA. Source https://bio.libretexts.org/TextMaps/Genetics/Book%3A_Working_with_Molecular_Genetics_(Hardison)/.

GATC motif, which can be as far a few thousand base pairs away. Until this point, the nuclease function of MutH has been dormant, but it is activated in the presence of ATP at a hemi-methylated GATC. It cleaves the unmethylated DNA strand, leaving a nick 5' to the G on the strand containing the unmethylated GATC (i.e., the new DNA strand). The same strand is nicked on the other side of the mismatch. Enzymes involved in other processes of repair and replication catalyze the remaining steps.

The SOS response

Photoreactivation, excision repair and post reactive recombination repair are generally error free repair mechanism. However, there also exist an error prone and mutation inducing repair called SOS repair.

Error in both complementary strand of DNA would be lethal for cell. Thus SOS repair reconstruct the chemical structure of DNA but the heredity information is lost. Hence SOS repair causes mutation along with repair of DNA. DNA polymerase V help in SOS repair.

It is also known as inducible repair. It involves more than 40 genes which encode protein responsible for protection and replication of DNA as well as repair and mutation.

SOS response has been found in *E. coli, Salmonella typhimurium, Mycobacterium tuberculosis* etc, but not in eukaryotic cell.

It is not a single discrete mechanism but includes diverse responses such as the ability to repair thymine dimers, to induce various prophages, to shut off respiration, to delay septum formation during cell division. These all responses are regulated coordinately.

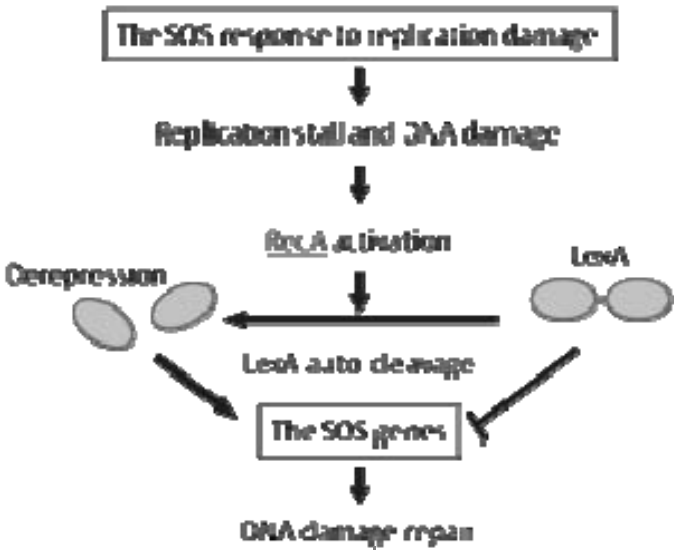
Mechanism of SOS response:

SOS response is induced when DNA is damaged or when replication of DNA stops and single stranded DNA accumulates.

Agents such as UV radiations, methyl methanesulphonate, as well as chemicals that damages DNA induces SOS response. Rec A and Lex A gene are major in SOS response induction.

lexA encodes lexA protein which is repressor. It binds to SOS box near promotor of SOS gene and prevents gene expression.

RecA protein is a nucleoprotein and is



Source: http://www.onlinebiologynotes.com/ repair-mechanism-of-mutation/

always high in bacterial cell. It form nucleoprotein filaments on single stranded DNA and protects further damage of DNA. Rec A protein acquires proteases activities and activates self-cleavage of lexA protein from SOS box.
Now the operator and promoter site become free and facilitates gene expression of SOS box gene.

Genomic imprinting is an epigenetic phenomenon that causes genes to be expressed in a manner specific to their parent of origin. Forms of genomic imprinting have been demonstrated in fungi, plants and animals. As of 2014, there are about 150 imprinted genes known in the mouse and about half that in humans.
Genomic imprinting is an inheritance process independent of the classical Mendelian inheritance. It is an epigenetic process that involves DNA methylation and histone methylation without altering the genetic sequence. These epigenetic marks are established ("imprinted") in the germline (sperm or egg cells) of the parents and are maintained through mitotic cell divisions in the somatic cells of an organism.

Imprinting mechanisms
Imprinting is a dynamic process. It must be possible to erase and re-establish imprints through each generation so that genes that are imprinted in an adult may still be expressed in that adult's offspring. (For example, the maternal genes that control insulin production will be imprinted in a male but will be expressed in any of the male's offspring that inherit these genes.) The nature of imprinting must therefore be epigenetic rather than DNA sequence dependent. In germline cells, the imprint is erased and then reestablish according to rhe sex of the individual, i.e., in the developing sperm (during spermatogenesis), a paternal imprint is established, whereas in developing oocytes (oogenesis), a maternal imprint is established. This process of erasure and reprogramming is necessary such that the germ cell imprinting status is relevant to the sex of the individual. In both plants and mammals, there are two major mechanisms that are involved in establishing the imprint; these are DNA methylation and histone modifications.

Recently, a new study has suggested a novel inheritable imprinting mechanism in humans that would be specific of placental tissue and that is independent of DNA methylation (the main and classical mechanism for genomic imprinting). Among the hypothetical explanations for this exclusively human phenomenon, two possible mechanisms have been proposed: either a histone modification that confers imprinting at novel placental-specific imprinted loci or, alternatively, a recruitment of DNMTs to these loci by a specific and unknown transcription factor that would be expressed during early trophoblast differentiation.

Imprinted genes in plants

A similar imprinting phenomenon has also been described in flowering plants (angiosperms). During fertilization of the egg cell, a second separate fertilization event gives rise to the endosperm, an extra embryonic structure that nourishes the embryo in a manner analogous to the mammalian placenta. Unlike the embryo, the endosperm is often formed from the fusion of two maternal cells with a male gamete. This results in a triploid genome. The 2:1 ratio of maternal to paternal genomes appears to be critical for seed development. Some genes are found to be expressed from both maternal genomes while others are expressed exclusively from the lone paternal copy. It has been suggested that these imprinted genes are responsible for the triploid block effect in flowering plants that prevents hybridization between diploids and autotetraploids.

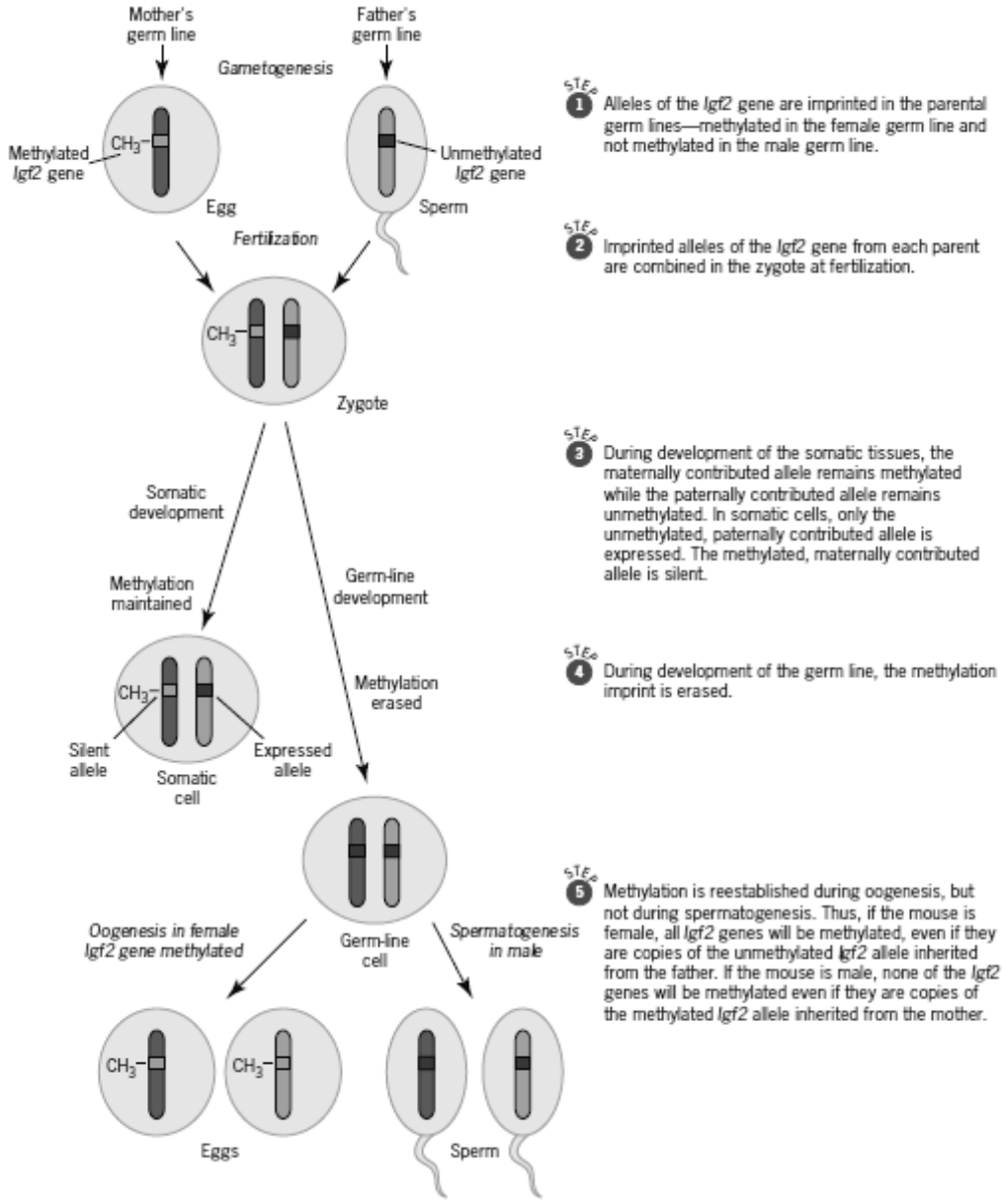


Figure Genomic imprinting. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Prion particles:

Prions are infectious agents composed entirely of a protein material that can fold in multiple, structurally abstract ways, at least one of which is transmissible to other prion proteins, leading to disease in a manner that is epidemiologically comparable to the spread of viral infection. Prions composed of the prion protein (PrP) are believed to be the cause of transmissible spongiform encephalopathies (TSEs) among other diseases.

Prions were initially identified as the causative agent in animal TSEs derived from scrapie in sheep and later bovine spongiform encephalopathy (BSE)—known popularly as "mad cow disease". Human prion diseases include Creutzfeldt–Jakob disease (CJD) and its variant (vCJD), Gerstmann Sträussler Scheinker syndrome, fatal familial insomnia, and kuru. All known prion diseases in mammals affect the structure of the brain or other neural tissue. No effective medical treatment is known. The illnesses are progressive and always fatal.

A 2015 study concluded that multiple system atrophy (MSA), a rare human neurodegenerative disease, is caused by a misfolded version of a protein called alpha-synuclein, and is therefore also classifiable as a prion disease. Several yeast proteins have also been identified as having prionogenic properties.

A protein as a stand-alone infectious agent stands in contrast to all other known infectious agents such as viruses, bacteria, fungi, and parasites, all of which contain nucleic acids (DNA, RNA, or both). For this reason, a minority of researchers still consider the prion/TSE hypothesis unproven.

Prions may propagate by transmitting their misfolded protein state. When a prion enters a healthy organism, it induces existing, properly folded proteins to convert into the misfolded prion form. In this way, the prion acts as a template to guide the misfolding of more proteins into prion form. In yeast, this refolding is assisted by chaperone proteins such as Hsp104. These refolded prions can then go on to convert more proteins themselves, leading to a chain reaction resulting in large amounts of the prion form. All known prions induce the formation of an amyloid fold, in which the protein polymerises into an aggregate consisting of tightly packed beta sheets. Amyloid aggregates are fibrils, growing at their ends, and replicate when breakage causes two growing ends to become four growing ends. The incubation period of prion diseases

is determined by the exponential growth rate associated with prion replication, which is a balance between the linear growth and the breakage of aggregates. The propagation of the prion depends on the presence of normally folded protein in which the prion can induce misfolding; animals that do not express the normal form of the prion protein can neither develop nor transmit the disease.

Prion aggregates are extremely stable and accumulate in infected tissue, causing tissue damage and cell death. This structural stability means that prions are resistant to denaturation by chemical and physical agents, making disposal and containment of these particles difficult. Prion structure varies slightly between species, but nonetheless prion replication is subject to epimutation and natural selection just like other forms of replication.

Site-directed mutagenesis

Site-directed mutagenesis is a molecular biology method that is used to make specific and intentional changes to the DNA sequence of a gene and any gene products. Also called site specific mutagenesis or oligonucleotide directed mutagenesis, it is used for investigating the structure and biological activity of DNA, RNA, and protein molecules, and for protein engineering.

Site-directed mutagenesis is one of the most important techniques in laboratory for introducing a mutation into a DNA sequence. There are numerous methods for achieving site directed mutagenesis, but with decreasing costs of oligonucleotide synthesis, artificial gene synthesis is now occasionally used as an alternative to site-directed mutagenesis. Since 2013, the development of the CRISPR/Cas9 technology, based on a prokaryotic viral defense system, has also allowed for the editing of the genome, and mutagenesis may be performed *in vivo* with relative ease

Basic mechanism

The basic procedure requires the synthesis of a short DNA primer. This synthetic primer contains the desired mutation and is complementary to the template DNA around the mutation site so it can hybridize with the DNA in the gene of interest. The mutation may be a single base change (a point mutation), multiple base changes, deletion, or insertion. The single strand primer is then extended using a DNA polymerase, which copies the rest of the gene. The gene thus copied contains the mutated site, and is then introduced into a host cell as a vector and cloned. Finally, mutants are selected by DNA sequencing to check that they contain the desired

mutation. The original method using single primer extension was inefficient due to a low yield of mutants. This resulting mixture contains both the original unmutated template as well as the mutant strand, producing a mixed population of mutant and non-mutant progenies. Furthermore, the template used is methylated while the mutant strand is unmethylated, and the mutants may be counter-selected due to presence of mismatch repair system that favors the methylated template DNA, resulting in fewer mutants. Many approaches have since been developed to improve the efficiency of mutagenesis.

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Complementation

Complementation is the phenomenon where two strains of organisms, each homozygous-recessive for mutations in different genes contributing to the same phenotype, are crossed to give an offspring with the wild-type phenotype. This is due to the fact that the offspring can inherit the mutant alleles from each parent, so long as it also inherits the complementary wild-type alleles from the other parent.

Simply put: for two genes, A and B, that contribute to a phenotype, if individual 1 has the genotype aaBB and a mutant phenotype, and individual 2 has the genotype AAbb and has a mutant phenotype, then a cross between individual 1 and individual 2 can potentially give rise to an offspring, AaBb, who has the wild type phenotype.

Complementation testing (also cis-trans testing), then, allows us to ascertain whether mutant phenotypes in two (or more) individuals are caused by mutations in the same genes or in different genes for that phenotype, by doing crosses and seeing whether the wild type phenotype can be restored in the offspring.

If a pairwise crossing between two individuals with the same mutant phenotype only bears offspring with that mutant phenotype, then there are three possibilities:

The mutations in the parent individuals are in the same gene (i.e., the parents are in the same complementation group)

There is an epistatic interaction going on (i.e., the mutant allele of one gene is epistatic to the wild type allele of another)

The mutant allele of one gene encodes an inhibitory product that prevents expression of the wildtype allele of another

A hypothetical example of purple flowers

The easiest way to understand a complementation test is by example. The pigment in a purple flower could depend on a biochemical pathway much like the biochemical pathways leading to the production of arginine in *Neurospora*. A plant that lacks the function of gene A (genotype aa) would produce mutant, white flowers that looked just like the flowers of a plant that lacked the function of gene B (genotype bb). Both A and B are enzymes in the same pathway that leads from a colorless compound#1, thorough colorless compound#2, to the purple pigment. Blocks at either step will result in a mutant white, not wild type purple, flower.

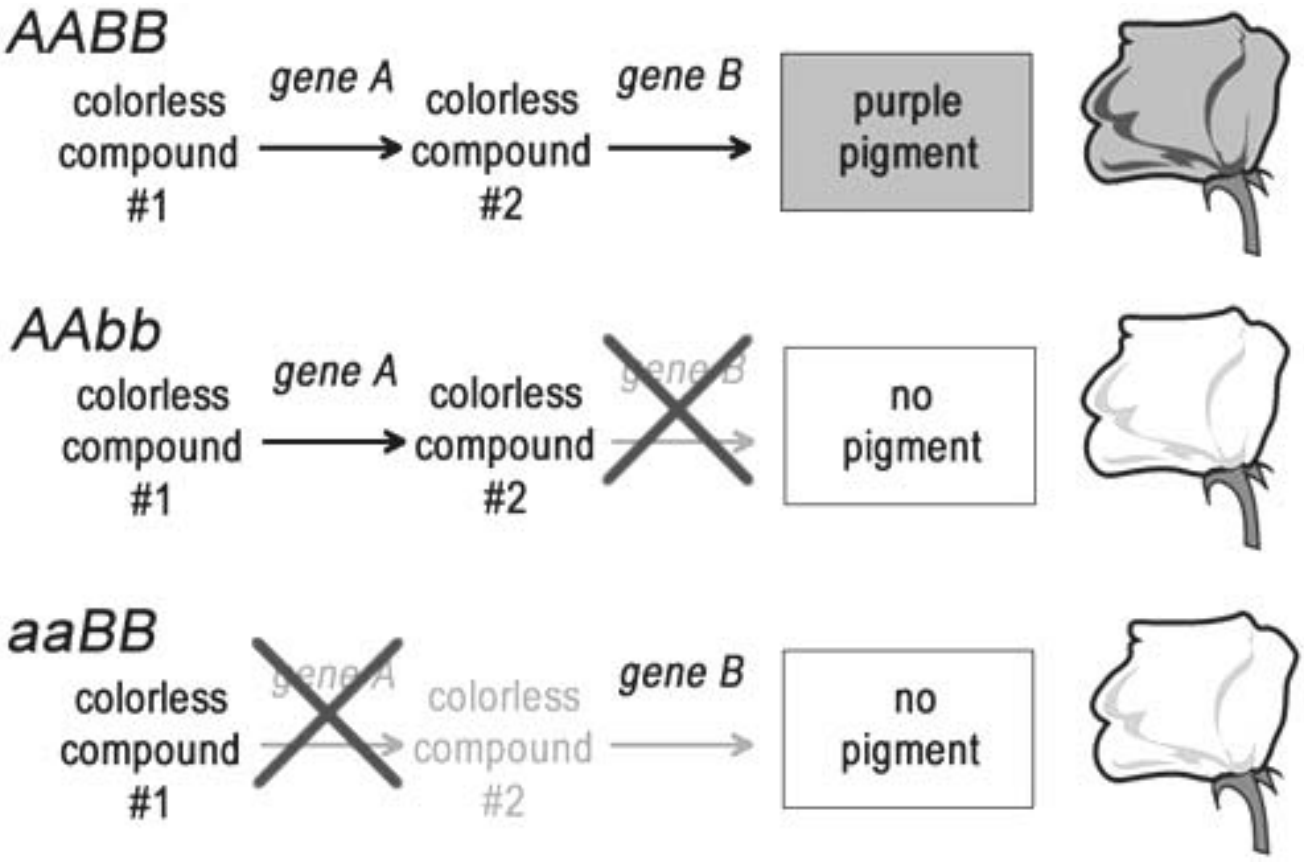


Figure In this simplified biochemical pathway, two enzymes encoded by two different genes modify chemical compounds in two sequential reactions to produce a purple pigment. Loss of either of the enzymes disrupts the pathway and no pigment is produced Source: http://cubocube.com/dashboard.php?a=1182&b=1267&c=103

In this simplified biochemical pathway, two enzymes encoded by two different genes modify chemical compounds in two sequential reactions to produce a purple pigment. Loss of either of the enzymes disrupts the pathway and no pigment is produced.

Mapping the rII Locus in T4

The wild type T4 phage produces small plaques with rough edges on both strains B and K of E. coli. The r mutants (rapid lysis) are easily distinguished by their large, sharp edged plaques. The wild type phages do not lyse and release progeny phage as rapidly as the r mutants. The r mutants are classified into groups depending upon their behavior in *E. coli* strains other than B.

The rII group of mutants analysed by Benzer differ from the wild type as they do not produce plaques on strain *E. coli* K which carries phage λ (lysogenic for λ). The wild type T4 (rII+) grows on *E. coli* K (λ).

Thus an rII mutant produces r-type plaques on strain B of *E. coli*, wild type plaques on strain K12S (designated strain S) of *E. coli*, (that is one which does not harbour A,) and it forms no plaques on strain K(λ). The wild type T4 produces similar plaques on all the 3 strains B, S and K.

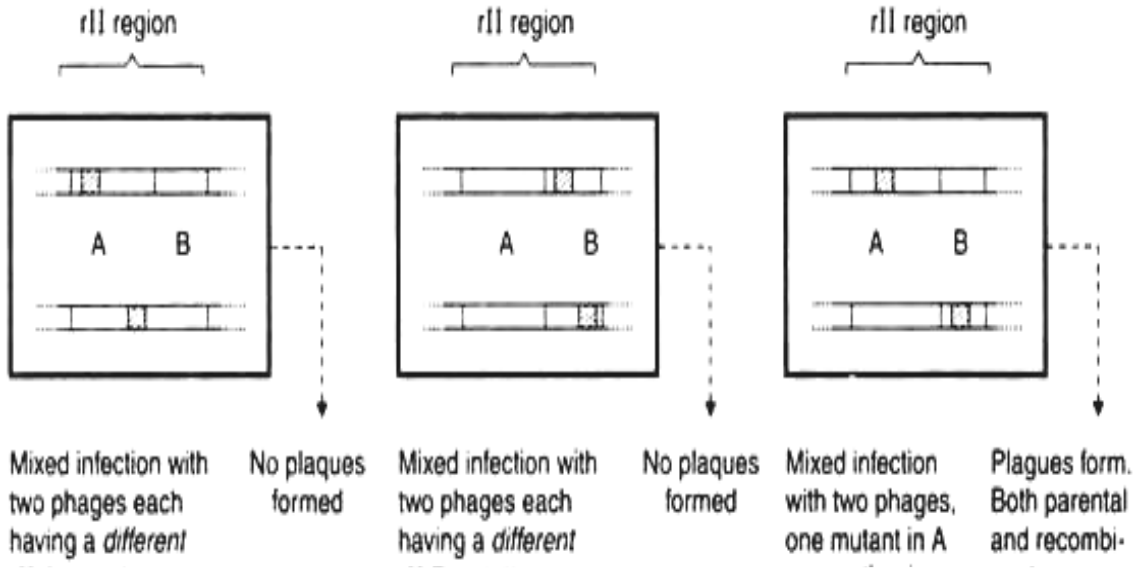
The rII mutants proved favourable for this study because it is possible to detect even a very small number of wild type particles among a very large group of mutants. The r group of mutants produces a distinct plaque type on strain B, and rII mutants are identified by testing on strain K.

On strain K(λ) only wild type will grow. Thus when the progeny of a genetic cross between two different rII mutants are added to *E. coli* K(λ) only the wild-type recombinants will form plaques and will be detected even when their frequency is as low as one per 106 progeny.

The cis-trans complementation tests first devised by Lewis for studying the gene in the lower eukaryotes were applied by Benzer to phage. He crossed pairs of rII mutants and found that they belonged to two functional groups, due to presence of two separate segments rII A and rII B in the rII region of phage chromosome. The two groups of rII mutants, namely rII A and rII B can be distinguished from their behavior after mixed infection of strain K (λ).

When mixed infection is done using two different mutants one belonging to rII A, the other to rII B group, the two phages multiply, cause lysis of host cell and plaques are formed.

This means that normal polypeptides of both mutants are required to produce plaques—that is to say, the two complement each other. But if the two mutants used for mixed infection of K(λ) both come from the same group, either rII A or rII B, they will not be able to form plaques (non-complementing).



Source: http://www.biologydiscussion.com/genetic-engineering/mapping-the-rii-locus-in-t4-genetics/67866

The fact that segments A and B show complementation demonstrates that A and B are independent, separate units. Each performs a different function resulting in distinct polypeptide end products. Polypeptides from both segments A and B are necessary for phage multiplication in strain K (λ). Thus A and B are two separate genes or cistrons (the term cistron was coined by Benzer).

The entire rII region is a single functional unit in the sense that all mutations within this region (both segments A and B) produce the rII phenotype. The polypeptide product of A is not fully known; however B codes for a cell membrane protein.

The following procedure was used by Benzer for mapping the rII region. Strain B cells were infected with a 1:1 mixture of two rII mutants. After cell lysis, the progeny recovered consisted of the two parental types and recombinants of two types—double mutants and wild type.

When progeny from many cells was considered, the two recombinant types were found in equal proportions. The progeny phages were made to infect K(λ) cells and B cells. The double rll mutants will not form plaques on K(λ), only the wild recombinant type will. Thus the total number of recombinants in the progeny is obtained simply by doubling the wild type plaques on K (λ).

On strain B all types of progeny, parentals and recombinants will form plaques. The proportion of parentals to recombinants is thus determined. In this way by crossing rII mutants in pairs, Benzer was able to map a large number of mutations in rII region.

The method described above is sensitive enough to detect one wild type phage in a million particles. The lowest frequency of recombination between two loci is an index of the smallest distance separating two loci within which recombination could occur. But r mutants can revert to the wild type as first found by Hershey.

Thus although Benzer had expected to detect a recombination frequency as low as 10-6, the lowest he actually found was 10-4 (0.01 %). Since this represents half of the recombinant types, the lowest frequency would be 0.02 %.

Therefore, the lowest frequency of recombination that can be accurately determined between two rII mutants is 0.02 per cent (that is a distance of 0.02 map units). Now the entire circular genetic map of T4 is 1500 map units containing about 2 x 10-5 nucleotide pairs.

Therefore a map distance of 0.02 map units would proportionately be 1.3 x 10-5 of the entire phage genome. Thus the minimum distance between two points within which recombination can occur would be (1.3 x 10-5) x (2 x 105) which amounts to about 3 nucleotide pairs. Later work on phage DNA by Yanofsky has shown that recombination can occur even between adjacent nucleotides.

With this technique Benzer mapped about 2000 mutations in rII region. Obviously it became difficult to cross such a large number of mutants in mixed infections. Fortunately there was a second class of mutations called deletion mutations which had a deficiency in either the A or B segment.

In phage deletions are identified by their stability and their failure to have second mutations which reverts them to the wild type. Further, recombination does not occur in the region of the deletion.

In deletion mapping a phage with a deleted segment and another phage carrying a mutation at the identical site as the deletion are used in mixed infection of strain *E. coli* B cells. Their progeny is plated on K(λ).

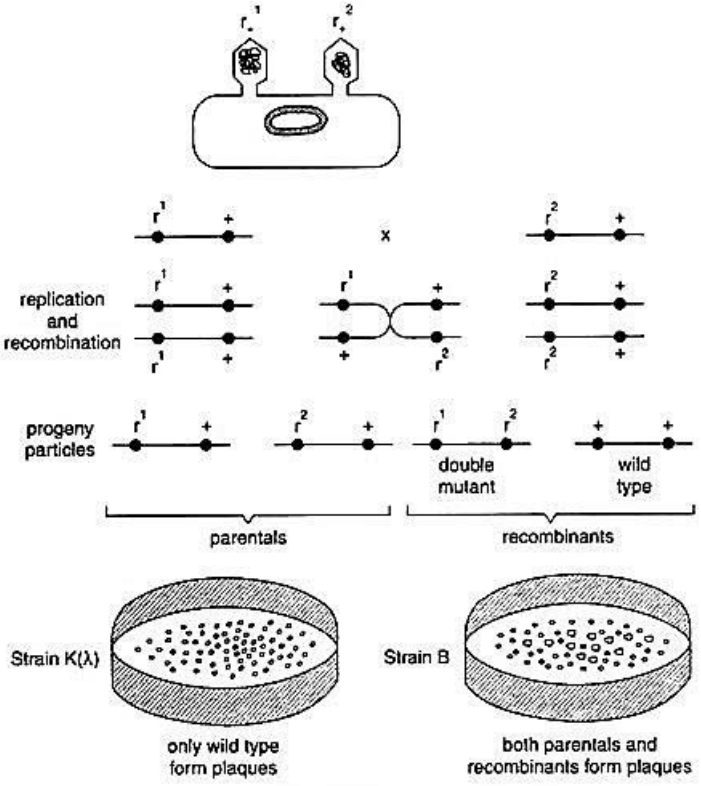


Fig. 22.3 Benzer's technique for fine structure genetic mapping of rII region of *T4*.

Source: http://www.biologydiscussion.com/genetic-engineering/mapping-the-rii-locus-in-t4-genetics/67866

If the deletion and the known mutation occupy the same site, then no wild type recombinants will occur. But if the sites are different then wild type recombinants would appear (Fig. 22.4). By testing a deletion with a number of known mutations, the length of a deleted segment can be determined.

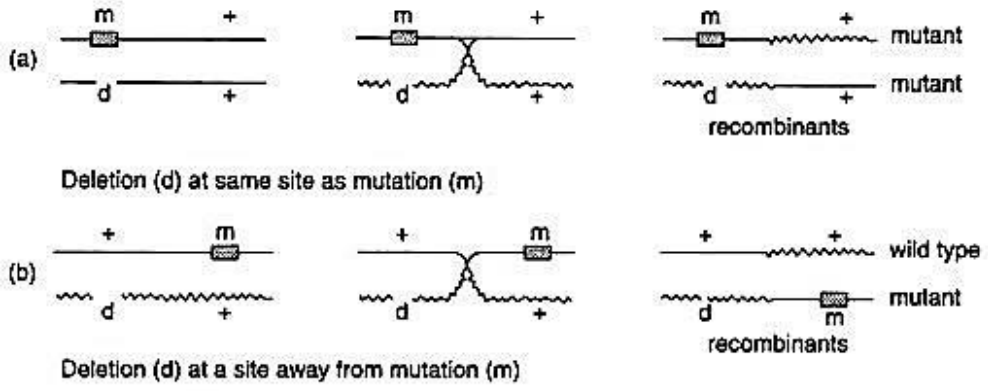


Fig. 22.4 Method of deletion mapping; mixed infections using phages with deletions and mutations at (*a*) same, and (*b*) at different sites.

Source: http://www.biologydiscussion.com/genetic-engineering/mapping-the-rii-locus-in-t4-genetics/67866

In this way a series of deletions showing gradations in length can be mapped. They are then used to map an unknown rII mutation by crossing a mutant with a series of deletions and observing whether recombination occurs or not (Fig. 22.5). The method involves selecting a

group of deletion mutants whose lengths are known and crossing an unknown mutant with each one of them.

As shown in the hypothetical Fig. 22.5, crosses between the unknown mutant M and deletion mutants d2, d3, d4 and d6, no recombinants are recovered because the mutant site lies opposite a deleted segment of the second phage DNA.

But when the unknown mutant M is crossed with d1, d5 and dl, recombinants are produced. In this way we can map the exact location of mutant M. The length of the DNA in rIIA gene is calculated to be 6 map units (800 base pairs) and in rII B region 4 map units (500 base pairs).

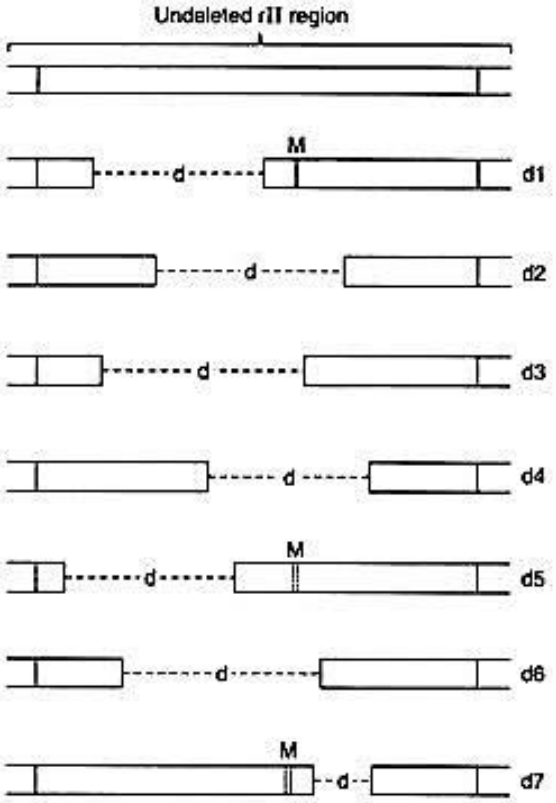


Fig. 22.5 Mapping an unknown rII mutation *M* by crossing the mutant with a series of delections.

Source: http://www.biologydiscussion.com/genetic-engineering/mapping-the-rii-locus-in-t4-genetics/67866

11. Biology of DNA and RNA: DNA forms; DNA replication; transcription and translation processes; RNA types; characterization of rRNA; pre mRNA processing.

DNA forms

The most common form of DNA which has right handed helix and proposed by Watson and Crick is called B-form of DNA or B-DNA. In addition, the DNA may be able to exist in other forms of double helical structure. These are A and C forms of double helix which vary from B- form in spacing between nucleotides and number of nucleotides per turn, rotation per base pair, vertical rise per base pair and helical diameter.

Table 5.3 : Forms of double stranded DNA helix

Parameters	*Forms of DNA*				
	A	*B*	*C*	*D*	*Z*
Conditions	75% rel. Humidity, Na^+ K^+, Cs^+ ions	92% rel. humidity low ions	66% rel. humidity Li^+ ions	—	low high salt conc.
Base pair per turn	11	10	9.33	8	12 (6 dimers)
Rotation per bp	+32.7°	+36.0°	+38.6°	—	-30.0°
Vertical rise per bp	2.56Å	3.38Å	3.32Å	3.03Å	3.71Å
Helical diameter	23Å	20Å	19Å	—	18Å
Pitch of the helix	28.15Å	34Å	31Å	—	45 Å
Tilt of bp Sugar puckering	20.2Å	6.3°	-7.8°	-16.7°	7°

1. The B-Form of DNA (B-DNA):

Structure of B-form of DNA has been proposed by Watson and Crick. It is present in every cell at a very high relative humidity (92%) and low concentration of ions. It has antiparallel double helix, rotating clockwise (right hand) and made up of sugar- phosphate back bone combined with base pairs or purine-pyrimidine.

The base pairs are perpendicular to longitudinal axis of the helix. The base pairs tilt to helix by 6.3°. The B-form of DNA is metabolically stable and undergoes changes to A, C or D forms depending on sequence of nucleotides and concentration of excess salts.

2. The A-Form of DNA (A-DNA):

The A-form of DNA is found at 75% relative humidity in the presence of Na^+, K^+ or Ca^+ ions. It contains eleven base pairs as compared to ten base pairs of B-DNA which tilt from

the axis of helix by 20.2°. Due to this displacement the depth of major groove increases and that of minor groove decreases. The A-form is metastable and quickly turns to the D-form.

3. The C-Form DNA (C-DNA):

The C-form of DNA is found at 66% relative humidity in the presence of lithium (Lit^+) ions. As compared to A-and B-DNA, in C-DNA the number of base pairs per turn is less i.e., 28/3 or 91/3. The base pairs show pronounced negative tilt by 7.8°.

4. The D-Form of DNA (D-DNA):

The D-form of DNA is found rarely as extreme variants. Total number of base pairs per turn of helix is eight. Therefore, it shows eight-fold symmetry. This form is also called poly (dA-dT) and poly (dG-dC) form. There is pronounced negative tilt of base pairs by 16.7° as compared to C form i.e., the base pairs are displaced backwardly with respect to the axis of DNA helix.

5. The Z-Form of DNA (Z-DNA) or Left Handed DNA:

In 1979, Rich and coworkers at MIT (U.S.A.) obtained Z-DNA by artificially synthesizing d (C-G) 3 molecules in the form of crystals. They proposed a left handed (synistral) double helix model with zig-zag sugar-phosphate back bone running in antiparallel direction. Therefore, this DNA has been termed as Z-DNA. The Z-DNA has been found in a large number of living organisms including mammals, protozoans and several plant species.

There are several similarities with B-DNA in having:

(i) Double helix,

(ii) Two antiparallel strands, and

(iii) Three hydrogen bonds between G-C pairing.

In addition, the Z-DNA differs from the B-DNA in the following ways:

(a) The Z-DNA has left handed helix, while the B-DNA has right handed helix.

(b) The Z-DNA contains zigzag sugar phosphate back bone as compared to regular back bone of the B-DNA.

(c) The repeating unit in Z-DNA is a dinucleotide due to alternating orientation of sugar residues, whereas in B-DNA the repeating unit is a mononucleotide, and sugar molecules do not have the alternating orientation.

(d) In the Z-DNA one complete turn contains 12 base pairs of six repeating dinucleotide, while in B- DNA one full turn consists of 10 base pairs i.e., the 10 repeating units.

(e) Due to the presence of high number (12) of base pairs in one turn of Z-DNA, the angle of twist per repeating unit i.e., dinucleotide is 60° as compared to 36° of B-DNA molecule.

(f) In Z-DNA the distance of twist making one turn of 360° is 45Å as against 34Å in B-DNA.

(g) The Z-DNA has fewer diameters (18Å) as compared to the B-DNA (20Å diameter).

DNA replication

Why Replicate DNA?

DNA is the genetic material that defines every cell. Before a cell duplicates and is divided into new daughter cells through mitosis or meiosis, biomolecules and organelles must be copied to be distributed among the cells. DNA, found within the nucleus, must be replicated in order to ensure that each new cell receives the correct number of chromosomes. The process of DNA duplication is called DNA replication. Replication follows several steps that involve multiple proteins called replication enzymes and RNA. In eukaryotic cells, such as animal cells and plant cells, DNA replication occurs in the S phase of interphase during the cell cycle. The process of DNA replication is vital for cell growth, repair, and reproduction in organisms.

DNA Structure

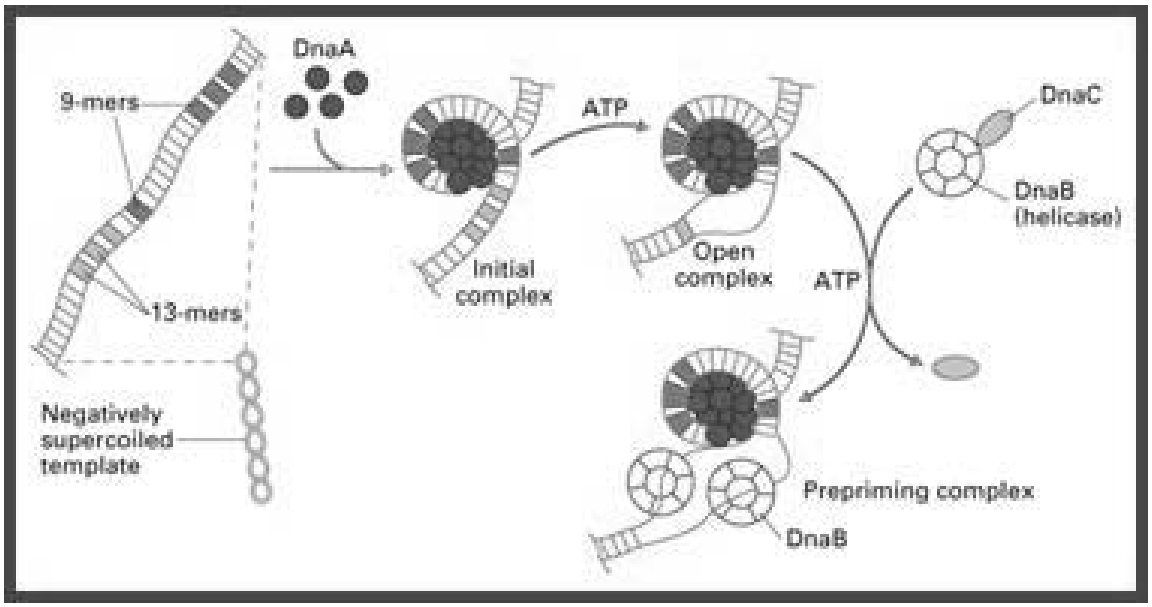
DNA or deoxyribonucleic acid is a type of molecule known as a nucleic acid. It consists of a 5-carbon deoxyribose sugar, a phosphate, and a nitrogenous base. Double-stranded DNA consists of two spiral nucleic acid chains that are twisted into a double helix shape. This twisting allows DNA to be more compact. In order to fit within the nucleus, DNA is packed into tightly coiled structures called chromatin. Chromatin condenses to form chromosomes during cell division. Prior to DNA replication, the chromatin loosens giving cell replication machinery access to the DNA strands.

DNA replication in prokaryotes

Initiation:

DNA replication begins from origin. In E coli, replication origin is called OriC which consists of 245 base pair and contains DNA sequences that are highly conserved among

bacterial replication origin. Two types of conserved sequences are found at OriC, three repeats of 13 bp (GATRCTNTTNTTTT) and four/five repeats of 9 bp (TTATCCACA) called 13 mer and 9 mer respectively.

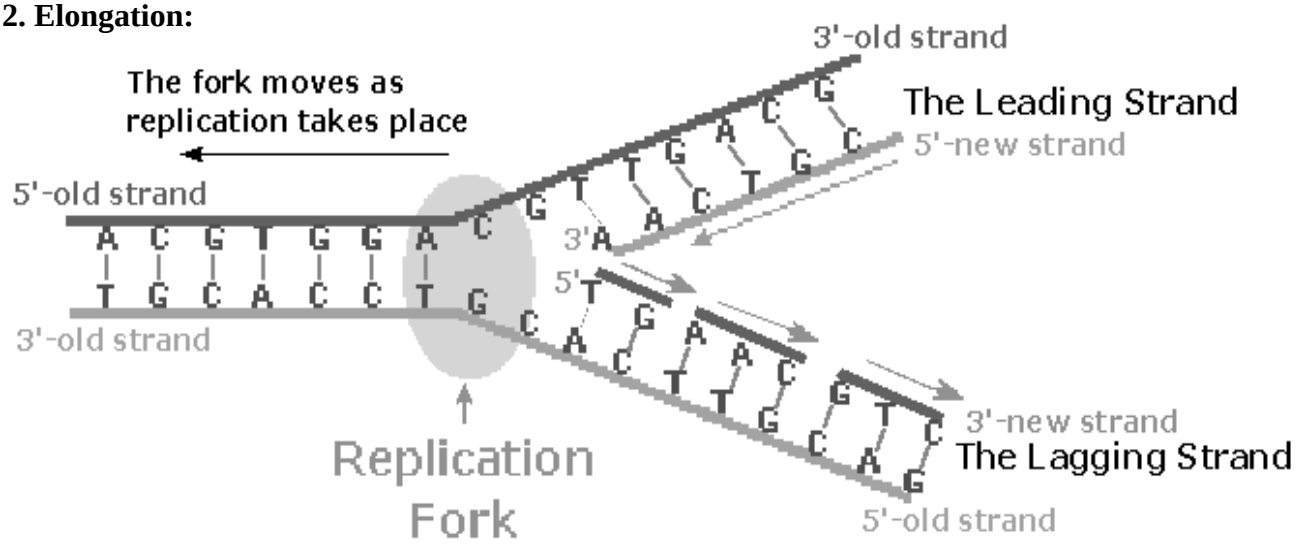


Source: http://www.onlinebiologynotes.com/dna-replication/

- About 20 molecules of Dna A proteins binds with 9 mer repeats along with ATP which causes DNA to wraps around dnaA protein forming initial complex. The dna A protein and ATP trigger the opening of 13 mer repeats froming open complex.
- Two copies of dnaB proteins (helicase) binds to 13 mer repeats. This binding is facilitated by another molecule called dnaC. The dnaB-dnaC interaction causes dnaB ring to open which binds with each of the DNA strand. The hydrolysis of bound ATP release dnaC leaving the dnaB bound to the DNA strand.
- The binding of helicase is key step in replication initiation. dnaB migrates along the single stranded DNA in 5'-3' direction causing unwinding of the DNA.
- The activity of helicase causes the topological stress to the unwinded strand forming supercoiled DNA. This stress is relieved by the DNA topoisomerase (DNA gyrase) by negative supercoiling. Similarly, single stranded binding protein binds to the separated strand and prevents reannealing of separated strand and stabilizes the strand.
- The DNA polymerase cannot initiate DNA replication. So, at first primase synthesize 10±1 nucleotide (RNA in nature) along the 5'-3' direction. In case of *E.coli* primer

synthesized by primase starts with ppp-AG-nucleotide. Primer is closely associated with dnaB helicase so that it is positioned to make RNA primer as ssDNA of lagging strand.

2. Elongation:



Source: http://www.onlinebiologynotes.com/dna-replication/

i. Leading strand synthesis:

Leading strand synthesis is more a straight forward process which begins with the synthesis of RNA primer by primase at replication origin.

DNA polymerase III then adds the nucleotides at 3'end. The leading strand synthesis then proceed continuously keeping pace with unwinding of replication fork until it encounter the termination sequences.

ii. Lagging strand synthesis:

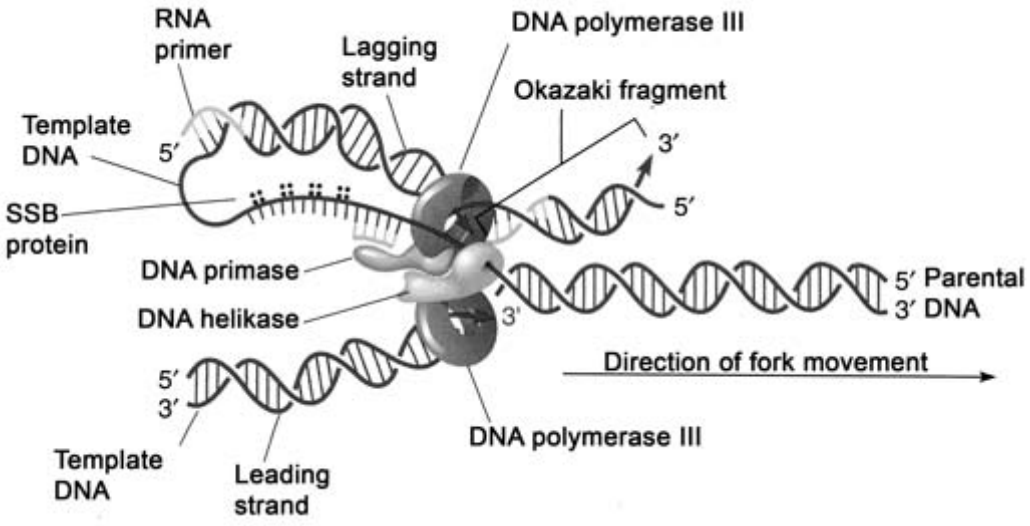
The lagging strand synthesized in short fragments called Okazaki fragments. At first RNA primer is synthesized by primase and as in leading strand DNA polymerase III binds to RNA primer and adds dNTPS.

On this level the synthesis of each okazaki fragments seems straight forward but the reality is quite complex.

Mechanism of Lagging strand synthesis

- The complexicity lies in the co-ordination of leading and lagging strand synthesis. Both the strands are synthesized by a single DNA polymerase III dimer which accomplished the looping of template DNA of lagging strand synthesizing Okazaki fragments.
- Helicase (dnaB) and primase (dnaG) constitute a functional unit within replication complex called primosome.

- DNA pol III use one set of core sub unit (Core polymerase) to synthesize leading strand and other set of core sub unit to synthesize lagging strand.
- In elongation steps, helicase in front of primase and pol III, unwind the DNA at the replication fork and travel along lagging strand template along 5'-3' direction.



Source: http://www.onlinebiologynotes.com/dna-replication/

- DnaG primase occasionally associated with dnaB helicase synthesizes short RNA primer. A new B-sliding clamp is then positioned at the primer by B-clamp loading complex of DNA pol III.
- When the Okazaki fragments synthesis is completed, the replication halted and the core sub unit dissociates from their sliding clamps and associates with new clamp. This initiates the synthesis of new Okazaki fragments.
- Both leading and lagging strand are synthesized co-ordinately and simultaneously by a complex protein moving in 5'-3' direction. In this way both leading and lagging strand can be replicated at same time by a complex protein that move in same direction.
- Every so often the lagging strands must dissociates from the replicosome and reposition itself so that replication can continue.
- Lagging strand synthesis is not completes until the RNA primer has been removed and the gap between adjacent Okazaki fragments are sealed. The RNA primer are removed by exonuclease activity (5'-3') of DNA pol-I and replaced by DNA. The gap is then sealed by DNA ligase using NAD as co-factor.

Termination:

Evantually the two replication fork of circular *E. coli* chromosome meet at termination recognizing sequences (ter).

The Ter sequence of 23 bp are arranged on the chromosome to create trap that the replication fork can enter but cannot leave. Ter sequences function as binding site for TUS protein.

Ter-TUS complex can arrest the replication fork from only one direction. Ter-TUS complex encounter first with either of the replication fork and halt it. The other opposing replication fork halted when it collide with the first one. This seems that the Ter-TUS sequences are not essential for termination but it may prevents over replication by one fork if other is delayed or halted by a damage or some obstacle.

When either of the fork encounter Ter-TUS complex, replication halted.

Final few hundred bases of DNA between these large protein complexes are replicated by not yet known mechanism forming two interlinked (cataneted) chromosome.

In *E. coli* DNA topoisomerase IV (type II) cut the two strand of one circular DNA and segrate each of the circular DNA and finally join the strand. The DNA finally transfer to two daughter cell.

Replication Fork Formation

Before DNA can be replicated, the double stranded molecule must be “unzipped” into two single strands. DNA has four bases called adenine (A), thymine (T), cytosine (C) and guanine (G) that form pairs between the two strands. Adenine only pairs with thymine and cytosine only binds with guanine. In order to unwind DNA, these interactions between base pairs must be broken. This is performed by an enzyme known as DNA helicase. DNA helicase disrupts the hydrogen bonding between base pairs to separate the strands into a Y shape known as the replication fork. This area will be the template for replication to begin.

DNA is directional in both strands, signified by a 5' and 3' end. This notation signifies which side group is attached the DNA backbone. The 5' end has a phosphate (P) group attached,

Replication Enzymes

DNA polymerase is an enzyme that synthesizes DNA.DNA replication would not occur without enzymes that catalyze various steps in the process. Enzymes that participate in the eukaryotic DNA replication process include:

DNA helicase - unwinds and separates double stranded DNA as it moves along the DNA. It forms the replication fork by breaking hydrogen bonds between nucleotide pairs in DNA.

DNA primase - a type of RNA polymerase that generates RNA primers. Primers are short RNA molecules that act as templates for the starting point of DNA replication.

DNA polymerases - synthesize new DNA molecules by adding nucleotides to leading and lagging DNA strands.

Topoisomerase or DNA Gyrase - unwinds and rewinds DNA strands to prevent the DNA from becoming tangled or supercoiled.

Exonucleases - group of enzymes that remove nucleotide bases from the end of a DNA chain.

DNA ligase - joins DNA fragments together by forming phosphodiester bonds between nucleotides.

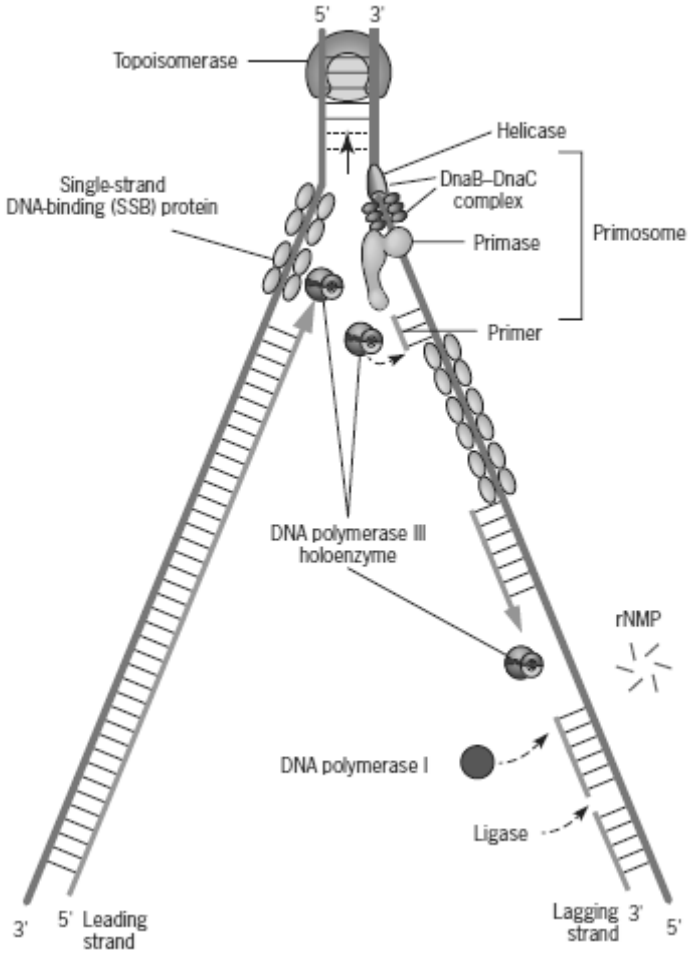


Figure Diagram of a replication fork in *E. coli* showing the major components of the replication apparatus, Source Snustad and Simmons, 2012, Principles of Genetics -6th ed.

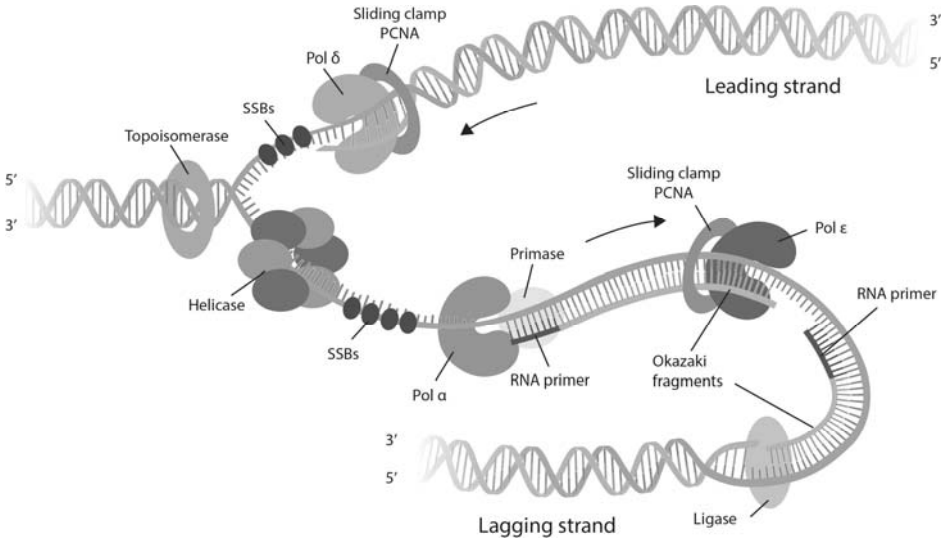
DNA replication in eukaryotes

DNA replication in eukaryotes occurs only in S-phase of cell cycle. However pre-initiation occur in G1 phase. Due to sheer size of chromosome in eukaryotes, chromosome contains multiple origin of replication. ARS (autonomously replicating sequence) in case of yeast is origin for replication.

Steps in DNA replication

1. Initiation

- The first step is the formation of pre-initiation replication complex (pre-RC). It occurs in two stages. 1st stage requires, there are no CDK activities. It occurs in early G1 phase. It is known as licensing but licensed pre-RC cannot initiate replication at G1 phase. 2nd stage is binding of ORC (origin recognition complex).
- The replication begins with binding of ORC to the origin. ORC is a hexamer of related protein and remains bounded even after DNA replication occurs. Furthermore ORC is analogue of prokaryotic dnaA protein.
- After binding of ORC to origin, cdc6/cdc18 and cdtl coordinate the loading of MEM (mini chromosome maintainance) to origin.
- MEM complex is thought to be major eukaryotic helicase.
- After binding of MEM complex to pre-RC, cdtl get displaced. Then DdK phosphorylates MEM, which activates its helicase activity. Again DdK and CdK recruit another protein called cdc45 which then recruit all the DNA replicating protein such that the origin get fired and replication begins.

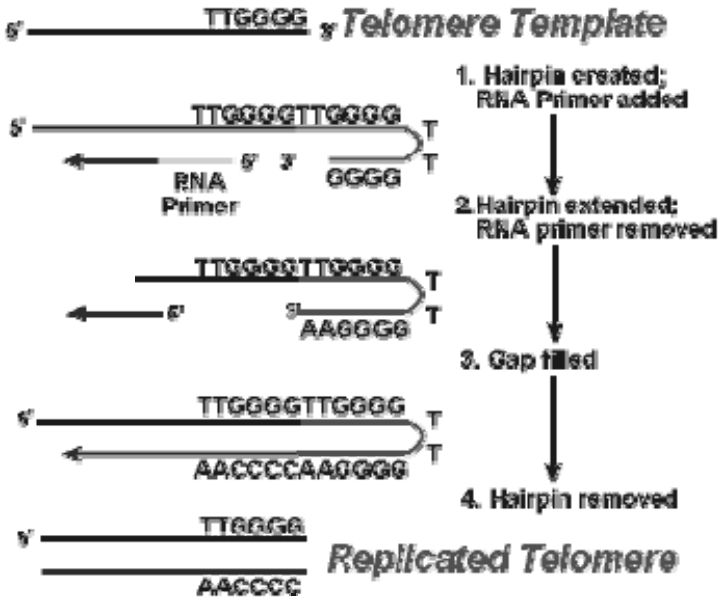


Source: http://www.onlinebiologynotes.com/dna-replication/

3. Termination:

- At the end of DNA replication the RNA primer are replaced by DNA by 5'-3'exonuclease and polymerase activity of DNA polymerase ε.

- Exonuclease activity of DNA polymerase removes the RNA primer and polymerase activity adds dNTPs at 3'-OH end preceding the primer.
- In case of bacteria with circular genome, the replacement of RNA primer with DNA is not a problem because there is always a preceding 3'-OH in a circular DNA.



Source: http://www.onlinebiologynotes.com/dna-replication/

- But in eukaryotic organism with linear DNA, there is a problem. When RNA primer at 5' end of daughter strand is removed, there is not a preceding 3'-OH such that the DNA polymerase can use it to replace by DNA. So, at 5' end of each daughter strand there is a gap (missing DNA). This missing DNA cause loss of information contain in that region. This gap must be filled before next round of replication.
- For solving this end replication problem; studies have found that linear end of DNA called telomere has G:C rich repeats. These sequences are known as telomere sequences. These repeats of telomere sequence are different among different organisms. Telomere in human cell consists of repeats of TTAGGG/AATCCC. Each species has its own species specific telomere repeats. These telomere sequence do not codes anything but it is essential to fill the gap in daughter strand and maintain the integrity of DNA.
- Telomere replication: end replication problem in Eukaryotic DNA
- There is an enzyme found in eukaryotic cell called telomerase.
- Telomerase is a DNA polymerase (RNA dependent DNA polymerase) which adds many copies of telomere sequence at 3'-OH end of template strand. Like other DNA

polymerase, telomerase also adds deoxyribonucleotide at 3'-OH end. Unlike other DNA polymerase, telomerase adds DNA at 3'-OH end of parent strand not at the daughter strand and also it synthesizes the same sequences over and over in absence of template strand.

- First telomerase binds to 3'-OH end of parent strand by hybridization between its AACCCCAAC RNA sequences and TTGGGG DNA sequences (telomere sequences of Tetrahymena).
- The telomerase adds TTG at 3' end of parent strand. After adding TTG sequences, telomerase translocates along 5'-3' end of parent strand. Now the telomerase adds GGGTTG to 3' end by using its CCCAAC sequence. Again telomerase translocates and adds GGGTTA sequence. This process is continued for many times. The parent strand become more longer than daughter strand. Now RNA polymerase (PRIMASE) synthesizes RNA primer by copying the parent strand in 5'-3' direction using telomere sequence as template.
- The DNA polymerase can now extend the primer in 5'-3' direction by adding deoxyribonucleotide to 3' end.
- The primer is now removed and it won't be replaced because it is an extra sequence added by copying telomere sequence.
- Finally the integrity of daughter strand is maintained.

Transcription

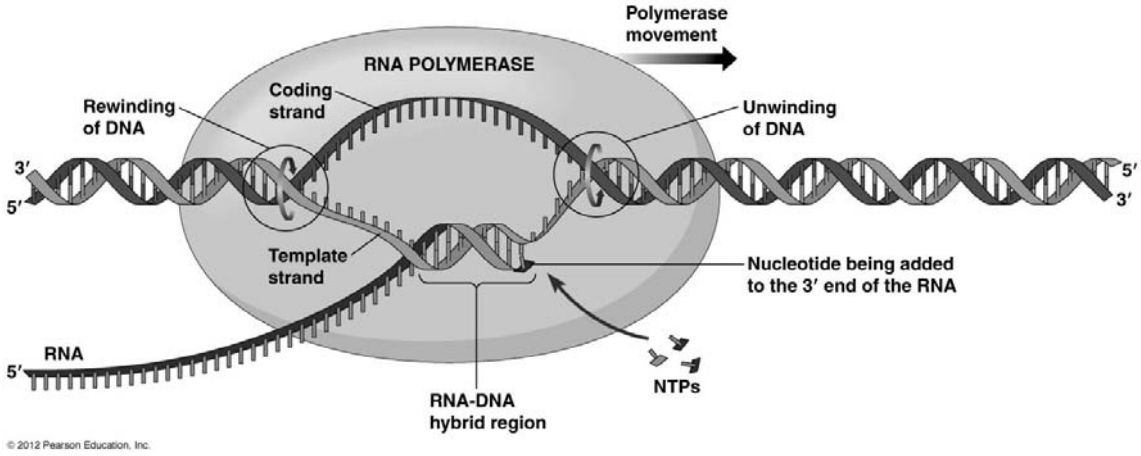
Introduction:

Transcription is a process in which ribonucleic acid (RNA) is synthesized from DNA. The word gene refers to the functional unit of the DNA that can be transcribed. Thus, the genetic information stored in DNA is expressed through RNA. For this purpose, one of the two strands of DNA serves as a template (non-coding strand or sense strand) and produces working copies of RNA molecules.

Transcription in Prokaryotes

- The process of synthesis of RNA by copying the template strand of DNA is called transcription.
- During replication entire genome is copied but in transcription only the selected portion of genome is copied.

- The enzyme involved in transcription is RNA polymerase. Unlike DNA polymerase it can initiate transcription by itself, it does not require primase. More exactly it is a DNA dependent RNA polymerase.



Source: http://www.onlinebiologynotes.com

The steps of transcription:

Transcription is an enzymatic process. The mechanism of transcription completes in three major steps-

1. Initiation:

Closed complex formation

Open complex formation

Tertiary complex formation

2. Elongation

3. Termination:

Rho- dependent

Rho-independent

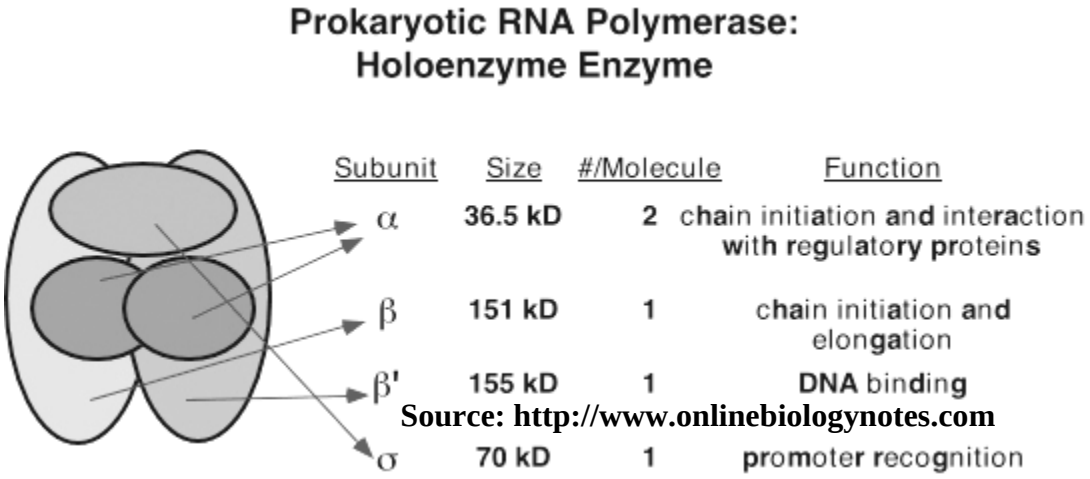
1. Initiation:

The transcription is initiated by RNA polymerase holoenzyme from a specific point called promoter sequence.

Bacterial RNA polymerase is the principle enzyme involved in transcription.

Single RNA polymerase is found in a bacteria which is called core polymerase and it consists of α, β, β' and ω sub units.

The core enzyme bind to specific sequence on template DNA strand called promoter. The binding of core polymerase to promoter is facilitates and specified by sigma (σ) factor. (σ70 in case of *E. coli*).

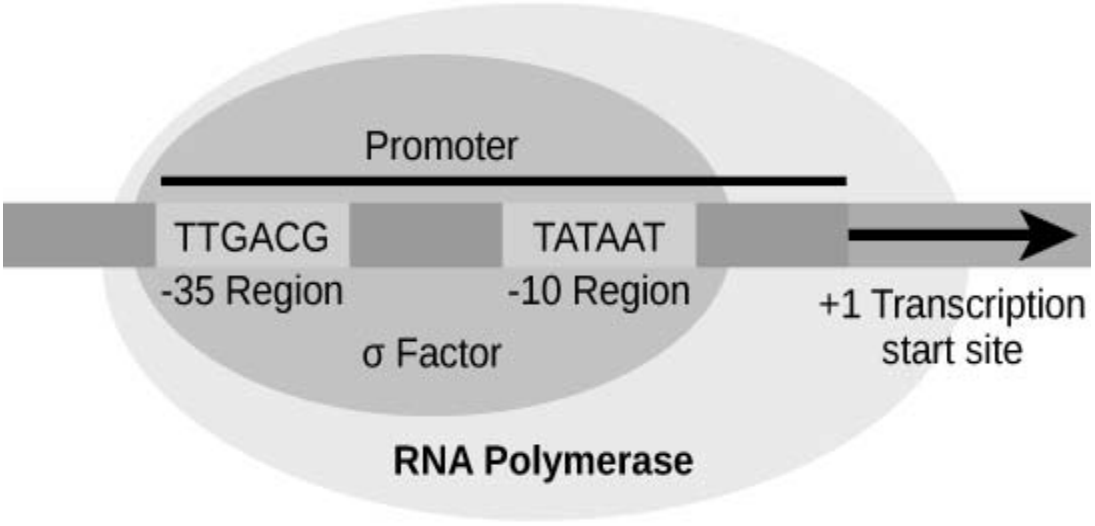


Source: http://www.onlinebiologynotes.com

The core polymerase along with σ-factor is called Holoenzyme i.e., RNA polymerase holoenzyme.

In case of *E. coli*, promoter consists of two conserved sequences 5'-TTGACA-3' at -35 element and 5'-TATAAT-3' at -10 element. These sequences are upstream to the site from which transcription begins. Binding of holoenzyme to two conserve sequence of promoter forms close complex.

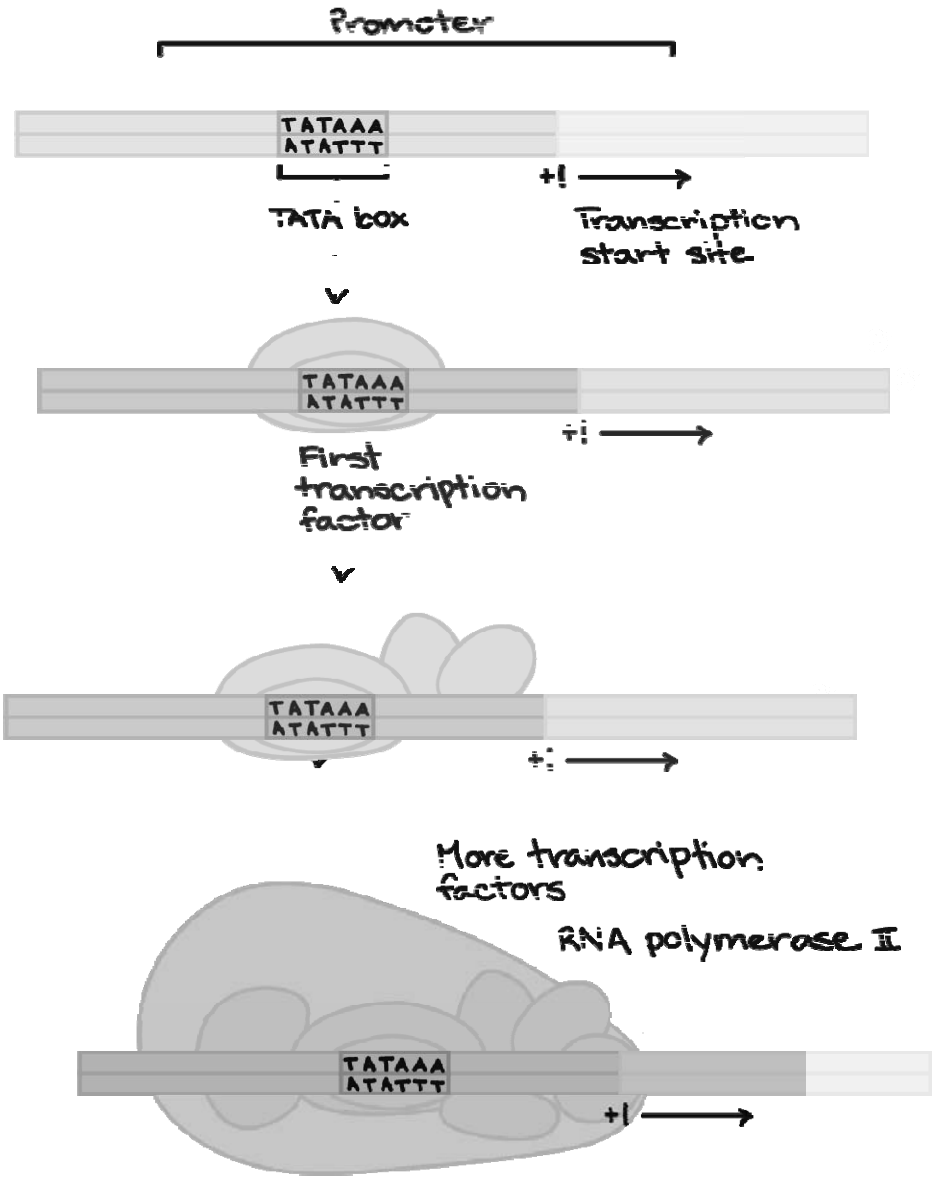
In some bacteria, the altered promoter may exist which contain UP-element and some may contain extended -10 element rather than -35 element.



Source: http://www.onlinebiologynotes.com

The binding of the enzyme RNA polymerase to DNA is the prerequisite for the transcription to start. The specific region on the DNA where the enzyme binds is known as promoter

region. There are two base sequences on the coding DNA strand which the sigma factor of RNA polymerase can recognize for initiation of transcription.



Source: https://www.khanacademy.org/science/biology/gene-regulation

1. Pribnow box (TATA box):

This consists of 6 nucleotide bases (TATAAT), located on the left side about 10 bases away (upstream) from the starting point of transcription.

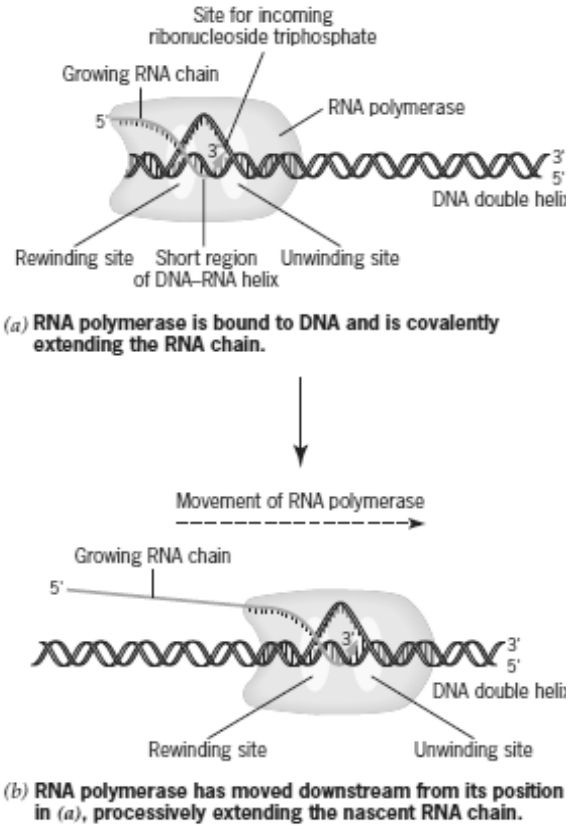
2. The '-35' sequence:

This is the second recognition site in the promoter region of DNA. It contains a base sequence TTGACA, which is located about 35 bases (upstream, hence of transcription start.

Elongation:

As the holoenzyme, RNA polymerase recognizes the promoter region, the sigma factor is released and transcription proceeds. RNA is synthesized from 5' end to 3' end (5’ ➔ 3') antiparallel to the DNA template. RNA polymerase utilizes ribonucleotide triphosphates (ATP, GTP, CTP and UTP) for the formation of RNA. For the addition of each nucleotide to the growing chain, pyrophosphate moiety is released. The sequence of nucleotide bases in the mRNA is complementary to the template DNA strand. It is however, identical to that of coding strand except that RNA contains U in place of T in DNA.

RNA polymerase differs from DNA polymerase in two aspects. No primer is required for RNA polymerase and, further, this enzyme does not possess endo the latter function (proof-reading the RNA synthesized.



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

This is in contrast to DNA replication which is carried out with high fidelity. It is, however, fortunate that mistakes in RNA synthesis are less dangerous, since they cells. The double helical structure of DNA unwinds as the transcription goes on, resulting in supercoils. The problem of supercoils is overcome by topoisomerases (more details given under replication). Due to lack of activity, RNA polymerase has no ability to repair the mistakes.

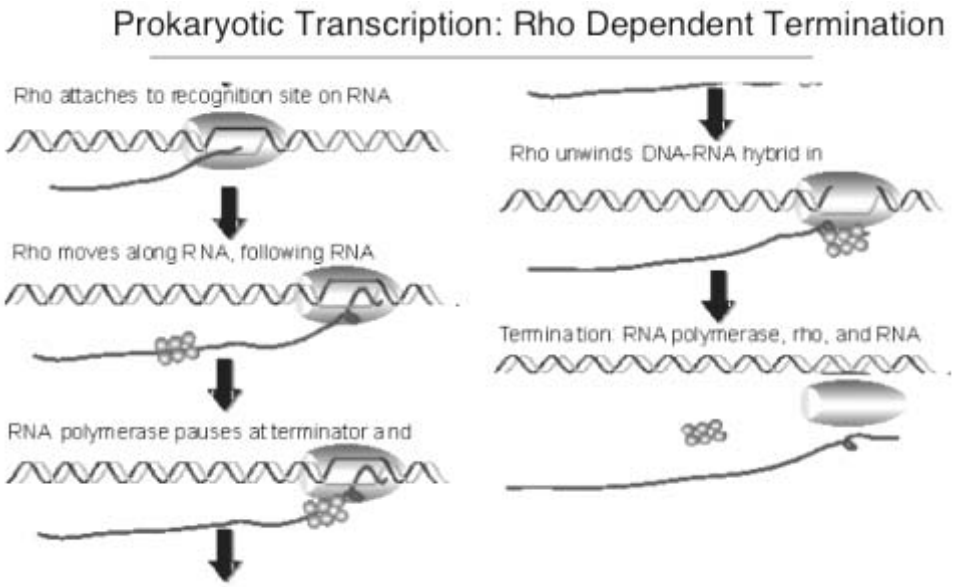
Termination:

The process of transcription stops by termination signals. Two types of termination are identified.

1. Rho (ρ) dependent termination:

Termination of RNA chains occurs when RNA polymerase encounters a termination signal. When it does, the transcription complex dissociates, releasing the nascent RNA molecule. There are two types of transcription terminators in *E. coli*. One type results in termination

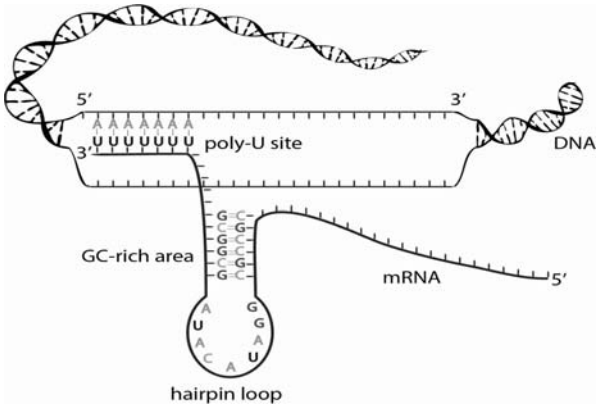
only in the presence of a protein called rho (ρ); therefore, such termination sequences are called rhodependent terminators.



Source: http://www.onlinebiologynotes.com

Rho (p) independent termination:

The other type results in the termination of transcription without the involvement of rho; such sequences are called rho-independent terminators. Rho-independent terminators contain a GC-rich region followed by six or more AT base pairs, with the A's present in the template strand. The nucleotide sequence of the GC-rich region contains inverted repeats—sequences of nucleotides in each DNA strand that are inverted and complementary. When transcribed, these inverted repeat regions produce single stranded RNA sequences that can base-pair and form hairpin structures. The RNA hairpin structures form immediately after the synthesis of the participating regions of the RNA chain and retard the movement of RNA polymerase molecules along the DNA, causing pauses in chain extension. Since AU base pairing is weak, requiring less energy.



Source: http://www.onlinebiologynotes.com

Transcription in Eukaryotes:

RNA synthesis in eukaryotes is a much more complicated process than the transcription described above for prokaryotes. As such, all the details of eukaryotic transcription (particularly about termination) are not clearly known. The salient features of available information are given here.

RNA Polymerases:

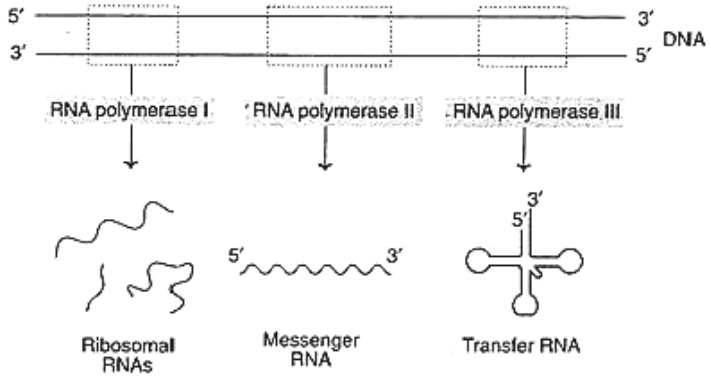


Fig. 4.7 : An overview of transcription in eukaryotes.

The nuclei of eukaryotic cells possess three distinct RNA polymerases.

1. RNA polymerase I is responsible for the synthesis of precursors for the large ribosomal RNAs.
2. RNA polymerase II synthesizes the precursors for mRNAs and small nuclear RNAs.
3. RNA polymerase III participates in the formation of tRNAs and small ribosomal RNAs.

Besides the three RNA polymerases found in the nucleus, there also exists a mitochondrial RNA polymerase in eukaryotes. The latter resembles prokaryotic RNA polymerase in structure and function.

Promoter Sites:

In eukaryotes, a sequence of DNA bases—which is almost identical to pribnow box of prokaryotes— is identified (Fig. 4.8). This sequence, known as Hogness box (or TATA box), is located on the left about 25 nucleotides away (upstream) from the starting site of mRNA synthesis.

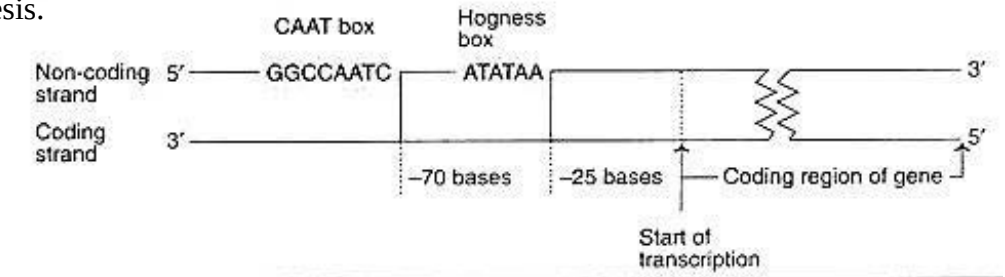


Fig. 4.8 : Promoter regions of DNA in eukaryotes.

There also exists another site of recognition between 70 and 80 nucleotides upstream from the start of transcription. This second site is referred to as CAAT box. One of these two sites (or sometimes both) helps RNA polymerase II to recognize the requisite sequence on DNA for transcription.

Initiation of Transcription:

The molecular events required for the initiation of transcription in eukaryotes are complex, and broadly involve three stages:

1. Chromatin containing the promoter sequence made accessible to the transcription machinery.

2. Binding of transcription factors (TFs) to DNA sequences in the promoter region.

3. Stimulation of transcription by enhancers.

A large number of transcription factors interact with eukaryotic promoter regions. In humans, about six transcription factors have been identified (TFIID, TFIIA, TFIIB, TFIIF, TFIIE, TFIIH). It is postulated that the TFs bind to each other, and in turn to the enzyme RNA polymerase.

Enhancer can increase gene expression by about 100 folds. This is made possible by binding to enhancers to transcription factors to form activators. If is believed that the chromatin forms a loop that allows the promoter and enhancer to be close together in space to facilitate transcription.

Translation:

Translation involves “decoding” a messenger RNA (mRNA) and using its information to build a polypeptide, or chain of amino acids. For most purposes, a polypeptide is basically just a protein (with the technical difference being that some large proteins are made up of several polypeptide chains).

The genetic code

In an mRNA, the instructions for building a polypeptide come in groups of three nucleotides called codons. Here are some key features of codons to keep in mind as we move forward:

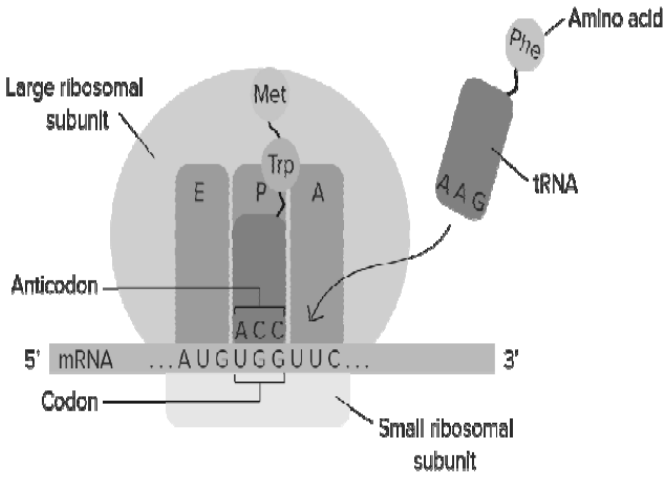
There are 61different codons for amino acids.

Three “stop” codons mark the polypeptide as finished.

One codon, AUG, is a “start” signal to kick off translation (it also specifies the amino acid methionine)These relationships between mRNA codons and amino acids are known as the genetic code .Each three-letter sequence of mRNA nucleotides corresponds to a specific amino acid, or to a stop codon. UGA, UAA, and UAG are stop codons. AUG is the codon for methionine, and is also the start codon.

Codons to amino acids

- In translation, the codons of an mRNA are read in order (from the 5' end to the 3' end) by molecules called transfer RNAs, or tRNAs.
- Each tRNA has an anticodon, a set of three nucleotides that binds to a matching mRNA codon through base pairing. The other end of the tRNA carries the amino acid that's specified by the codon.

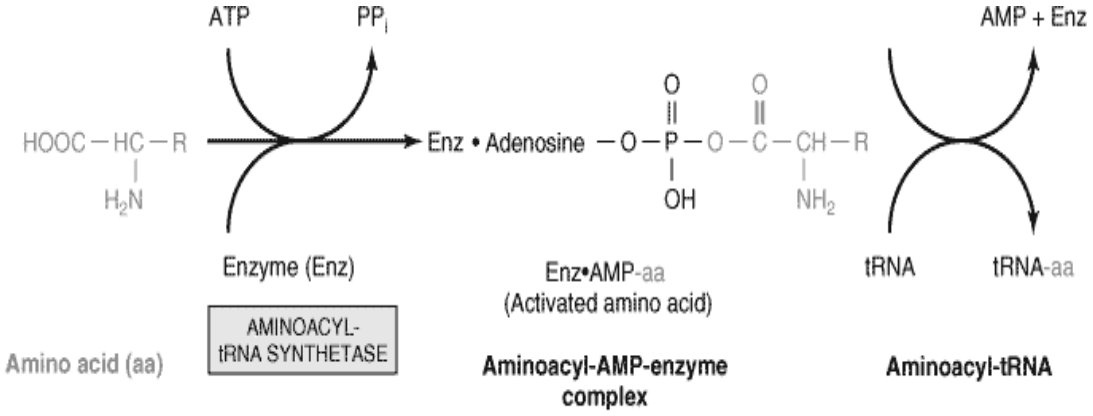


Source: https://www.khanacademy.org/science/biology/gene-regulation

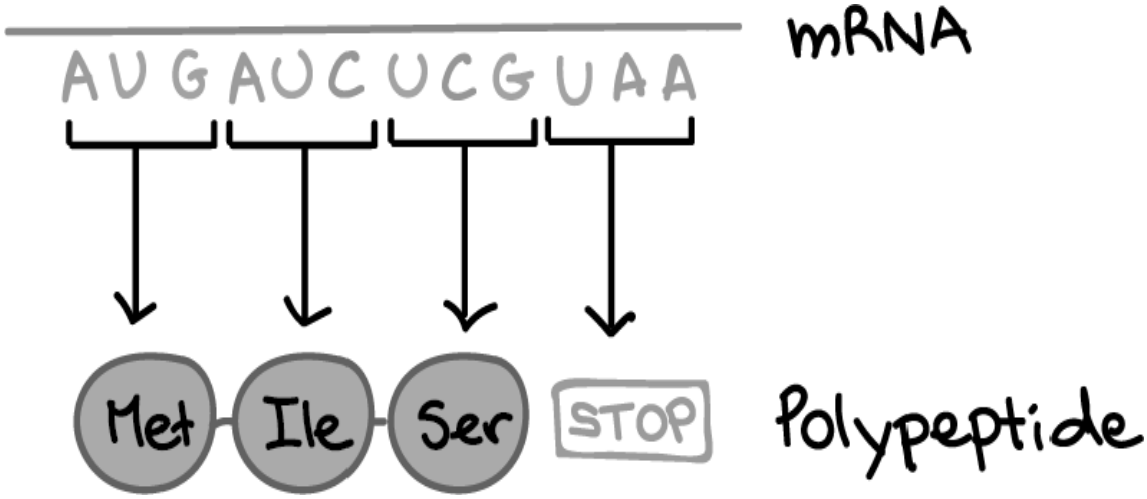
Activation of amino acids:

The activation of amino acids takes place in cytosol. The activation of aminoacids is catalyzed by their aminoacyl tRNA synthetases. All the 20 aminoacids are activated and bound to 3’ end of their specific tRNA in the presence of ATP and Mg++.

The N-formylated methionine is chain initiating aminoacid in bacteria whereas methionine is chain initiating aminoacid in eukaryotes. Methionine is activated by methionyl-tRNA synthetase. For N-formylmethionine two types of tRNA are used ie. tRNAmet and tRNAfmet. Similarly, all 2o aminoacids are activated (amino acyl-AMP enzyme complex) and then bound to their specific tRNA forming Aminoacyl tRNA.



tRNAs bind to mRNAs inside of a protein-and-RNA structure called the ribosome. As tRNAs enter slots in the ribosome and bind to codons, their amino acids are linked to the growing polypeptide chain in a chemical reaction. The end result is a polypeptide whose amino acid sequence mirrors the sequence of codons in the mRNA.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Initiation

In order for translation to start, we need a few key ingredients. These include:

- A ribosome (which comes in two pieces, large and small)

- An mRNA with instruc ions for the protein we'll build
- An "initiator" tRNA carrying the first amino acid in the protein, which is almost always methionine (Met).

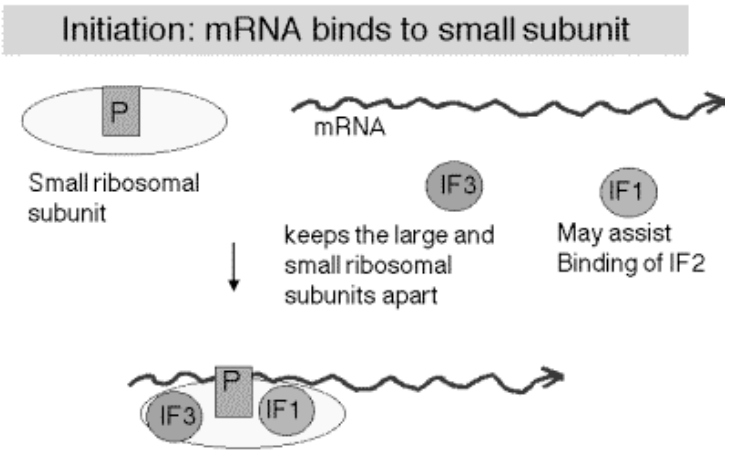
In the first step, initiation factor 3 (IF3) binds to 30S ribosomal unit.

Then mRNA binds to 30S ribosomal subunit in such a way that AUG codon lie on the peptidyl (P) site and the second codon lies on aminoacyl (A) site.

The tRNA carrying formylated methionine i.e., FMet–tRNAFMet is placed at P-site. This specificity is induced by IF-2 with utilization of GTP. The IF1 prevent binding of FMet–tRNAFMet is in A-site.

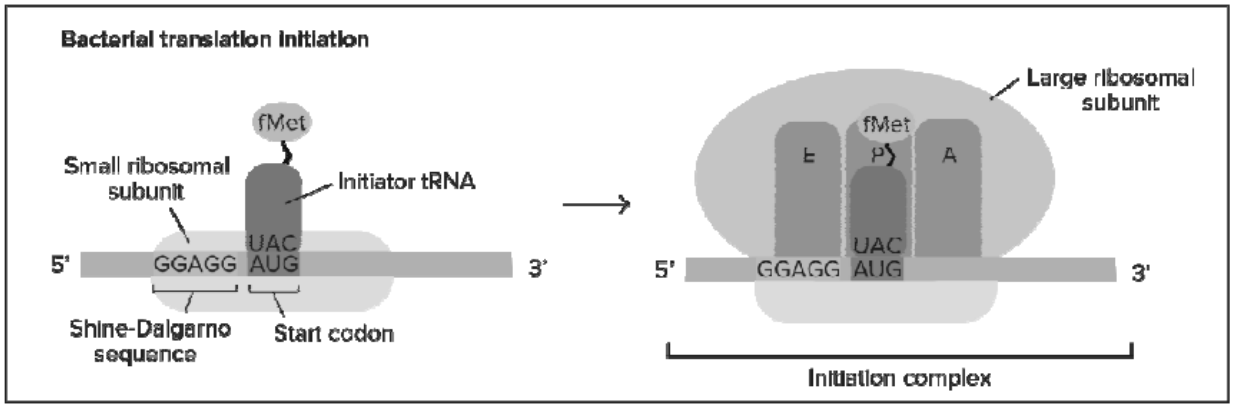
Shinedalgrno sequence in the mRNA guide correct positioning of AUG codon at P-site of 30S ribosome.

After binding of FMet–tRNAFMet on P-site, IF-3, IF-2 and IF-1 are released so that 50S ribosomal unit binds with 30S forming 70S ribosome. The exit site is located in 50S.



Source: http://www.onlinebiologynotes.com/translation-in-prokaryotes/

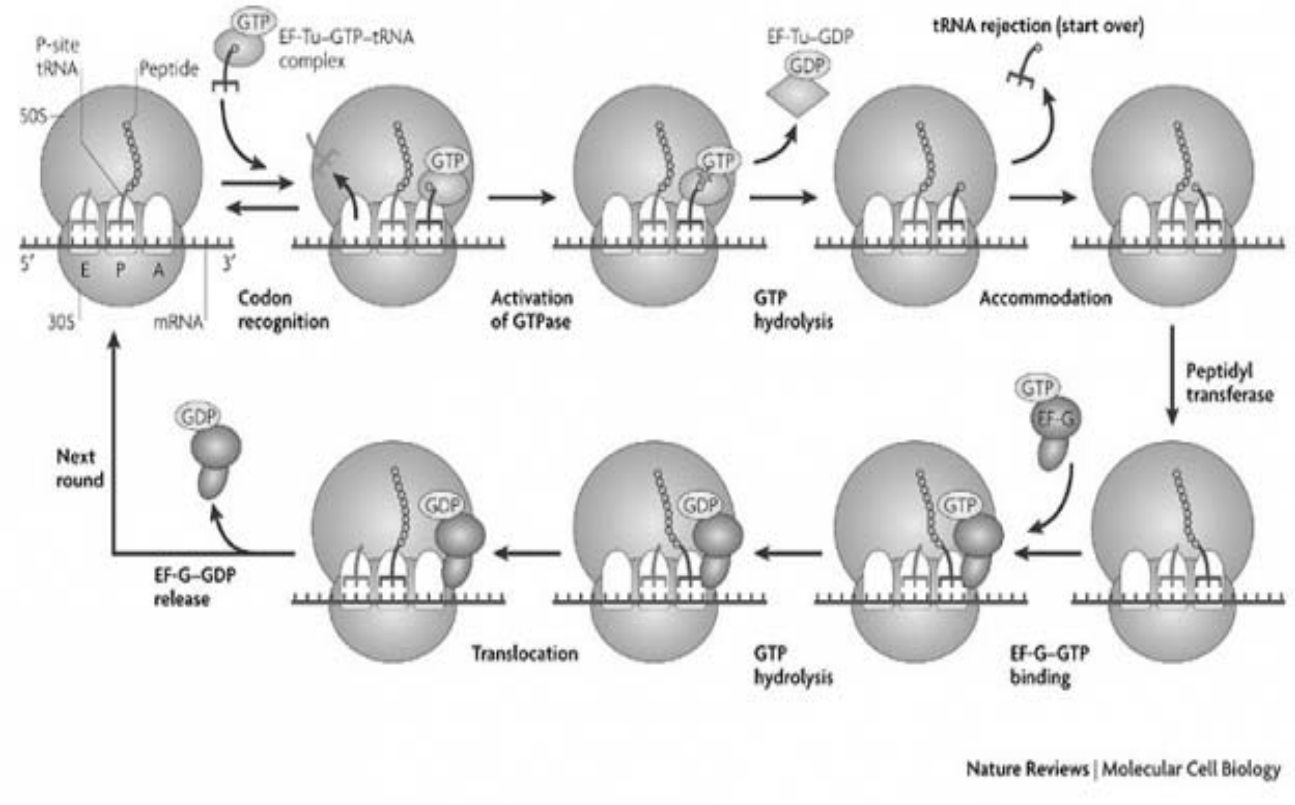
In bacteria, the situation is a little different. Here, the small ribosomal subunit doesn't start at the 5' end of the mRNA and travel toward the 3' end. Instead, it attaches directly to certain sequences in the mRNA. These Shine-Dalgarno sequences come just before start codons and "point them out" to the ribosome. A Shine-Dalgarno sequence marks the start of each coding ribosome find the right start codon for each gene.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Elongation

Methionine-carrying tRNA starts out in the middle slot of the ribosome, called the P-site. Next to it, a fresh codon is exposed in another slot, called the A site. The A-site will be the "landing site" for the next tRNA, one whose anticodon is a perfect (complementary) match for the exposed codon.



Source: http://www.onlinebiologynotes.com/translation-in-prokaryotes/

Binding of AA-tRNA at A-site:

The 2nd tRNA carrying next amino acid comes into A-site and recognizes the codon on mRNA. This binding is facilitated by EF-TU and utilizes GTP.

After binding, GTP is hydrolyzed and EF-TU-GDP is released.

EF=TU-GDP then and enter into EF-TS cycle.

ii. Peptide bond formation:

The amino acid present in t-RNA of P-site ie Fmet is transferred to t-RNA of A-site forming peptide bond. This reaction is catalyzed by peptidyl transferase.

Now, the t-RNA at P-site become uncharged

iii. Ribosome translocation:

After peptide bond formation ribosome moves one codon ahead along 5'-3' direction on mRNA, so that dipeptide-tRNA appears on P-site and next codon appear on A-site.

The uncharged tRNA exit from ribosome and enter to cytosol.

The ribosomal translocation requires EF-G-GTP (translocase enzyme) which changes the 3D structure of ribosome and catalyze 5'-3' movement.

The codon on A-site is now recognized by other aminoacyl-tRNA as in previous.

The dipeptide on P-site is transferred to A-site forming tripeptide.

This process continues giving long polypeptide chain of amino acids.

Termination

Polypeptides, like all good things, must eventually come to an end. Translation ends in a process called termination. Termination happens when a stop codon in the mRNA (UAA, UAG, or UGA) enters the A site.

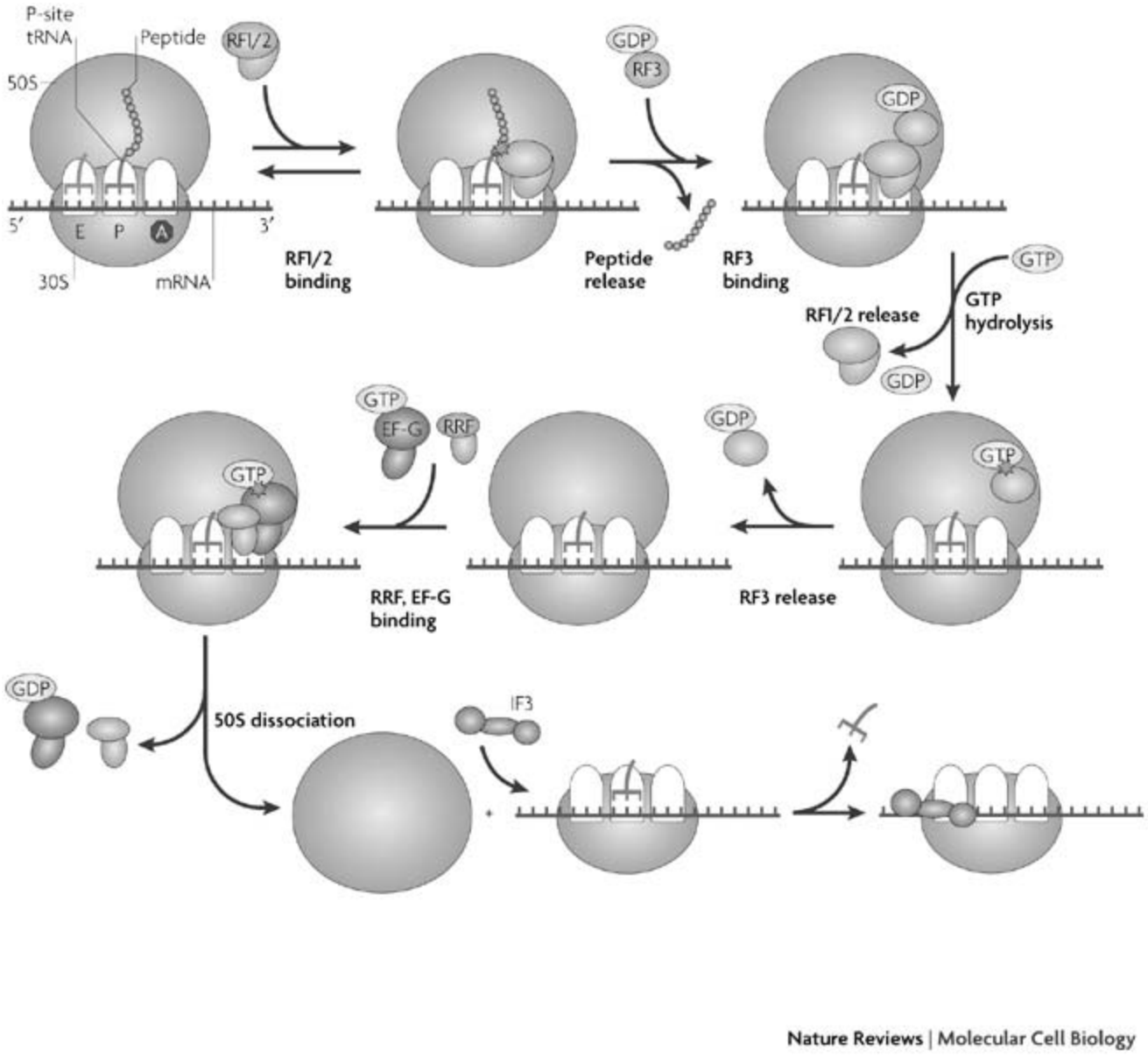
The peptide bond formation and elongation of polypeptide continues until stop codon appear on A-site.

If stop codon appear on A-site it is not recognized by t-RNA carrying amino acids because stop codon do not have anticodon on mRNA.

The stop codon are recognized by next protein called release factor (Rf-1, RF-2 and RF-3) which hydrolyses and cause release of all component ie 30s, 50S, mRNA and polypeptide separates.

RF-1 recognizes UAA and UAg while RF-2 recognizes UAA and UGA while RF-3 dissociate 30S and 50S subunits.

In case of eukaryotes, only one release actor eRF causes dissociation.

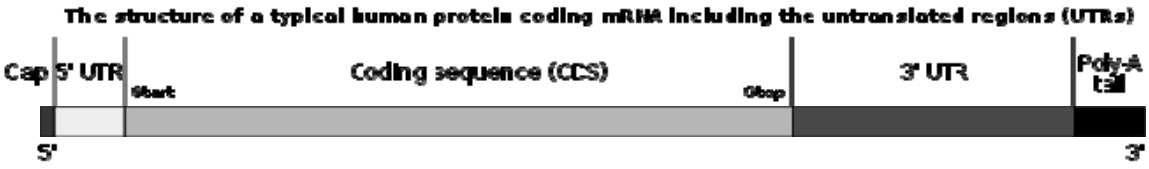


Source: http://www.onlinebiologynotes.com/translation-in-prokaryotes/

RNA types:

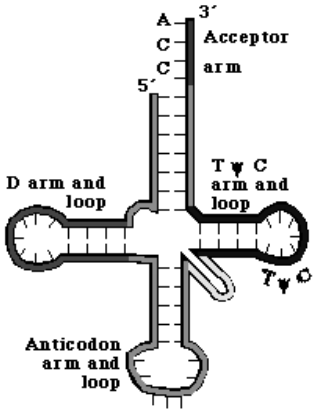
1. **Messenger RNA (mRNA)** carries information about a protein sequence to the ribosomes, the protein synthesis factories in the cell. It is coded so that every three nucleotides (a codon) correspond to one amino acid. In eukaryotic cells, once precursor mRNA (pre-mRNA) has been transcribed from DNA, it is processed to mature mRNA. This removes its introns—non-coding sections of the pre-mRNA. The mRNA is then exported from the nucleus to the cytoplasm, where it is bound to ribosomes and translated into its corresponding protein form with the help of tRNA. In prokaryotic

cells, which do not have nucleus and cytoplasm compartments, mRNA can bind to ribosomes; while, it is being transcribed from DNA. After a certain amount of time, the message degrades into its component nucleotides with the assistance of ribonucleases.



Source: https://en.wikipedia.org/wiki/Messenger_RNA

2. **Transfer RNA (tRNA)** is a small RNA chain of about 80 nucleotides that transfers a specific amino acid to a growing polypeptide chain at the ribosomal site of protein synthesis during translation. It has sites for amino acid attachment and an anticodon region for codon recognition that binds to a specific sequence on the messenger RNA chain through hydrogen bonding.



Source: http://www.proteinsynthesis.org/trna/

3. **Ribosomal RNA (rRNA)** is the catalytic component of the ribosomes. Eukaryotic ribosomes contain four different rRNA molecules: 18S, 5.8S, 28S and 5S rRNA. Three of the rRNA molecules are synthesized in the nucleolus, and one is synthesized elsewhere. In the cytoplasm, ribosomal RNA and protein combine to form a nucleoprotein called a ribosome. The ribosome binds mRNA and carries out protein synthesis. Several ribosomes may be attached to a single mRNA at any time. Nearly all the RNA found in a typical eukaryotic cell is rRNA.

4. **MicroRNAs (miRNA; 21–22 nt)** are found in eukaryotes and act through RNA interference (RNAi), where an effector complex of miRNA and enzymes can cleave complementary mRNA, block the mRNA from being translated, or accelerate its degradation.

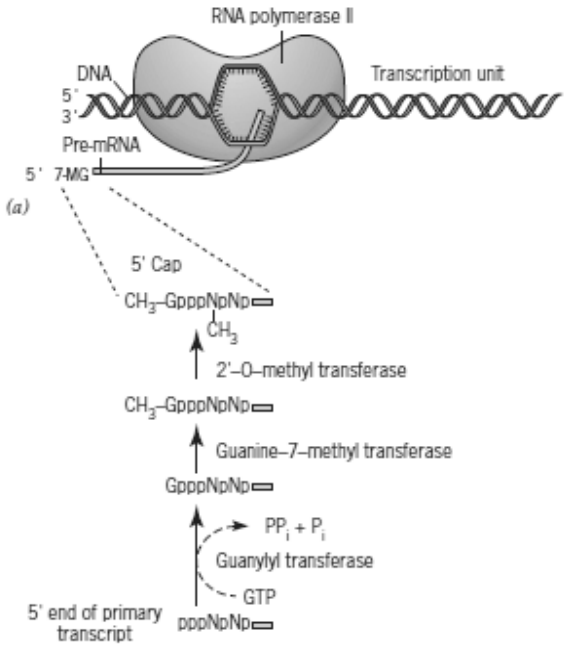
5. **Small interfering RNAs (siRNA; 20–25 nt)** are often produced by breakdown of viral RNA, there are also endogenous sources of siRNAs. siRNAs act through RNA interference in a fashion similar to miRNAs. Some miRNAs and siRNAs can cause genes they target to be methylated, thereby decreasing or increasing transcription of those genes.

6. **Small nuclear RNAs (snRNA):** Many RNAs are involved in modifying other RNAs. Introns are spliced out of pre-mRNA by spliceosomes, which contain several **small nuclear RNAs (snRNA)**, or the introns can be ribozymes that are spliced by themselves.

7. **Small nucleolar RNAs (snoRNA):** RNA can also be altered by having its nucleotides modified to nucleotides other than A, C, G and U. In eukaryotes, modifications of RNA nucleotides are in general directed by **small nucleolar RNAs (snoRNA; 60–300 nt)**, found in the nucleolus and cajal bodies. snoRNAs associate with enzymes and guide them to a spot on an RNA by base pairing to that RNA. These enzymes then perform the nucleotide modification. rRNAs and tRNAs are extensively modified, but snRNAs and mRNAs can also be the target of base modification. RNA can also be methylated.

Pre-mRNA processing:

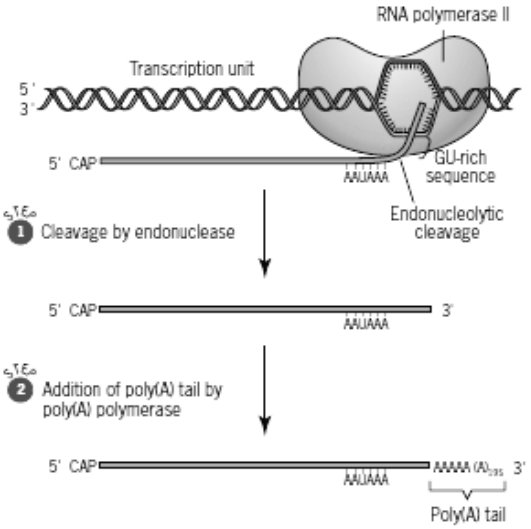
Capping: Early in the elongation process, the 5' ends of eukaryotic pre-mRNAs are modified by the addition of 7-methyl guanosine (7-MG) caps. These 7-MG caps are added when the growing RNA chains are only about 30 nucleotides long. The 7-MG cap contains an unusual 5-5' triphosphate linkage and two or more methyl groups. These 5' caps are added co-transcriptionally by the biosynthetic pathway. The 7-MG caps are recognized by protein factors involved in the initiation of translation and also help protect the growing RNA chains from degradation by nucleases.



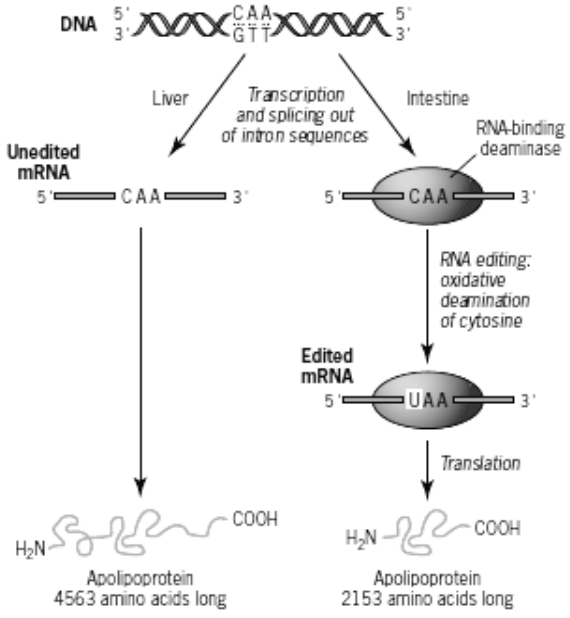
(b) **Pathway of biosynthesis of the 7-MG cap.**

Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

Poly A tail: The cleavage event that produces the 3 ' end of a transcript usually occurs at a site 11 to 30 nucleotides downstream from a conserved polyadenylation signal, consensus AAUAAA, and upstream from a GU-rich sequence located near the end of the transcript. After cleavage, the enzyme **poly (A) polymerase** adds poly(A) tails, tracts of adenosine monophosphate residues about 200 nucleotides long, to the 3' ends of the transcripts. The addition of poly(A) tails to eukaryotic mRNAs is called **polyadenylation.** To examine the polyadenylation signal of the human *HBB* (globin) gene, check out Solve It: Formation of the 3'-Terminus of an RNA Polymerase II Transcript. The formation of poly (A) tails on transcripts requires a specificity component that recognizes and binds to the AAUAAA sequence, a stimulatory factor that binds to the GU-rich sequence, an endonuclease, and the poly(A) polymerase. These proteins form a multimeric complex that carries out both the cleavage and the polyadenylation in tightly coupled reactions. The poly (A) tails of eukaryotic mRNAs enhance their stability and play an important role in their transport from the nucleus to the cytoplasm.



RNA editing: Normally, the genetic information is not altered in the mRNA intermediary. However, the discovery of **RNA editing** has shown that exceptions do occur. RNA editing processes alter the information content of gene transcripts in two ways: (1) by changing the structures of individual bases and (2) by inserting or deleting uridine monophosphate residues.



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

The first type of RNA editing, which results in the substitution of one base for another base, is rare. This type of editing was discovered in studies of the apolipoprotein-B (*apo-B*) genes and mRNAs in rabbits and humans. Apolipoproteins are blood proteins that transport certain types of fat

molecules in the circulatory system. In the liver, the *apo-B* mRNA encodes a large protein 4563 amino acids long. In the intestine, the *apo-B* mRNA directs the synthesis of a protein only 2153 amino acids long. Here, a C residue in the pre-mRNA is converted to a U, generating an internal UAA translation– termination codon, which results in the truncated apolipoprotein.

Splicing:

This process takes place in the nucleus and involves the removal of noncoding intron sequences from pre-mRNAs to produce mature mRNAs in which the coding sequences, corresponding to the exons, are continuous. The mature spliced mRNA, an accurate template for protein synthesis, is then exported to the cyto-plasm where it acts as a template for protein synthesis.

Splicing depends on the presence of signal sequences in the pre-mRNA. In almost all genes, the first two nucleotides at the 5′ end of an intron are GT and the last two at the 3′ end are AG. These are part of larger signal sequences present at the 5′ and 3′ ends of the introns. The complete 5′ signal sequence is 5′ AGGTAAGT 3′ and the 3′ sequence is 5′ YYYYYYNCAG 3′ (Y = pyrimidine, N = any nucleotide).

A branch point sequence is present in vertebrates, in the introns 10-40 bases upstream of the 3′ signal sequence. A more specific sequence 5′ UACUAAC 3′, occurs in introns of yeast. Splicing occurs in two steps (Fig. 16.8A). In the first step, the 2′ hydroxyl group of the adenine of the branch point sequence attacks the phosphodiester bond 5′ to the G of the GT (5′ splice site).

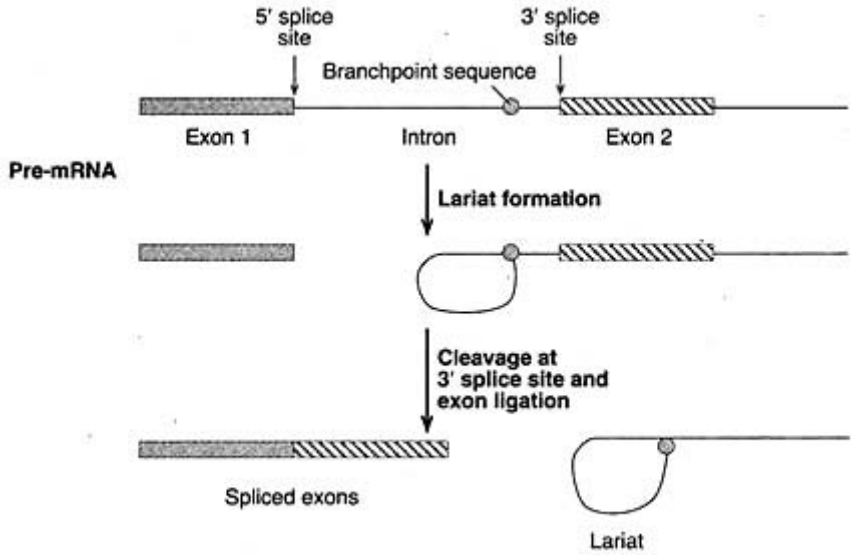


Fig. 16.8A: Splicing of pre-mRNA in eukaryotes

Source: http://www.biologydiscussion.com

Splicing a pre-mRNA in Eukaryotes

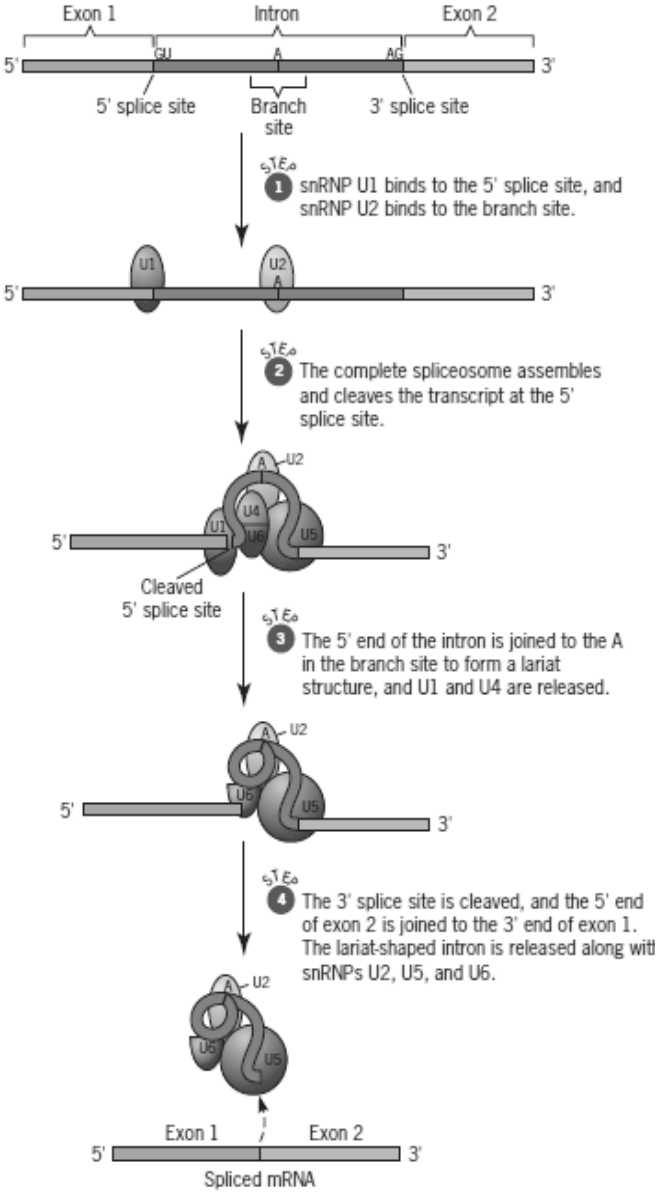
The bond is bro-ken releasing the 5′ end of the intron and attach-ing it to the branch point sequence. The intron now forms a tailed loop structure called a lariat. In the second step, the 3′ end of the Intron is cleaved after G of the AG (3′ splice site), the intron is released and the two exon sequences are joined together.

Splicing is catalyzed by a group of molecules called small nuclear ribonucleoproteins (snRNPs) – U1, U2, U4, U5 and U6. These are composed of small RNA molecules rich in uracil, called U RNAs or small nuclear RNAs (snRNAs) that exist complexed with proteins. The U1 snRNP binds to the 5′ splice site and the U2 snRNP binds to the branch point sequence.

The remaining snRNPs, U5 and U4/U6, then form a complex with U1 and U2 causing the intron to loop out and the exons to be brought together. The combination of the pre-mRNA and the snRNPs is called the spliceosome and this is responsible for folding the pre-mRNA into the correct conformation for splicing (Fig. 16.8B).

The spliceosome also catalyzes the cutting and joining reactions that excise the intron and ligate the exons. Once splicing is completed the spliceosome dissociates.

Spliceosome Formation



Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.

12. Genetic regulation: Regulation of prokaryotic gene expression – lac, *trp* and *ara* operons; regulation of eukaryotic gene expression – brief account.

Regulation of Gene Expression in Prokaryotes

In bacteria the expression of genes is controlled by extracellular signals often present in the medium in which bacteria are grown. These signals are carried to the genes by regulatory proteins. Regulatory proteins are of two types. They are positive regulators called activators and negative regulators called repressors. These activators and repressors are DNA binding proteins.

Negative Regulators or Repressors:

The repressor or inhibitor protein binds to the target site (operator) on DNA. These block the RNA polymerase enzyme from binding to the promoter, thus preventing the transcription. The repressor binds to the site where it overlaps the polymerase enzyme. Thus, activity of the genes is turned off. It is called negative control mechanism.

An anti-repressor or anti-inhibitor called inducer is needed to inactivate the repressor and thereby activating the genes. Thus, the genes are switched on. This is demonstrated by lactose operon.

Positive Regulators or Activators:

To activate the transcription by the promoter, the activator helps polymerase enzyme to bind to the promoter.

Genes under positive control mechanism are expressed only when an activator or stimulator or active regulator is present.

Operon:

The Operon:

1. Operons are segments of genetic material (DNA) that function as regulated unit that can be switched on or off.
2. An operon consists of minimum four types of genes: regulator, operator, promoter and structural.
3. Regulator gene is a gene which forms a biochemical for suppressing the activity of operator gene.
4. Operator gene is a gene which receives the product of regulator gene. It allows the functioning of the operon when it is not covered by the biochemical produced by regulator gene.

5. The functioning of operon is stopped when operator gene is covered.

6. Promoter gene is the gene which provides point of attachment to RNA polymerase required for transcription of structural genes.

7. Structural genes are genes which transcribe mRNA for polypeptide synthesis.

8. An operon may have one or more structural genes, e.g., 3 in lac operon, 5 in tryptophan operon, 9 in histidine operon.

9. The polypeptides may become component of structural proteins, enzymes, transport proteins, hormones, antibodies, etc. Some structural genes also form non-coding RNAs.

In bacteria cistrons or structural genes, producing enzymes of a metabolic pathway are organized in a cluster whose functions are related. Poly cistronic genes of prokaryotes along with their regulatory genes constitute a system called operon. Operon is a unit of expression and regulation.

1. Lactose Operon or Lac Operon:

This is a negative control mechanism. In 1961 Francois Jacob and Jacques Monod proposed operon model for the regulation of gene expression in *E. coli*. The synthesis of enzyme (3-galactosidase has been studied in detail. This enzyme causes the breakdown of lactose into glucose and galactose.

The lac Operon controls three genes responsible for synthesis of β-galactosidase (lac Z gene), β- galactosidase permease (lac Y gene) and transacetylase (lac A gene) enzymes. Therefore, mRNA produced by this Operon is known as polycistronic mRNA. Lac Operon contains a regulator gene called as lac I, which is responsible for synthesis of a protein referred to as repressor protein. The repressor protein binds to as specific DNA sequence and inhibits β- galactosidase synthesis.

What makes the lac operon turn on?

E. coli bacteria can break down lactose, but it's not their favorite fuel. If glucose is around, they would much rather use that. Glucose requires fewer steps and less energy to break down than lactose. However, if lactose is the only sugar available, the *E. coli* will go right ahead and use it as energy source. To use lactose, the bacteria must express the lac operon genes, which encode key enzymes for lactose uptake and metabolism. To be as efficient as possible, *E. coli* should express the lac operon only when two conditions are met:

I. Lactose is available, and

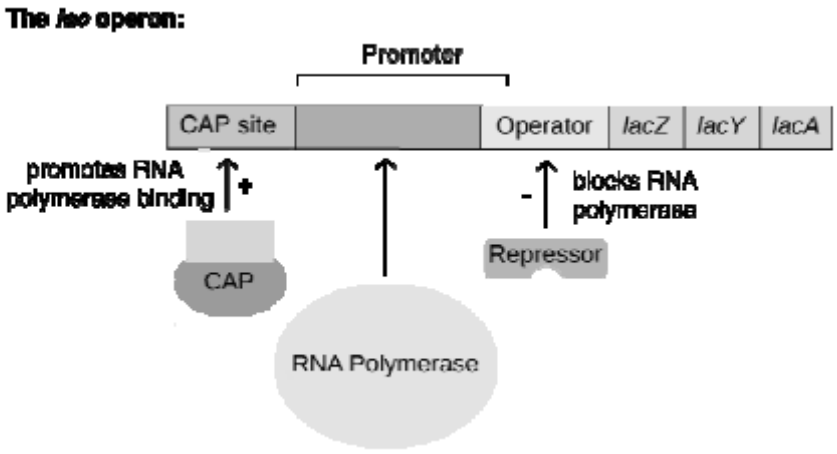
II. Glucose is not available

How are levels of lactose and glucose detected, and how do changes in levels affect lac operon transcription? Two regulatory proteins are involved:

One, the lac repressor, acts as a lactose sensor. The other, catabolite activator protein (CAP), acts as a glucose sensor. These proteins bind to the DNA of the lac operon and regulate its transcription based on lactose and glucose levels. Let's take a look at how this works.

Structure of the lac operon

The lac operon contains three genes: lacZ, lacY, and lacA. These genes are transcribed as a single mRNA, under control of one promoter. Genes in the lac operon specify proteins that help the cell utilize lactose. lacZ encodes an enzyme that splits lactose into monosaccharides (single unit sugars) that can be fed into glycolysis. Similarly, lacY encodes a membrane-embedded transporter that helps bring lactose into the cell. In addition to the three genes, the lac operon also contains a number of regulatory DNA sequences. These are regions of DNA to which particular regulatory proteins can bind, controlling transcription of the operon.



Source: https://www.khanacademy.org/science/biology/gene-regulation

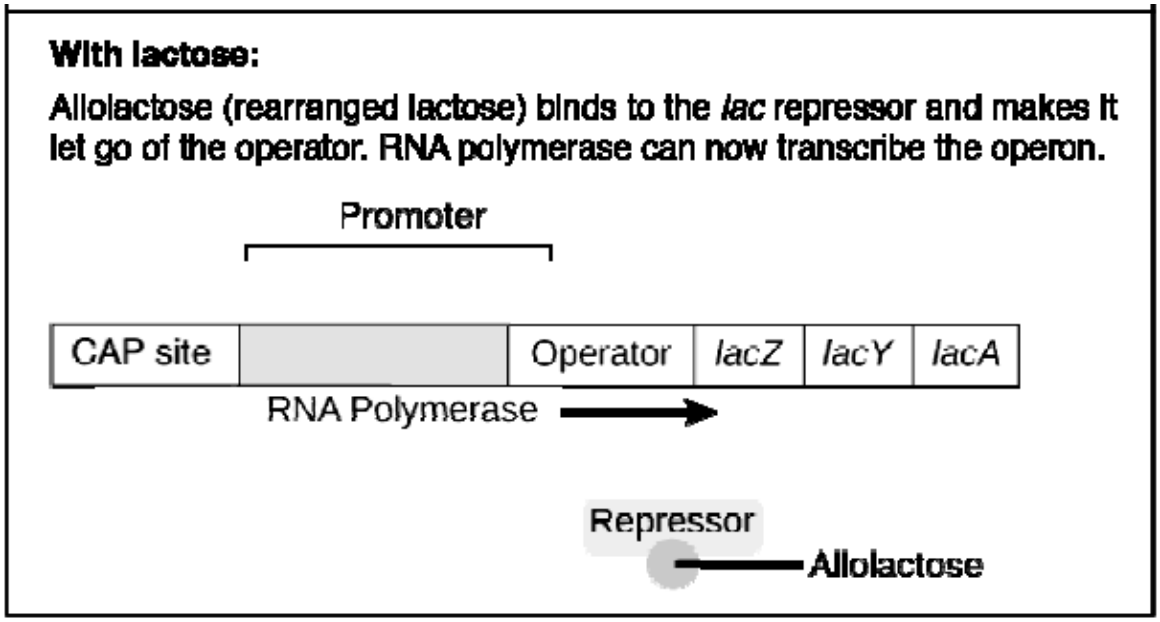
The promoter is the binding site for RNA polymerase, the enzyme that performs transcription. The operator is a negative regulatory site bound by the lac repressor protein. The operator overlaps with the promoter, and when the lac repressor is bound, RNA polymerase cannot bind to the promoter and start transcription. The CAP binding site is a positive regulatory site that is bound by catabolite activator protein (CAP). When CAP is bound to this site, it promotes transcription by helping RNA polymerase bind to the promoter.

The lac repressor

The lac repressor is a protein that represses (inhibits) transcription of the lac operon. It does this by binding to the operator, which partially overlaps with the promoter. When bound, the lac repressor gets in RNA polymerase's way and keeps it from transcribing the operon. [Where does the lac repressor come from?]

When lactose is not available, the lac repressor binds tightly to the operator, preventing

transcription by RNA polymerase. However, when lactose is present, the lac repressor loses its ability to bind DNA. It floats off the operator, clearing the way for RNA polymerase to transcribe the operon.



Source: https://www.khanacademy.org/science/biology/gene-regulation

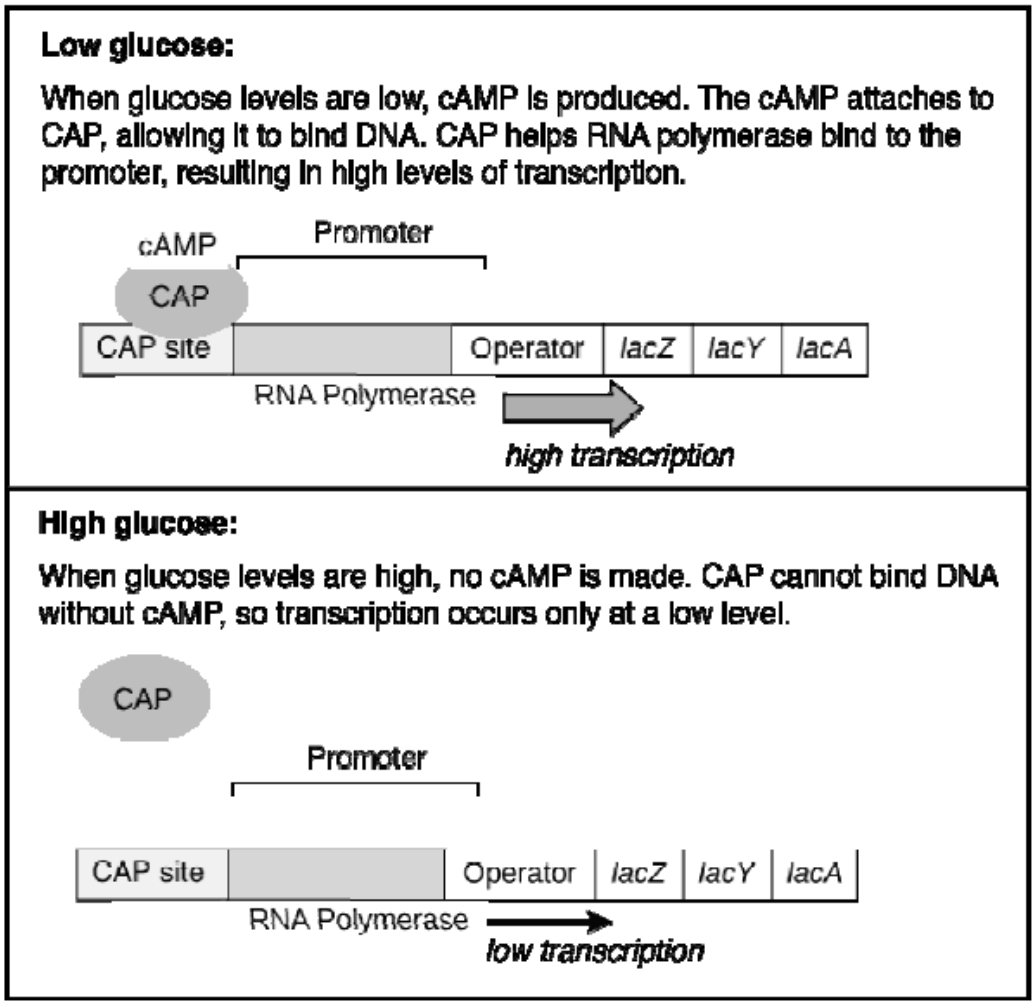
This change in the lac repressor is caused by the small molecule allolactose, an isomer (rearranged version) of lactose. When lactose is available, some molecules will be converted to allolactose inside the cell. Allolactose binds to the lac repressor and makes it change shape so it can no longer bind DNA. Allolactose is an example of an inducer, a small molecule that triggers expression of a gene or operon. The lac operon is considered an inducible operon because it is turn off (repressed), but can be turned on in the presence of the inducer allolactose.

Catabolite activator protein (CAP)

When lactose is present, the lac repressor loses its DNA-binding ability. This clears the way for RNA polymerase to bind to the promoter and transcribe the lac operon. That sounds like the end of the story, right?

Well. But not quite. As it turns out, RNA polymerase alone does not bind very well to the lac operon promoter. It might make a few transcripts, but it won't do much more unless it gets extra help from catabolite activator protein (CAP). CAP binds to a region of DNA just before the lac operon promoter and helps RNA polymerase attach to the promoter, driving high levels of transcription.

CAP isn't always active (able to bind DNA). Instead, it's regulated by a small molecule called cyclic AMP (cAMP). cAMP is a "hunger signal" made by *E. coli* when glucose levels are low. cAMP binds to CAP, changing its shape and making it able to bind DNA and promote transcription. Without cAMP, CAP cannot bind DNA and is inactive.



Source: https://www.khanacademy.org/science/biology/gene-regulation

[How is cAMP made, and how does it report glucose levels?]

CAP is only active when glucose levels are low (cAMP levels are high). Thus, the lac operon can only be transcribed at high levels when glucose is absent. This strategy ensures that bacteria only turn on the lac operon and start using lactose after they have used up the entire preferred energy source (glucose).

So, when does the lac operon really turn on?

The lac operon will be expressed at high levels if two conditions are met:

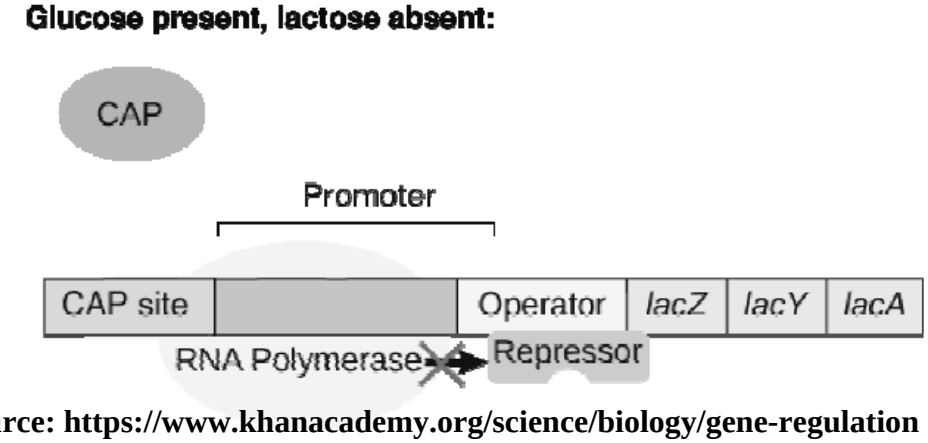
Glucose must be unavailable: When glucose is unavailable, cAMP binds to CAP, making CAP able to bind DNA. Bound CAP helps RNA polymerase attach to the lac operon promoter. Lactose must be available: If lactose is available, the lac repressor will be released

from the operator (by binding of allolactose). This allows RNA polymerase to move of toward on the DNA and transcribe the operon.

These two events in combination – the binding of the activator and the release of the repressor – allow RNA polymerase to bind strongly to the promoter and give it a clear path for transcription. They lead to strong transcription of the lac operon and production of enzymes needed for lactose utilization.

Glucose present, lactose absent:

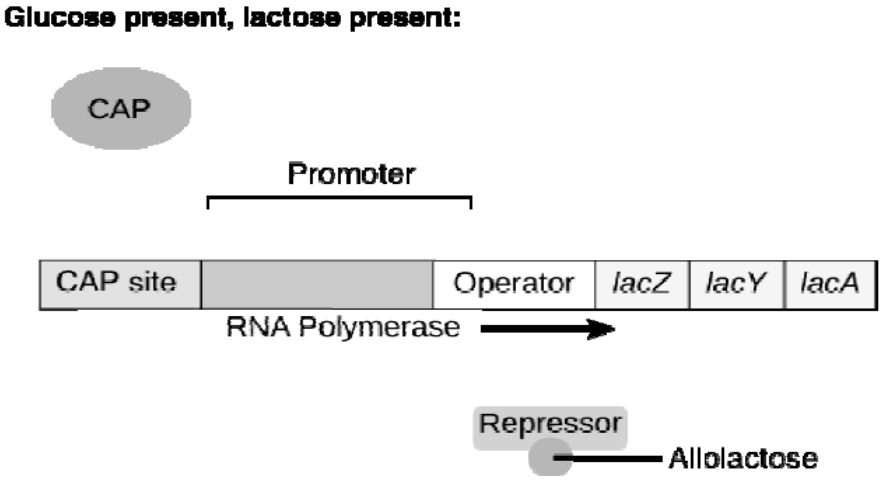
No transcription of the lac operon occurs. That's because the lac repressor remains bound to the operator and prevents transcription by RNA polymerase. Also, cAMP levels are low because glucose levels are high, so CAP is inactive and cannot bind DNA.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Glucose present, lactose present:

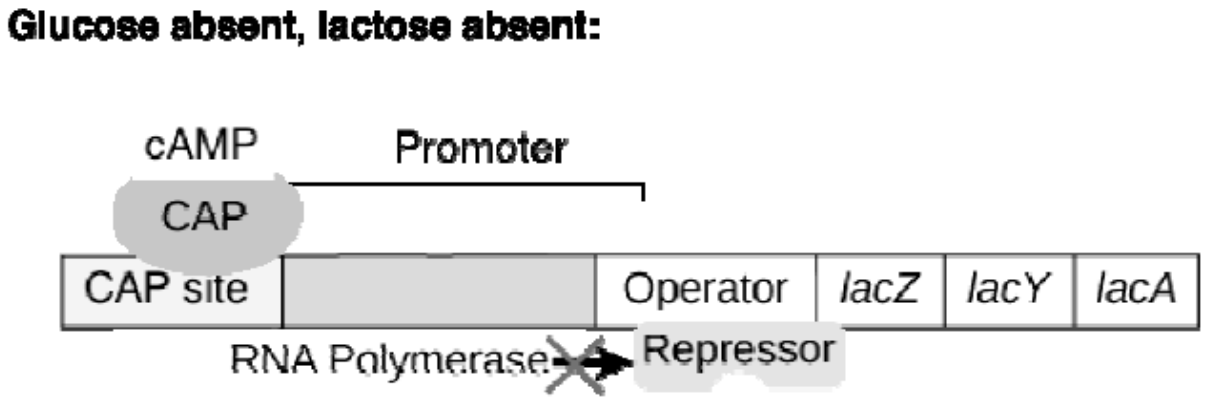
Low level transcription of the lac operon occurs. The lac repressor is released from the operator because the inducer (allolactose) is present. cAMP levels, however, are low because glucose is present. Thus, CAP remains inactive and cannot bind to DNA, so transcription only occurs at a low, leaky level.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Glucose absent, lactose absent:

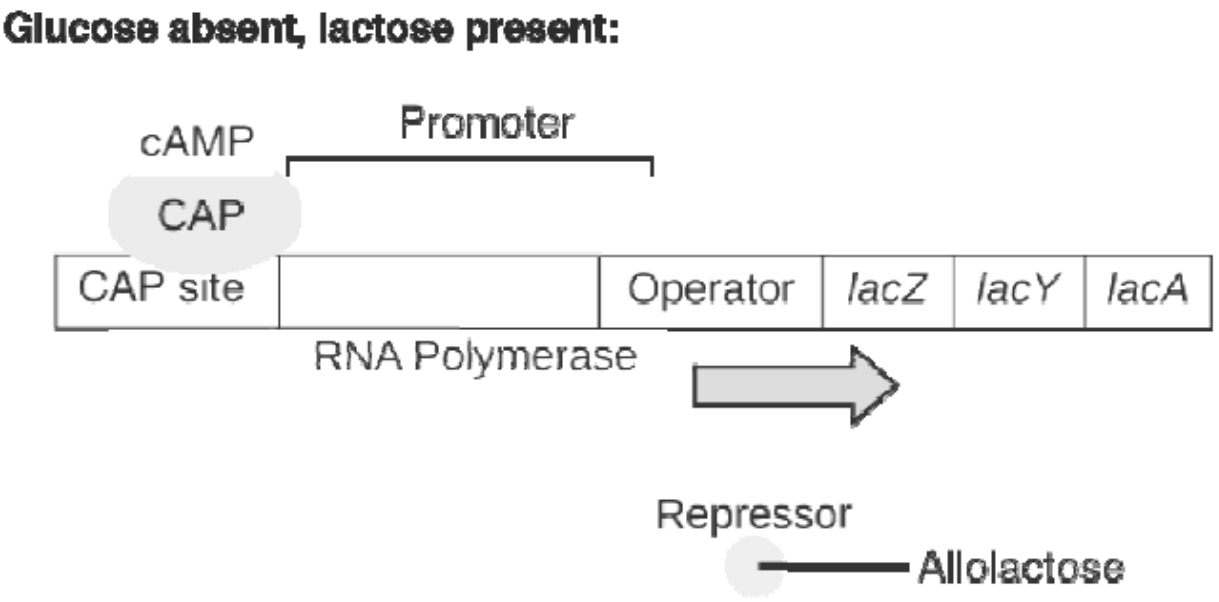
No transcription of the lac operon occurs. cAMP levels are high because glucose levels are low, so CAP is active and will be bound to the DNA. However, the lac repressor will also be bound to the operator (due to the absence of allolactose), acting as a roadblock to RNA polymerase and preventing transcription.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Glucose absent, lactose present:

Strong transcription of the lac operon occurs. The lac repressor is released from the operator because the inducer (allolactose) is present. cAMP levels are high because glucose is absent, so CAP is active and bound to the DNA. CAP helps RNA polymerase bind to the promoter, permitting high levels of transcription.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Tryptophan operon:

The trp of *E. coli* controls the synthesis of the enzymes that catalyze the biosynthesis of the amino acid tryptophan. The functions of the five structural genes and the adjacent regulatory sequences of the trp operon have been analyzed in detail by Charles Yanofsky and colleagues. The five structural genes encode enzymes that convert chorismic acid to tryptophan. The expression of the trp operon is regulated at two levels: repression, which controls the initiation of transcription, and attenuation, which governs the frequency of premature transcript termination.

Repression

The trp operon of *E. coli* is a negative repressible operon. The organization of the trp operon and the pathway of biosynthesis of tryptophan are shown in Figure.

The trpR gene, which encodes the trp repressor, is not closely linked to the trp operon. The operator (O) region of the trp operon lies within the primary promoter (P1) region. There is also a weak promoter (P2) at the operator distal end of the trpD gene. The P2 promoter increases the basal level of transcription of the trpC, trpB, and trpA genes. Two transcription termination sequences (t and t´) are located downstream from trpA. The trpL region specifies a 162 nucleotide long mRNA leader sequence. The regulation of transcription of the trp operon is diagrammed in Figure.

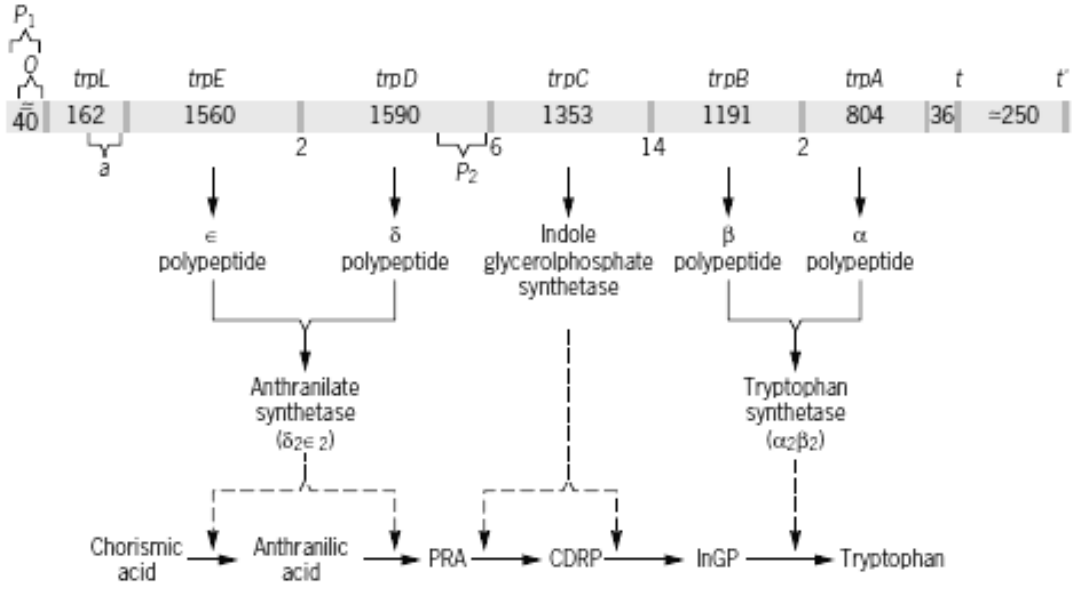


Figure Organization of the trp operon in *E. coli.* Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

In the absence of tryptophan (the co-repressor), RNA polymerase binds to the promoter region and transcribes the structural genes of the operon. In the presence of tryptophan, the co-repressor/repressor complex binds to the operator region and prevents RNA polymerase from initiating transcription of the genes in the operon. The rate of transcription of the trp operon in the de-repressed state (absence of tryptophan) is 70 times the rate that occurs in the repressed state (presence of tryptophan). In trpR mutants, which lack a functional repressor, the rate of synthesis of the tryptophan biosynthetic enzymes is still reduced about tenfold by the addition of tryptophan to the medium.

Attenuation:

Deletions that remove part of the trpL region result in increased rates of expression of the trp operon. However, these deletions have no effect on the repressibility of the trp operon; that is, repression and de-repression occur just as in $trpL^{+}$ strains. These results indicate that the synthesis of the tryptophan biosynthetic enzymes is regulated at a second level by a mechanism that is independent of repression/de-repression and requires nucleotide sequences present in the trpL region of the trp operon.

This second level of regulation of the trp operon is called attenuation, and the sequence within trpL that controls this phenomenon is called the attenuator.

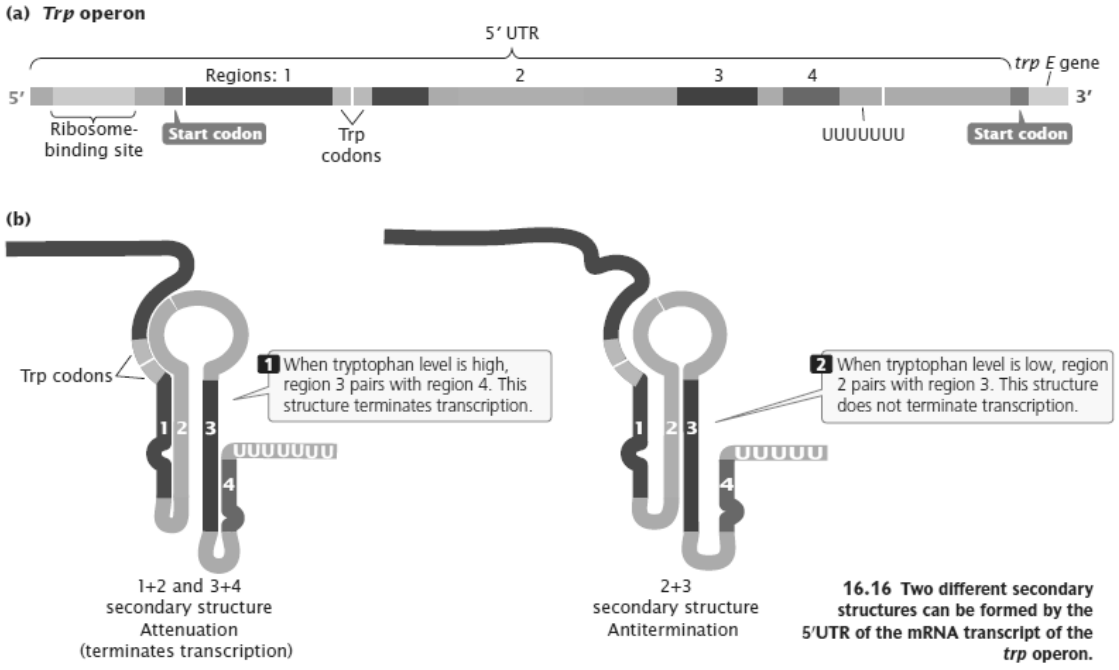


Figure Attenuation of the trp operon. Source: Benjamin A. Pierce, 2012, Genetics: A conceptual apporach -4th ed.)

Attenuation occurs by control of the termination of transcription at a site near the end of the mRNA leader sequence. This “premature” termination of trp operon transcription occurs only in the presence of tryptophan-charged tRNATrp. When this premature termination or attenuation occurs, a truncated (140 nucleotides) trp transcript is produced. The attenuator region has a nucleotide-pair sequence essentially identical to the transcription–termination signals found at the ends of most bacterial operons.

These termination signals contain a G:C-rich palindrome followed by several A:T base pairs. Transcription of these termination signals yields a nascent RNA with the potential to form a hydrogen-bonded hairpin structure followed by several uracils.

***ara* operon:**

The **L-arabinose operon**, also called the ***ara*** or ***araBAD* operon**, is an operon required for the breakdown of the five-carbon sugar, L-arabinose, in *Escherichia coli*. The L-arabinose operon contains three structural genes: *araB, araA, araD* (collectively known as *araBAD*), which encode for three metabolic enzymes that are required for the metabolism of L-arabinose. AraB (ribulokinase), AraA (an isomerase), AraD (an epimerase) produced by these genes catalyze conversion of L-arabinose to an intermediate of the pentose phosphate pathway, D-xylulose-5-phosphate.

The structural genes of the L-arabinose operon are transcribed from a common promoter into a single transcript, a mRNA. The expression of the L-arabinose operon is controlled as a single unit by the product of regulatory gene *araC* and the catabolite activator protein (CAP)-cAMP complex. The regulator protein AraC is sensitive to the level of arabinose and plays a dual role as both an activator in the presence of arabinose and a repressor in the absence of arabinose to regulate the expression of *araBAD*. AraC protein not only controls the expression of *araBAD* but also auto-regulates its own expression at high AraC levels.

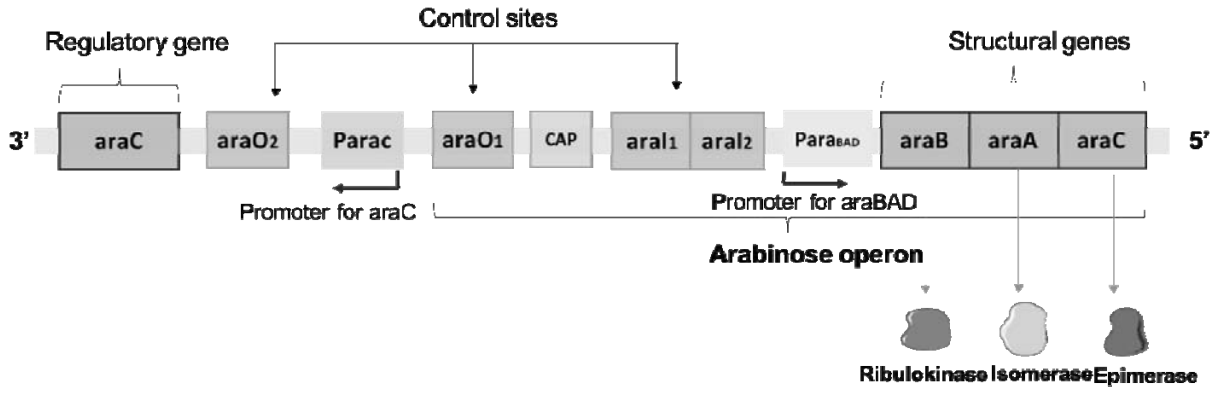


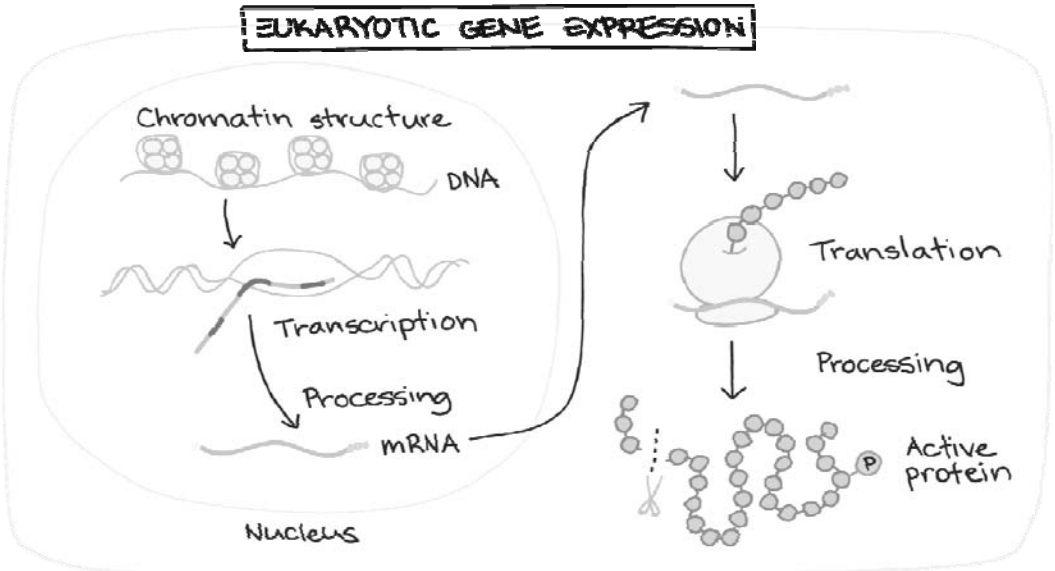
Figure Structure of the ara operon. Source: https://en.wikipedia.org/wiki/L-arabinose_operon

Gene regulation is how a cell controls which genes, out of the many genes in its genome, are "turned on" (expressed). Thanks to gene regulation, each cell type in your body has a different set of active genes – despite the fact that almost all the cells of your body contain the exact same DNA. These different patterns of gene expression cause your various cell types to have different sets of proteins, making each cell type uniquely specialized to do its job.

Eukaryotic gene expression can be regulated at many stages

In the articles that follow, we'll examine different forms of eukaryotic gene regulation. That is, we'll see how the expression of genes in eukaryotes (like us!) can be controlled at various stages, from the availability of DNA to the production of mRNAs to the translation and processing of proteins. Eukaryotic gene expression involves many steps, and almost all of them can be regulated. Different genes are regulated at different points, and it's not uncommon for a gene (particularly an important or powerful one) to be regulated at multiple steps.

- **Chromatin accessibility**: The structure of chromatin (DNA and its organizing proteins) can be regulated. More open or "relaxed" chromatin makes a gene more available for transcription.
- **Transcription**: Transcription is a key regulatory point for many genes. Sets of transcription factor proteins bind to specific DNA sequences in or near a gene and promote or repress its transcription into an RNA.
- **RNA processing**: Splicing, capping, and addition of a poly A tail to an RNA molecule can be regulated, and so can exit from the nucleus. Different mRNAs may be made from the same pre-mRNA by alternative splicing.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Transcription: The key control point

Transcription is the process where a gene's DNA sequence is copied (transcribed) into an RNA molecule. Transcription is a key step in using information from a gene to make a protein. If you're not familiar with those ideas yet, you might consider watching the central dogma video for a solid intro from Sal.

Gene expression is when a gene in DNA is "turned on," i.e.,, used to make the protein it specifies. Not all the genes in your body are turned on at the same time, or in the same cells or parts of the body.

For many genes, transcription is the key on/off control point:

- If a gene is not transcribed in a cell, it can't be used to make a protein in that cell.
- If a gene does get transcribed, it is likely going to be used to make a protein (expressed). In general, the more a gene is transcribed, the more protein that will be made.

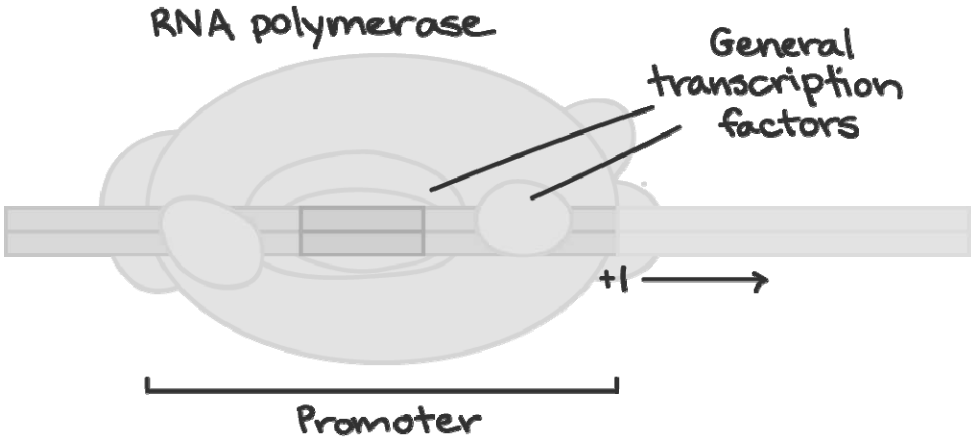
Various factors control how much a gene is transcribed. For instance, how tightly the DNA of the gene is wound around its supporting proteins to form chromatin can affect a gene's availability for transcription.

Proteins called transcription factors, however, play a particularly central role in regulating transcription. These important proteins help determine which genes are active in each cell of your body.

Transcription factors

What has to happen for a gene to be transcribed? The enzyme RNA polymerase, which makes a new RNA molecule from a DNA template, must attach to the DNA of the gene. It attaches at a spot called the promoter.

- In bacteria, RNA polymerase attaches right to the DNA of the promoter. You can see how this process works, and how it can be regulated by transcription factors, in the lac operon and trp operon videos.
- In humans and other eukaryotes, there is an extra step. RNA polymerase can attach to the promoter only with the help of proteins called basal (general) transcription factors. They are part of the cell's core transcription toolkit, needed for the transcription of any gene.



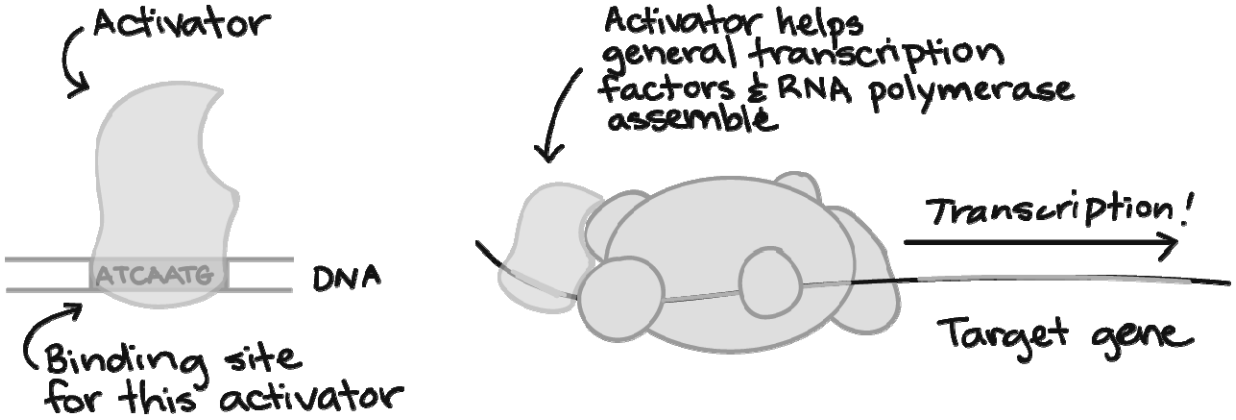
Source: https://www.khanacademy.org/science/biology/gene-regulation

How do transcription factors work?

A typical transcription factor binds to DNA at a certain target sequence. Once it's bound, the transcription factor makes it either harder or easier for RNA polymerase to bind to the promoter of the gene.

Activators

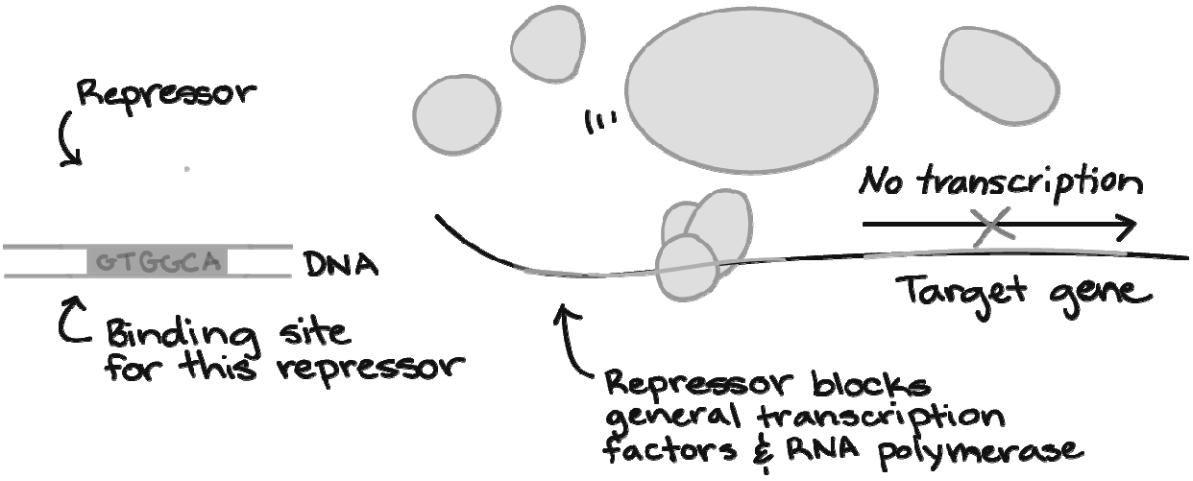
Some transcription factors activate transcription. For instance, they may help the general transcription factors and/or RNA polymerase bind to the promoter, as shown in the diagram below.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Repressors

Other transcription factors repress transcription. This repression can work in a variety of ways. As one example, a repressor may get in the way of the basal transcription factors or RNA polymerase, making it so they can't bind to the promoter or begin transcription.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Regulation of RNA processing

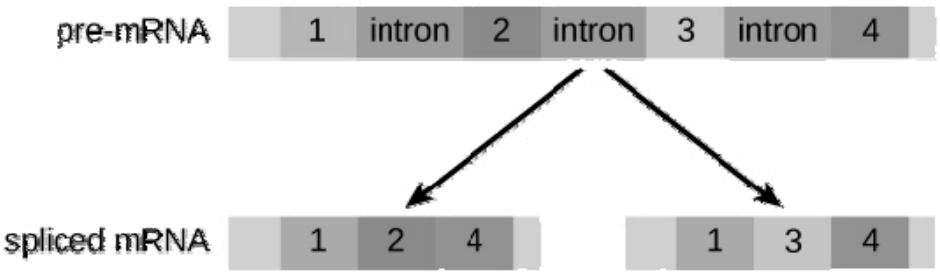
When a eukaryotic gene is transcribed in the nucleus, the primary transcript (freshly made RNA molecule) is not yet considered a messenger RNA. Instead, it's an "immature" molecule called a pre-mRNA.

The pre-mRNA has to go through some modifications to become a mature mRNA molecule that can leave the nucleus and be translated. These include splicing, capping, and addition of a poly A tail, all of which can potentially be regulated – sped up, slowed down, or altered to result in a different product.

Alternative splicing

Most pre-mRNA molecules have sections that are removed from the molecule, called introns, and sections that are linked or together to make the final mRNA, called exons. This process is called splicing.

In the process of alternative splicing, different portions of an mRNA can be selected for use as exons. This allows either of two (or more) mRNA molecules to be made from one pre-mRNA.



Alternative splicing, Source: https://www.khanacademy.org/science/biology/gene-regulation

Alternative splicing is not a random process. Instead, it is typically controlled by regulatory proteins. The proteins bind to specific sites on the pre-mRNA and "tell" the splicing factors

which exons should be used. Different cell types may express different regulatory proteins, so different exon combinations can be used in each cell type, leading to the production of different proteins.

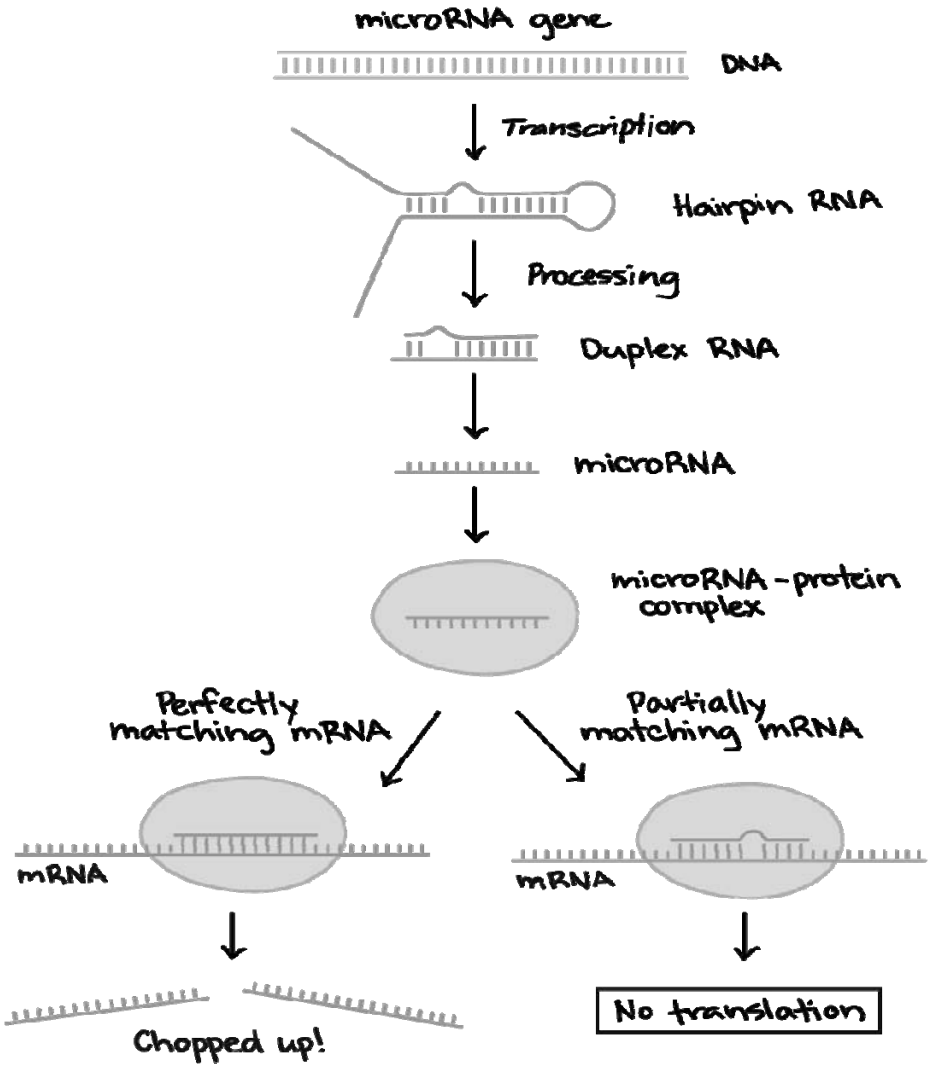
Small regulatory RNAs

Once an mRNA has left the nucleus, it may or may not be translated many times to make proteins. Two key determinants of how much protein is made from an mRNA are its "lifespan" (how long it floats around in the cytosol) and how readily the translation machinery, such as the ribosome, can attach to it.

A recently discovered class of regulators, called small regulatory RNAs, can control mRNA lifespan and translation. Let's see how this works.

microRNAs

MicroRNAs (miRNAs) were among the first small regulatory RNAs to be discovered. A miRNA is first transcribed as a long RNA molecule, which forms base pairs with itself and folds over to make a hairpin.



Source: https://www.khanacademy.org/science/biology/gene-regulation

Next, the hairpin is chopped up by enzymes, releasing a small double-stranded fragment of about 22nucleotides .One of the strands in this fragment is the mature miRNA, which binds to a specific protein to make an RNA-protein complex.

The miRNA directs the protein complex to "matching" mRNA molecules (ones that form base pairs with the miRNA). When the RNA-protein complex binds.

- If the miRNA and its target match perfectly, an enzyme in the RNA-protein complex will typically chop the mRNA in half, leading to its breakdown.
- If the miRNA and its target have some mismatches, the RNA-protein complex may instead bind to the mRNA and keep it from being translated.

These are not the only ways that miRNAs inhibit expression of their targets, and scientists are still investigating their many modes of action.

What do miRNAs actually do in organisms? Their direct role is to reduce the expression of their target genes, but they may play this role to produce many different outcomes.

For instance, in mice, a specific miRNA plays a key role in the development and function of the vascular (circulatory) system. Mice without function of this miRNA had defects in heart development and were unable to survive. Changes in expression levels of miRNAs are also associated with human diseases, including various types of cancer and cardiac hypertrophy.

Temperature: the heat shock genes

When organisms are subjected to the stress of high temperature, they respond by synthesizing a group of proteins that help to stabilize the internal cellular environment. These **heat shock proteins**, found in both prokaryotes and eukaryotes, are among the most conserved polypeptides known.

In *Drosophila*, for example, one of the heat-shock proteins called HSP70 (for heat-shock protein, molecular weight 70 kilodaltons) is encoded by a family of genes located in two nearby clusters on one of the autosomes. Altogether, there are five to six copies of these hsp70 genes in the two clusters. When the temperature exceeds 33ºC, each of the genes is transcribed into RNA, which is then processed and translated to produce HSP70 polypeptides. This heat induced transcription of the hsp70 genes is mediated by a polypeptide called the heat-shock transcription factor, or HSTF, which is present in the nuclei of *Drosophila* cells. When *Drosophila* is heat stressed, the HSTF is chemically altered by phosphorylation. In this altered state, it binds specifically to nucleotide sequences upstream of the hsp70 genes and makes the genes more accessible to RNA polymerase II, The

sequences to which the phosphorylated HSTF binds are called heat-shock response elements (HSEs).

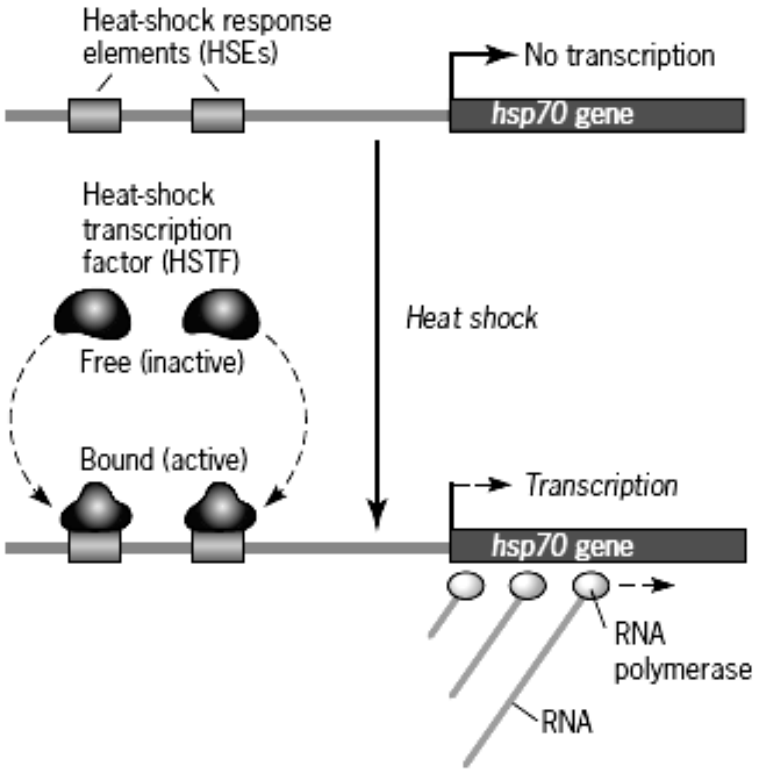


Figure Induction of transcription from the Drosophila hsp70 gene by heat shock Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

Signal molecules: genes that respond to hormones

In multicellular eukaryotes, one type of cell can signal another by secreting a **hormone.** Hormones circulate through the body, make contact with their target cells, and then initiate a series of events that regulate the expression of particular genes. In animals there are two general classes of hormones. The first class, **the steroid hormones**, are small, lipid-soluble molecules derived from cholesterol. Because of their lipid nature, they have little or no trouble passing through cell membranes, examples are estrogen and progesterone. Once these hormones have entered a cell, they interact with cytoplasmic or nuclear proteins called hormone receptors. The receptor/hormone complex that is formed then interacts with the DNA where it acts as a transcription factor to regulate the expression of certain genes.

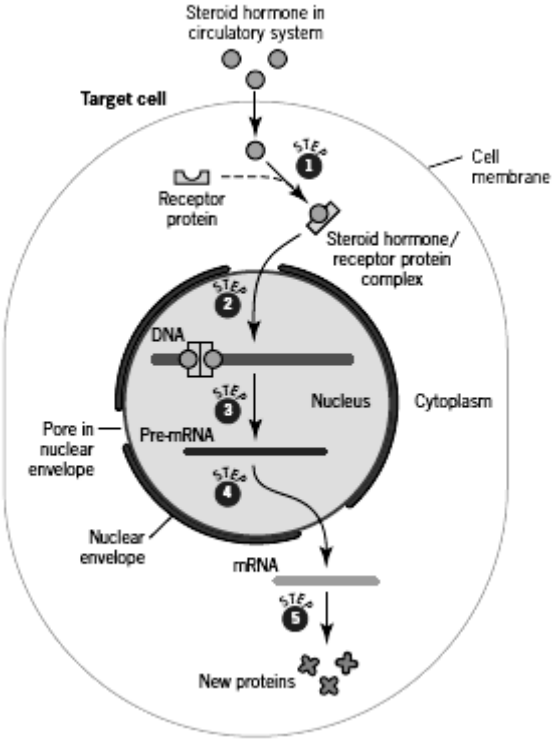


Figure Signal transduction pathway. Source: Snustad and Simmons, 2012, Principles of Genetics -6th ed.)

DNase I Hypersensitivity

Several types of changes are observed in chromatin structure when genes become transcriptionally active. As genes become transcriptionally active, regions around the genes become highly sensitive to the action of DNase I. These regions, called **DNase I hypersensitive sites**, frequently develop about 1000 nucleotides upstream of the start site of transcription, suggesting that the chromatin in these regions adopts a more open configuration during transcription. This relaxation of the chromatin structure allows regulatory proteins access to binding sites on the DNA. Indeed, many DNase I hypersensitive sites correspond to known binding sites for regulatory proteins. At least three different processes affect gene regulation by altering chromatin structure: (1) the modification of histone proteins; (2) chromatin remodeling; and (3) DNA methylation. Each of these mechanisms will be discussed in the sections that follow.

Histone Modification

Histones in the octamer core of the nucleosome have two domains: (1) a globular domain that associates with other histones and the DNA and (2) a positively charged tail domain that probably interacts with the negatively charged phosphate groups on the backbone of DNA. The tails of histone proteins are often modified by the addition or removal of phosphate groups, methyl groups, or acetyl groups. These modifications have sometimes been called the **histone code**, because they encode information that affects how genes are expressed.

Methylation of histones: One type of histone modification is the addition of methyl groups to the tails of histone proteins. These modifications can bring about either the activation or the repression of transcription, depending on which particular amino acids in the histone tail are methylated. A common modification is the addition of three methyl groups to lysine 4 in the tail of the H3 histone protein, abbreviated H3K4me3 (K is the abbreviation for lysine). The H3K4me3 modification is frequently found in promoters of transcriptionally active genes in eukaryotes.

Acetylation of histones: Another type of histone modification that affects chromatin structure is acetylation, the addition of acetyl groups (CH3CO) to histone proteins. The acetylation of histones usually stimulates transcription. For example, the addition of a single acetyl group to lysine 16 in the tail of the H4 histone prevents the formation of the 30 nm chromatin fiber causing the chromatin to be in an open configuration and available for transcription.

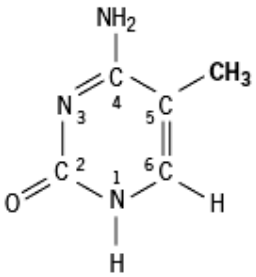
Chromatin remodeling

Experiments that assess the sensitivity of DNA to digestion with DNase I have established that transcribed DNA is more accessible to in transcribed DNA, the nucleosomes are altered by multiprotein complexes that ultimately facilitate the action of the RNA polymerase. This alteration of nucleosomes in preparation for transcription is called **chromatin remodeling.**

DNA Methylation

Another change in chromatin structure associated with transcription is the methylation of cytosine bases, which yields 5-methylcytosine. The methylation of cytosine in DNA is distinct from the methylation of histone proteins mentioned earlier. Heavily methylated DNA is associated with the repression of transcription in vertebrates and plants, whereas transcriptionally active DNA is usually unmethylated in these organisms. Abnormal patterns of methylation are also associated with some types of cancer.

5′ mCpG 3′
3′ GpCm 5′



Structure of 5-methylcytosine

DNA methylation is most common on cytosine bases adjacent to guanine nucleotides (CpG, where p represents the phosphate group in the DNA backbone); so two methylated cytosines sit diagonally across from each other on opposing strands: DNA regions with many CpG sequences are called **CpG islands** and are commonly found near transcription start sites. While genes are not being transcribed, these CpG islands are often methylated, but the methyl groups are removed before the initiation of transcription. CpG methylation is also associated with long term gene repression, such as on the inactivated X chromosome of female mammals.

Imprinting

DNA methylation in mammals is also responsible for unusual cases in which the expression of a gene is controlled by its parental origin. For example, in mice, the Igf 2 gene, which encodes an insulin-like growth factor, is expressed when it is inherited from the father but not from the mother. By contrast, a gene known as H19 is expressed when it is inherited from the mother but not from the father. Whenever the expression of a gene is conditioned by its parental origin, geneticists say that the gene has been **imprinted**—a term intended to convey the idea that the gene has been marked in some way so that it “remembers” which parent it came from.

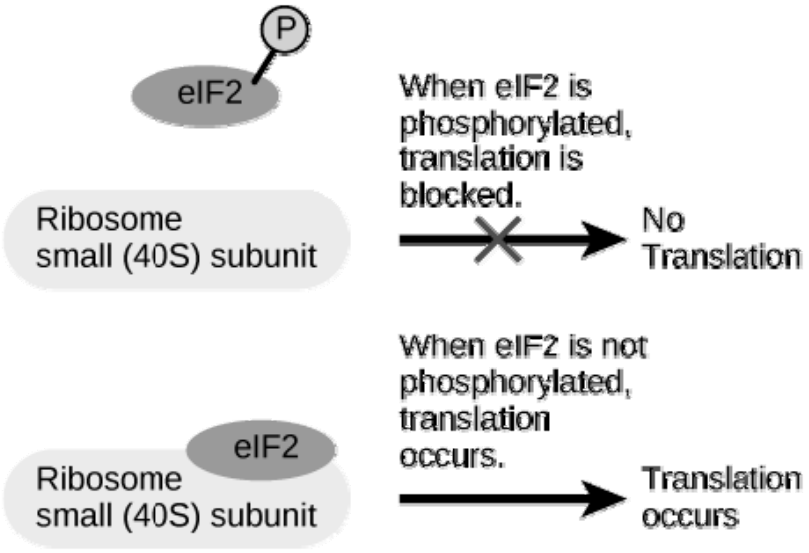
Regulation of translation

We already saw how miRNAs can inhibit translation, but there are a number of other ways that translation of an mRNA can also be regulated in a cell. One key step for regulation is translation initiation.

In order for translation to begin, the ribosome, an RNA-and-protein complex that houses translation, must assemble on the mRNA. This process involves many “helper” proteins, which make sure the ribosome is correctly positioned. Translation can be regulated globally (for every mRNA in the cell) through changes in the availability or activity of the “helper” proteins.

For example, in order for translation to begin, a protein called eukaryotic initiation factor-2 (eIF-2) must bind to a part of the ribosome called the small subunit. Binding of eIF-2 is controlled by phosphorylation, or addition of a phosphate group to the protein.

When eIF-2 is phosphorylated, it's turned "off"—it undergoes a shape change and can no longer play its role in initiation, so translation cannot begin. When eIF-2 is not phosphorylated, in contrast, it's "on" and can carry out its role in initiation, allowing translation to proceed.



Source: https://www.khanacademy.org/science/biology/gene-regulation

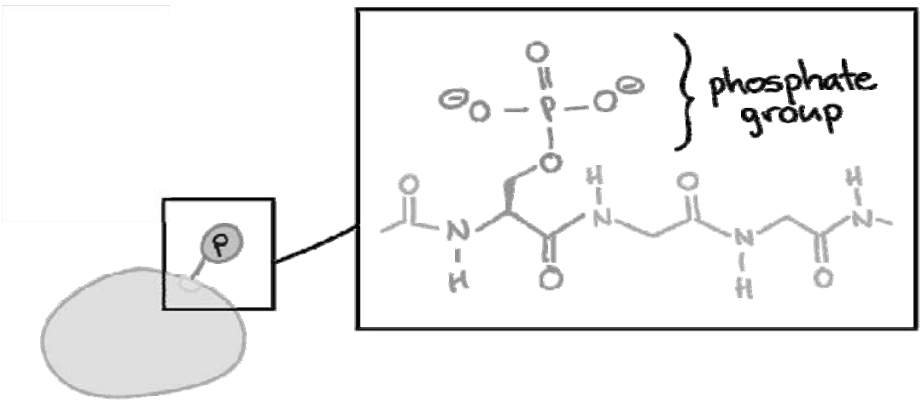
In this way, phosphorylation of eIF-2 acts as a switch, turning translation on or off. Inactivation of translation can be a good strategy in periods when the cell can't "afford" to make new proteins (e.g., when the cell is starved for nutrients).

Proteins can be regulated after translation

There are also regulatory mechanisms that act on proteins that have already been made. In these cases, an "edit" to the protein – such as removal of amino acids, or addition of a chemical modification – can lead to a change in its activity or behavior. These processing and modification steps can be targets for regulation

Phosphorylation

One of the most common post-translational modifications is phosphorylation, in which a phosphate group is attached to a protein. The effect of phosphorylation varies from protein to protein: some are activated by phosphorylation, while others are deactivated, and others yet simply change their behavior (interacting with a different partner, or going to a different part of the cell).

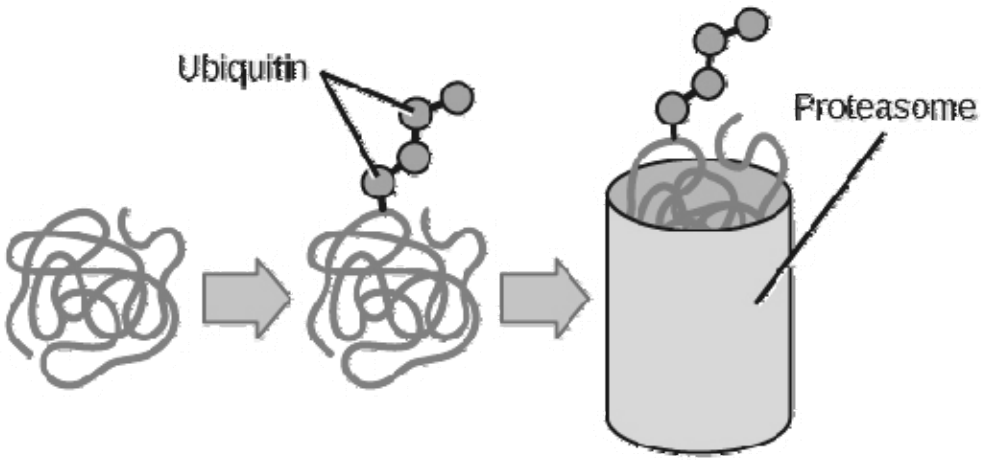


Source: https://www.khanacademy.org/science/biology/gene-regulation

We saw one example of this above, when we examined how eIF-2 is inactivated by addition of a phosphate group (blocking translation). However, many different proteins can be selectively phosphorylated, producing various effects depending on the protein's role in the cell.

Ubiquitination

Proteins can be tagged for degradation by the addition of a chemical marker called ubiquitin. Ubiquitin-tagged proteins are taken to the proteasome, or “recycling center” of the cell, and broken down into their component parts. Ubiquitination is an important way of controlling the persistence of a protein in the cell.



Source: https://www.khanacademy.org/science/biology/gene-regulation

13. Transposomal elements: Ac-Ds, IS elements, P-elements and their role in genetics.

Transposable elements are mobile DNA sequences found in the genomes of all organisms. In many genomes, they are quite abundant: for example, they make up at least 45% of human DNA. Most transposable elements are able to insert at many different locations, relying on mechanisms that are distinct from homologous recombination. They often cause mutations, either by inserting into another gene and disrupting it or by promoting DNA rearrangements

Definition of Transposons: Transposons are found to encode a special protein named as transposase which catalyses the process of transposition. Transposons are particular to different groups of organisms. They constitute a fairly accountable fraction of genome of organisms like fungi, bacteria, plants, animals and humans. Transposons have had a major impact on changing or altering the genetic composition of organisms.

Transposons or transposable genetic elements are often referred to as 'mobile genetic elements' also. They can be categorized on different bases like their mode of transposition or on the basis of the organisms in which they are present.

Types of Transposons:

Different transposons may change their sites by following different transposition mechanisms.

On the basis of their transposition mechanism, transposons may be categorized into following types:

(i) Cut-and-Paste Transposons:

They transpose by excision (cutting) of the transposable sequence from one position in the genome and its insertion (pasting) to another position within the genome.

Presence of transposable elements was first predicted by Barbara McClintock in maize (corn) in late 1940s. After several careful studies, she found that certain genetic elements were moving from one site to an entirely different site in the chromosome. She called this phenomenon of changing sites of genetic elements as transposition and those genetic elements were called by her as controlling elements.

These controlling elements were later on called as transposable elements by Alexander Brink. In late 1960s this phenomenon was also discovered in bacteria.

Consequently, the molecular biologists called them as Transposons. A transposon may be defined as: "a DNA sequence that is able to move or insert itself at a new location in the

genome." The phenomenon of movement of a transposon to a new site in the genome is referred to as transposition.

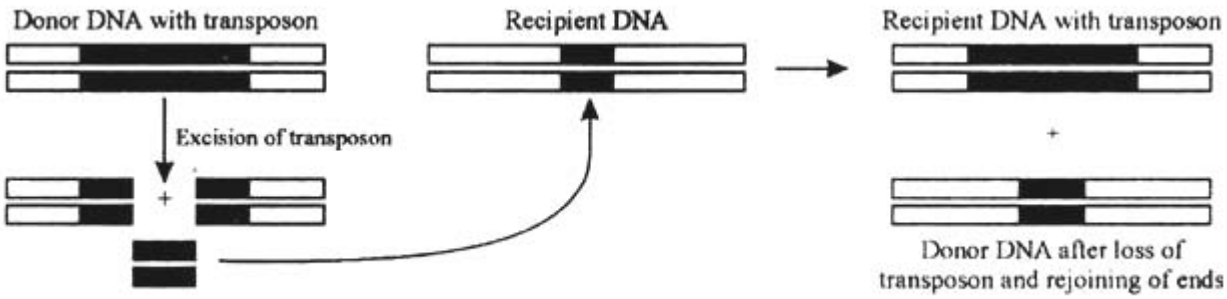


Fig. 1. Cut and Paste Transposons.

Source: http://www.biologydiscussion.com/biotechnology/transposons

The cut-and-paste transposition involves two transposase subunits. Each transposase submit binds to the specific sequences at the two ends of transposon. These subunits of transposase protein then come together and lead to the excision of transposon.

This excised 'transposon-Transposase Complex' then gets integrated to the target recipient site. In this manner, the transposon is cut from one site and then pasted on other site by a mechanism mediated by transposase protein.

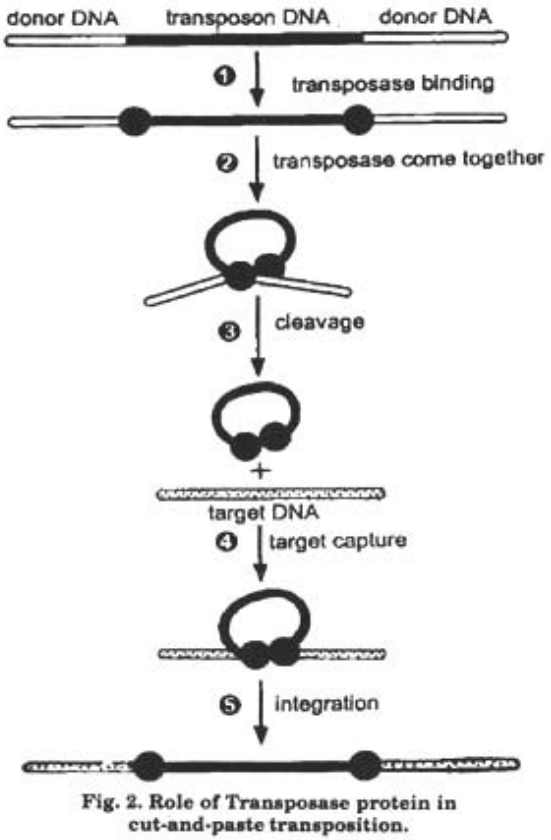


Fig. 2. Role of Transposase protein in cut-and-paste transposition.

Source: http://www.biologydiscussion.com/biotechnology/transposons

Examples of cut-and-paste type of transposons are IS-elements, P-elements in maize, hobo-elements in *Drosophila* etc.

(ii) Replicative Transposons:

They transpose by a mechanism which involves replication of transposable sequence and this copy of DNA, so formed, is inserted into the target site while the donor site remains unchanged. Thus, in this type of transposition, there is a gain of one copy of transposon and both the donor and the recipient DNA molecule are having one-one transposable sequence each, after transposition.

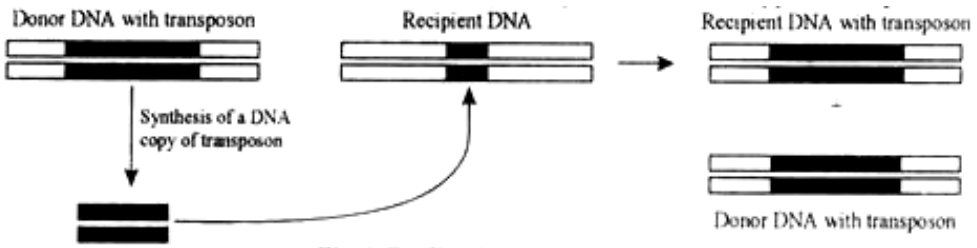


Fig. 3. Replicative Transposons.

Source: http://www.biologydiscussion.com/biotechnology/transposons

Tn3-elements found in bacteria are good examples of such type of transposons.

(iii) Retro Elements:

Their transposition is accomplished through a process which involves the synthesis of DNA by reverse transcription (i.e., RNA DNA) by using elements RNA as the template. This type of transposition involves an RNA intermediate; the transposable DNA is transcribed to produce an RNA molecule.

This RNA is then used as a template for producing a complementary DNA by the activity of enzyme reverse transcriptase. This single stranded DNA copy so formed is then made double stranded and then inserted into the target DNA site. The transposable elements which require reverse transcriptase tor their movement are called retro transposons.

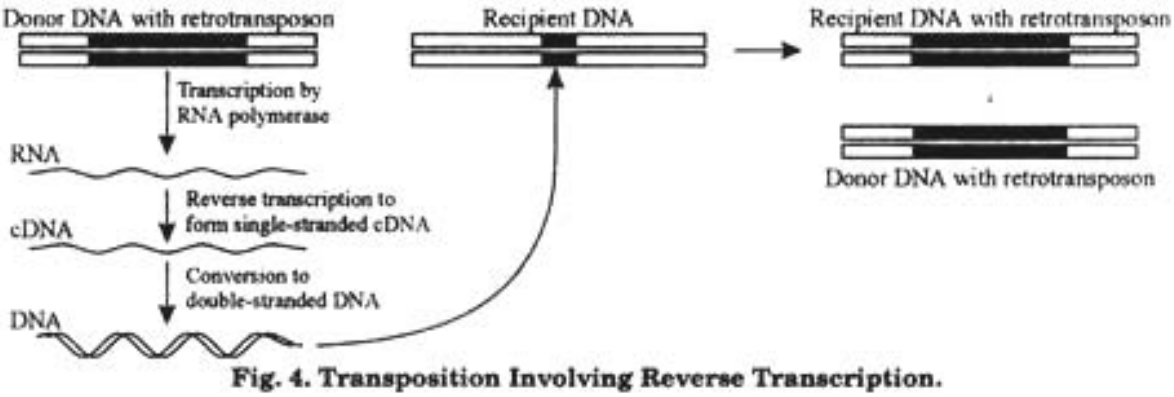


Fig. 4. Transposition Involving Reverse Transcription.

Source: http://www.biologydiscussion.com/biotechnology/transposons

The Retro elements may be viral or non-viral. Out of these two, the non-viral retro elements are important and may further be classified as:

(A) Retrovirus like elements:

They carry long terminal repeats (LTR). Examples are copia, gypsy elements in *Drosophila*.

Retroposons:

LTR are absent. Examples are LINEs and SINEs in humans.

Transposable Elements in Prokaryotes:

Although the presence of transposons was predicted in eukaryotes but first observation at molecular level was done in bacteria, which is a prokaryote.

Bacterial transposable elements are of the following types:

(a) Insertion Sequences or IS Elements:

They are the transposable sequences which can insert at different sites in the bacterial chromosomes.

IS-elements contain ITRs (Inverted Terminal Repeats), these were first observed in *E.coli*. IS elements are relatively short usually not exceeding 2500 bp. The ITRs present at the ends of IS-elements are an important feature which enables their mobility. The ITRs present in the IS-elements of *E.coli* usually range between 18-40 bp.

The term 'Inverted Terminal Repeat' (ITR) implies that the sequence at 5 end of one strand is identical to the sequence at 5′ end of the other strand but they run in inverse opposite direction (Fig. 5). In Ecoli chromosome, a number of copies of several IS-elements like IS1, IS2, IS3, IS4 and IS5 are present.

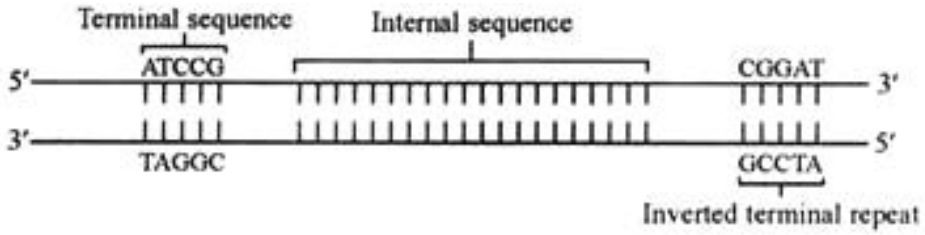


Fig. 5. An Insertion sequence (IS-element) with inverted terminal repeats (ITRs)

Source: http://www.biologydiscussion.com/biotechnology/transposons

(b) Prokaryotic Transposon Element:

These are also called composite transposons and are shown by the symbol Tn. It is made up of two IS elements, one present at each end of a DNA sequence which contains genes whose functions are not related to the transposition process. These transposons have been found to

have inverted repeats at the ends. The length of these inverted repeats ranges from a few nucleotides to about 1500 bp.

It can be said that these are the large transposons which are formed by capturing of an immobile DNA sequence within two insertion sequences thus enabling it to move. Examples of such transposons include the members of Tn series like Tn1, Tn5, Tn9, Tn10, etc.

Transposable Elements in Eukaryotes:

(a) Transposons in Maize:

Different types of transposons present in maize are described below:

Ac-Ds system:

This system of transposable elements in maize was analyzed and given by Barbara Mc. Clintock. Here Ac stands for Activator and Ds for Dissociation. Barbara found that Ds and Ac genes were sometimes mobile and moved to different chromosomal locations thus resulting in different kernel phenotypes.

Ds element is activated by Ac and on activation it serves as the site provider for breakage in chromosome. Ac can move autonomously while Ds can move only in the presence of Ac. The transposition involving this Ac-Ds system produces altered kernel phenotypes.

Other transposable elements of maize are:

i. spm (suppressor mutator) system,

ii. dt (dotted) system,

iii. Mu (Mutator) system, etc.

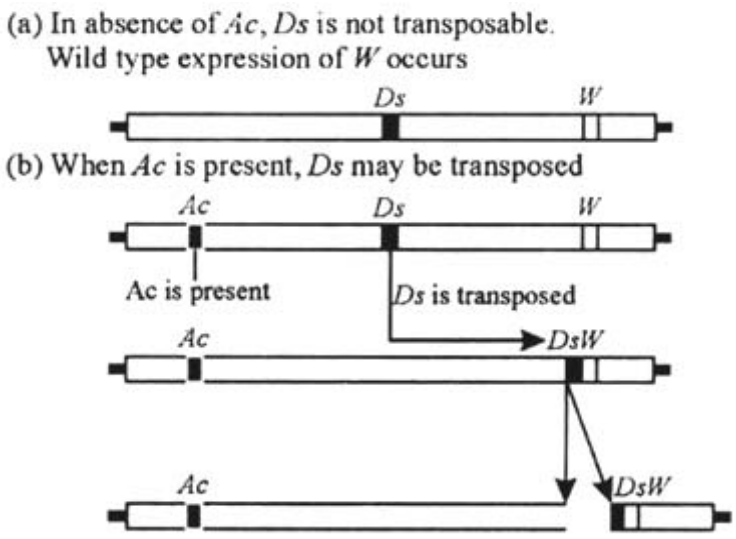


Fig. 6. Effects of Transposition involving *Ac-Ds* system in maize.

Source: http://www.biologydiscussion.com/biotechnology/transposons

(b) Transposons in *Drosophila*:

A number of transposable elements are found in *Drosophila* which are of different types and account for a quite high fraction of *Drosophila* genome.

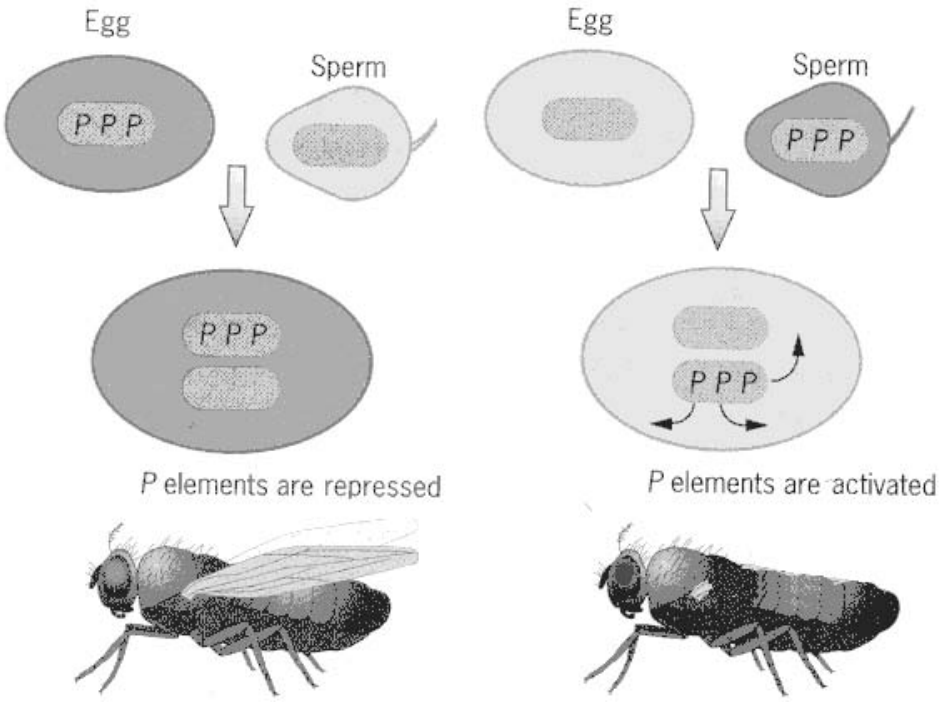
P-elements:

These were discovered during the study of 'hybrid-dysgenesis' which is a sterility causing condition. They are 2.9 kb long and contain 31 bp long inverted terminal repeats High rate of P-element transposition causes hybrid dysgenesis. P-elements encode transposase enzyme which helps in their transposition. These are also useful as vectors for introducing foreign genes into *Drosophila*.

P-elements are transposable elements that carry genes for transposase activity that cause the elements to move, and repressor activity that prevents expression of transposase.

In a cross between a P-element-carrying female and a laboratory male [left], repressors in the maternally derived cytoplasm repress expression of the maternally inherited P elements. The resulting offspring show the wild-type phenotype.

In a cross between a P-element-carrying male and a laboratory female [right], repressors are absent in the maternally - derived cytoplasm. The two zygotes are chromosomally identical but cytoplasmically different. In the right-hand cross, P elements are activated and undergo transposition in the genome, causing release of mutator activity and a variety of dysgenic phenotypes in the offspring.



Source: http://www.mun.ca/biology/scarr/P-element_hybrid_dysgenesis.htm

14. Population genetics: Hardy-Weinberg principle; gene frequency in a population, genetic equilibrium, factors affecting gene frequency.

Populations

A population is a group of organisms of the same species that are found in the same area and can interbreed. A population is the smallest unit that can evolve—in other words, an individual can't evolve.

Alleles

An allele is a version of a gene, a heritable unit that controls a particular feature of an organism.

For instance, Mendel studied a gene that controls flower color in pea plants. This gene comes in a white allele, w, and a purple allele, W. Each pea plant has two gene copies, which may be the same or different alleles. When the alleles are different, one—the dominant allele, W—may hide the other—the recessive allele, w. A plant's set of alleles, called its genotype, determines its phenotype, or observable features, in this case flower color.

Population genetics is the branch of genetics that deals with frequencies of alleles and genotypes in breeding populations. Population genetics examines allelic variation among individuals, the transmission of allelic variants from parents to offspring generation after generation, and the temporal changes that occur in the genetic makeup of a population because of systematic and random evolutionary forces.

Allele frequency

Allele frequency refers to how frequently a particular allele appears in a population. For instance, if all the alleles in a population of pea plants were purple alleles, W, the allele frequency of W would be 100%, or 1.0. However, if half the alleles were W and half were w, each allele would have an allele frequency of 50%, or 0.5.

Estimation of allelic frequencies:

Frequency of the M-N Blood Types in a Sample of 6129 Individuals

Blood Type	Genotype	Number of Individuals
M	$L^M L^M$	1787
MN	$L^M L^N$	3039
N	$L^N L^N$	1303

Because an entire population is usually too large to study, we resort to analyzing a representative sample of individuals from it. The blood types are determined by two alleles of a gene on chromosome 4: *LM*, which produces the M blood type, and *LN*, which produces the

N blood type. People who are *LMLN* heterozygotes have the MN blood type. To estimate the frequencies of the *LM* and *LN* alleles, we simply calculate the incidence of each allele among all the alleles sampled:

1. Because each individual in the sample carries two alleles of the blood-type locus, the total number of alleles in the sample is two times the sample size: 2 × 6129 = 12,258.

2. The frequency of the *LM* allele is two times the number of *LMLM* homozygotes plus the number of *LMLN* heterozygotes, all divided by the total number of alleles sampled: [(2 × 1787) + 3039]/12,258 = 0.5395.

3. The frequency of the *LN* allele is two times the number of *LNLN* homozygotes plus the number of *LMLN* heterozygotes, all divided by the total number of alleles sampled: [(2 × 1303) + 3039]/12,258 = 0.4605.

Thus, letting *p* represent the frequency of the *LM* allele and letting *q* represent the frequency of the *LN* allele, we estimate that in the population from which the sample was taken, $p = 0.5395$ and $q = 0.4605$. Furthermore, because *LM* and *LN* represent 100 percent of the alleles of this particular gene, $p + q = 1$.

The Hardy–Weinberg principle:

In the first decade of the twentieth century, these questions were posed independently by G. H. Hardy, a British mathematician, and by Wilhelm Weinberg, a German physician. In 1908 Hardy and Weinberg each published papers describing a mathematical relationship between allele frequencies and genotype frequencies. This relationship, now called the **Hardy–Weinberg principle,** allows us to predict a population's genotype frequencies from its allele frequencies.

The Law states that gene frequencies in a population remain constant from generation to generation if no evolutionary processes like migration, mutation, selection and drift are operating.

Thus if matings are random, and no other factors disturb the reproductive abilities of any genotype, the equilibrium genotypic frequencies are given by the square of the allelic frequencies.

Let's suppose that in a population a particular gene is segregating two alleles, *A* and *a*, and that the frequency of *A* is *p* and that of *a* is *q*. Thus, on the assumption of random mating, the predicted frequencies of the three genotypes in the population are:

Genotype	Frequency
AA	p^2
Aa	$2pq$
aa	q^2

These predicted frequencies can be obtained by expanding the binomial expression $(p + q)^2 = p^2 + 2pq + q^2$. Population geneticists refer to them as the Hardy–Weinberg genotype frequencies.

Hardy–Weinberg equilibrium:

Random mating and no differential survival or reproduction among the members of the population, the Hardy–Weinberg genotype frequencies—and, of course, the underlying allele frequencies—persist generation after generation. This condition is referred to as the Hardy–Weinberg equilibrium.

Factor affecting gene frequencies:

Some of the major factors which affect the genetic equilibrium and induce the variability in population are as follows:

(A) Mutations (B) Recombinations during Sexual Reproduction (C) Genetic Drift (D) Gene Migration (Gene Flow) (E) Natural Selection.

(A) Mutations:

These are characterized by:

(i) These are sudden, large and inheritable changes in the genetic material.

(ii) Mutations are random (indiscriminate) and occur in all directions.

(iii) Most mutations are harmful or neutral. It is estimated that only one out of 1,000 mutations is useful.

(iv) Rate of mutation is very low, i.e., one per million or one per several million genie loci. But rate of mutation is sufficient to produce considerable genetic variability.

(v) Certain mutations are pre-adaptive and appear even without exposure to a specific environment. These express and become advantageous only when after exposure to new environment which only selects the pre-adaptive mutations that occurred earlier.

(viii) Significance of mutations:

(a) Mutations create and maintain variations within a population.

(b) These also introduce new genes and alleles in a gene pool.

(c) Accumulation of mutations over a number of generations may lead to speciation.

(B) Recombinations during Sexual Reproduction:

Recombination involves reshuffling of genes of chromosomes. Chances of recombination are more in those organisms which undergo sexual reproduction which involves gametogenesis followed by fertilization.

Sexual reproduction involves recombinations during three stages:

(i) Crossing over

(ii) By independent assortment of chromosomes

(iii) By random fertilization

Significance:

Due to recombination's, though only reshuffling of already existing characters takes place and no new genes are produced but it leads to redistribution of different traits to different individuals of a population. Different combinations bring diversity in genotype and phenotype of different organisms. So recombination is an agent of evolution.

(C) Genetic Drift:

It is the random change in the frequency of alleles occurring by chance fluctuations. It is characterized by:

(i) It is a binomial sampling error of the gene pool, i.e., that alleles which form the gene pool of the next generation are a sample of the alleles of present population.

(ii) Genetic drift always influences frequencies of alleles and is inversely proportional to the size of population. So genetic drift is most important in very small populations in which there are increased chances of inbreeding which increases the frequency of individuals homozygous for recessive alleles, many of which maybe deleterious.

(iii) Genetic drift occurs when a small group separates from a larger population and may not have all the alleles or may differ from the parental population in the frequencies of certain genes. This explains for the difference between island populations and mainland population.

(iv) In a small population, a chance event (e.g. snow storm) may increase the frequency of a character having little adaptive value.

(v) Genetic drift can also operate through founder effect. In this, genetic drift can cause dramatic changes in the allele frequencies in a population derived from small groups of colonisers, called founders, to a new habitat.

These founders do not have all of the alleles found in their source population. These founders become quickly different from the parental population and may form a new species, e.g. evolution of Darwin finches on Galapagos Islands which were probably derived from a few initial founders.

(vi) Population bottleneck:

It is reduction in allele frequencies caused by drastic reduction in population size called population crash e.g. decrease in cheetah population in Africa due to over-hunting. As the given gene pool is limited, population bottleneck often prevents the species to reestablish its former richness so new population has a much restricted gene pool than the larger parent population.

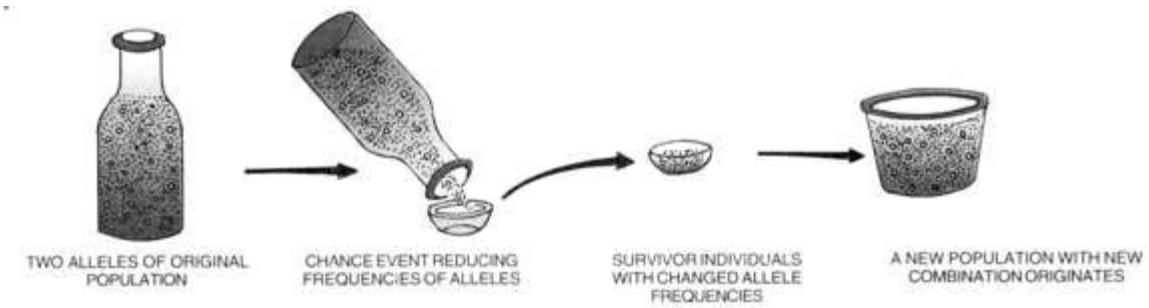


Fig. 7.47. Bottleneck effect on the frequencies of two alleles.

Source: http://www.yourarticlelibrary.com/biology/

(D) Gene Migration (Gene Flow):

Most populations are only partially isolated from other populations of same species. Usually some migration-emigration (moving out of some individuals out of a population) or immigration (entry of some members of a population into another population of same species) occurs between the populations.

Immigration results in the addition of new alleles into the existing gene pool and changes the allele frequencies. Degree of changes in allele frequencies depends upon the differences between the genotypes of immigrants and native population.

If there is no much genetic differences, then entry of a small number of migrants will not change the allele frequencies much. However, if the populations are genetically quite different, a small amount of immigration can result in large changes in allele frequencies.

If the migrating individuals interbreed with the members of local population, called hybridization, these may bring many new alleles into the local gene pool of the host population. This is called gene migration. If the inter specific hybrids are fertile, then these may initiate a new trend in evolution which lead to formation of new species.

(E) Natural Selection:

The process by which comparatively better adapted individuals out of a heterogeneous population are favored by the Nature over the less adapted individuals is called natural selection.

Types of Natural selection:

The three different types of natural selections observed are:

1. Stabilizing or balancing selection:

It leads to the elimination of organisms having overspecialized characters and maintains homogenous population which is genetically constant. It favors the average or normal phenotypes, while eliminates the individuals with extreme expressions. In this, more individuals acquire mean character value.

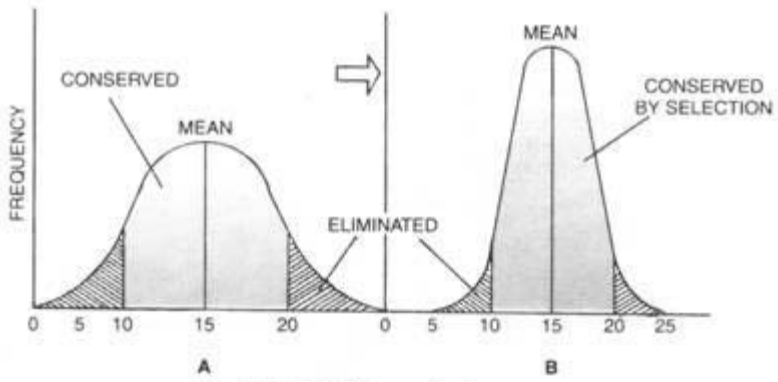


Fig. 7.49. Stabilizing selection.

Source: http://www.yourarticlelibrary.com/biology/

It reduces variation but does not change the mean value. It results very slow rate of evolution. If we draw a graphical curve of population, it is bell-shaped. The bell-shaped curve narrows due to elimination of extreme variants.

Example:

Sickle-cell anaemia in human beings (Explained in Neo-Darwinism).

2. Directional or Progressive selection:

In this selection, the population changes towards one particular direction along with change in environment. As environment is undergoing a continuous change, the organisms having acquired new characters survive and others are eliminated gradually.

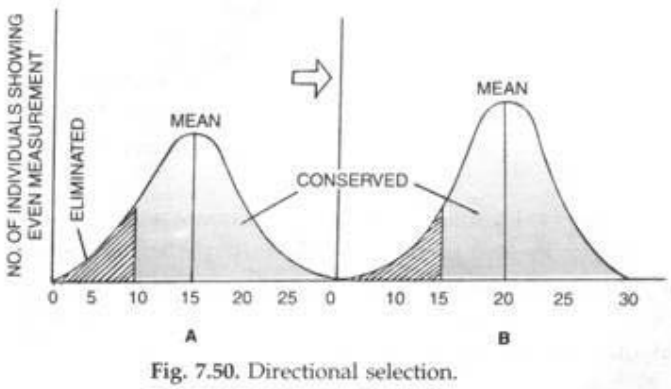


Fig. 7.50. Directional selection.

Source: http://www.yourarticlelibrary.com/biology/

In this, individuals at one extreme (less adapted) are eliminated while individuals at other extreme (more adapted) are favored. This produces more and more adapted individuals in the population when such a selection operates for many generations. In this type of selection, more individuals acquire value other than mean character value.

Examples:

Industrials melanism (Explained in Neo-Darwinism):

In this, number of the light coloured moths {*Biston betularia*) decreased gradually while that of the melanic moths (*B. carbonaria*) increased showing directional selection.

3. Disruptive selection:

It is a type of natural selection which favors extreme expressions of certain traits to increase variance in a population. It breaks a homogeneous population into many adaptive forms. It results in balanced polymorphism.

In this type of selection, more individuals acquire peripheral character value at both ends of the distribution curve. This kind of selection is rare and eliminates most of the members with mean expression so producing two peaks in the distribution of a trait.

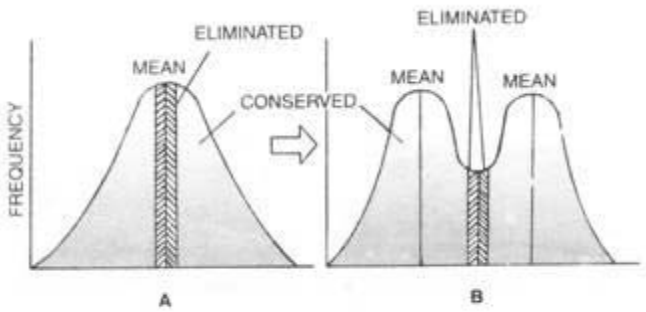


Fig. 7.51. Disruptive selection.

Source: http://www.yourarticlelibrary.com/biology/

Example:

In sea, the three types of snails i.e., white colored; brown colored and black colored are present. The white colored snails are invisible when covered by barnacles. The black colored snails are invisible when rock is bare. But brown colored snails are eaten by predators in both the conditions. So these are eliminated gradually.

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15. Cell cycle regulation and cancer: Role of proteins in controlling cell cycle; apoptosis; oncogenes and protooncogenes; tumour suppressor genes; role of E2F and p53 in controlling cell cycle; cancer therapy.

Cell cycle regulators

Introduction

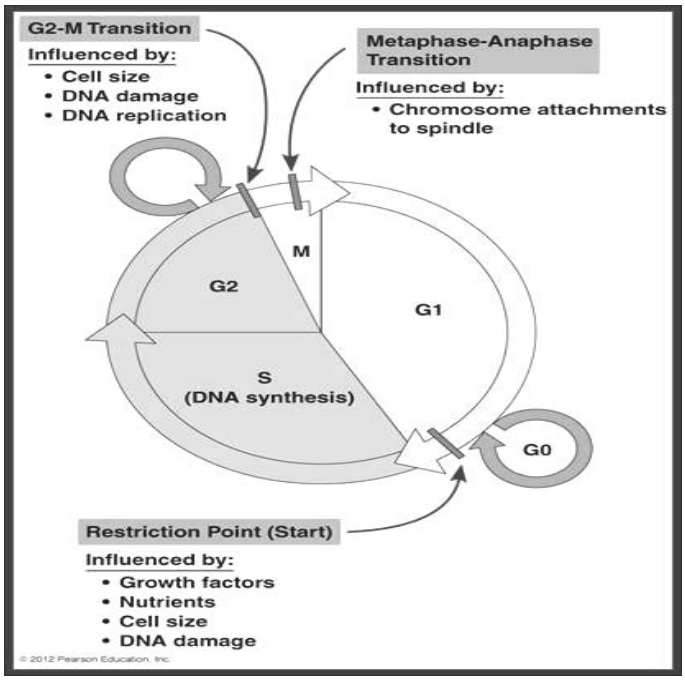
As cells move through the cell cycle, do they breeze through from one phase to the next? If they're cancer cells, the answer might be yes. Normal cells, however, move through the cell cycle in a regulated way. They use information about their own internal state and cues from the environment around them to decide whether to proceed with cell division. This regulation makes sure that cells don't divide under unfavorable conditions (for instance, when their DNA is damaged, or when there isn't room for more cells in a tissue or organ).

Cell cycle checkpoints

A checkpoint is a stage in the eukaryotic cell cycle at which the cell examines internal and external cues and "decides" whether or not to move forward with division.

There are a number of checkpoints, but the three most important ones are:

- The G1 checkpoint, at the G1 /S transition.
- The G2 checkpoint, at the G2 /M transition.
- The spindle checkpoint, at the transition from metaphase to anaphase.



Source: https://www.mun.ca/biology/desmid/brian/BIOL2060/BIOL2060-19

The G_1 checkpoint

The G_1 checkpoint is the main decision point for a cell – that is, the primary point at which it must choose whether or not to divide. Once the cell passes the G_1 checkpoint and enters S phase, it becomes irreversibly committed to division. That is, barring unexpected problems, such as DNA damage or replication errors, a cell that passes the G_1 checkpoint will continue the rest of the way through the cell cycle and produce two daughter cells.

At the At the G_1 checkpoint, a cell checks whether internal and external conditions are right for division. Here are some of the factors a cell might assess:

- **Size.** Is the cell large enough to divide?
- **Nutrients.** Does the cell have enough energy reserves or available nutrients to divide?
- **Molecular signals.** Is the cell receiving positive cues (such as growth factors) from neighbors?
- **DNA integrity**. Is any of the DNA damaged?

These are not the only factors that can affect progression through the G_1checkpoint, and which factors are most important depend on the type of cell. For instance, some cells also need mechanical cues (such as being attached to a supportive network called the extracellular matrix) in order to divide

If a cell doesn't get the go-ahead cues it needs at the G_1 checkpoint, it may leave the cell cycle and enter a resting state called G_0 phase. Some cells stay permanently in G_0 , while others resume dividing if conditions improve. Checkpoint, a cell checks whether internal and external conditions are right for division. Here are some of the factors a cell might assess:

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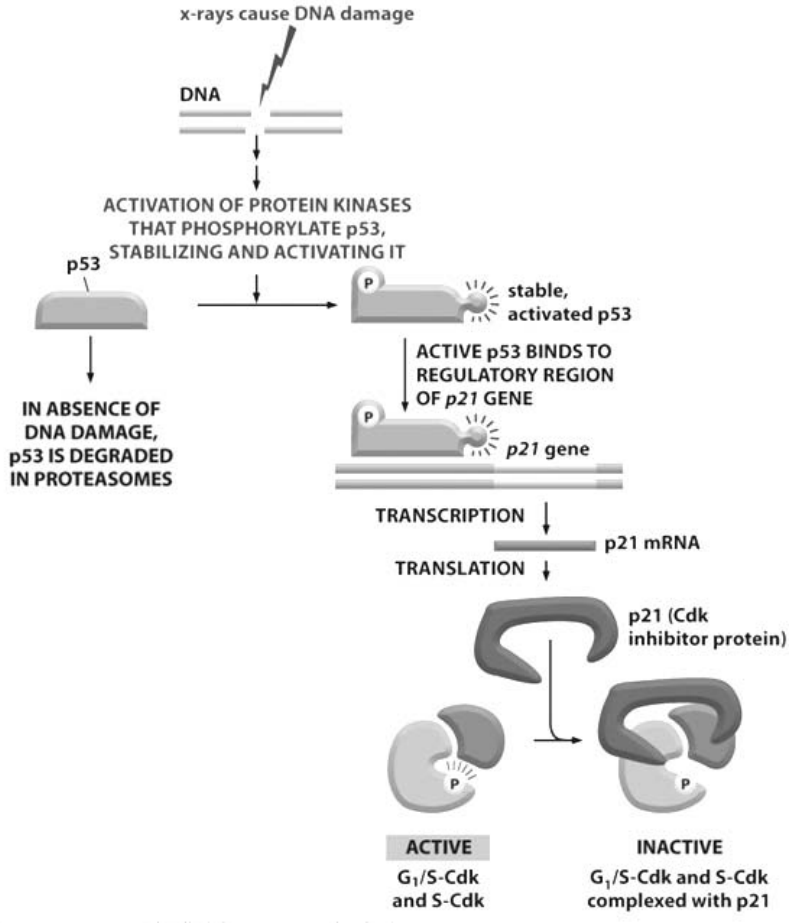
If a cell doesn't get the go-ahead cues it needs at the G_1 checkpoint, it may leave the cell cycle and enter a resting state called G_0 phase. Some cells stay permanently in G_0, while others resume dividing if conditions improve.

This checkpoint monitors damaged DNA. If DNA damage is detected, checkpoint protein will prevent the formation of active S-Cdk complex and blocks the progression of S-phase. In mammals, a protein of p53 gene cause delay in entry of cells with damaged DNA into S-phase by following ways:



Source: Bruce Alberts Molecular Biology of the Cell. 2015. 6th ed:

The G_2 checkpoint

To make sure that cell division goes smoothly (produces healthy daughter cells with complete, undamaged DNA), the cell has an additional checkpoint before M phase, called the G_2 checkpoint. At this stage, the cell will check:

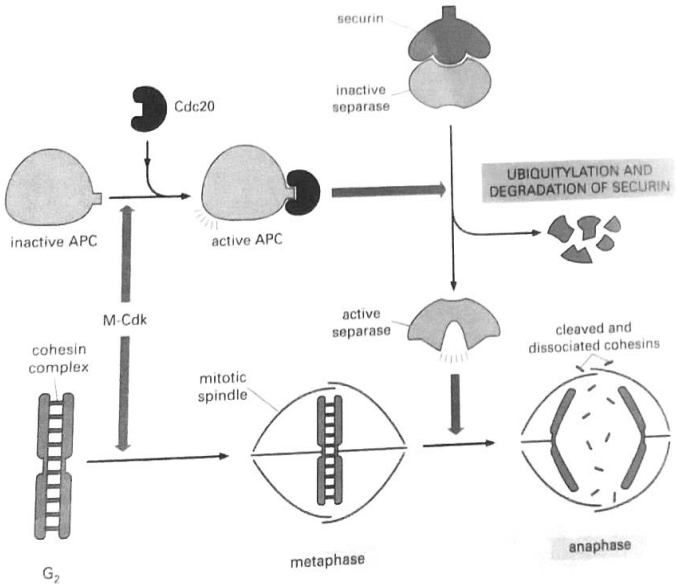
- **DNA integrity.** Is any of the DNA damaged?
- **DNA replication.** Was the DNA completely copied during S phase?

If errors or damage are detected, the cell will pause at the G_2 checkpoint to allow for repairs. If the checkpoint mechanisms detect problems with the DNA, the cell cycle is halted, and the cell attempts to either complete DNA replication or repair the damaged DNA.

If the damage is irreparable, the cell may undergo apoptosis, or programmed cell death. This self-destruction mechanism ensures that damaged DNA is not passed on to daughter cells and is important in preventing cancer.

The spindle checkpoint

The M checkpoint is also known as the spindle checkpoint: here, the cell examines whether all the sister chromatids are correctly attached to the spindle microtubules. Because the separation of the sister chromatids during anaphase is an irreversible step, the cycle will not proceed until all the chromosomes are firmly attached to at least two spindle fibers from opposite poles of the cell.



Source: Bruce Alberts Molecular Biology of the Cell. 2015. 6th ed:

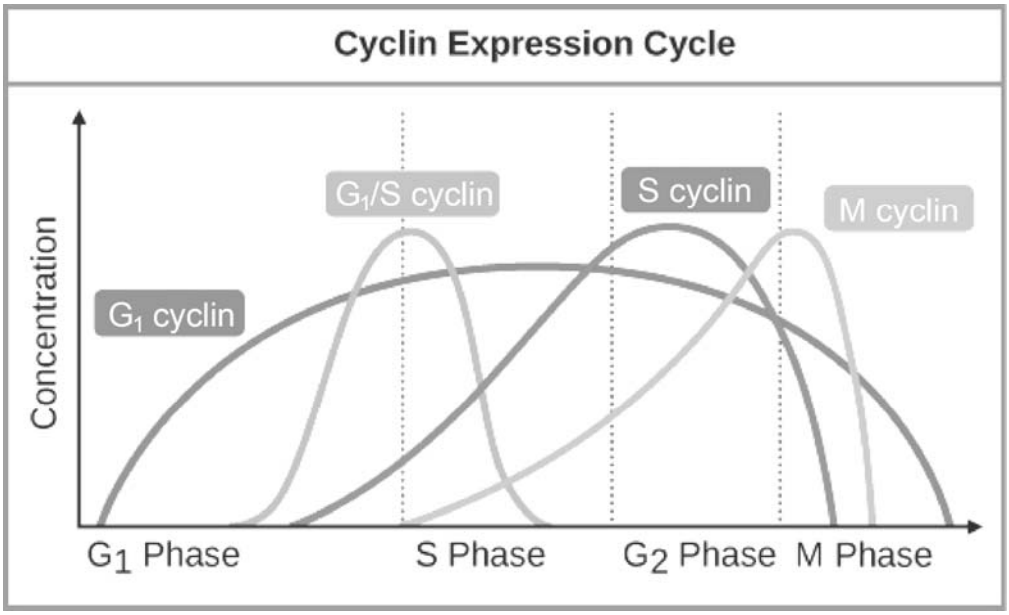
The tension created by this bipolar attachment, which initiates the anaphase entry. To do this, the sensing mechanism ensures that the **anaphase-promoting complex (APC)** is no longer inhibited, which is now free to degrade cyclin B and to break down **securin.** The latter is a protein whose function is to inhibit **separase**, which in turn cuts the **cohesins**, the protein composite responsible for cohesion of sister chromatids. Once this inhibitory protein is degraded via ubiquitination and subsequent proteolysis. Separase then causes sister chromatid separation.

Mutants of mad2 and BUB genes of mammals and yeast inactivate this spindle separation.

Cyclins

Cyclins are among the most important core cell cycle regulators. Cyclins are a group of related proteins, and there are four basic types found in humans and most other eukaryotes: G_1 cyclins, G_1/S cyclins, S cyclins, and M cyclins.

As the names suggest, each cyclin is associated with a particular phase, transition, or set of phases in the cell cycle and helps drive the events of that phase or period. For instance, M cyclin promotes the events of M phase, such as nuclear envelope breakdown and chromosome condensation.



Source: https://www.khanacademy.org/

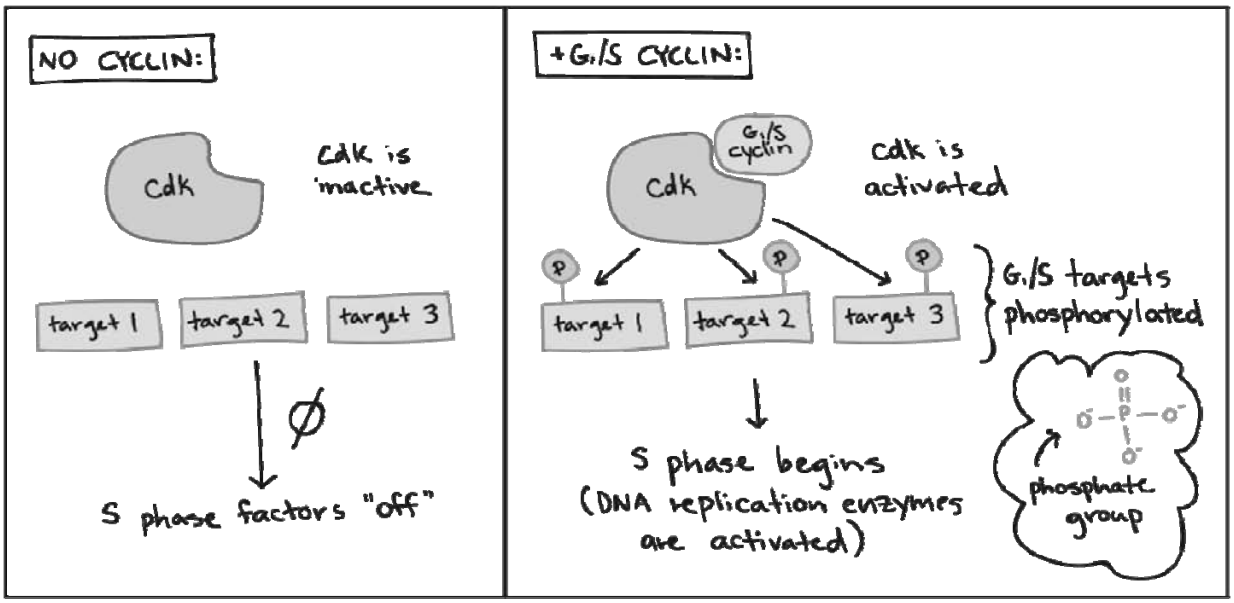
The levels of the different cyclins vary considerably across the cell cycle, as shown in the diagram at right. A typical cyclin is present at low levels for most of the cycle, but increases strongly at the stage where it's needed. M cyclin, for example, peaks dramatically at the transition from G_2 to M phase. G_1 cyclins are unusual in that they are needed for much of the cell cycle.

Cyclin-dependent kinases

In order to drive the cell cycle forward, a cyclin must activate or inactivate many target proteins inside of the cell. Cyclins drive the events of the cell cycle by partnering with a family of enzymes called the cyclin-dependent kinases (Cdks). A lone Cdk is inactive, but the binding of a cyclin activates it, making it a functional enzyme and allowing it to modify target proteins.

How does this work? Cdks are kinases, enzymes that phosphorylate (attach phosphate groups to) specific target proteins. The attached phosphate group acts like a switch, making the target protein more or less active. When a cyclin attaches to a Cdk, it has two important effects: it activates the Cdk as a kinase, but it also directs the Cdk to a specific set of target proteins, ones appropriate to the cell cycle period controlled by the cyclin. For instance, G_1/S

cyclins send Cdks to S phase targets (e.g., promoting DNA replication), while M cyclins send Cdks to M phase targets (e.g., making the nuclear membrane break down).



Source: https://www.khanacademy.org/

In general, Cdk levels remain relatively constant across the cell cycle, but Cdk activity and target proteins change as levels of the various cyclins rise and fall. In addition to needing a cyclin partner, Cdks must also be phosphorylated on a particular site in order to be active (not shown in the diagrams in this article), and may also be negatively regulated by phosphorylation of other sites

Cyclins and Cdks are very evolutionarily conserved, meaning that they are found in many different types of species, from yeast to frogs to humans. The details of the system vary a little: for instance, yeast has just one Cdk, while humans and other mammals have multiple Cdks that are used at different stages of the cell cycle. (Yes, this kind of an exception to the "Cdks don't change in levels" rule!) But the basic principles are quite similar, so that Cdks and the different types of cyclins can be found in each species

MPF and its role in cell cycle regulation:

Maturation-promoting factor (abbreviated MPF, also called mitosis-promoting factor or M-Phase-promoting factor) is the cyclin-Cdk complex that was discovered first in frog eggs. MPF promotes the entrance into mitosis (the M phase) from the G_2 phase by phosphorylating multiple proteins needed during mitosis. MPF is composed of two subunits:

Cyclin-dependent kinase 1 (CDK1 catalytic subunit) It uses ATP to phosphorylate specific serine and threonine residues of target proteins which is necessary for cell cycle progression.

Cyclin, a regulatory subunit. The cyclins are necessary for the kinase subunit to function with the appropriate substrate. In the absence of cyclin, the kinases are inactive.

G_1 phase progression by Cdk activity:

As the cell exists from mitosis, M-Cdk inactivation is initiated through M-cyclin degradation. Increase Hct1 and Sic1 activities results in stable CDK inactivation during G_1.

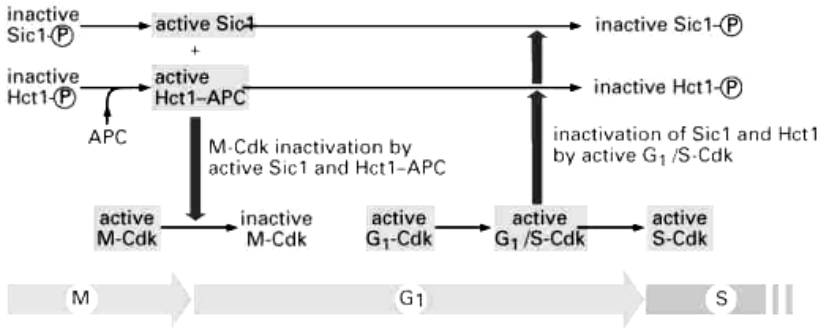


Figure 17–29. Molecular Biology of the Cell, 4th Edition.

Accumulation of G_1-cyclin that is resistant to Sic-1 and Hct-1-APC

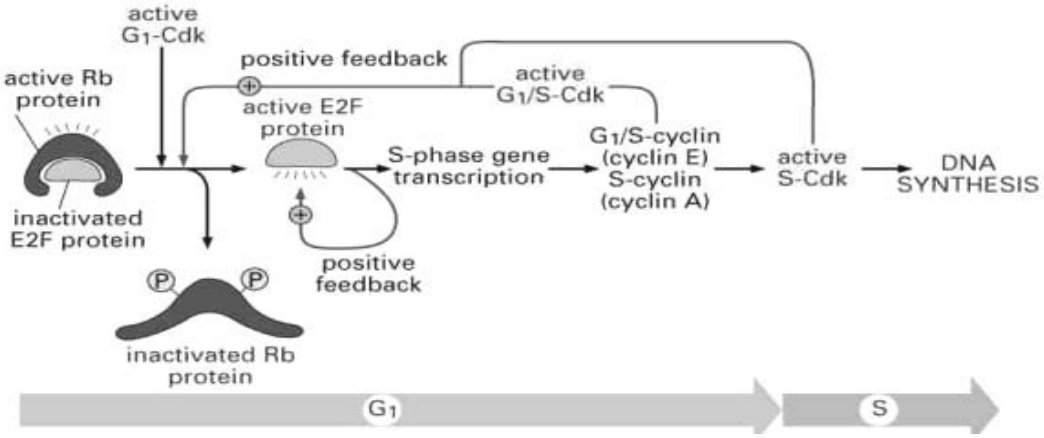
G_1-Cdk stimulates G_1/S-cyclin synthesis

G1/S-Cdk stimulates S-cyclin synthesis, and increase in S-Cdk activity

G1/S-Cdk phosphorylates Sic1 and Hct-1 and blocks their activity

Initiation of S-phase:

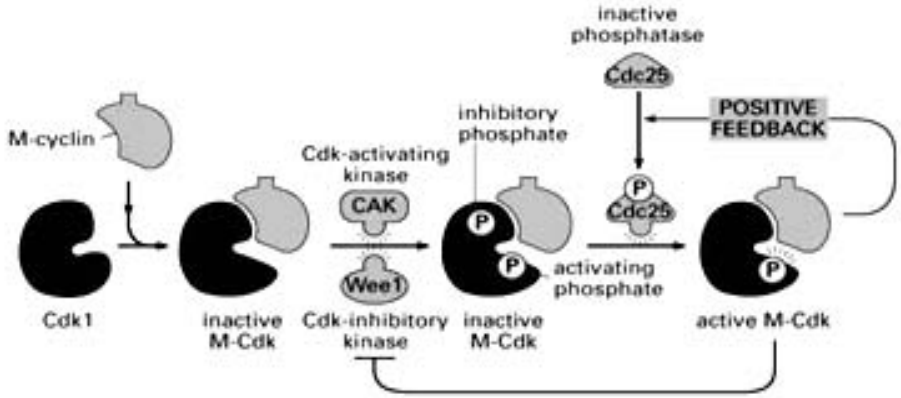
G_1-Cdk activity initiates Rb phosphorylation. This inactive Rb and freeing E2F to activate the transcription of gene for a G_1/S-cyclin, and S-cyclin. Enhancing S-Cdk Activity initiates DNA replication at ORC, complexed with Cdc6 and Mcm proteins.



Source: Bruce Alberts Molecular Biology of the Cell. 2015. 6th [ed]:

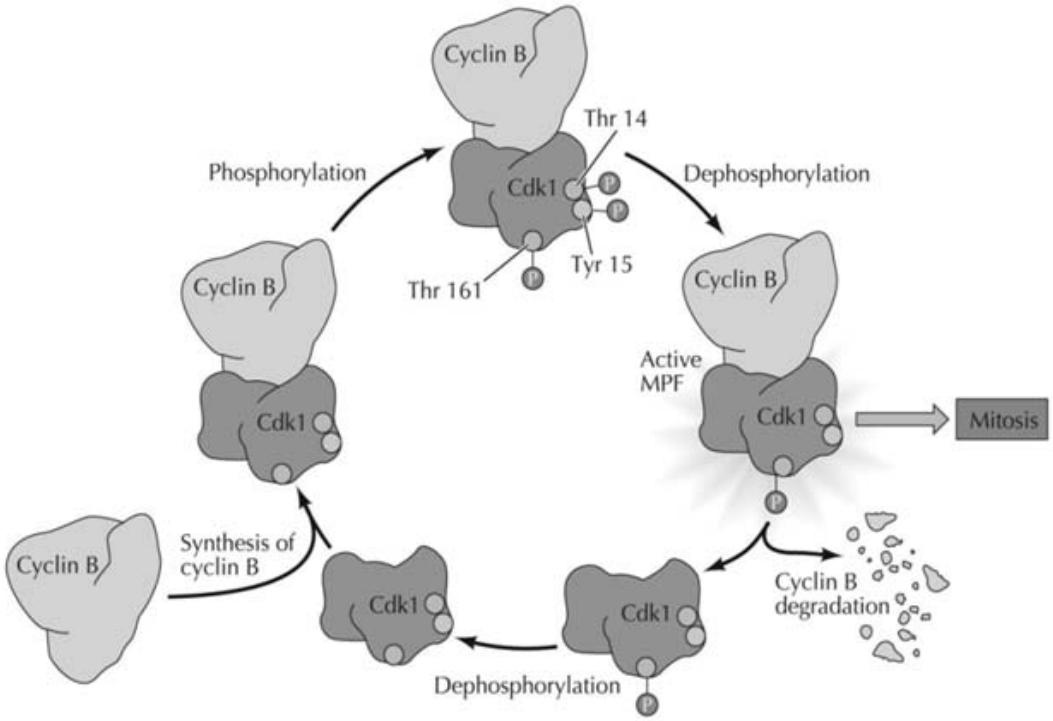
Entry into M-phase:

Cdk1 associates with M-cyclin as the levelsof M-cyclin gradually rise. The resulting M-cdk complex is phosphorylated on an activating site by the CAK (Cdk-activating kinase) and a pair of inhibitory sites by the Wee1 kinase. M-Cdk complex is then activated at the end of G_2 by the phosphatase Cdc25 and inhibit Wee1 activity. The cell enters into mitosis.



Source: Bruce Alberts Molecular Biology of the Cell. 2015. 6th ed:

The mechanism of phosphorylation and dephosphorylation of M-phase kinase actually control the cell cycle. Cdc2 forms complexes with cyclin B during S and G2. Cdc2 is then phosphorylated on threonine-161, which is required for Cdc2 activity, as well as on tyrosine-15 (and threonine-14 in vertebrate cells), which inhibits Cdc2 activity. Dephosphorylation of Thr14 and Tyr15 activates MPF at the G2 to M transition. MPF activity is then terminated toward the end of mitosis by proteolytic degradation of cyclin B.



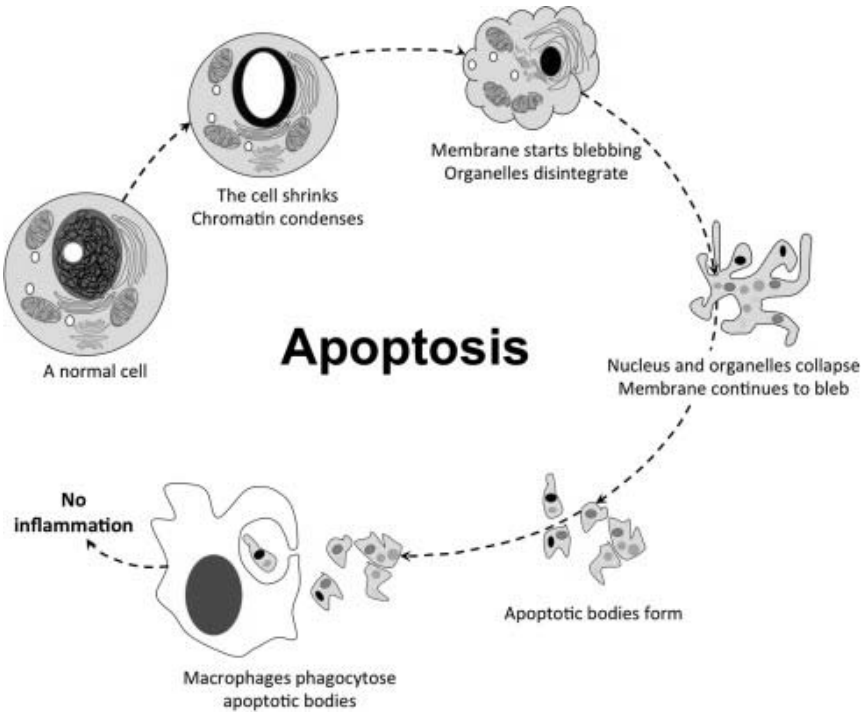
Mechanism of cell cycle regulation Source: Copper The cell 4ed

Apoptosis:

Apoptosis (Greek word meaning "falling off") is a process of programmed cell death that occurs in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay. Apoptosis can be triggered by a variety of stimuli, including withdrawal of essential growth factors, treatment with glucocorticoids, gamma irradiation etc.

Cellular events:

A cell that undergoes apoptosis dies neatly, without damaging its neighbors. The cell shrinks and condenses. The cytoskeleton collapses, the nuclear envelope disassembles, and the nuclear DNA breaks up into fragments. Most importantly, the cell surface is altered, displaying properties that cause the dying cell to be rapidly phagocytosed, either by a neighboring cell or by a macrophage before any leakage of its contents occurs This not only avoids the damaging consequences of cell necrosis but also allows the organic components of the dead cell to be recycled by the cell that ingests it.



Cytology of apoptosis. Source: https://www.researchgate.net/publication/274013152_Regulation_of_Ceramide_Channel_Formation_and_Disassembly_Insights_on_the_Initiation_of_Apoptosis

Mechanism:

There are 3 different caspase dependent pathways. This are-

1. **Extrinsic pathway:**

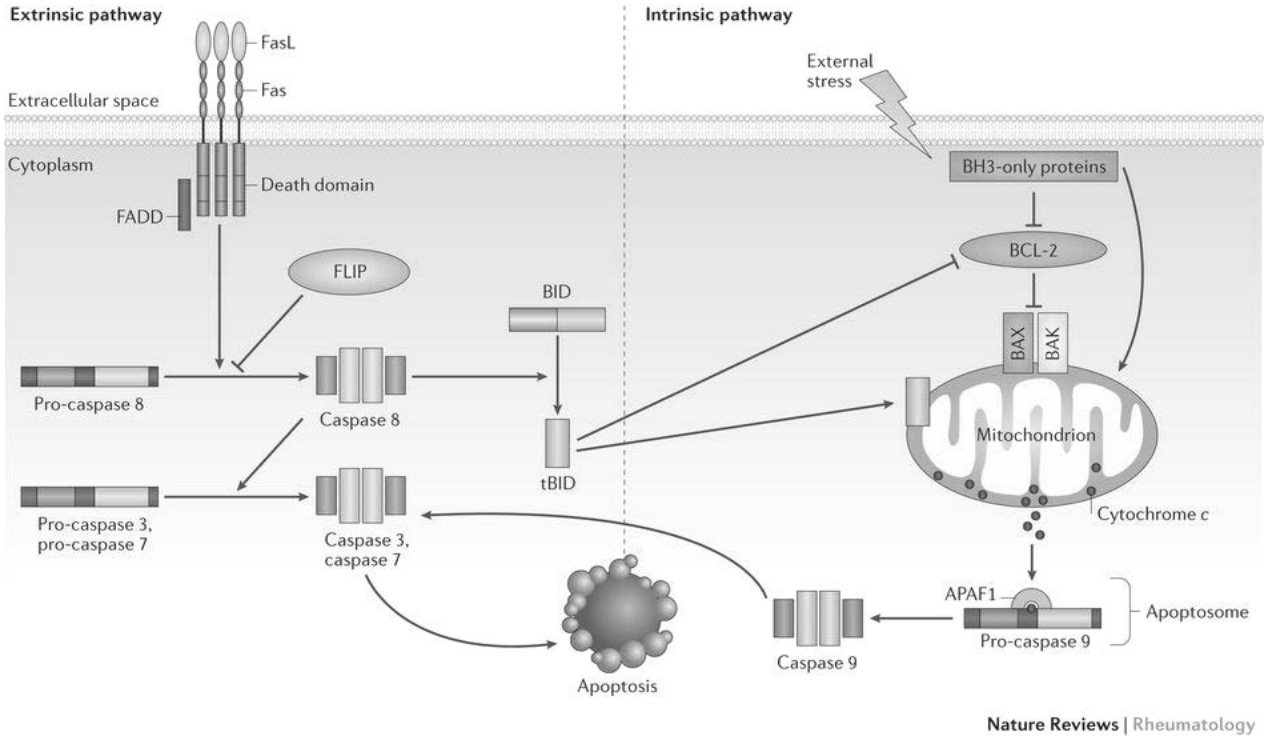
The fas receptor (First apoptosis signal) is a transmembrane protein of the TNF familywhich binds the Fas ligand (FasL). The interaction between Fas and FasL results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, caspase-8. The activated caspase-8 molecules the activated caspase-3 to induce apoptosis.

2. Intrinsic pathway:

Activation of Bcl-2 gene results mitochondrial change which leads release of cytochrome c. Once cytochrome c is released it binds with Apoptotic protease activating factor – 1 (Apaf-1) and ATP, which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector caspase-3.

3. Granazyme B pathway:

Granazyme B, a cytosolic T cell product, directly cleaves and activates several caspase, resulting in apoptosis.



Apoptotic pathway, Source: https://www.studyread.com/apoptosis-pathway/ Nature reviews

A number of genes have been identified which play role in the regulation of apoptosis. Such as-Ced-1-10, egl1

Ced 9 − Ced 3 − Ced 4 −Cell death

Caspase (A proteolytic enzyme**)** are cysteine proteases which cleaves the substrate at ehe C-terminal of an amino acid residue. Two caspase classes are-

Initiators (Caspase 2, 8, 9, 10)

Effectors (Caspase 3, 6, 7)

Caspase independent apoptotic pathway There also exist a caspase independent apoptotic pathway that is mediated by AIF (apoptosis-inducing factor).

Importance:

1. It is necessary mechanism complementary to proliferation to ensure homeostasis in all tissue.
2. Removal of a number of vestigial structure.
3. It is a necessary anticancer mechanism.

Cancer:

Cancer is a group of disease characterized by uncontrolled cell growth and division. Cancers arise when critical genes are mutated. These mutations can cause biochemical processes to go awry and lead to the unregulated proliferation of cells.Without regulation, cancer cells divide ceaselessly, piling up on top of each other to form tumors. When cells detach from a tumor and invade the surrounding tissues, the tumor is **malignant**. When the cells do not invade the surrounding tissues, the tumor is **benign**. Malignant tumors may spread to other locations in the body, forming secondary tumors. This process is called **metastasis.**

Possible causes:

1. Clonally inherited – genetic basis.
2. Some viruses can induce tumors experimentally.
3. Mutagenic agents, chemicals, ionizing radiations can induce tumor in experimental animals.
4. Colon cancer / cancer of eye – simple dominant inheritance.
5. Leukemias and lymphomas are associated with chromosomal aberrations.
6. Accumulation of spontaneous mutations in the somatic tissues.

Characteristics of cancer cells

1. Immortalization.
2. Loss of contact inhibition

3. Reduced cellular adhesion
4. Invasiveness – Malignant cells generally secrete Proteases that digest extra cellular matrix components, allowing the cancer cells to invade adjacent normal tissues. eg. Collagenase digests and penetrates through basal laminae to invade the underlying connective tissue.
5. Fail to differentiate – (Loss of size and shape of cells)
6. Autostimulation of cell division
7. Apoptosis – loss
8. High nucleus to cytoplasm ratio
9. Depolymerization of cytoskeleton – microtubules
10. Chromosomal changes – high degree of ploidy and aneuploidy
11. Interaction with Immune System.

Genetic basis for cancer

The recent great advances in understanding cancer have come through application of molecular genetic techniques. However, before these techniques were available to researchers, there was strong evidence that the underlying causes of cancer are genetic. First, it was known that the cancerous state is clonally inherited. When cancer cells are grown in culture, their descendants are all cancerous. The cancerous condition is therefore transmitted from each cell to its daughters at the time of division—a phenomenon indicating that cancer has a genetic (or epigenetic) basis. Second, it was known that certain types of viruses can induce the formation of tumors in experimental animals. The induction of cancer by viruses implies that the proteins encoded by viral genes are involved in the production of the cancerous state. Third, it was known that cancer can be induced by agents capable of causing mutations. Mutagenic chemicals and ionizing radiation had been shown to induce tumors in experimental animals. In addition, a wealth of epidemiological data had implicated these agents as the causes of cancer in humans. Fourth, it was known that certain types of cancer tend to run in families. In particular, susceptibility to retinoblastoma, a rare cancer of the eye, and susceptibility to some forms of colon cancer appeared to be inherited as simple dominant conditions, albeit with incomplete penetrance and variable expressivity. Because susceptibility to these special types of cancer is inherited, it seemed plausible that all cancers might have their basis in genetic defects—either inherited mutations or somatic mutations acquired during a person's lifetime. Finally, it was known that certain types of white blood cell cancers (leukemias and lymphomas) are associated with particular chromosomal

aberrations. Collectively, these diverse observations strongly suggested that cancer is caused by genetic malfunctions.

Oncogenes

Genes that promote autonomous cell growth in cancer cells are called **oncogenes**, and their normal cellular counterparts are called **proto-oncogenes**. Proto-oncogenes are physiologic regulators of cell proliferation and differentiation while oncogenes are characterized by the ability to promote cell growth in the absence of normal mitogenic signals. Their products, oncoproteins, resemble the normal products of proto-oncogenes with the exception that oncoproteins are devoid of important regulatory elements. Their production in the transformed cells becomes constitutive, that is, not dependent on growth factors or other external signals. Proto-oncogenes can be converted to oncogenes by several mechanisms, such as,

1. Mutational change in the protein.
2. Constitutive activation.
3. Gene amplification resulting in over expression of gene(s)
4. Reciprocal translocation and position effect.
5. Mutation in tumour suppressor gene(s).

resulting in:

- Overproduction of growth factors
- Flooding of the cell with replication signals
- Uncontrolled stimulation in the intermediary pathways
- Cell growth by elevated levels of transcription factors

Tumour suppressor genes

Tumour suppressor genes encode proteins that are:

- receptors for secreted hormones that function to inhibit cell proliferation
- negative regulators of cell cycle entry or progression
- negative regulators of growth signaling pathways (e.g. APC or PTEN)
- checkpoint-control proteins that arrest the cell cycle if DNA is damaged or chromosomes are abnormal

- proteins that promote apoptosis DNA repair enzymes.

The transformation of a normal cell to a cancer cell is accompanied by the loss of function of one or more tumour suppressor genes and both gene copies must be defective in order to promote tumour development.

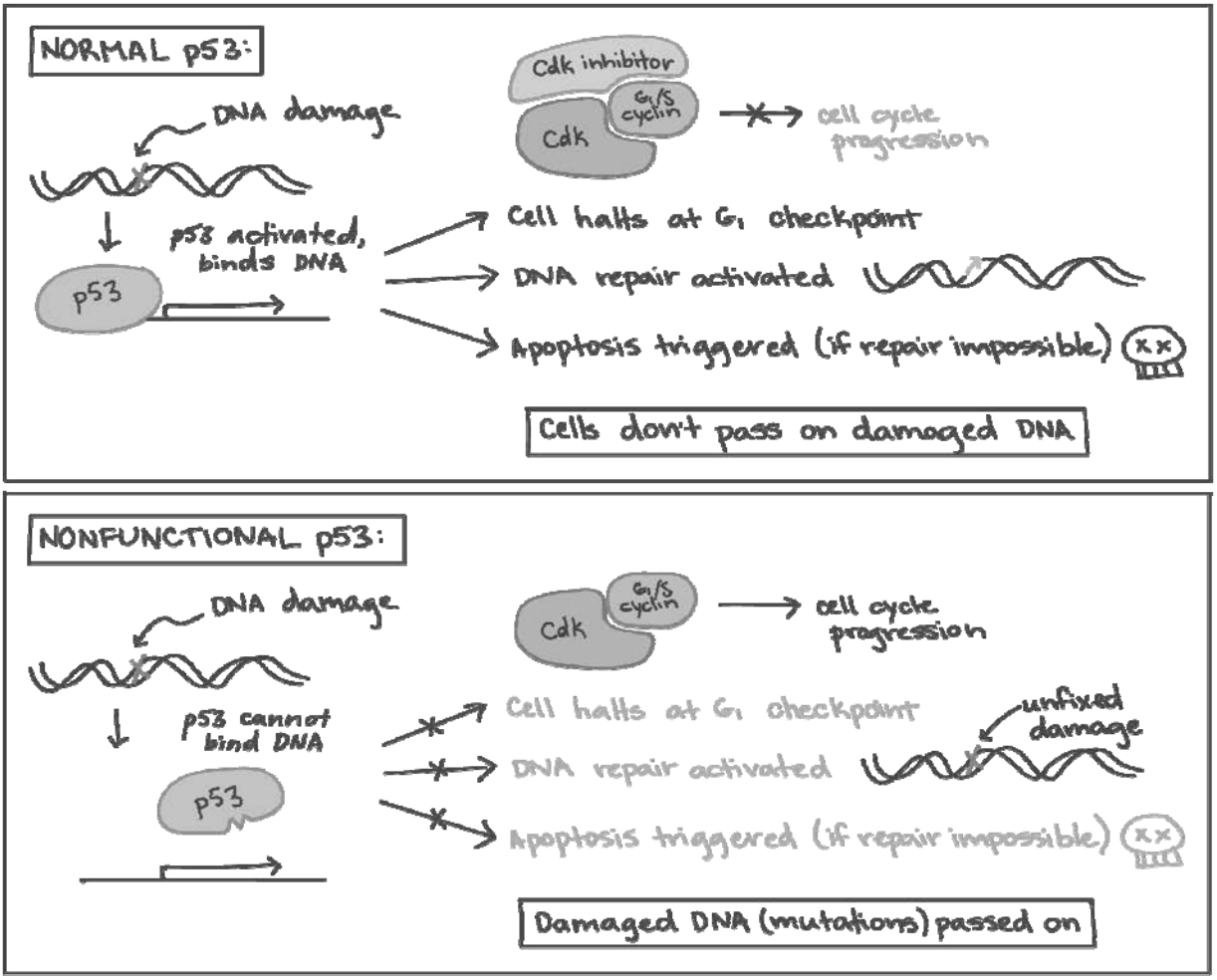
GENES	**TYPES OF CANCER**
APC	Colon / Rectum carcinoma
p^{53}	Brain tumors, lungs carcinoma, breast, liver, leukemia etc.
BRCA I	Breast and ovarian
Rb	Retinoblastoma, bladder, breast, lungs etc.
VHL	Renal

p53

The 53-kilodalton tumor suppressor protein p53 was discovered through its role in the induction of cancers by certain DNA viruses. This protein is encoded by a tumor suppressor gene called TP53. Inherited mutations in TP53 are associated with the Li-Fraumeni syndrome, a rare dominant condition in which any of several different types of cancer may develop. Somatic mutations that inactivate both copies of the TP53 gene are also associated with a variety of cancers. In fact, such mutations are found in a majority of all human tumors. Loss of p53 function is therefore a key step in carcinogenesis.

The p53 protein plays a key role in cellular responses to stress. In normal cells the level of p53 is low, but when the cells are treated with a DNA damaging agent such as radiation, the level of p53 increases dramatically. This response to DNA damage is mediated by a pathway that decreases the degradation of p53. In response to DNA damage, p53 is phosphorylated, converting it into a stable and active form. Once activated, p53 either stimulates the

transcription of genes whose products arrest the cell cycle, thereby allowing the damaged DNA to be repaired, or it activates another set of genes whose products ultimately cause the damaged cell to die.



Source: https://www.khanacademy.org/

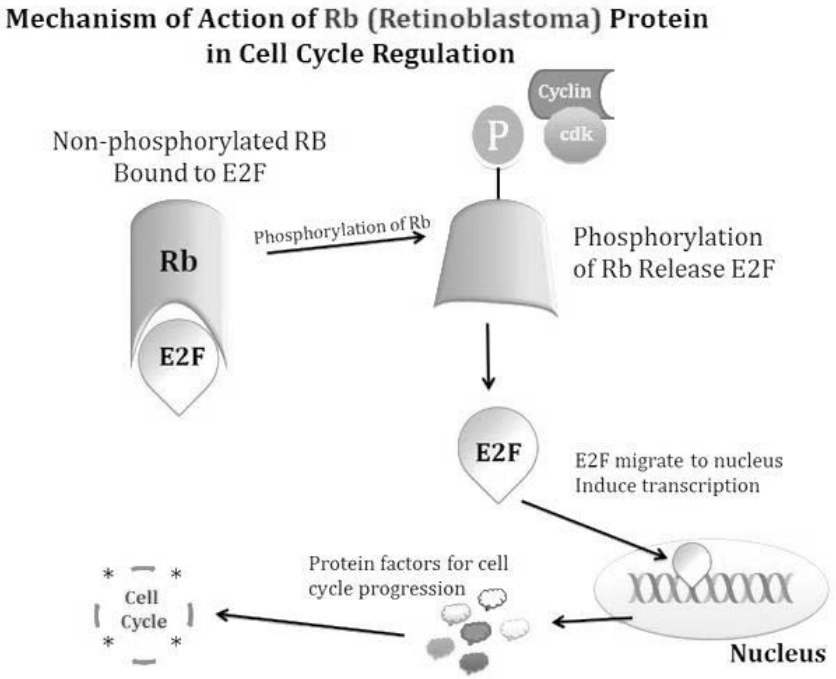
In cancer cells, p53 is often missing, non-functional, or less active than normal. For example, many cancerous tumors have a mutant form of p53 that can no longer bind DNA. Since p53 acts by binding to target genes and activating their transcription, the non-binding mutant protein is unable to do its job.

When p53 is defective, a cell with damaged DNA may proceed with cell division. The daughter cells of such a division are likely to inherit mutations due to the unrepaired DNA of the mother cell. Over generations, cells with faulty p53 tend to accumulate mutations, some of which may turn proto-oncogenes to oncogenes or inactivate other tumor suppressors.

p53 is the gene most commonly mutated in human cancers, and cancer cells without p53 mutations likely inactivate p53 through other mechanisms (e.g., increased activity of the proteins that cause p53 to be recycled).

Role of Rb/E_2F:

The retinoblastoma (Rb) protein is a tumour suppressor gene that controls the cell cycle transition from G_1 to S Phase. Rb protein binds regulatory transcription factor E_2F which is required for the synthesis of DNA replication enzymes. When Rb is bound to E_2F, transcription/replication is blocked. The presence of growth factors (via the Ras pathway) activates cyclin dependent kinase 4/6 Active CDK4/6- phosphorylates and inhibits Rb, taking the brakes off E2F, and transition to S phase occurs. Disruption/deletion of the Rb gene therefore leads to uncontrolled cell proliferation.



Source: https://www.easybiologyclass.com/tumor-suppressor-gene-rb-and-its-role-in-cell-cycle-and-cancer/

Cancer therapy:

Cancer can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible.

Examples of surgical procedures for cancer:

mastectomy for breast cancer and

prostatectomy for prostate cancer.

Chemotherapy

Chemotherapy is the treatment of cancer with drugs ("anticancer drugs") that can destroy cancer cells. It interferes with cell division in various possible ways, e.g. with the duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy target all rapidly dividing cells and are not specific for cancer cells. Hence, chemotherapy has the potential to harm healthy tissue, especially those tissues that have a high replacement rate (e.g. intestinal lining). These cells usually repair themselves after chemotherapy.

The majority of chemotherapeutic drugs can be divided in to:

1. alkylating agents,
2. antimetabolites,
3. anthracyclines,
4. plant alkaloids,
5. topoisomerase inhibitors,
6. monoclonal antibodies, and
7. other antitumour agents.

All of these drugs affect cell division or DNA synthesis and function in some way.

Side-effects

Important common side-effects include (dependent on the agent):

- Hair loss
- Nausea and vomiting
- Diarrhea or constipation
- Anaemia
- Malnutrition
- Depression of the immune system, hence (potentially lethal) infections and sepsis
- Hemorrhage
- Secondary neoplasms
- Cardiotoxicity
- Hepatotoxicity
- Nephrotoxicity
- Ototoxicity
- Death

Common drug:

Cyclophosphamide (Cytoxan),

Methotrexate,

5-Fluorouracil (5-FU) and

Doxorubicin (Adriamycin).

Monoclonal antibody therapy:

Immunotherapy is the use of immune mechanisms against tumors. These are used in various forms of cancer, such as breast cancer (trastuzumab/Herceptin®) and leukemia (gemtuzumab ozogamicin/Mylotarg®).

The agents are monoclonal antibodies directed against proteins that are characteristic to the cells of the cancer in question, or cytokines that modulate the immune system's response.

Radiation therapy

Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of ionizing radiation to kill cancer cells and shrink tumors.

Radiation therapy can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy.

Radiation therapy injures or destroys cells in the area being treated (the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly.

Immunotherapy

Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the tumor. Contemporary methods for generating an immune response against tumours include intravesical BCG immunotherapy for superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients. Cancer vaccines to generate specific immune responses are the subject of intensive research for a number of tumours, notably malignant melanoma and renal cell carcinoma. Sipuleucel-T is a vaccine like strategy in late clinical trials for prostate cancer in which dendritic cells from the patient are loaded with prostatic acid phosphatase peptides to induce a specific immune response against prostate derived cells.

Hormonal therapy

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.

16. Recombinant DNA technology – brief account

Recombinant DNA technology is a set of molecular techniques for locating, isolating, altering, and studying DNA segments. The term recombinant is used because, frequently, the goal is to combine DNA from two distinct sources. Genes from two different bacteria might be joined, for example, or a human gene might be inserted into a viral chromosome. Commonly called **genetic engineering**, recombinant DNA technology now encompasses many molecular techniques that can be used to analyze, alter, and recombine virtually any DNA sequences from any number of sources.

Basic steps involved in recombinant DNA technology:

The basic steps of recombinant DNA technology using the bacterial plasmid as cloning vector

i. Selection and isolation of DNA insert

ii. Selection of suitable cloning vector

iii. Introduction of DNA-insert into vector to form recombinant DNA molecule

iv. Recombinant DNA molecule is introduced into a suitable host.

v. Selection of transformed host cells.

vi. Expression and multiplication of DNA-insert in the host.

vii. Selection and isolation of DNA insert:

First step in recombinant DNA technology is the selection of a DNA segment of interest which is to be cloned. This desired DNA segment is then isolated enzymatically. This DNA segment of interest is termed as DNA insert or foreign DNA or target DNA or cloned DNA.

(ii) Selection of suitable cloning vector:

A cloning vector is a self-replicating DNA molecule, into which the DNA insert is to be integrated. A suitable cloning vector is selected in the next step of rec DNA technology. Most commonly used vectors are plasmids and bacteriophages.

(iii) Introduction of DNA-insert into vector to form recombinant DNA molecule:

The target DNA or the DNA insert which has been extracted and cleaved enzymatically by the selective restriction endonuclease enzymes [in step (i)] are now ligated (joined) by the enzyme ligase to vector DNA to form a rec DNA molecule which is often called as cloning-vector-insert DNA construct.

(iv) Recombinant DNA molecule is introduced into a suitable host:

Suitable host cells are selected and the rec DNA molecule so formed [in step (iii)] is introduced into these host cells. This process of entry of rec DNA into the host cell is called transformation. Usually selected hosts are bacterial cells like *E. coli*; however, yeast, fungi may also be utilized.

(v) Selection of transformed host cells:

Transformed cells (or recombinant cells) are those host cells which have taken up the recombinant DNA molecule. In this step the transformed cells are separated from the non-transformed cells by using various methods making use of marker genes.

(vi) Expression and Multiplication of DNA insert in the host:

Finally, it is to be ensured that the foreign DNA inserted into the vector DNA is expressing the desired character in the host cells. Also, the transformed host cells are multiplied to obtain sufficient number of copies. If needed, such genes may also be transferred and expressed into another organism.

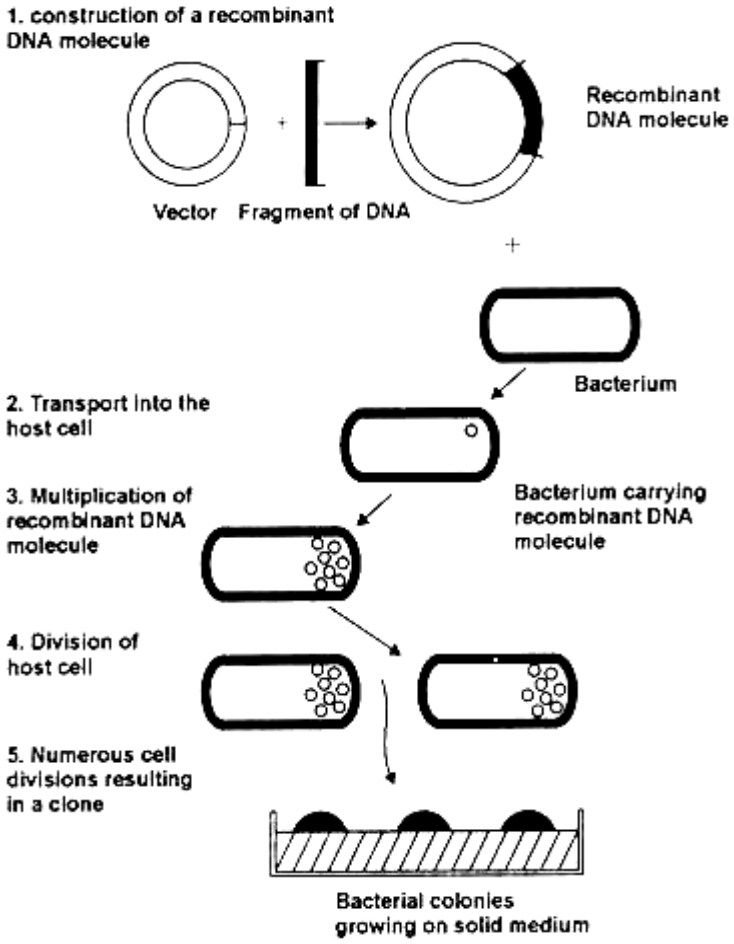


Fig. 1. The basic steps of rec DNA Technology using the bacterial plasmid as cloning vector.

Source: http://cdn.biologydiscussion.com

(A) ENZYMES:

A number of specific enzymes are utilized to achieve the objectives of recombinant DNA technology.

(a) Restriction Endonuclease:

These enzymes serve as important tools to cut DNA molecules at specific sites, which is the basic need for recombinant DNA technology.

These are the enzymes that produce internal cuts (cleavage) in the strands of DNA, only within or near some specific sites called recognition sites/recognition sequences/ restriction sites 01 target sites. Such recognition sequences are specific for each restriction enzyme. Restriction endonuclease enzymes are the first necessity for recombinant DNA technology.

ENZYME	ORGANISM	RECOGNITION SEQUENCE*	BLUNT OR STICKY END
*Eco*RI	*Escherichia coli*	GAATTC	Sticky
*Bam*H	*Bacillus amyloliquefaciens*	GGATCC	Sticky
*Bgl*II	*Bacillus globigii*	AGATCT	Sticky
*Pvu*I	*Proteus vulgaris*	CGATCG	Sticky
*Pvu*II	*Proteus vulgaris*	CAGCTG	Blunt
*Hind*III	*Haemophilus influenzae* R_c	AAGCTT	Sticky
*Hin*fI	*Haemophilus influenzae* R_f	GANTC	Sticky
*Sau*3A	*Staphylococcus aureus*	GATC	Sticky
*Alu*I	*Arthrobacter luteus*	AGCT	Blunt
*Taq*I	*Thermus aquaticus*	TCGA	Sticky
*Hae*III	*Haemophilus aegyptius*	GGCC	Blunt
*Not*I	*Nocardia otitidis-caviarum*	GCGGCCGC	Sticky
*Sfi*I	*Streptomyces fimbriatus*	GGCCNNNNNGGCC	Sticky

Some of the most frequently used restriction endonucleases.

Types of Restriction Endonucleases:

There are 3 main categories of restriction endonuclease enzymes:

Type-I Restriction Endonucleases:

These are the complex type of endonucleases which cleave only one strand of DNA. These enzymes have the recognition sequences of about 15 bp length.

They require Mg++ ions and ATP for their functioning. Such types of restriction endonucleases cleave the DNA about 1000 bp away from the 5′ end of the sequence ‘TCA’ located within the recognition site. Important examples of Type-I restriction endonuclease enzyme are EcoK, EcoB, etc.

Type-II Restriction Endonucleases:

These are most important endonucleases for gene cloning and hence for rec DNA technology. These enzymes are most stable. They show cleavage only at specific sites and therefore they produce the DNA fragments of a defined length. These enzymes show cleavage in both the strands of DNA, immediately outs.de then- recognition sequences. They require Mg++ ions for their functioning.

The recognition sequences for Type-II Restriction Endonuclease enzymes are in the form of palindromic sequences with rotational symmetry, i.e., the base sequence in the first half of one strand of DNA is the mirror image of the second half of other strand of that DNA double helix. Important examples of Type-II Restriction endonucleases include Hinfl, EcoRI, PvuII, Alul, Haelll etc.

Type-III Restriction Endonucleases:

These are not used for gene cloning. They are the intermediate enzymes between Type-I and Type-II restriction endonuclease. They require Mg++ ions and ATP for cleavage and they cleave the DNA at well-defined sites in the immediate vicinity of recognition sequences, e.g. Hinf III, etc.

(c) DNA ligase:

The function of these enzymes is to join two fragments of DNA by synthesizing the phosphodiester bond. They function to repair the single stranded nicks in DNA double helix and in rec DNA technology they are employed for sealing the nicks between adjacent nucleotides. This enzyme is also termed as molecular glue.

(d) DNA polymerases:

These are the enzymes which synthesize a new complementary DNA strand of an existing DNA or RNA template. A few important types of DNA polymerases are used routinely in genetic engineering. One such enzyme is DNA polymerase, which prepared from *E coli*. The Klenow fragment of DNA polymerase I is employed to make the protruding ends double stranded by extension of the shorter strand.

Reverse transcriptase is also an important type of DNA polymerase enzyme for genetic engineering. It uses RNA as a template for synthesizing a new DNA strand called as cDNA and complementary DNA. Its main use is in the formation of cDNA libraries. Apart from all these above mentioned enzymes, a few other enzymes also mark their importance in genetic engineering.

(a) Terminal deoxynucleotidyl transferase enzyme:

It adds single stranded sequences to 3′-terminus of the DNA molecule. One or more deoxyribonucleotides (dATP, dGTP, dTTP, dCTP) are added onto the 3′-end of the blunt ended fragments.

(b) Alkaline Phosphatase Enzyme:

It functions to remove the phosphate group from the 5′-end of a DNA molecule.

(c) Polynucleotide Kinase Enzyme:

It has an effect reverse to that of Alkaline Phosphatase, i.e., it functions to add phosphate group to the 5′-terminus of a DNA molecule.

(B) Cloning Vectors:

It is another important natural tool which geneticists use in rec DNA technology. The cloning vector is the DNA molecule capable of replication in a host organism, into which the target DNA is introduced producing the rec DNA molecule.

A cloning vector may also be termed as a cloning vehicle or earner DNA or simply as a vector or a vehicle a great variety of cloning vectors are present for use with *E. coli* is the host organism.

However under certain circumstances it becomes desirable to use different host for cloning experiments. So, various cloning vectors have been developed based on other bacteria like *Bacillus, Pseudomonas, Agrobacterium*, etc. and on different eukaryotic organisms like yeast and other fungi.

Applications of Recombinant DNA Technology:

Some important applications of recombinant DNA technology are enlisted below:

(1) Production of Transgenic Plants:

By utilizing the tools and techniques of genetic engineering it is possible to produce transgenic plants or the genetically modified plants. Many transgenic plants have been developed with better qualities like resistance to herbicides, insects or viruses or with expression of male sterility, etc.

(2) Production of Transgenic Animals:

By the use of rec DNA technology, desired genes can be inserted into the animal so as to produce the transgenic animal. The method of recombinant DNA technology aids the animal breeders to increase the speed and range of selective breeding in case of animals. It helps for the production of better farm animals so as to ensure more commercial benefits.

(3) Production of Hormones:

By the advent of techniques of rec DNA technology, bacterial cells like *E.coli* are utilized for the production of different fine chemicals like insulin, somatostatin, somatotropin and p-endorphin. Human Insulin Hormone i.e., Humulin is the first therapeutic product which was produced by the application of recombinant DNA technology.

(4) Production of Vaccines:

Vaccines are the chemical preparations containing a pathogen in attenuated (or weakened) or inactive state that may be given to human beings or animals to confer immunity to infection. A number of vaccines have been synthesized biologically through recombinant DNA technology.

(5) Biosynthesis of Interferon:

Interferons are the glycoproteins which are produced in very minute amounts by the virus infected cells. Interferons have antiviral and even anticancerous properties. By recombinant DNA technology method, the gene of human fibroblasts (which produce interferons in human beings) is inserted into the bacterial plasmid.

(6) Production of Antibiotics:

Antibiotics produced by microorganisms are very effective against different viral, bacterial or protozoan diseases. Some important antibiotics are tetracyclin, penicillin, streptomycin, novobiocin, bacitracin, etc.

Recombinant DNA technology helps in increasing the production of antibiotics by improving the microbial strains through modification of genetic characteristics.

(7) Production of Commercially Important Chemicals:

Various commercially important chemicals can be produced more efficiently by utilizing the methods of recombinant DNA technology. A few of them are the alcohols and alcoholic beverages obtained through fermentation; organic acids like citric acid, acetic acid, etc. and vitamins produced by microorganisms.

(8) Application in Enzyme Engineering:

As we know that the enzymes are encoded by genes, so if there are changes in a gene then definitely the enzyme structure also changes. Enzyme engineering utilizes the same fact and can be explained as the modification of an enzyme structure by inducing alterations in the genes which encode for that particular enzyme.

(9) Prevention and Diagnosis of Diseases:

Genetic engineering methods and techniques have greatly solved the problem of conventional methods for diagnosis of diseases. It also provides methods for the prevention of a number of diseases like AIDS, cholera, etc. Monoclonal antibodies are useful tools for disease diagnosis. Monoclonal antibodies are produced by using the technique called hybridoma technology.

(10) Gene Therapy:

Gene therapy is undoubtedly the most beneficial area of genetic engineering for human beings. It involves delivery of specific genes into human body to correct the diseases. Thus it is the treatment of diseases by transfer and expression of a gene into the patients' cells so as to ensure the restoration of a normal cellular activity.

(11) Practical Applications of Genetic Engineering:

Recombinant DNA technology has an immense scope in Research and Experimental studies. It is applied for:

a. Localizing specific genes.

b. Sequencing of DNA or genes.

c. Study of mechanism of gene regulation.

d. Molecular analysis of various diseases.

e. Study of" mutations in DNA, etc.

(12) Applications in forensic science:

The applications of recombinant DNA technology (or genetic engineering) in forensic sciences largely depend on the technique called DNA profiling or DNA fingerprinting. It enables us to identify any person by analyzing his hair roots Wood stains, serum, etc. DNA fingerprinting also helps to solve the problems of parentage and to identify the criminals.

(13) Biofuel Production:

Biofuels are derived from biomass and these are renewable and cost effective. Genetic engineering plays an essentially important role in a beneficial and large scale production of biofuels like biogas. biohydrogen biodiesel bio-ethanol., etc. Genetic engineering helps to improve organisms for obtaining higher product yields and product tolerance.

(14) Environment Protection:

Genetic engineering makes its contributions to the environment protection in various ways. Most important to mention are the new approaches utilized for waste treatments and bioremediation Environment protection means the conservation of resources and hence to limit the degradation of environment.

19. Let's sum up

- Each eukaryotic chromosome contains a long linear DNA molecule packaged into 11-nm nucleosomes. Folding of nucleosomes produce 30-nm solenoid that finally makes the chromatid.

- Eukaryotic DNA exhibits three classes of sequences. Unique-sequence DNA exists in very few copies. Moderately repetitive DNA consists of moderately long sequences that are repeated from hundreds to thousands of times. Highly repetitive DNA consists of very short sequences that are repeated in tandem from many thousands to millions of times.

- Centromeres are chromosomal regions where spindle fibers attach and telomeres consists tandemly repeated sequences that stabilize the ends of chromosomes. Telomerase is an enzyme makes DNA using RNA as a template.

- Karyotype study helps to identify a particular chromosomal complement with characteristic number and morphology. The banding pattern for each chromosome is specific allowing identification of each of the chromosomes. In FISH, specific probe tagged with fluorescence microscope is often used for finding specific features in DNA whereas in GISH, entire genome is tagged.

- The mechanism by which sex is specified is termed sex determination. Sex may be determined by differences in specific chromosomes, genotypes, or environment. In Drosophila melanogaster, sex is determined by a balance between genes on the X chromosomes and genes on the autosomes. In humans, sex is ultimately determined by the presence or absence of the SRY gene located on the Y chromosome.

- Sex-linked characteristics are determined by genes on the sex chromosomes. Dosage compensation equalizes the amount of protein produced by X-linked genes in males and females.

- A polytene chromosome of *Drosophila* salivary glands has eukaryotic gene activity that is regulated at the level of RNA synthesis. The B chromosomes suppress homologous pairing which reduces multiple pairing between homologous chromosomes in allopolyploids. Deletion mapping has been used to reveal the chromosomal location of a gene.

- Linked genes do not assort independently. In a testcross for two completely linked genes (no crossing over), only nonrecombinant progeny are produced. When two genes assort independently, recombinant progeny and nonrecombinant progeny are produced in equal proportions. When two genes are linked with some crossing over between them, more nonrecombinant progeny than recombinant progeny are produced.

- In translocation heterozygotes, the chromosomes form cross like structures in meiosis, and the segregation of chromosomes produces unbalanced gametes. Aneuploidy usually causes drastic phenotypic effects because it leads to unbalanced gene dosage. All the chromosomes in an autopolyploid derive from one species; chromosomes in an allopolyploid come from two or more species.

- Organellar DNA controls plastidial inheritance in four o'clock plant, *Oenothera* and mitochondrial inheritance in yeast, maize.

- The rate at which individual genes are transferred during conjugation provides information about the order of the genes and the distances between them on the bacterial chromosome. Frequencies of the cotransformation of genes provide information about the physical distances between chromosomal genes. Phage genes can be mapped by infecting bacterial cells with two different phage strains and counting the number of recombinant plaques produced by the progeny phages.

- Mutations are heritable changes in genetic information. Most damage to DNA is corrected by DNA-repair mechanisms.

- A DNA nucleotide consists of a deoxyribose sugar, a phosphate group, and a nitrogenous base. RNA consists of a ribose sugar, a phosphate group, and a nitrogenous base.

- Genetic material must contain complex information, be replicated accurately, and have the capacity to be translated into the phenotype. The central dogma of molecular biology proposes that information flows in a one-way direction, from DNA to RNA via transcription and RNA to protein via translation.

- Gene expression can be controlled at different levels, including the alteration of DNA or chromatin structure, transcription, mRNA processing, RNA stability, translation, and posttranslational modification. Much of gene regulation is through the action of regulatory proteins binding to specific sequences in DNA.

- Transposable elements are mobile DNA sequences that insert into many locations within a genome and often cause mutations and DNA rearrangements. Transposons have played an important role in genome evolution.
- A population's genetic composition can be described by its genotypic and allelic frequencies. The Hardy–Weinberg law describes the effects of reproduction and Mendel's laws on the allelic and genotypic frequencies of a population.
- The cell cycle is controlled by cyclins and cyclin-dependent kinases. Mutations in genes that control the cell cycle are often associated with cancer. Cancer is fundamentally a genetic disorder, arising from somatic mutations in multiple genes that affect cell division and proliferation.
- Recombinant DNA technology is a set of molecular techniques for locating, isolating, altering, and studying DNA segments.

20. Suggested Readings

1. Snustad, D.P. & Simmons, M.J. Principles of Genetics, John Wiley & Sons.
2. Pierce, Benjamin A. Genetics (2nd ed.), 2005, W.H. Freeman & Company.
3. Klug, W.S. & Cummings, M.R. Concepts of Genetics, 2003, Pearson Education.
4. Griffiths, A.I.F., Miller, J.H., Suzuki, D.T., Lewentin, C.R. & Gilbert, M.W. An Intrduction to Genetic Analysis, 2005 (8th ed.), W.H. Freeman & Company.
5. Russell, Peter J. 2006. iGenetics: A Molecular Approach. San Francisco: Benjamin Cummings.
6. B.D. Singh 1996. Fundamentals Of Genetics, Kalyani Publishers.
7. Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, Peter Walter. Molecular Biology of the Cell. 2015. 6th Ed: Garland Science.
8. Gerald Karp. Cell Biology. 2013. 7th Ed. International Student Version. Wiley.
9. Lewin, B. Genes VIII, 2004, Pearson Educational International.
10. Cooper, G.M. The Cell, A molecular approach (2nd ed.), 2000, ASM Press.
11. Gupta, P.K. Genetics, 2007, Rastogi Publications.

12. Kar, D.K. and Halder, S. Cell Biology, Genetics and Molecular Biology 2008, New Central Book Agency.
13. http://www.biologydiscussion.com/
14. https://en.wikipedia.org/

Assignment

Explain molecular organization of centromeres and telomeres.

Give an account of chromosome banding and its applications.

What are chromosomal structural aberrations ? Describe meiosis in heterozygotes for such aberrations.

Write short notes on any four of the following:

a. Robertsonian translocation b. Paramutation c. FISH d. Polytene chromosomes e. Nucleosome

Describe meiotic behaviour of trisomics. How are they useful in assigning genes to chromosomes?

Describe meiosis in an autotetraploid.

Describe the molecular model of genome organization.

Write short notes on any four of the following:

a. Uses of allopolyploids b. RNA editing c. Primary and secondary trisomics d. complex structural heterozygotes e. Allotetraploid and amphidiploid

Explain multiple allelic inheritance and its significance

Give an account of three-point testcross method of gene maping.

Describe the molecular basis of DNA damage and the repair mechanisms.

Write short notes on any four of the following:

a. Correlation of genetic and physical maps. b. C-value paradox c. Hollidays model d. Telomere e. sex-influenced characters

Describe the gene complementation test

Describe the methods of gene mapping in bacteriophages.

With the help of one suitable example for each, describe the genetic basis of mitochondrial and chloroplast related characters.

Write short notes on any four of the following:

a. site-directed mutagenesis b. Mechanism of transposition c. Genetic complementation d. Deletion mapping e. Distinction between cytoplasmic and nuclear types of inheritance.

Write short notes on any four of the following:

a. attenuation at trp operon b. types of RNAs c. experimental proof of Okazaki fragments d. gene imprinting e. Gene silencing.

Describe the process of RNA maturation in eukaryotes.

All the materials are self-written and collected from ebook, journals and websites.

NOTE