CELL AS A FUNDAMENTAL UNIT OF LIFE AND ORGANISM

The biological science which deals with the study of structure, function, molecular organization, growth, reproduction and genetics of the cells, is called **cytology** (Gr., *kytos* = hollow vessel or cell; *logous* = to discourse) or **cell biology**. The word 'cell' is derived from Latin cella to cover i.e., it covers and encompasses the basic unit of life.

The cell, itself, is considered to be the basic structural and functional unit of life as it is the smallest unit of all living organisms capable of carrying out all the activities necessary for life. Cells are the most basic building units of life. It can also be said that the cell is a complete unit of metabolism because it has all the chemical and physical factors necessary for its growth and maintenance.

A cell is capable of independent existence and can carry out all the functions which are necessary for a living being. A cell carries out nutrition, respiration, excretion, transportation and reproduction; the way an individual organism does. Unicellular organisms are capable of independent existence which shows a cell's capability to exist independently. Due to this, a cell is called the fundamental and structural unit of life.

HISTORY OF DISCOVERY OF CELLS

- Cell was first discovered by Robert Hooke in 1665 in a simple microscope.
- In 1674, Leeuwenhoek, with the help of developed microscope, discovered the free living cells in pond water.
- In 1831, Robert Brown had discovered the **nucleus** in the cell.
- In 1839, Purkinje used the term 'protoplasm' for the fluid substance found in the cell.
- The cell theory was proposed by Schleiden (1838) and Schwann (1839).
- In 1855, Virchow further expanded the cell theory and suggested that all cells arise from pre-existing cells.
- In 1940, the discovery of electron microscope made possible to observe and understand the complex structure of the cell.

The cell theory of Schleiden and Schwann states that:

a. All living things are composed of one or more cells;
b. New cells are formed from the pre-existing ones through divisions;
c. There are basic similarities in chemical compositions and metabolic functions of all cells;
d. The activity of an organism is the collective activities and interactions of its cellular structures, i.e., cell is the functional unit of tissues and organs.

Unicellular Organisms

- The single cellular organisms, such as *Amoeba, Chlamydomonas, Paramoecium*, and bacteria, are known as unicellular organisms.

Multicellular Organisms

- The organisms consisting of many cells are known as multicellular organisms. E.g. human being, animals, birds, etc.

Significant Characteristics of Cells

- Each living cell has the aptitude to perform certain basic functions that are characteristic of all living forms.
- Each such cell has certain specific components within it known as cell organelles.
- Different types of cells have different function and each cell organelle performs a special function.
- These organelles collectively constitute the basic unit of life known as cell.
- All cells are found to have the same organelles, irrespective of their different functions and the organism they found in.

STRUCTURE OF CELL

Recently with the introduction of electron microscope and the use of new biological techniques for an analysis of the parts of a cell, the finer detailed structures, properties and functions of the cell have come to light.

Shape and Size of Cells: Cells come in all shapes and sizes. While most of the cells are spherical in shape, cells of various other shapes are also found. Most of the cells are microscopic in size, i.e. it is impossible to see them with naked eyes. Some cells are fairly large, e.g. a **neuron in human body** can be as long as 1 meter. The **egg of an ostrich** is the largest known cell of a living animal and an average egg is 15 cm long and 13 cm wide. **Xylem**, the vascular tissue in plants which conducts water and nutrients, is the largest plant cell.

Xylem elements are many millimetre(mm) long and they have a narrow lumen. **Ovules of *Cycas*** is the largest plant cell in multicellular plants. A unicellular marine algae, ***Acetabularia*.** It can reach upto 10 cm in height.

Structural Organization of Cell

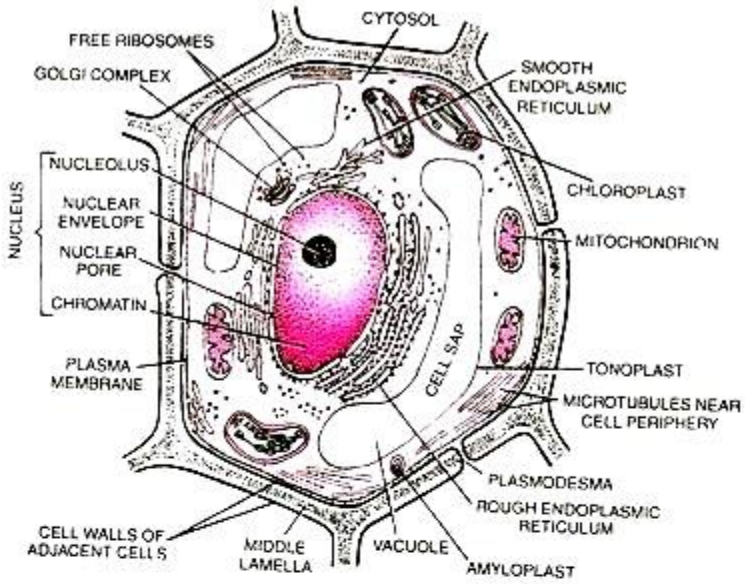


Fig. 8.11. A generalised ultra structure of an eucaryotic plant cell.

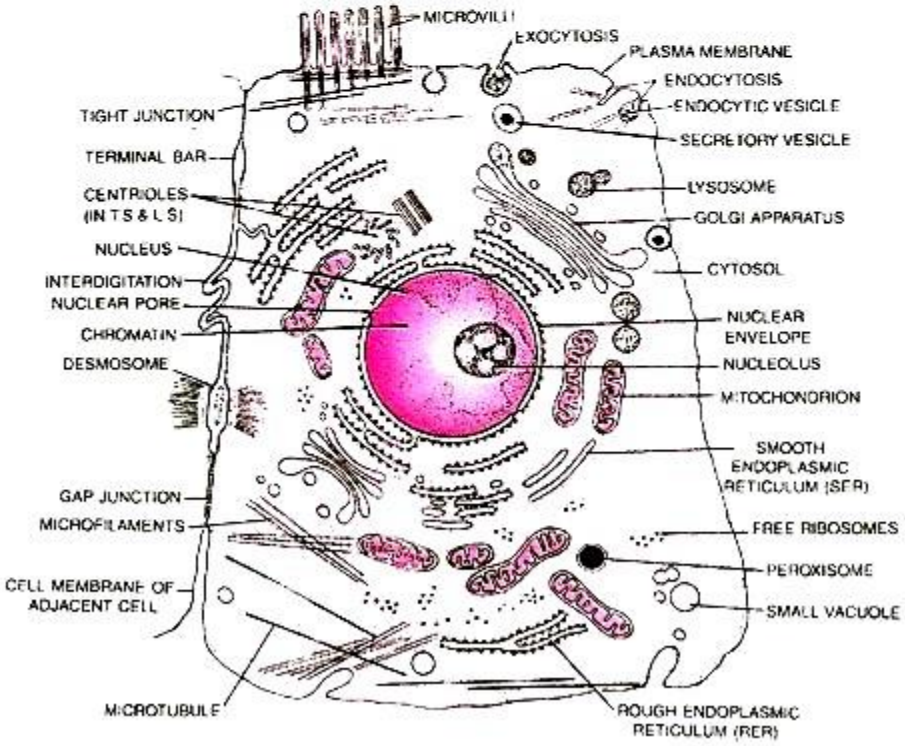


Fig. 8.12. A generalised ultrastructure of an eucaryotic animal cell.

Following are the three basic features that every cell possesses −

- Plasma Membrane/Cell Membrane
- Nucleus
- Cytoplasm

Plasma Membrane/Cell Membrane

- Plasma membrane is the **outermost covering** layer of the cell.
- Plasma membrane allows certain materials to enter inside the cell and come out from the cell; therefore, it is known as **selectively permeable membrane**.
- The movement of water molecules through the selectively permeable membrane is known as **osmosis**.
- **Cell Wall**

- Plant cells have an addition protecting cover known as **cell wall** (absent in animal cell).
- The cell wall lies outside the plasma membrane; likewise, it also covers plasma membrane.
- The **cell wall** is essentially composed of **cellulose**.
- **Molecular Structure of the Plasma Membrane:**
- The plasma membrane is composed of two layers of protein molecules and two layers of lipid molecules. The lipid molecules occur in chains. In plasma membrane, two molecular chains of lipids remain parallel to each other and form a bimolecular or double-layered structure.
- Both lipid layers remain linked with each other by the inner ends of lipid molecules which are non-polar and hydrophobic (Gr., hydr= water; phobe=hate) in nature. Both the layers of lipids are able to be held together due to Vanderwaal's forces at these non-polar ends.
- The lipid layers are enclosed by an outer and inner layer of proteins. The lipid molecules remain linked with the molecules of protein layers by their outer, polar and hydrophilic (Gr., hydr – water; phil-loving) ends. In the hydrogen bonds, ionic linkages or electrostatic forces bind the molecules of lipids and proteins together.
- The carbohydrate molecules occur in the association of protein molecules and provide stability to lipoprotein complex. The protein layers provide elasticity and mechanical resistance to the plasma membrane.

Nucleus

- Nucleus or nuculeus is a Latin term and its meaning is **kernel** or seed.
- The nucleus has a **double layered covering**, which is known as **nuclear membrane**.
- The **nuclear membrane** has **some pores**, which allow certain materials come inside (in nucleus) and go outside (in the cytoplasm).
- The most significant feature of nucleus is – it contains **chromosomes**.
- **Chromosomes** are **rod-shaped structures** and it is **visible only** when the **cell is about to divide**.
- Chromosomes are composed of **DNA** and **protein**.
- DNA (**Deoxyribo Nucleic Acid**) molecules contain inheritance features from parents to next generation.
- DNA molecules also contain the information essential for constructing and organizing cells.
- **Functional segments of DNA** are known as **genes**.
- DNA is present as the part of chromatin material.
- Chromatin material is visible as entangled mass of thread like structures.
- Whenever the cell is about to divide, the chromatin material gets organized into chromosomes.
- The nucleus plays a central and significant role in cellular reproduction.
- The cell, which has no nuclear membrane, is known as **prokaryotes** (i.e. Pro = primitive or primary; karyote ≈ karyon = nucleus).
- The cell, which has a nuclear membrane, is known as **eukaryotes**.
- Prokaryotic cell does not have many other cytoplasmic organelles those are present in eukaryotic cells.

Cytoplasm

- Cells consist of cytoplasm inside the cell membrane, which contains many biomolecules including proteins and nucleic acids.
- There are many structures found in the cytoplasm known as cell organelles.

Cell Organelles

- Following are the major cell organelles that play a major role in the functioning of cell −
  - Nucleus
  - Endoplasmic Reticulum
  - Ribosome
  - Golgi apparatus
  - Lysosomes
  - Mitochondria
  - Plastids
  - Vacuoles

Endoplasmic Reticulum

- The endoplasmic reticulum (or simply ER) is a **large network of membrane-bound tubes and sheets**.

- Based on visual structure, ER is categorized as **rough endoplasmic reticulum** (RER) and **smooth endoplasmic reticulum** (SER).
- When the **ribosome attached** on the surface of ER, it is known as **Rough Endoplasmic Reticulum** and **without ribosome**, it is known as **Smooth Endoplasmic Reticulum**.
- The **SER** helps in the manufacturing of **fat** molecules, or **lipids**, which is important for cell functioning.
- One of the significant **functions** of **ER** is to serve as **channels** for the **transportation of materials** (**especially proteins**) in **various regions of the cytoplasm** and also **between** the **cytoplasm** and the **nucleus**.

Ribosome

- The ribosomes, normally, present in all active cells.
- **Ribosome** are the **sites** of **protein** manufacturing.

Golgi Apparatus

- The Golgi Apparatus is named after the name of its discover **Camillo Golgi**.
- Golgi Apparatus **consists** of a system of **membrane-bound vesicles** arranged roughly **parallel** to each other in **stacks** known as **cisterns**.
- The significant **functions** of Golgi Apparatus are the **storage**, **modification**, and **packaging of products** in vesicles.
- The Golgi apparatus also helps in the **formation of lysosomes**.

Lysosomes

- Lysosomes are a sort of **waste disposal system** of the **animal cell**.
- Lysosomes help in keeping the cell clean by **digesting** the **foreign material** as well as **worn-out cell organelles**.
- Lysosomes **contain** powerful **digestive enzymes** capable of **breaking down** all sorts of **organic materials**.
- Lysosome has a typical feature i.e. when the cell gets damaged lysosome most likely bursts and the released enzymes digest their own cell. Because of this reason, lysosome is also known as the '**suicide bags**' of a cell.

Mitochondria

- Mitochondria, commonly, are known as the **powerhouses** of the cell.
- Mitochondria **release** the **energy** required **for various chemical activities** (essential for the life).
- Mitochondria release energy in the form of **ATP** (Adenosine Triphosphate) molecules.
- **ATP** is popular as the **energy currency** of the cell.
- Mitochondria have their **own DNA** and **ribosomes**; hence, they are **capable** to **make** some of **their own proteins.**

Plastids

- Plastids are present only in the **plant cells**.
- Plastid is categorized as – **Chromoplasts** (it is colored plastids) and **Leucoplasts** (It is either white or colorless plastids).
- Plastids contain **chlorophyll pigment**, which are known as **Chloroplasts**.
- **Chloroplasts** play important role in the **photosynthesis** in plants.
- **Chloroplasts** also contain various types of **yellow or orange pigments**.
- **Leucoplasts** are the **organelles** in which some important materials such as **starch**, **oils**, and **protein** granules get **stored**.
- **Plastids** look **like mitochondria** (in terms of **external structure**).
- Like the mitochondria, **plastids** also possess their **own DNA** and **ribosomes**.

Vacuoles

- Vacuoles are commonly the **storage sacs** that contain **solid** or **liquid materials**.
- In **animal cell**, **vacuoles** are **small**; whereas in **plant cell**, vacuoles are of **large** size.
- **Plant cells vacuoles** are filled with **cell sap** and provide **turgidity** and **rigidity** to the cell.

INTRODUCTION TO CHROMOSOMES:

Chromosomes are threadlike deeply stained compact DNA protein complex that carry genetic information in a linear sequence of genes.

They are the physical basis of heredity or hereditary vehicles as they store, replicate, transcribe and transmit the genetic information.

Though the scope of the term include the bacterial nucleoid (prochromosome), organelle genomes, viral genomes and eukaryotic nuclear chromosome, but only the last one considered as typical chromosome.

HISTORY:

W. Hofmeister (1848): First discovered chromosomes in the dividing pollen mother cells of *Tradescantia*.

Strasburger (1875): First observed chromosome during cell division.

W. Flemming (1879): Described the splitting of dark stained rod-like structures during cell division which he called as chromatin.

Beneden and Boveri (1887): First discovered that the number of chromosome in a given species remains constant.

W. Waldyer (1888): Coined the term chromosome.

Sutton and Boveri (1902): Proposed the chromosome theory of inheritance.

Boveri (1932): He described chromosomes as bearer of hereditary traits.

Kornberg (1974): Proposed the concept of chromosome ultra-structure in the form of nucleosome.

CHROMOSOME NUMBER:

Each species contain a fixed number of chromosomes (Beneden and Boveri, 1087). However, change in chromosome number can be seen in a species and is called as polyploidy (euploidy and aneuploidy). Normally gamete or gametophyte cells contain one set of chromosomes called genome and the cells are called haploid. The somatic cells of animals and sporophytes have two haploid sets or genomes and are said to be diploid cells.

Minimum:

Mucor hiemalis (bread mould), n-2

Haplopappus gracilis (Family-Asteraceae), 2n=2

Ascaris megalocephala (horse roundworms), 2n = 2

Maximum:

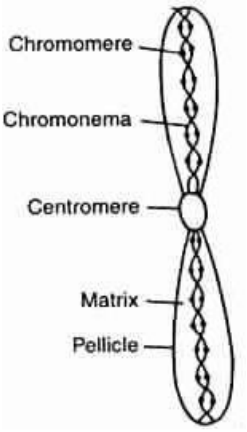
Ophioglossum reticulum (Adders tongue fern) 2n = 1272

Aulocantha sp (protozoa), 2n = 1600.

CHROMOSOME SIZE:

The size of chromosome is normally measured at mitotic metaphase. The length may vary from 0.1 – 50.0/µm while the diameter may vary from 0.2-3/µm. In general monocots among plants have large chromosomes while Orthoptera (Grasshopper) and Amphibia among animals have larger chromosomes.

STRUCTURE OR MORPHOLOGY OF CHROMOSOME:



The shape and size of chromosomes change along with cell cycle. During interphase the chromosomes remain in form of chromatin reticulum. During cell division, the chromatin reticulum condenses, so that by the end of prophase distinct thread like structures appear called chromonemata (sing, chromonema). In metaphase and anaphase the chromonemeta become fully condensed and take the shapes of chromatids in eukaryotic nuclear chromosomes. This cyclic change in shape and size of chromosomes during cell cycle is called chromosomal cycle.

The morphology of chromosome is best studied at metaphase and anaphase because of their high degree of condensation. The sample taken for this purpose, is mainly from shoot or root apex containing meristematic tissue or from pollen mother cells of plants, and tissue from sex organs and WBC in animals.

In eukaryotes the chromosomes are multiple large, linear and are present in the nucleus of the cell.

- Each chromosome typically has one centromere and one or two arms that project from the centromere.
- Structurally, each chromosome is differentiated into three parts—
  1. Pellicle
  2. Matrix
  3. Chromonemata

Pellicle

- It is the outer envelope around the substance of chromosome.

- It is very thin and is formed of achromatic substances.

Matrix

- It is the ground substance of chromosome which contains the chromonemata.
- It is also formed of non-genic materials.

Chromonemata

- Embedded in the matrix of each chromosome are two identical, spirally coiled threads, the chromonemata.
- The two chromonemata are also tightly coiled together that they appear as single thread of about 800Å thickness.
- Each chromonemata consists of about 8 microfibrils, each of which is formed of a double helix of DNA.

A typical chromosome has following parts:

(a) Centromere (Primary constriction):

A metaphase chromosome has two identical sister chromatids, which are attached to each other at a point called centromere or primary constriction. At anaphase the centromere splits the sister chromatids separate to become two anaphasic chromosomes. Therefore, anaphase chromosome is a half metaphase chromosome. The parts of chromosome on either side of centromere are called arms.

Thus, metaphase chromosome has four arms while anaphase chromosome has two arms. The two arms are equal in isobrachial chromosomes and unequal in heterobrachial chromosomes. When the arms are unequal, the short arm is designated as 'p' and the long arm is designated as 'q'.

Based on the position of centromere, chromosomes are called:

(i) Telocentric (centromere terminal),
(ii) Acrocentric (centromere subterminal and capped by telomere),
(iii) Sub-metacentric (centromere is submedian),
(iv) Metacentric (centromere median).

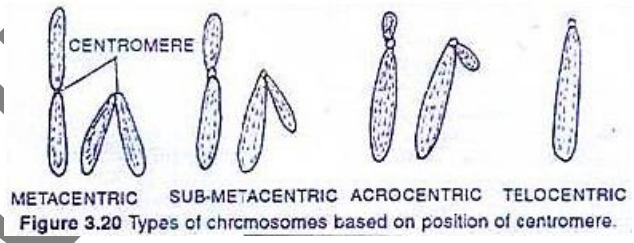


Figure 3.20 Types of chromosomes based on position of centromere.

Normally chromosomes are monocentric (one centromere); sometimes chromosomes may be dicentric (e.g., in wheat, maize etc.) or polycentric (e.g., *Luzula*. *Ascaris* etc.). However, in some insects the centromere is diffused along the length of chromosome.

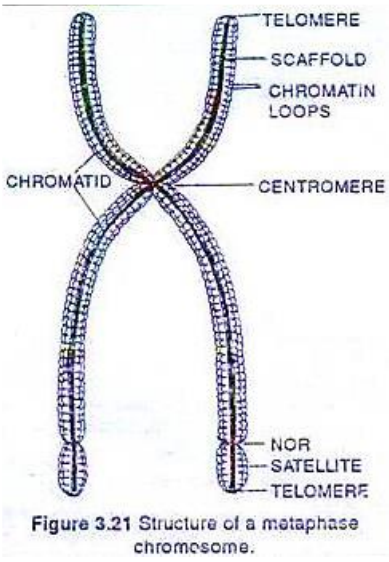


Figure 3.21 Structure of a metaphase chromosome.

Centromere has four important functions, viz:

(a) Orientation of chromosomes at metaphase,
(b) Movement of chromosomes during anaphase,
(c) Formation of chromatids, and
(d) Chromosome shape.

(b) Kinetochore:

The surface of centromere bears a specialized multi-protein complex called kinetochore to which spindle fibers (microtubules) attach. The centromere of a metaphase chromosome contains 2 kinetochores facing in opposite direction. Kinetochore is trilamilar type in lower plants while ball and cup type in higher plants.

(c) Secondary Constrictions:

Besides centromere a chromosome may have one or more secondary constrictions. The part of chromosome beyond secondary constriction is called satellite or trabant which remains attached to the main part of chromosomes by a thread of chromatin. The chromosome having satellite is called sat chromosome. Secondary constriction s are of two types NOR and Joint .They are always constant in their positions and often used as markers. The NOR (nucleolar organizer region) are specialized to produce nucleolus and rRNA. The joints sometimes develop due to breaking and fusion of chromosome segments.

(d) Telomeres:

The terminal ends of chromosomes are called telomeres. A telomere is a short-repeated DNA sequence (GC rich) complexed with proteins. They are synthesized separately and later add to the chromosomal tips.

The telomeres help is various ways:

(i) Provide stability by preventing end fusions of chromosomes,
(ii) Act as initiators of synapsis,

(iii) Shortening of telomeres causes senescence and aging.

(e) Chromo-meres:

Sometimes along the entire length of interphase chromosomes appear beaded due to accumulation of chromatin. These bead-like structures are called chromo-meres. At metaphase the chromo-meres are tightly coiled and are no longer visible. These are clearly visible in the polytene chromosomes. Available evidences indicate that chromomere represents a unit of DNA replication, chromosome coiling, RNA synthesis and RNA processing.

Ultra-structure of Chromosome:

A metaphase chromosome has two sister chromatids, each about 700nm in diameter. The electron microscopic studies have revealed that, each chromatid consists of a central non-histone core called scaffold or nuclear matrix, from which loop of 30 nm chromatin fiber fan out radially. Each lateral loop is about 300nm long and has up to 100 Kb of DNA.

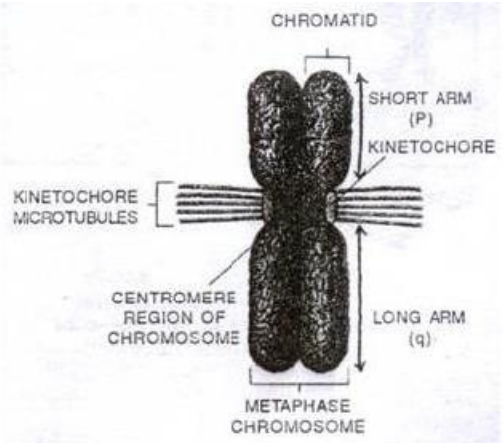
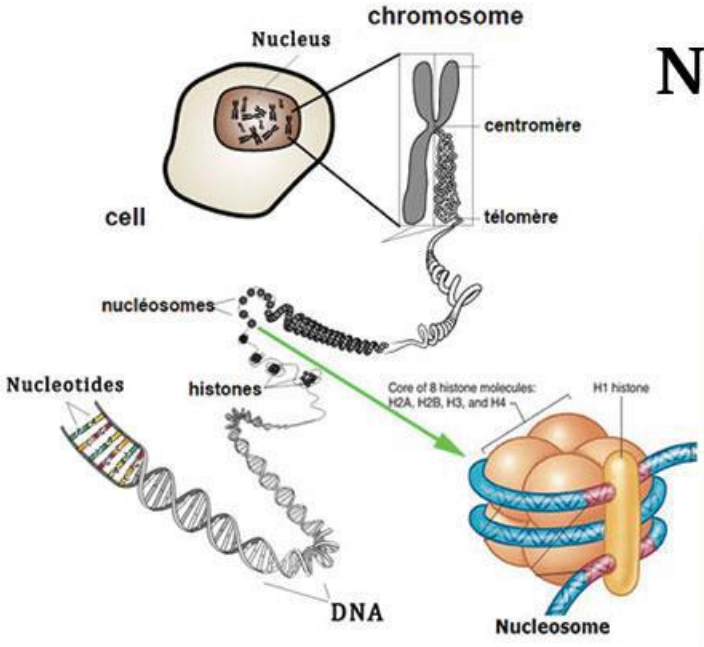
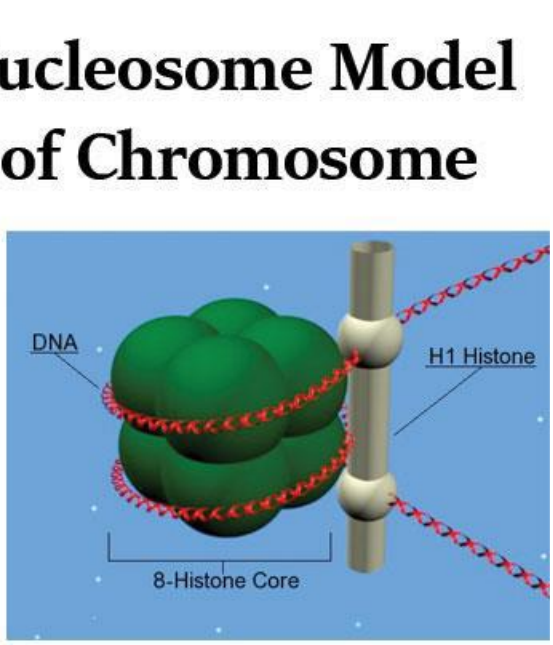


Figure 3.22 Structure of a mitotic chromosome. Kinetochore microtubules are anchored to proteins at the centromere.

NUCLEOSOME AND ITS IMPORTANCE IN THE ORGANIZATION OF EUKARYOTIC CHROMOSOME

- The **chromosomes** are the nuclear components of the special organization, individuality, and function that are capable of self-reproduction and play a vital role in heredity, mutation, variation and evolutionary development of the species.
- Each chromosome is made up of **DNA** tightly coiled many times around proteins that support its structure.
- The proteins that bind to the DNA to form eukaryotic chromosomes are traditionally divided into two classes: the histones and the non-histone chromosomal proteins.
- The complex of both classes of protein with the nuclear DNA of eukaryotic cells is known as chromatin.
- Chromatin are a highly compacted structure consisting of packaged DNA and necessary so as to fit DNA into the nucleus.
- The assembly of DNA into chromatin involves a range of events, beginning with the formation of the basic unit, the nucleosome, and ultimately giving rise to a complex organization of specific domains within the nucleus.
- In the first step of this process, DNA is condensed into an 11 nm fiber that represents an approximate 6-fold level of compaction. This is achieved through nucleosome assembly.
- The nucleosome is the smallest structural component of chromatin and is produced through interactions between DNA and histone proteins.
- Each nucleosome consists of histone octamer core, assembled from the histones H2A, H2B, H3 and H4 (or other histone variants in some cases) and a segment of DNA that wraps around the histone core. Adjacent nucleosomes are connected via "linker DNA".





Introduction

- Nucleosome model is a scientific model which explains the organization of DNA and associated proteins in the chromosome.
- It also further explains the exact mechanism of the folding of the DNA in the nucleus.
- The model was proposed by Roger Kornberg in 1974 and is the most accepted model of chromatin organization.
- It was confirmed and christened by P. Oudet et al., (1975).

Features of the Nucleosome Model of Chromosomes

- In eukaryotes, DNA is tightly bound to an equal mass of histones, which serve to form a repeating array of DNA-protein particles, called nucleosomes.
- If it was stretched out, the DNA double-helix in each human chromosome would span the cell nucleus thousands of time.
- Histones play a crucial role in packing this very long DNA molecule in an orderly way (i.e., nucleosome) into nucleus only a few micrometers in diameter.

- Thus, nucleosomes are the fundamental packing unit particles of the chromatin and give chromatin a "beads-on-a-string" appearance in electron micrographs taken after treatments that unfold higher-order packing.
- Each nucleosome is a disc-shaped particle with a diameter of about 11 nm and 5.7 nm in height containing 2 copies of each 4 nucleosome histones–H2A, H2B, H3, and H4.
- This histone octamer forms a protein core [(i.e., a core of histone tetramer (H3, H4)2 and the apolar regions of 2(H2A and H2B)] around which the double-stranded DNA helix is wound 1¾ time containing 146 base pairs.
- In chromatin, the DNA extends as a continuous thread from nucleosome to nucleosome.
- Each nucleosome bead is separated from the next by a region of linker DNA which is generally 54 base pair long and contains single H1 histone protein molecule.
- Generally, DNA makes two complete turns around the histone octamers and these two turns (200 bp long) are sealed off by H1 molecules.
- On average, nucleosomes repeat at intervals of about 200 nucleotides or base pairs. For example, a eukaryotic gene of 10,000 nucleotide pairs will be associated with 50 nucleosomes and each human cell with 6×10^9 DNA nucleotide pairs contains 3×10^7

The Folding of the DNA

- The first step is the assembly of the DNA with a newly synthesized tetramer (H3-H4), are specifically modified (e.g. H4 is acetylated at Lys5 and Lysl2 (H3-H4)), to form a sub-nucleosomal particle, which is followed by the addition of two H2A-H2B dimers.
- This produces a nucleosomal core particle consisting of 146 base pairs of DNA bind around the histone octamer. This core particle and the linker DNA together form the nucleosome.
- The next step is the maturation step that requires ATP to establish regular spacing of the nucleosome cores to form the nucleo-filament.
- During this step the newly incorporated histones are de-acetylated.
- Next, the incorporation of linker histones is accompanied by folding of the nucleo-filament into the 30 nm fiber, the structure of which remains to be elucidated.
- Two principal models exist- the solenoid model and the zig-zag.
- Finally, further successive folding events lead to a high level of organization and specific domains in the nucleus.

TYPES OF CHROMOSOMES

A. Autosomes and Sex Chromosomes

- Human chromosomes are of two types:- autosomes and sex chromosomes.
- Genetic traits that are linked to the sex of the person are passed on through the sex chromosomes. The rest of the genetic information is present in the autosomes.
- Humans have 23 pairs of chromosomes in their cells, of which 22 pairs are autosomes and one pair of sex chromosomes, making a total of 46 chromosomes in each cell.

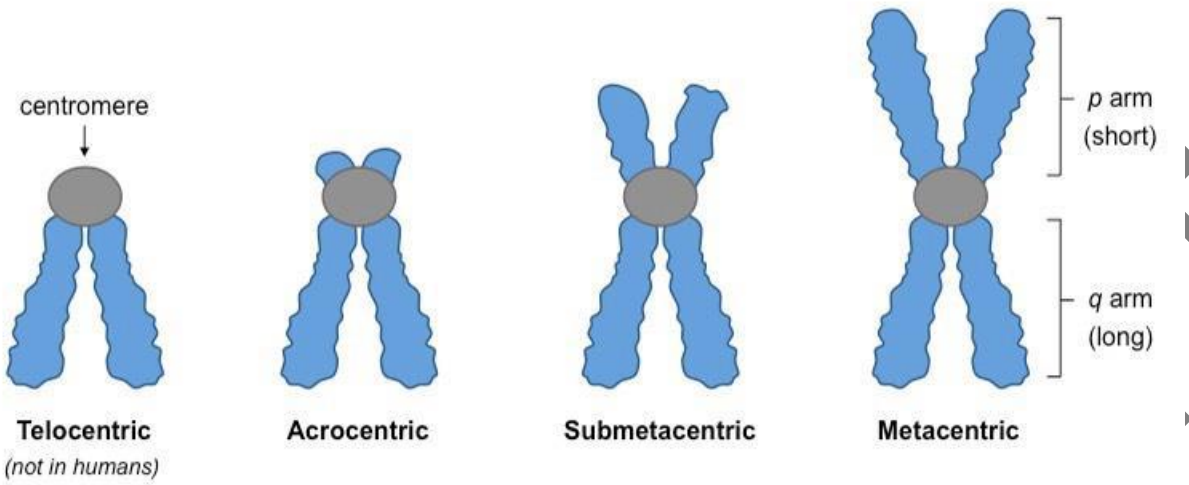
B. On the Basis of Number of Centromeres

1. **Monocentric** with one centromere.
2. **Dicentric** with two centromeres.
3. **Polycentric** with more than two centromeres
4. **Acentric** without centromere. Such chromosomes represent freshly broken segments of chromosomes which do not survive for long.
5. **Diffused or non-located** with indistinct centromere diffused throughout the length of chromosome.

C. On the Basis of Location of Centromere

1. **Telocentric** are rod-shaped chromosomes with centromere occupying the terminal position, so that the chromosome has just one arm.
2. **Acrocentric** are also rod-shaped chromosomes with centromere occupying a sub-terminal position. One arm is very long and the other is very short.
3. **Sub-metacentric** chromosomes are with centromere slightly away from the mid-point so that the two arms are unequal.

4. **Metacentric** are V-shaped chromosomes in which centromere lies in the middle of chromosome so that the two arms are almost equal.



HOLOCENTRIC CHROMOSOME

A chromosome with a diffused centromere is called holokinetic or holocentric chromosome. Such chromosomes do not have localized centromere, but the entire body of the chromosome exhibits centromeric activity.

The spindle fibres get attached to the entire body of such chromosomes. Sister chromatids of such chromosomes are not associated at any point by centric connection and have autonomous entity. Such chromosomes can move towards either pole during anaphase.

Function and Significance of Chromosomes

- The number of the chromosomes is constant for a particular species. Therefore, these are of great importance in the determination of the phylogeny and taxonomy of the species.
- **Genetic Code Storage**: Chromosome contains the genetic material that is required by the organism to develop and grow. DNA molecules are made of chain of units called genes. Genes are those sections of the DNA which code for specific proteins required by the cell for its proper functioning.
- **Sex Determination:** Humans have 23 pairs of chromosomes out of which one pair is the sex chromosome. Females have two X chromosomes and males have one X and one Y chromosome. The sex of the child is determined by the chromosome passed down by the male. If X chromosome is passed out of XY chromosome, the child will be a female and if a Y chromosome is passed, a male child develops.
- **Control of Cell Division:** Chromosomes check successful division of cells during the process of mitosis. The chromosomes of the parent cells insure that the correct information is passed on to the daughter cells required by the cell to grow and develop correctly.
- **Formation of Proteins and Storage:** The chromosomes direct the sequences of proteins formed in our body and also maintain the order of DNA. The proteins are also stored in the coiled structure of the chromosomes. These proteins bound to the DNA help in proper packaging of the DNA.

CELL DIVISION

Cell Division:

Cell division, cell reproduction or cell multiplication is the process of formation of new or daughter cells from the pre-existing or parent cells.

The importance of cell division can be appreciated by realizing the following facts:

1. Cell division is a pre-requisite for the continuity of life and forms the basis of evolution to various life forms.
2. In unicellular organisms, cell division is the means of asexual reproduction, which produces two or more new individuals from the mother cell. The group of such identical individuals is known as clone.
3. In multi-cellular organisms, life starts from a single cell called zygote (fertilized egg). The zygote transforms into an adult that is composed of millions of cells formed by successive divisions.
4. Cell division is the basis of repair and regeneration of old and worn out tissues.

It occurs in three ways:

1. Amitosis or Direct cell division.
2. Mitosis or Indirect cell division.
3. Meiosis or Reductional cell division

Phases of Cell Cycle:

Most cells divide one or more times during their life time. When they do, they pass through an ordered sequence of events that collectively forms the cell cycle. The duration of the cell cycle varies greatly from one cell to another.

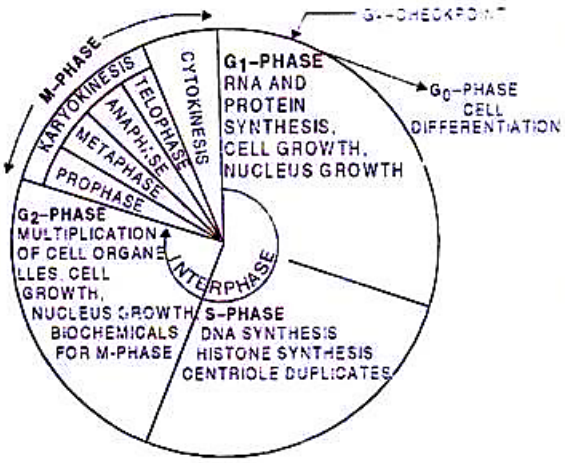


Figure 6.1 Cell cycle.

The shortest cell cycle occurs in early embryo and can last as little as 8 minutes. The cell cycle of growing eukaryotic cell lasts from 90 minutes to more than 24 hours, its duration varying considerably within a population of cells.

Howard & Pelc (1953) studied the details of cell cycle. The entire cell cycle may be divided into four phases: G_1 S, G_2 & M. The phases G_1, S and C_2 are together known as interphase (I-phase) which lasts more than 95% of the generation time. Though the interphase is called resting phase, it is actually an active or energy phase which is preparatory to cell division.

i. G_1– phase (= Gap-I or First growth phase or post-mitotic gap phase):

It is the most variable as well as longest phase of cell cycle during which RNA and proteins are synthesized. In a specific point of G_1 a cell decides whether to start anew cycle or to withdraw from the cycle. This point is called G_1 checkpoint. The cell that leaves the cell cycle to remain in a resting stage is said to be in Go state or quiscent phase.

ii. S-phase (= synthetic phase):

During this phase DNA synthesis or replication takes place. As a result the DNA content per cell doubles. In animal cells, centrioles duplicate in the cytoplasm. S-phase is also known as invisible stage of M-phase.

iii. G_2-phase (= Gap II or Second Growth Phase or Pre-mitotic phase).

In this phase, cell growth continues due to synthesis of RNA and proteins. However, DNA synthesis stops. Cell organelles like mitochondria and chloroplast divide.

iv. M-Phase (= Mitotic phase).

It is the final phase of cell cycle. It starts with nuclear division (Karyokinesis) and ends with division of cytoplasm (cytokinesis or C-phase). M-phase is of 3 types amitosis, mitosis & meiosis.

Control of Cell Cycle:

The events of cell cycle are genetically controlled and highly conserved through evolution. The control mechanism operates in the same manner in yeast, plants and animals.

Usually the cell cycle is controlled at three main checkpoints viz., G_1/S, G_2/M and spindle checkpoint (late metaphase). Checkpoints are the stages where a cell cycle may be stopped if the circumstances are not right for cell division.

i. G_1/S Checkpoint.

It is the primary checkpoint commonly known as 'start' (in yeast), restriction point or R-point (in animals). This checkpoint assesses extracellular growth factors or mitogens and intracellular nutritional state. Starvation, lack of mitogens can halt the cell cycle at this point and the cell enters the G_0-phase

ii. G_2/M Checkpoint:

It ensures the success of DNA-replication. DNA-dam aging agents can stop the cycle at this point.

iii. Spindle checkpoint:
It operates at the beginning of anaphase. It assesses whether all chromosomes are attached to the spindle or not. Passage through the above checkpoints is controlled by specific protein kinases that take part in phosphorylation and dephosphorylation. For the activity of protein kinases a number of protein stimulators are required which are called cyclins. The protein kinases are thus called Cyclin-dependent Kinases (Cdks) or mitosis promoting factor (MPF).

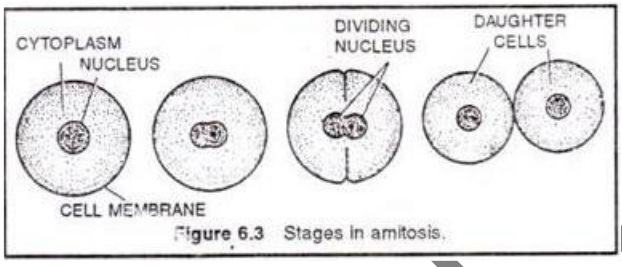


Figure 6.3 Stages in amitosis.

AMITOSIS (DIRECT CELL DIVISION): Remak (1955) discovered amitosis in RBCs of chick embryo, but the term was coined by Flemming (1882). (GK. a = no, mitosis = thread, osis = state)
Amitosis is a mode of division in which nucleus elongates, constricts in the middle and divides directly into two daughter nuclei. This is followed by centripetal constriction of cytoplasm to form two daughter cells.

Amitosis is characterized by:
i. Intact nuclear envelope is found through nut the division.
ii. Chromatin does not condense into definite chromosomes.
iii. A spindle is not formed.
iv. Chromatin distribution occurs unequally which causes abnormalities in metabolism and reproduction.
v. Cytokinesis may or may not follow karyokinesis.
Amitosis occurs in mega-nucleus of paramecium, nuclei of internodal cells of Cham, endosperm cells of seeds, cartilage cells and diseased cells.

MITOSIS

(Indirect Cell Division)/ (Equational Cell Division)/ (somatic or vegetative cell division)/ duplication division : (GK. Mitos = thread, osis = state)

Definition:
Mitosis is a type of cell division in which chromosomes are equally distributed resulting in two genetically identical daughter cells.

History:
Mitosis was first discovered in plant cells by Strasburger (1875). Later on, W. Flemming (1879) discovered it in animal cells. The term mitosis was coined by Flemming (1882).

Occurrence:
The cells undergoing mitosis are called mitocytes. In plants, the mitocytes are mostly meristematic cells. In animals, the mitocytes are stem cells, germinal epithelium & embryonic cells. It also occurs during regeneration. Root tip is the best material to study mitosis.

Duration:
It varies from 30 minutes to 3 hours.

Steps of Mitosis:
Mitosis is a continuous process and for better understanding the whole process is divided into following six stages:

1. Prophase
2. Prometaphase
3. Metaphase
4. Anaphase
5. Telophase

Karyokinesis

6. C-Phase or Cytokinesis

1. Prophase:

(i) Nucleus becomes spherical and cytoplasm becomes more viscous.
(ii) The chromatin slowly condenses into well-defined chromosomes- short, thick, helically coiled threads.
(iii) Each chromosome split into two sister chromatids joined at the centromere.
(iv) The spindle (microtubules) begins to form outside nucleus. In plants the spindle apparatus or mitotic spindle is anastral. In animals and brown algae the mitotic spindle is amphiastral which include two asters in opposite poles of the spindle. Each aster consists of two centrioles surrounded by astral rays.
(v) Nuclear membrane and nucleolus starts dissolving or disappearing.

2. Prometaphase:

(i) Nuclear envelop breaks down into membrane vesicles and the chromosomes set free into the cytoplasm.

(ii) Chromosomes are attached to spindle microtubules through kinetochores. Specialized protein complexes that mature on each centromere are called Kinetochores.

(iii) Nucleolus disappears.

3. Metaphase:

(i) Kinetochore microtubules align the chromosomes in one plane to form metaphasic plate or equatorial plate. The process of formation of metaphasic plate is called congression.

(ii) Centromeres lie on the equatorial plane while the chromosome arms are directed away from the equator called auto orientation.

(iii) Smaller chromosomes remain towards the centre while larger ones occupy the periphery.

4. Anaphase:

(i) Chromosomes split simultaneously at the centromeres so that the sister chromatids separate. They are now called daughter chromosomes. Where each one consists of single chromatid.

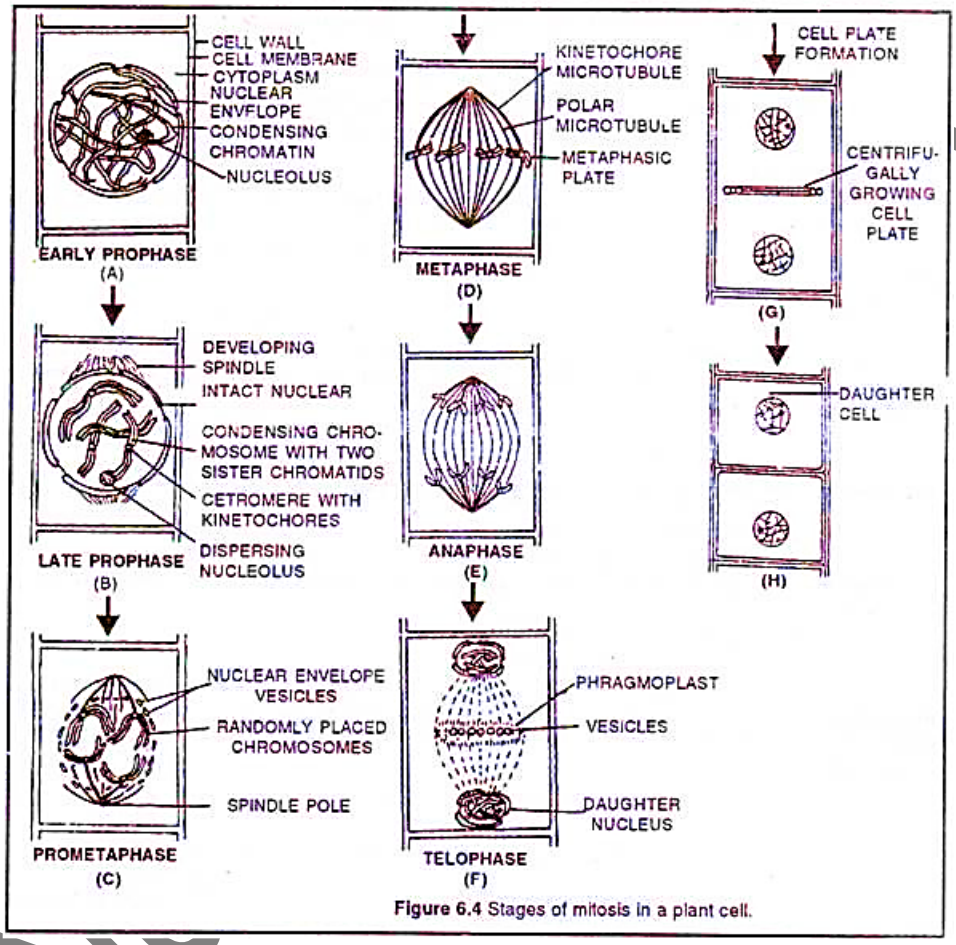


Figure 6.4 Stages of mitosis in a plant cell.

(ii) The separated sister chromatids move towards opposite poles at the speed of 1µm per minute.

(iii) Pole-ward movement of daughter chromosomes occurs due to shortening of kinetochore microtubules; appearance and elongation of inter-zonal fibers.

(iv)Daughter chromosomes appear V-shaped (metacentric), L-shaped (sub-metacentric), I-shaped (acrocentric) and I-shaped (telocentric).

(v) It is the shortest of all stages of mitosis.

5. Telophase:

(i) Daughter chromosomes arrive at the poles.

(ii) Kinetochore microtubules disappear.

(iii) Nuclear envelope reforms around each chromosome cluster of each pole.

(iv) Chromosomes uncoil into chromatin.

(v) Nucleolus re-appears.

(vi) It is considered as the reverse of prophase.

6. C-phase or Cytokinesis:

(i) It is the cytoplasmic division that starts during anaphase and completed by the end of telophase.

(ii) It takes place by two bare different methods i.e. cell plate method and cleavage or cell furrowing method.

i. Cell plate cytokinesis.

It occurs in plant cells. The spindle fibres persists at equatorial plane. The Golgi vesicles fuse at the centre to form barrel shaped phragmoplast. Further addition of vesicles causes the phragmoplast to grow centrifugally till it meets with plasma membrane of the mother cell. The contents of phragmoplast solidify to become cell plate or future middle lamella which separates the two daughter cells. The daughter protoplast secretes primary wall materials on both sides of the cell plate or middle lamella.

ii. Cleavage cytokinesis.

It occurs in animal cells and pollen mother cells of some angiosperms. In this process, a cleavage furrow appears at the middle, which gradually deepens and breaks the parent cell into two daughter cells. A special structure called mid body is formed in the centre, and it is a centripetal process.

MITOTIC APPARATUS

Notes on Microtubule and Spindle

During the process of cell division the cytoplasmic network of microtubules disappears and the microtubules are reassembled in the form of spindle in the dividing cell (in both mitotic and meiotic cell division). Though the controlling pattern of this disappearance and recognized is not very clear, but it has been realised that there are several regions which act as microtubule organising centres (MOCs or MTOCs).

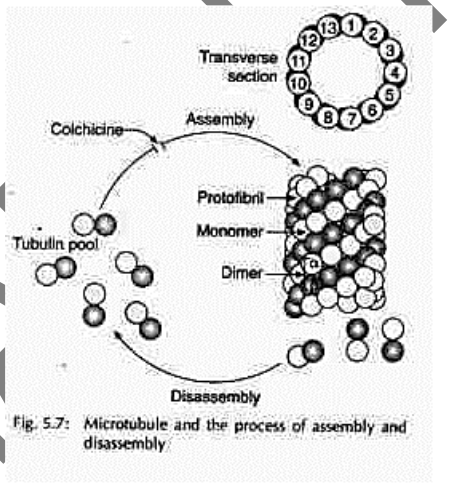


Fig. 5.7: Microtubule and the process of assembly and disassembly

The following are the examples of different microtubule organising centres involve in cell division:

(i) Centrioles:

They form the mitotic spindle and also the centriole satellites that form other centrioles.

(ii) Kinetochores:

These are the regions on the centromeres of chromosomes which are the attachment sites for microtubules of mitotic spindle.

(iii) Pericentriolar Cloud:

It has also been shown that the microtubules are assembled from the pericentriolar cloud and not from the centrioles.

(iv) Other MOCs:

In plant cells as they do not have the centrioles, so other centres play the role for organization of microtubule. Assembly of microtubules occurs in three steps. In the first step, free α-β tubulin dimers associate longitudinally to form short unstable protofilament. Next the short protofilaments associate laterally into more stable curved sheet.

In the final step, thirteen such protofilaments join laterally to form the cylinder (Fig. 5.7). Microtubule then grows by the addition of the subunits to the ends of protofilaments.

The process of microtubule assembly requires tubulin monomers bound to GTP, Mg^{2+} and ions. Though GTP binding is necessary for microtubule assembly but GTP hydrolysis does not provide the energy to drive the process. GTP remains bound to the tubulin in microfilaments.

When GTP is hydrolysed to GDP, the monomers become less stable and disaggregate. Polymerisation and disaggregation of microtubules may occur at either end, and may proceed independently. Microtubules have plus and minus ends. In the plus end microtubule assembly or disassembly occurs faster than in the minus end. Colchicine, vinblastine, vincristine and podophyllotoxin inhibit microtubule assembly whereas taxol promotes and stabilizes the microtubule formation.

Spindle Apparatus:

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 (Fig. 5.8A). The EM and polarization microscopic studies have revealed that in plant cells, which are devoid of centrioles and asters, the first spindle fibres appear at prophase in a clear zone surrounding the nucleus. Birefringence is strongest near the

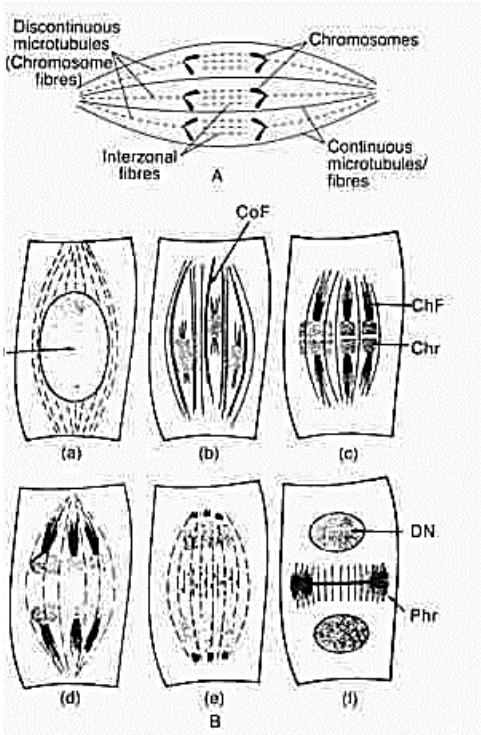


Fig. 5.8: A. Continuous and discontinuous microtubules in a mitotic anaphase cell; continuous microtubules are connected to the two poles at its two ends. While the discontinuous microtubules are connected to kinetochores of chromosomes at one end and to the pole on the other hand; B. Diagram of mitosis in a plant cell showing the changes in birefringence of the spindle fibers. Note the absence of centrioles and asters. Abbreviations: CoF, continuous fibres; ChF, chromosomal fibres; Chr, chromosome; DN, daughter nucleus; N, nucleus; Phr, phragmoplast (courtesy of S. Inoué)

kinetochores but becomes weaker towards the poles. During anaphase, the chromosomes are led by intensely birefringent chromosomal spindle fibres (Fig. 5.8B).

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.

A study on the number of microtubules has shown that there may be as few as single microtubule per chromosome in the spindle of yeast cell and as many as 5000 in the spindle of a higher plant cell. The chromosomal fibres are also called kinetochore tubules.

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. 5.9).

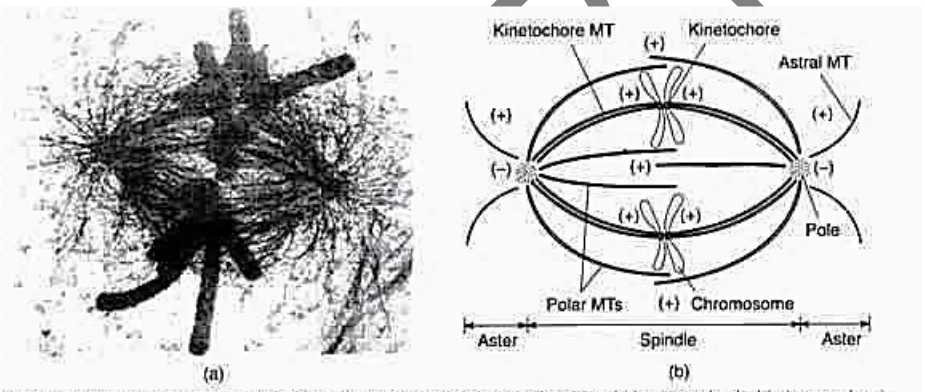


Fig. 5.9A: Mitotic apparatus in a metaphase cell. (a) To visualize the spindle microtubules more clearly, biotin-tagged anti-tubulin antibodies were added to make microtubules more massive. The large cylindrical objects are chromosomes. (b) Diagram showing the three sets of microtubules (MTs) in the 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)

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.

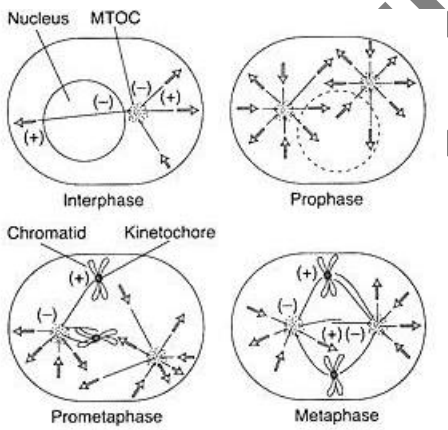


Fig. 5.10: Microtubule dynamics during mitosis. In both interphase and mitotic cells, most microtubules radiate from the microtubule organising centres (MTOCs), with the (–) ends of the microtubules closest to the MTOC and the (+) ends extending outward. A typical interphase cell has long microtubules; during mitotic prophase the microtubule-nucleating activity of the already replicated MTOCs increases, generating a larger number of shorter, more dynamic microtubules. In late prophase, some of the microtubules interact with kinetochores, causing the microtubules to be partially stabilized. In the metaphase mitotic apparatus, astral microtubules elongate and shorten (after Baltimore)

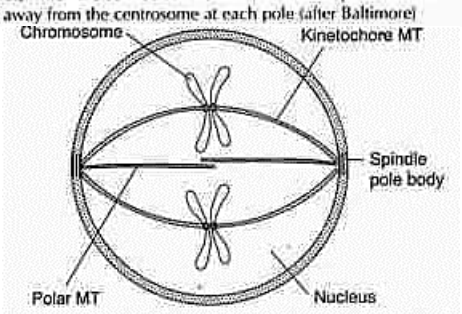


Fig. 5.9B: Mitotic apparatus in S. cerevisiae. In yeast, the nucleus remains intact during mitosis; thus the chromosomes are isolated from direct interaction with the cytosol. Spindle pole bodies, which are attached to the nuclear membrane, organize the spindle microtubules (MTs) (after Baltimore)

At metaphase, only the spindle microtubules are present; at anaphase with the movement -of the chromosomes, the spindle becomes de-polymerised; 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 (Fig. 5.10).

All microtubules situated between the poles and the kinetochores have the same polarity, i.e., with the fast growing ends distal to the poles. In dividing plant cells the plus ends of the microtubules are directed towards the phragmoplast, i.e., both kinetochore and phragmoplast may serve as positioning sites for microtubules.

MITOTIC INHIBITORS

A mitotic inhibitor is a drug that inhibits mitosis, or cell division. These drugs disrupt microtubules, which are structures that pull the chromosomes apart when a cell divides. Mitotic inhibitors are used in cancer treatment, because cancer cells are able to grow and eventually spread through the body (metastasize) through continuous mitotic division. Thus, cancer cells are more sensitive to inhibition of mitosis than normal cells. Mitotic inhibitors are also used in cytogenetics (the study of chromosomes), where they stop cell division at a stage where chromosomes can be easily examined.

Mitotic inhibitors are derived from natural substances such as plant alkaloids, and prevent cells from undergoing mitosis by disrupting microtubule polymerization, thus preventing cancerous growth. Microtubules are long, ropelike proteins that extend through the cell and move cellular components around. Microtubules are long polymers made of smaller units (monomers) of the protein tubulin. Microtubules are created during normal cell functions by assembling (polymerizing) tubulin components, and are disassembled when they are no longer needed. One of the important functions of microtubules is to move and separate chromosomes and other components of the cell for cell division (mitosis). Mitotic inhibitors interfere with the assembly and disassembly of tubulin into microtubule polymers. This interrupts cell division, usually during the mitosis (M) phase of the cell cycle when two sets of fully formed chromosomes are supposed to separate into daughter cells.

Examples of mitotic inhibitors frequently used in the treatment of cancer include paclitaxel, docetaxel, vinblastine, vincristine, and vinorelbine. Colchicine and griseofulvin are mitotic inhibitors used in the treatment of gout and toenail fungus, respectively.

Significance of-Mitosis:

1. Genetic Stability:

Mitosis maintains constant chromosome number and genetic stability in all somatic or vegetative cells of the body.

2. Growth:

Mitosis increases cell number so that a zygote transforms into a multicellular adult.

3. Surface-Volume ratio:

As the size (volume) of a cell increases, the surface area decreases accordingly. By mitosis, the cell becomes smaller in size and the surface volume ratio is restored.

4. Nucleo-plasmic ratio:

When a cell grows in size, nucleocytoplasmic ratio decreases. 11 are restored by mitosis.

5. Mitosis is a method of asexual reproduction and vegetative propagation.

6. Mitosis provides new cells for repair, regeneration and wound healing.

7. DNA content is reduced to half from parent cell to daughter cell.

Abnormal Mitosis:

(a) Intra nuclear mitosis (= Promitosis):

In this type of division nuclear envelope does not disappear and spindle developed within the nucleus, e.g., many protozoans, yeast, some fungi etc.

(b) Endomitosis (=Endopolyploidy):

Reduplication of chromatids within intact nucleus forming polytene chromosome, e.g. some liver cells in human.

(c) Dinomitosis:

In this mitosis, nuclear envelope remains intact and intra nuclear spindle is not formed. Condensed chromosomes are present even in interphase, e.g., Dinoflagellates.

(d) Free nuclear division:

In this case, repeated karyokinesis occurs without subsequent cytokinesis. This causes multinucleate condition called coenocyte (e.g… Rhizopus, Mucor etc.), Syncytium (e.g. Opalina) orplasmodium (e.g., slime moulds).

(e) C-Mitosis:

Doubling of chromosome number without cytokinesis by the application of alkaloid colchicine is known as C-Mitosis.

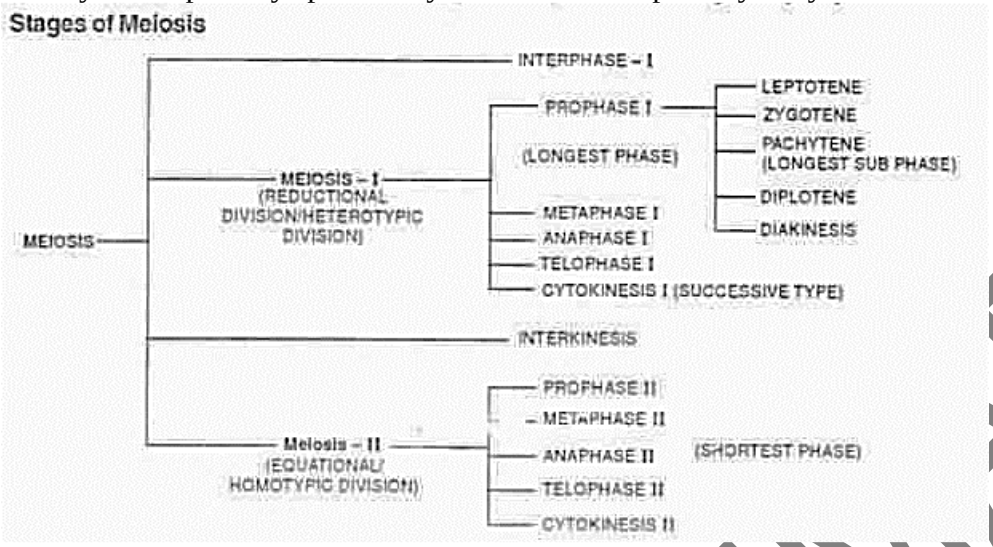
MEIOSIS

(Reductional OR dis-junctional Cell Division): (Gr. meioum = to reduce, osis = state)

Definition: Meiosis is a double division in which a diploid cell divides twice to form four haploid daughter cells.

History: Vanzeneden (1883)-First reported meiosis. Farmer & Moore (1905) coined the term meiosis.

Occurrence: The cells undergoing meiosis are called meiocytes. In plants, the meiocytes are microsporocytes (Pollen mother cell) of anthers and megasporocytes (megaspore mother cell) of ovules. In animals, the meiocytes are primary spermatocytes in testes and primary oocytes in ovaries.



lnterphase I:

(i) Physiologically most active stage

(ii) Nuclear envelop remains intact

(iii) Nucleoli is prominent

(iv) Chromosomes appear in form of chromatin reticulum

Prophase I:

It is typically longer in the first meiotic division and much longer than mitotic prophase and more complex phases. On the basis of chromosomal behaviour, it is divided into 5 sub-stages: Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.

(a) Leptotene (= Leptonema-thin thread) (short stage)

(i) Chromatin gradually condenses to form chromosomes.

(ii) Chromosomes appear as thin thread like structures with series of beads called Chromomeres also called Bouquet formation or synezesis.

(iii) Although chromosomes are replicated chromatids are not distinguished.

(iv) Both ends of each chromosome attached to nuclear envelope via specialized structures called attachment plaque.

(b) Zygotene (=Zygonema-paired\pairing)

(i) Pairing or synapsis of homologues chromosomes takes place in a zipper-like manner.

(ii) The pairing begins from the end away from the centromere and gradually extends along the entire length of the chromosome and it ends with the pairing of the centromeres.

(iii) Each synapsed chromosome pair is called a bivalent.

(iv) During synapsis, a ladder like proteinous structure appears called synaptonemal complex (SC) between the homologues of a bivalent. Synaptonemal complex = Bivalent + U-Protein

(v) Shortening of chromosomes continues.

(c) Pachytene (= Pachynema)

(i) The bivalents condense and each chromosome of the bivalent divides into two strands

(ii) At the end of pachytene Bivalents appear as tetrads.

(iii) Recombination nodules appear at intervals on the SC (synaptonemal complex)

(iv) The recombination nodules are thought to contain enzymes for crossing over or genetic recombination

(v) Between non-sister chromatids.

(vi) Nucleolus disappears.

(d) Diplotene (= Diplonema)

(i) The SC dissolves so that the homologues in a bivalent separate from each other except at the cross-over points or chiasmata, where two strands, one from each homologous chromosome, unite together to form Xs. The number of chiasma in a bivalent varies from 1-12. Two hypotheses are well known to explain the formation of chiasma.

According to the Classical hypothesis, on one side two paternal and two maternal chromatids are paired and on the other side a paternal chromatid pairs with a maternal and a maternal chromatid pairs with a paternal one. It means that formation of a chiasma may or may not give rise to a crossing-over.

According to Chiasma type hypothesis, two strands in a four-stranded bivalent, break and unite diagonally in a X-shaped fashion to form chiasma. It means that crossing-over precedes the formation of chiasma.

But this is known that occurrence of one chiasma prevents the formation of another chiasma at the nearby region. This phenomenon is called chiasma interference. At the end of diplotene, the chromosomes thicken and become short. In some forms, the chiasma slips and comes to the terminal end of chromosomes. This is called terminalisation.

(ii) Homologues condense and detach from the nuclear envelope.

(iii) This stage lasts for months or years.

(e) Diakinesis

(i) The chromosomes further contract.

(ii) Number of chiasmata reduce.

(iii) RNA synthesis stops

(iv) Bivalents move towards the inner side of the nucleus

(v) Spindle formation occurs.

(vi) By the end, the homologues are separated off but held together only at their ends, nucleolus disappears and the nuclear envelope breaks down.

Metaphase I:

(i) Bivalents arrange on two equatorial plates.

(ii) The centromeres are directed towards poles and the arms of chromosomes face the equatorial plate called co-orientation. The gap between two centromeres is dependent upon the position of chiasma.

(iii) The chromosomes become much condensed and gain a smooth appearance.

(iv) The microtubules (chromosomal fibers) from opposite poles of the spindle attach to the bivalents.

Anaphase I:

(i) Half of the homologues chromosome separate and move to opposite pole. This process is known as disjunction.

(ii) The behavior of chromosomes is the same as in mitosis. Only difference is that during mitosis, a half centromere and one chromatid migrates while here an entire chromosome having two chromatids and an intact centromere does the same behavior.

(iii) Chromosomal fibres contract causing attraction while interzonal spindle fibres elongate causing repulsion.

(iv) The movement ends the pairing of the bivalent which causes the chiasmata to slip off from the terminal end. It must be remembered that chromosomes which separate during anaphase are not the same which appeared during zygotene to form the bivalent. Due to chiasma formation and crossing- over, many parts of it are reorganized.

Telophase I:

(i) This phase resembles that of mitosis. Only difference is the orientation of chromatids. Two chromatids of each chromosome are arranged either like L or V. A narrow stem body persists between the nuclei at the two poles.

(ii) Each pole possesses a group of dyad chromosomes.

(iii) Spindle fibers disappear

(iv) Nucleoli reappear and nuclear envelope reformed.

(v) In *Trillium* telophase I is absent. The chromosomes pass from anaphase I lo prophase

Cytokinesis: The occurrence of cytoplasmic division may or may not follow the nuclear separation. In some cases, a resting stage or interphase or interkinesis appears, while in many instances the telophase nuclei pass directly into the prophase stage of second meiotic division.

Interkinesis:

(i) It is a very brief interphase between meiosis I and meiosis II.
(ii) There is no DNA replication i.e. S-phase absent.

Meiosis II (Equational or Homotypic D. vision):

The meiosis II is similar to mitosis in which chromosomes number remains constant.

Prophase II:

It is a brief stage and is similar to the mitotic prophase. No complication occurs as in the first meiotic division.
(i) Chromosomes shorten and thicken
(ii) Nucleoli disappear.
(iii) Nuclear envelope breaks down
(iv) The spindle fibres appear at right angles to the spindle of meiosis-I.
(v) In animal cells, centrosome divides and moves to opposite poles.

Metaphase-II:

i. Chromosomes aligned in one equatorial plate.
ii. Spindle fibres attached to kinetochores of sister chromatids.
iii. Centromeres remain on the metaphasic plate while the chromatids are extended towards the poles.

Anaphase-II:

(i) The centromere divides and the two chromatids of each chromosome separate and pulled towards opposite poles.
(ii) The separated chromatids are now called as daughter chromosomes.

Telophase II:

The process is same as that of mitotic telophase, with the only difference that the telophase nuclei here contain only half the number of chromosomes.
(i) The daughter chromosomes reach at the opposite poles.
(ii) The chromosomes uncoiled to form chromatin.
(iii) Nucleoli and nuclear envelope reappear.
(iv) Spindle fibers disappear.

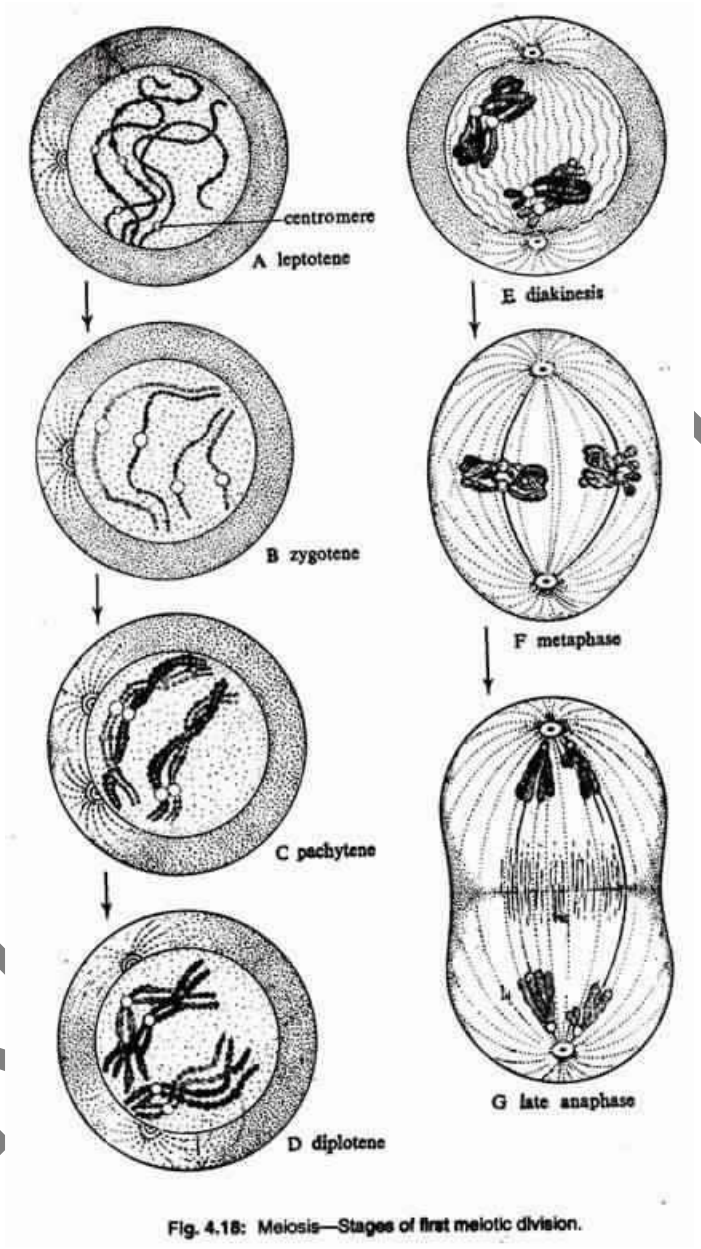


Fig. 4.18: Meiosis—Stages of first meiotic division.

Cytokinesis:

In meiosis, 2 types of cytokinesis can be seen.

(a) Successive type:

In this case, cytokinesis occurs after both meiosis I and meiosis 11. As a result, four haploid cells are formed. In plants the cells are arranged in form of isobilateral tetrad or in a linear manner.

(b) Simultaneous type:

In this case, cytokinesis occurs twice only after meiosis II. The four haploid cells arranged in form of a tetrahedral tetrad.

In plant cells, cytokinesis takes place by cell plate method, while in animal cells cleavage or furrowing method generally occurs.

Significance of Meiosis:

1. Meiosis essentially maintains constancy in chromosomes from generation to generation.
2. Crossing over and disjunction bring genetic variation within the species. The variations are important raw materials for evolution and also help in improvement of races.
3. Meiosis causes segregation and random assortment of genes.
4. Meiosis causes conversion from sporophytic generation to gametophytic generation in plants.

5. It leads to the formation of haploid gametes (n) which is an essential process in sexually reproducing organisms. Fertilization restores the normal somatic (2n) chromosome number.

Types of Meiosis:

There are three types of meiosis based on the variations in time and place of the division in the life-cycle of the plant.

1. Zygotic or Initial Meiosis (Haplontoic Pattern):

During the process of fertilization, the two gametes fuse to form zygote which represents the only diploid stage in the life-cycle. The zygote undergoes meiosis and forms four haploid cells which later on develop into haploid individuals, e.g., Thallophyta.

2. Gametic or Terminal Meiosis (Diplontoic Pattern):

This type of meiosis can be seen in animals and some lower plants. Here the meiotic division takes place immediately before gamete formation and the haploid cells thus formed are transformed into sperm (male gamete) and egg (female gamete).

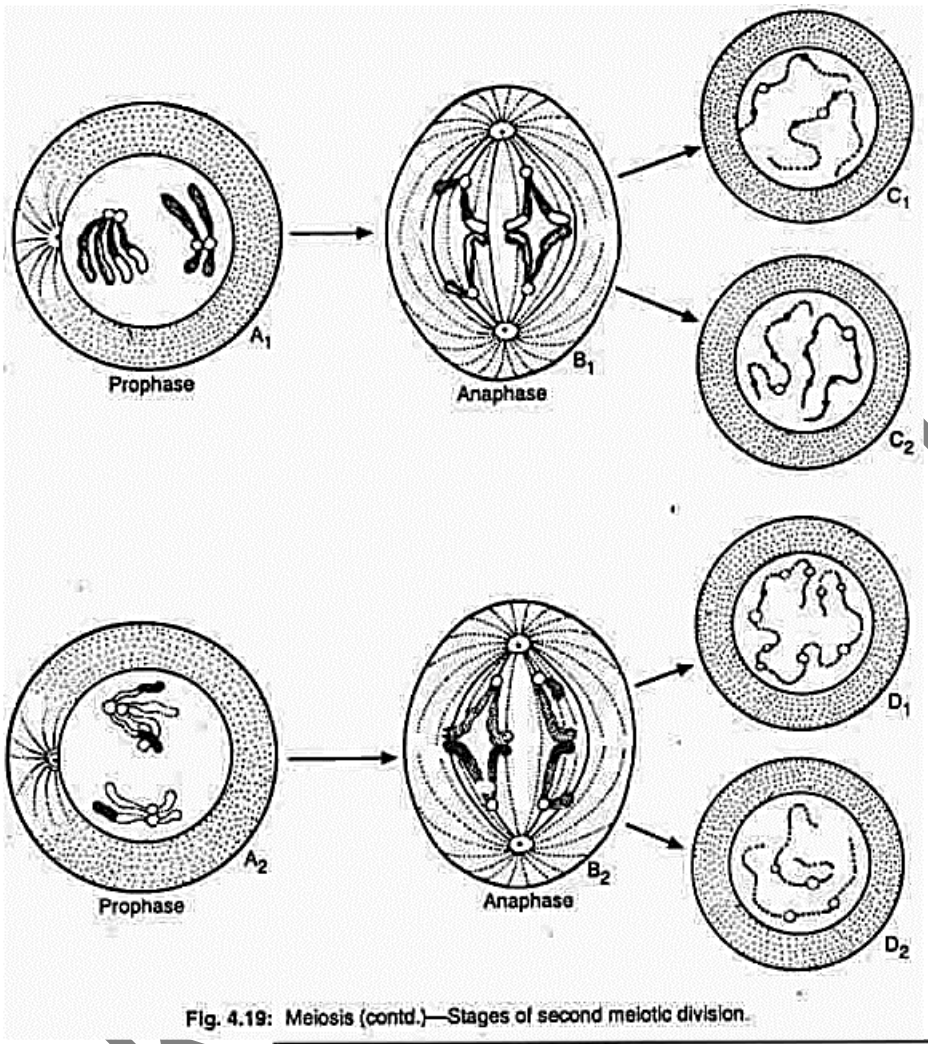


Fig. 4.19: Meiosis (contd.)—Stages of second meiotic division.

3. Sporic or Intermediate Meiosis (Diplo- haplontoic Pattern):

We come across this type of meiosis in higher plants and in some thallophyta but not in animals. The life-cycles of these organisms are characterised by alternation of haploid and diploid generations (i.e., gametophytic and sporophytic generations).

Meiosis occurs in the sporogenous cells (micro-and megaspore mother cells) of the sporophyte producing haploid spores. The spores on germination form gametophytes (male and female). Cells of the gametophyte form gametes. Fusion of these gametes again leads to diploid or sporophytic generation, and in this way alternation between gametophytic and sporophytic generations keeps on going.

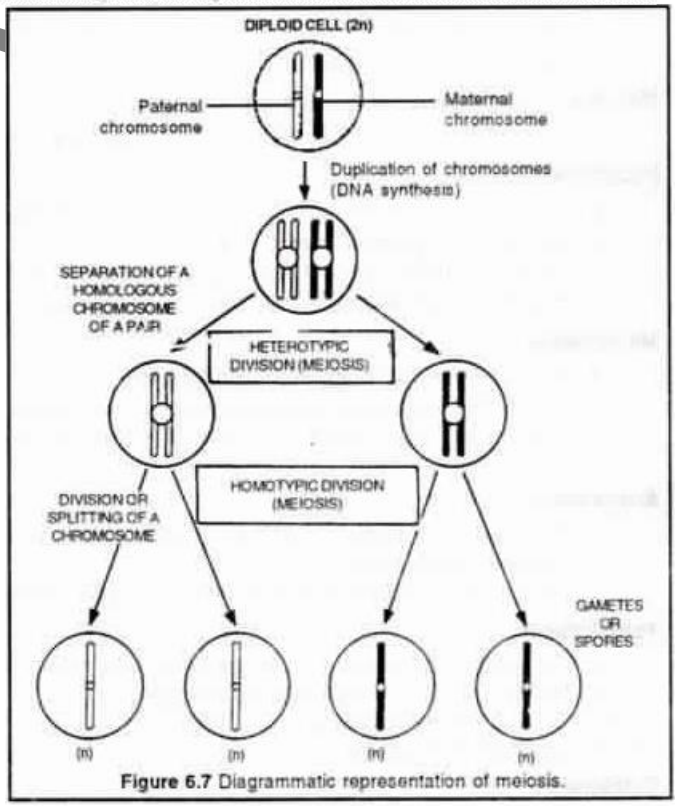


Figure 6.7 Diagrammatic representation of meiosis.

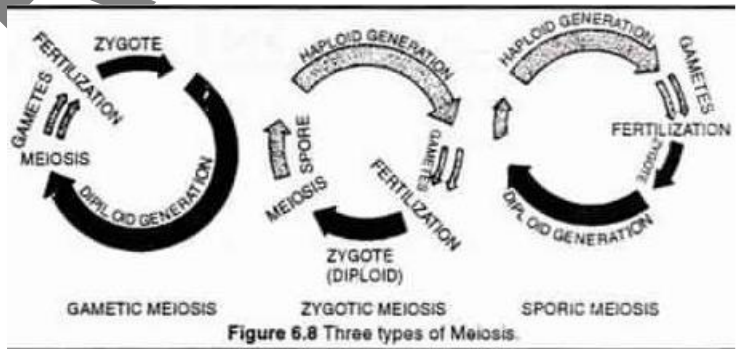


Figure 6.8 Three types of Meiosis.

CYTOLOGICAL PROOF OF CROSSING OVER:

Morgan and his collaborators established the genetic basis of crossing over and linkage describing the exchange of parts between the homologous chromosomes and linear arrangement of linked genes along chromosomes. This genetic inclination or bias could not be demonstrated cytologically since we cannot observe the homologous chromosomes (being all identical) under the microscope because of the following reasons.

(i) Crossing over occurs between homologous chromosomes. Such chromosomes are alike in appearance and it is not possible to distinguish between them in microscope.

(ii) During crossing over, the four chromatids are intimately coiled around one another.

(iii) In living cells, crossing over cannot be seen. In fixed and stained cells, one cannot say that chromatids have exchanged parts or not.

For nearly twenty years crossing over remained only a working hypothesis with geneticists. Finally, the cytological evidence which established beyond doubt the occurrence of crossing over, was given by S. Stern on *Drosophila* and H.B. Creighton and B. McClintock on Maize.

(1) Stern's Experiments or *Drosophila*:

Stern discovered a variety of *Drosophila* in which a part of Y chromosome had broken off and became attached to the end of one of the X-chromosome. Likewise, he described another variety in which one of the X-chromosomes was broken.

Usually in *Drosophila* normal fly has red round (++) eyes. Two mutant genes, one carnation (car) causing darkish red eyes and being recessive to red (+)eye colour and other bar (B) causing narrow eyes and dominant to round (+) eyes are both in X chromosome. The female fly is XX and male has XY chromosome.

In female (XX), one X chromosome was broken in to two by X-ray or other means and contained mutant genes car and B. The other X contains normal allele (+ for red and + for round eye i.e., normal) and its end a segment of Y chromosome was attached. This breaking of X chromosome and attaching Y segment is a plan or scheme to distinguish between cross-overs and non-cross-overs progeny.

The carnation barred female (car B+ +) produces four types of gametes, Out of which two are cross-overs and two non-cross overs formed without interchange of homologous segments of chromosomes. When these were mated with males (XY) having carnation round eyes (car +), the non-cross-overs are carnation bar and red round while cross-over contain red bar and carnation round.

Thus, cytological basis of crossing over can be established by distinguishing chromosomes under microscope. (Stern's experiment was an unique demonstration of the hypothesis that interchange of chromosomal material takes place between homologous chromosomes).

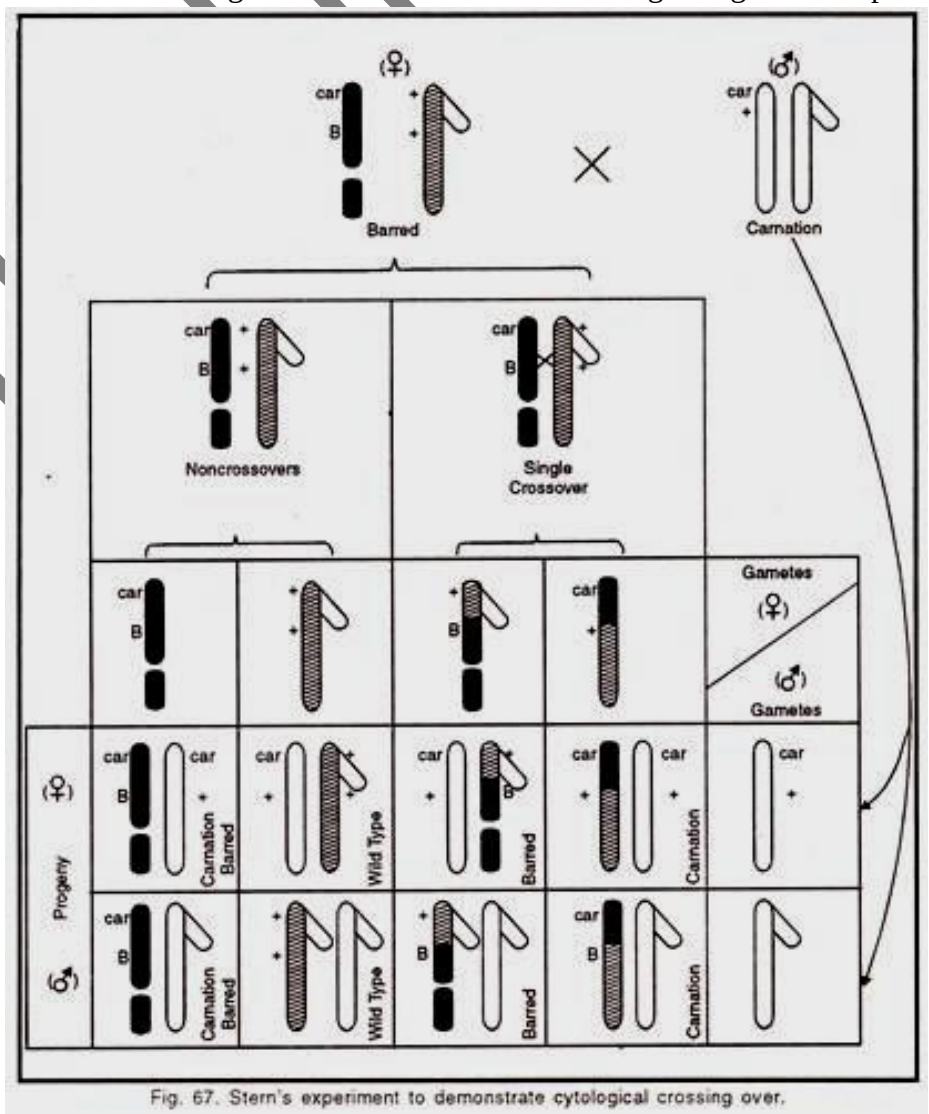


Fig. 67. Stern's experiment to demonstrate cytological crossing over.

2. Creighton and McClintock's Experiments on Maize:

Similar demonstration of cytological crossing over was demonstrated in maize by these workers. They observed a corn plant which had a pair of chromosomes whose two members could be held apart cytologically. Among them, one was normal and another had a trans-located piece of another chromosome at one end. The other end was like a knob (hard round protuberance).

The normal chromosome carried 'c' for colourless endosperm and W for starchy endosperm. Other knobbed chromosome had alleles 'C' for coloured and 'w' for waxy endosperm. Creighton and McClintock crossed this plant with a plant having homologous chromosome with recessive genes i.e., colourless waxy 'ccww'.

As a gametogenesis, two non-cross-overs and two cross-overs gametes will be formed which after union will form four kinds of off springs.

The non-crossover plants i.e., colourless starchy and colourless waxy obtained from parent either knobbed chromosome or normal but cross-over plants had one chromosome of this particular pair i.e., colourless waxy (ccww) contained a chromosome with trans-located piece but no knob, whereas coloured starchy (CcWw) showed knobbed chromosome but no trans-located piece.

Thus cross-overs showed cytological evidence of crossing over i.e., exchange of homologous chromosome parts during maturation of germ cells.

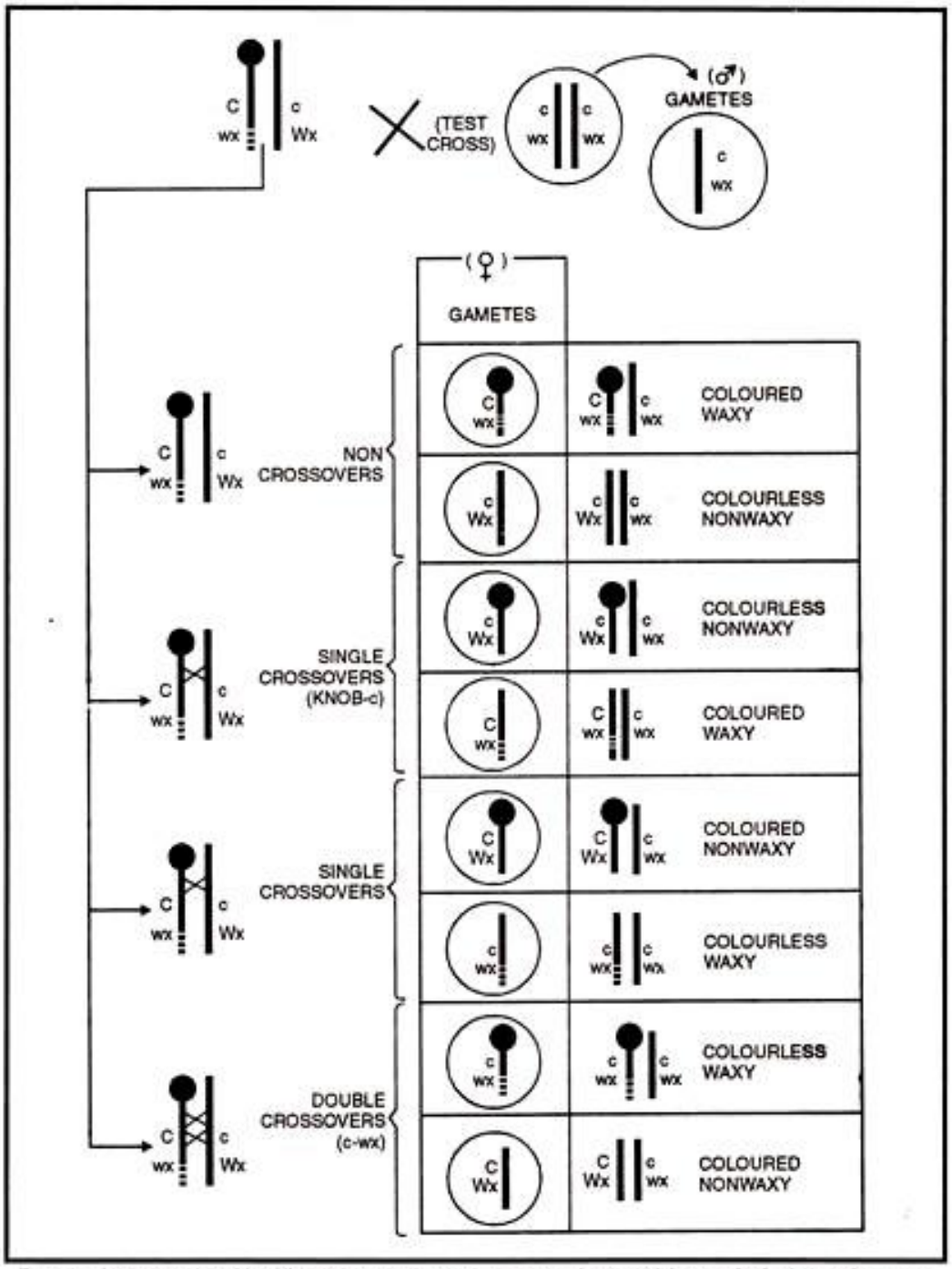
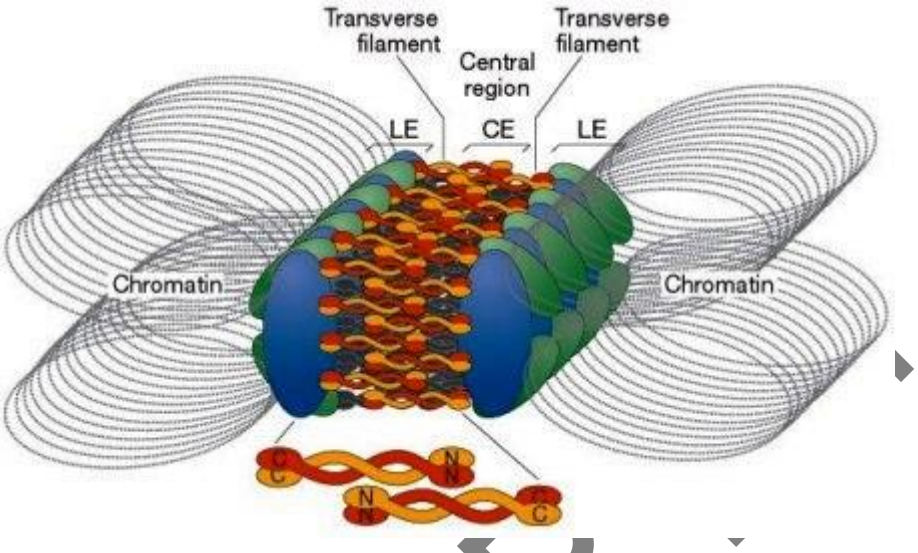


Fig. 68. Creighton and Mc Clintock's experiment in corn to give proof for cytological crossing over.

STRUCTURE AND FUNCTION OF SYNAPTONEMAL COMPLEX

In the process of crossing over three major happenings takes place- first breakage, then movement of these segments (transposition) and fusion by ligase in correct position. In the sea of genetic material, a process like crossing over to take place there should be a framework or a mechanism with all the necessary enzymes to carry out the process effectively.



Synaptonemal complex is the structure assigned with the duty of carrying out the process "Crossing over".

Synaptonemal complex is a highly organized structure of filaments seen between synapsed chromosome during zygotene and pachytene stages of prophase I of meiosis I. The structure is complex and can be studied only through electron microscope.

Synaptonemal complex consists of three parallel dense lines that are equally spaced or it is a tripartite ribbon like structure. It is a 0.2μM thick protein rich frame work. These lateral lines are flanked by chromatin. The two lateral lines are denser than the central line. Each lateral line is attached to the inner side of the homologous chromosome. The central and lateral elements are joined by LC fibers (L lateral to C central fibers). The ends of lateral elements are attached to the inner surface of nuclear membrane.

Lateral lines are rich in DNA, RNA and protein whereas central line has RNA and protein with little DNA. Synaptonemal complex is essential for chiasmata formation and crossing over. Recombination process is carries out by a large protein assembly of 90nm diameter called Recombination nodule (RNs) seen on the central element of Synaptonemal complex.

The major functions of Synaptonemal complex

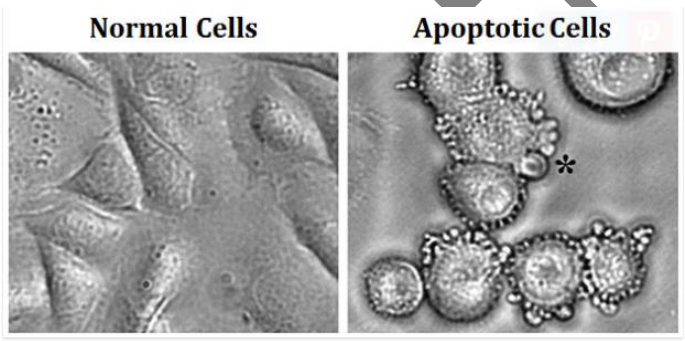
1. Maintenance of synapsis in fixed state for an extended period for crossing over to occur.
2. To provide a structural framework within which exchange of segments takes place.
3. To segregate recombination DNA from the rest of other chromosomal DNA.

BRIEF STUDY ON APOPTOSIS (PROGRAMMED CELL DEATH)

What is Apoptosis? Why apoptosis is known as the 'Programmed Cell Death?

The total number of cells in an organism is fundamentally fixed to a specific range in all multicellular organisms. In every multi-cellular organism, the cell number is effectively controlled by two strategies- (a) by regulating cell division and (b) by regulating cell death. If cells are no longer needed, they commit suicide (Self-destruction) by activating an intracellular death signaling programme. Thus, this death process is known as 'Programmed Cell Death'. This programmed cell death pathway is called Apoptosis.

The term apoptosis in Greek literally means 'falling off'. Just like the old leaves 'fall off' from the trees without affecting the life of the plant, the apoptotic cell death will not interfere with the functioning of the organ and organism. The most striking feature of apoptosis is that if a cell undergoes the programmed cell death, the neighbouring cells are not at all damaged. Apoptotic death of a cell and its subsequent phagocytosis by a neighbouring cell or by a macrophage allow the organic components of the dead cell to be effectively recycled. The apoptosis is better known as the 'Programmed Cell Death'. It is a natural well-orchestrated, well sequenced and timely executed chain of events leads to the death of a cell.



What are the characteristics of Apoptotic Cell Death?

An apoptotic cell death is characterized by:

1. Shrinkage of the cell
2. Shrinkage of the nuclear membrane
3. Loss of adhesion to the neighbouring cells
4. Formation of membrane blebs (externalization of inner leaflet of membrane)
5. Decay of mRNA
6. Condensation and fragmentation of the chromatin (DNA).
7. Formation of small fragmented chromatin in membrane bounded structure called apoptotic bodies.
8. Rapid engulfment of the apoptotic cell debris by the process of phagocytosis.

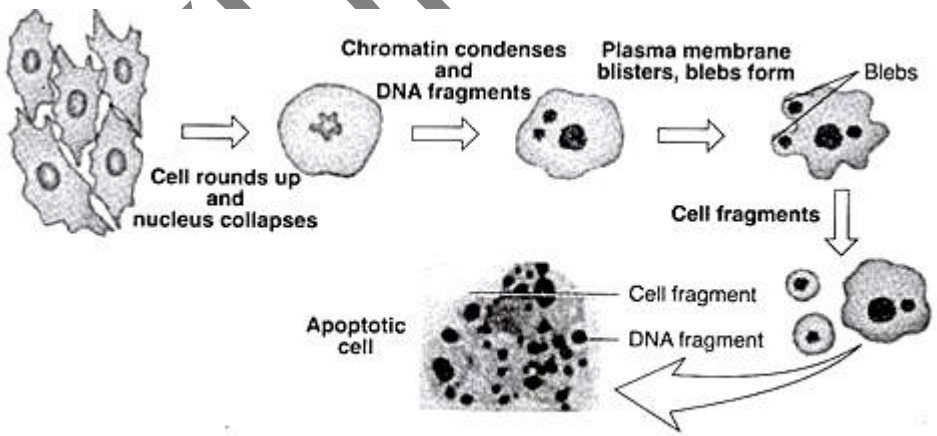


Fig. 5.33A: Sequence of cellular events during apoptosis

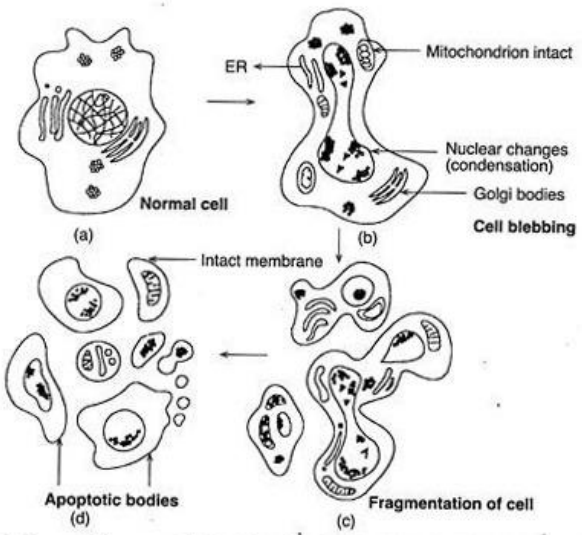
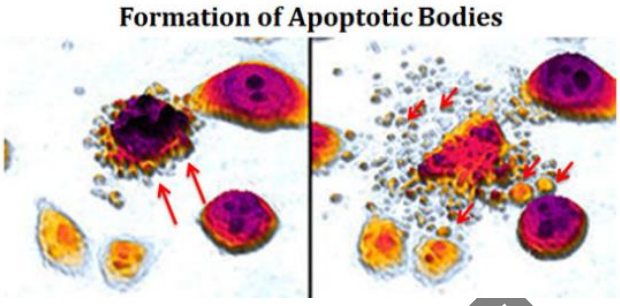


Fig. 5.33B: Cytological changes during apoptosis. Normal cell undergoes shrinkage showing condensation of chromatin and cell blebbing. Thereafter the cell is fragmented to produce apoptotic bodies, but the membranes remain intact (from Rastogi)

What are 'apoptotic bodies'?



During apoptotic cell death, the nucleus gets fragmented into many discrete chromatin bodies due to degradation of nuclear DNA. Each such nuclear fragment is surrounded by blebbed plasma membrane and these units were bud-off from the apoptotic cell. Thus, by the completion of apoptosis, the cell content is converted into many small vesicles called 'apoptotic bodies. Apoptotic bodies are immediately phagocytosed by the macrophages or surrounding healthy cells.

Does apoptosis be natural or pathogenic?

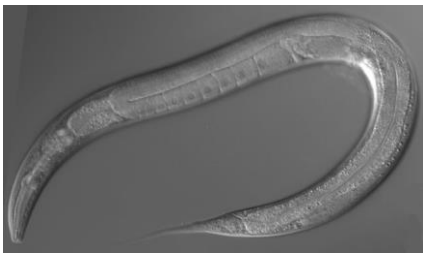
Webbed toes formation due to the lack of apoptotic cell death during embryonic development.

The apoptosis is a natural process. About 1010 to 1011 cells in the human body dies every day by the process of apotosis. Apoptosis is essential for the proper embryonic development in higher orgnaims. For example, the separation of fingers and toes in a developing human embryo occurs because cells between the digits undergo apoptosis during the embryonic development. Apoptosis also helps to prevent the perpetuation of lethal genetic damages in the body. The apoptotic cell death can occur in a cell when its genetic material is severely damaged and it cannot be rectified by the inbuilt DNA repair mechanism. Sometimes, the apoptosis can be pathogenic such as the death of healthy neurons which leads to the alzheimer's disease.

How was apoptosis discovered?

German scientist Karl Vogt was first to describe the principle of apoptosis in 1842. In 1885, anatomist Walther Flemming delivered a more precise description of the process of programmed cell death. However, it was not until 1965 that the topic was resurrected. While studying tissues using electron microscopy, John Foxton Ross Kerr was able to distinguish apoptosis from traumatic cell death.[8] Kerr was invited to join Alastair R. Currie, as well as Andrew Wyllie. These three people coined apoptosis. The 2002 Nobel Prize in Medicine was awarded to Sydney Brenner, Horvitz and John E. Sulston for their work identifying genes that control apoptosis. The molecular basis of apoptosis was elucidated for the first time in a nematode *Caenorhabditis elegans*. The worm *C. elegans* constantly maintain their cell number in its embryonic and adult stages. During the embryonic development, the worm produces exactly 1090 cells. Among these 1090 cells, 131 cells are precisely destined to die by apoptosis during the development. Further studies in the worm identified a specific gene involved in controlling the apoptosis process and it is named as **CED-3**. A worm with inactive CED-3 gene by mutation fails to induce the apoptotic cell death in the embryonic development stage. This shows that CED-3 plays a crucial role in executing the process of programmed cell death. Later scientists identified genes homologous to the CED-3 of *C. elegans* in other organisms including humans and subsequently named as **Caspases**.

What are caspase? What is the importance of caspases in apoptosis?



Caspases are a family of protein present in human and other animals which are homologous to the CED-3 gene product of *C. elegans*. Caspases are cysteine proteases involved in the execution of apoptotic cell death. Cysteine proteases are a category of protease enzymes with a cysteine residue at its active site. The caspases are produced as inactive zymogens called procaspases. Pro-caspases are activated to caspases during the early stages of apoptosis. Activation of procaspase to caspases is achieved by the catalytic removal of a part of the peptide chain. Activated caspases are responsible for most of the molecular events in the apoptosis signaling pathway.

How caspases execute apoptosis? What are the targets of caspases during apoptosis?

Caspases execute the apoptosis by selectively targeting and cleaving a large array of keymolecules in the cells. Most important target molecules of caspases during apoptosis are given below:

1. **Protein Kinases:** Protein kinases such as Focal Adhesion Kinase (FAK), Protein Kinase B (PKB), Protein Kinase C (PKC) and Raf1. Inactivation of the FAK cause detachment of the apoptotic cells from its neighboring cells due to the inhibition of cell adhesion.
2. **Lamin:** Lamin form the inner lining of the nuclear membrane and thus the cleavage of lamins lead to the disintegration of nuclear lamina (nuclear membrane) and breakge of the nucleus.
3. **Cytoskeleton proteins:** The cleavage of cytoskeleton proteins such as actin, tubulin and intermediate filaments lead to the shrinkage of the cells.
4. **CAD** (Caspase Activated DNases): CAD is an endonuclease. In a normal cell, the CAD endonuclease exists in an inactive stage. The cleavage of CAD by caspase actvates the CAD enzyme. Activated CAD then translocated into the nucleus and it cleaves and degrades the DNA.

What are apoptotic signals?

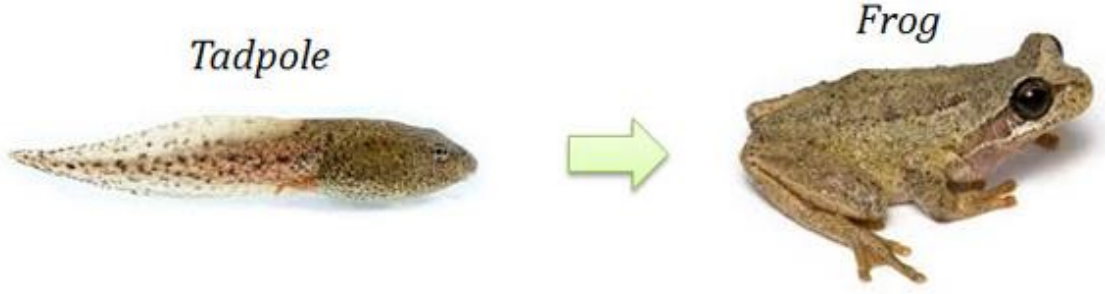
Any stimuli that can induce and initiate the programmed cell death patheay are called apoptotic signals. The source of apoptotic signals can be of two different types such as those from the external sources and those signals originated in the cell itself. Based on the source of signals, there are esentially two types of apoptotic signaling pathways. They are:

1. **Intrinsic Apoptotic Pathway:** Here the apoptotic stimuli are originated internally in the target cell itself. The most important internal signal that induces intrinsic signaling is sever DNA damage that cannot be rectified by the DNA repair mechanism.
2. **Extrinsic Apoptotic Pathway:** Here the stimuli are from the external source (not from the cell itself). The most important external apoptotic signals are cytokines such as Tumour Necrosis Factor (TNF). Even though the signalling cascades of extrinsic and intrinsic pathways are separate, there is always cross-talk between these two pathways. The extrinsic pathway can induce the activation of the intrinsic pathway of apoptosis.

What are the significance of apoptosis?

Apoptosis is a beneficial event. Moreover, failure to regulate apoptosis can result in the damage of organs or organisms. The main significance of apoptosis are given below:

1. Apoptosis help to maintain the homeostasis in multicellular organisms.
2. Apoptosis also helps to maintain the proper body size.
3. Apoptosis maintains the constancy of cell number in an organ or organism.
4. Apoptotic cell death is a pre-request for the proper embryonic development.
5. By the process of apoptosis, the body can eliminate unwanted cells such as:
   i. A cell with severely damaged DNA.
   ii. A cell with fatal mutation.
   iii. A pathogen (virus) infected cell
   iv. Unwanted cells formed during embryonic development.
6. Cells that to be killed during proper neuronal architecture development.
7. Apoptosis also helps to kill T lymphocytes with receptors for the proteins present on the normal cell. These T cells are produced during the embryonic development. These dangerous T lymphocytes are eliminated by apoptotic cell death.
8. Apoptotic cell death can be pathogenic in some cases.
9. Apoptosis is involved in some neurodegenerative diseases such as Alzheimer's, Parkinson's disease and Huntingtons's disease by the elimination of essential neurons.
10. Failure to induce apoptosis is the main reason for most of the cancers.



Development of Tadpole to Frog require Apoptotic Cell Death

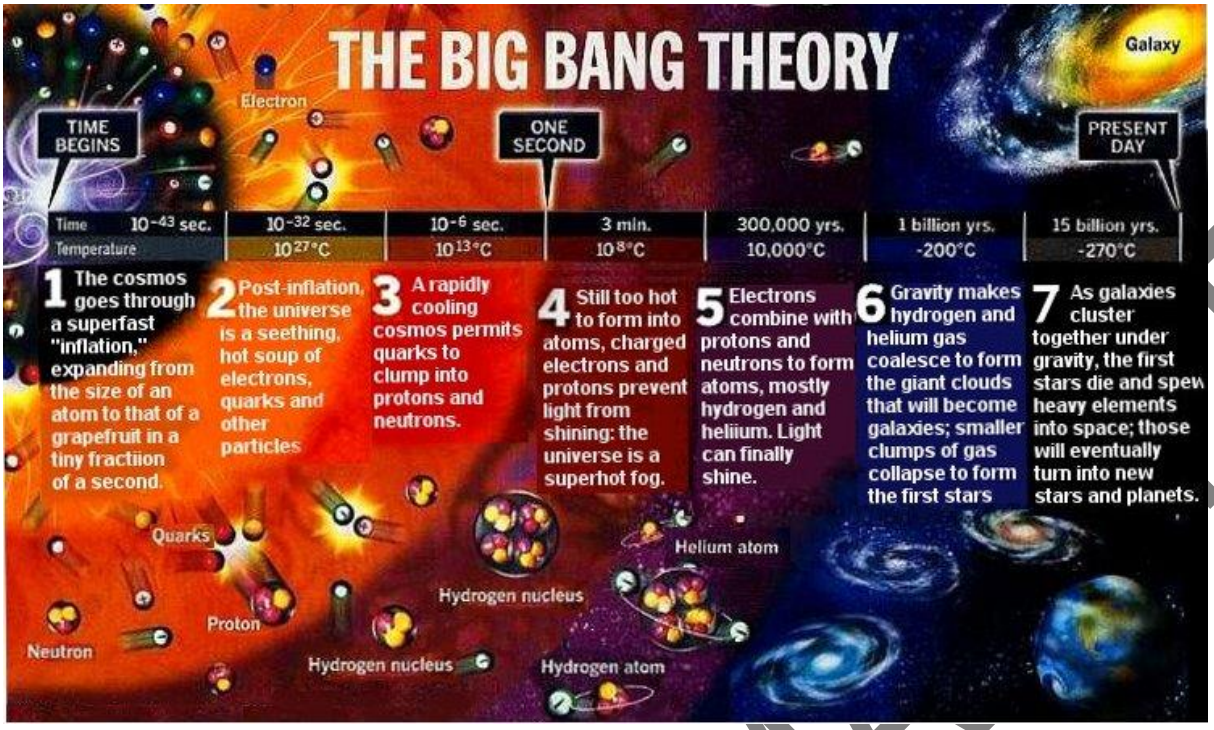
How the macrophages specifically recognize the apoptotic cells for phagocytosis?

Both the intrinsic and extrinsic pathway of apoptosis converges by activating the same executioner caspases, i.e, caspase-3. As the apoptotic signalling proceeds the cell loses its contact with the neighbouring cell and starts to shrink. The cell ultimately shrinks into one or more condensed membrane-enclosed structures called the apoptotic body. The apoptotic bodies are characterized by the presence of phosphatidyl serine on their outer surface. Phosphatidyl serine is a membrane flipping occurs which results in the externalization of phosphatidyl serine residues. These externalized phosphatidyl serine molecules are the eat me signals for the macrophages. The macrophages recognize these 'eat me' signal and they completely phagocytosis the apoptotic bodies. Thus, the apoptotic cell death is completed without spilling the cellular content into the extracellular environment. This is very significant because the release of cell debris can trigger inflammatory responses which ultimately cause severe tissue damage.

What is the relationship between Apoptosis and Cancer?

Cancer is a pathological process of uncontrolled division of cells leading to tumor development. Some cancerous cells also have the potential to invade healthy tissues by a process called metastasis. The cancer is essentially the uncontrolled division of an abnormal cell with mutations of genetic damage. If the apoptotic signalling is properly working, these unwanted cells can be eliminated from the body by programmed cell death pathway. Thus the main reason for cancer is the failure to induce apoptosis in an unwanted cell and as a result of this, the unwanted cell perpetuates without any control.

ORIGIN OF UNIVERSE- BIG BANG THEORY



The best-supported theory of our universe's origin centers on an event known as the big bang. This theory was born of the observation that other galaxies are moving away from our own at great speed in all directions, as if they had all been propelled by an ancient explosive force.

A Belgian priest named Georges Lemaître first suggested the big bang theory in the 1920s, when he theorized that the universe began from a single primordial atom. The idea received major boosts from Edwin Hubble's observations that galaxies are speeding away from us in all directions, as well as from the 1960s discovery of cosmic microwave radiation—interpreted as echoes of the big bang—by Arno Penzias and Robert Wilson.

Further work has helped clarify the big bang's tempo. Here's the theory: In the first 10^-43 seconds of its existence, the universe was very compact, less than a million billion billionth the size of a single atom. It's thought that at such an incomprehensibly dense, energetic state, the four fundamental forces—gravity, electromagnetism, and the strong and weak nuclear forces—were forged into a single force, but our current theories haven't yet figured out how a single, unified force would work. To pull this off, we'd need to know how gravity works on the subatomic scale, but we currently don't.

It's also thought that the extremely close quarters allowed the universe's very first particles to mix, mingle, and settle into roughly the same temperature. Then, in an unimaginably small fraction of a second, all that matter and energy expanded outward more or less evenly, with tiny variations provided by fluctuations on the quantum scale. That model of breakneck expansion, called inflation, may explain why the universe has such an even temperature and distribution of matter.

After inflation, the universe continued to expand but at a much slower rate. It's still unclear what exactly powered inflation.

As time passed and matter cooled, more diverse kinds of particles began to form, and they eventually condensed into the stars and galaxies of our present universe.

By the time the universe was a billionth of a second old, the universe had cooled down enough for the four fundamental forces to separate from one another. The universe's fundamental particles also formed. It was still so hot, though, that these particles hadn't yet assembled into many of the subatomic particles we have today, such as the proton. As the universe kept expanding, this piping-hot primordial soup—called the quark-gluon plasma—continued to cool. Some particle colliders, such as CERN's Large Hadron Collider, are powerful enough to re-create the quark-gluon plasma.

Radiation in the early universe was so intense that colliding photons could form pairs of particles made of matter and antimatter, which is like regular matter in every way except with the opposite electrical charge. It's thought that the early universe contained equal amounts of matter and antimatter. But as the universe cooled, photons no longer packed enough punch to make matter-antimatter pairs. So like an extreme game of musical chairs, many particles of matter and antimatter paired off and annihilated one another.

Somehow, some excess matter survived—and it's now the stuff that people, planets, and galaxies are made of. Our existence is a clear sign that the laws of nature treat matter and antimatter slightly differently. Researchers have experimentally observed this rule imbalance, called CP violation, in action. Physicists are still trying to figure out exactly how matter won out in the early universe.

Within the universe's first second, it was cool enough for the remaining matter to coalesce into protons and neutrons, the familiar particles that make up atoms' nuclei. And after the first three minutes, the protons and neutrons had assembled into hydrogen and helium nuclei. By mass, hydrogen was 75 percent of the early universe's matter, and helium was 25 percent. The abundance of helium is a key prediction of big bang theory, and it's been confirmed by scientific observations.

Despite having atomic nuclei, the young universe was still too hot for electrons to settle in around them to form stable atoms. The universe's matter remained an electrically charged fog that was so dense, light had a hard time bouncing its way through. It would take another 380,000 years or so for the universe to cool down enough for neutral atoms to form—a pivotal moment called recombination. The cooler universe made it transparent for the first time, which let the photons rattling around within it finally zip through unimpeded. We still see this primordial afterglow today as cosmic microwave background radiation, which is found throughout the universe. The radiation is similar to that used to transmit TV signals via antennae. But it is the oldest radiation known and may hold many secrets about the universe's earliest moments.

There wasn't a single star in the universe until about 180 million years after the big bang. It took that long for gravity to gather clouds of hydrogen and forge them into stars. Many physicists think that vast clouds of dark matter, a still-unknown material that outweighs visible matter by more than five to one, provided a gravitational scaffold for the first galaxies and stars.

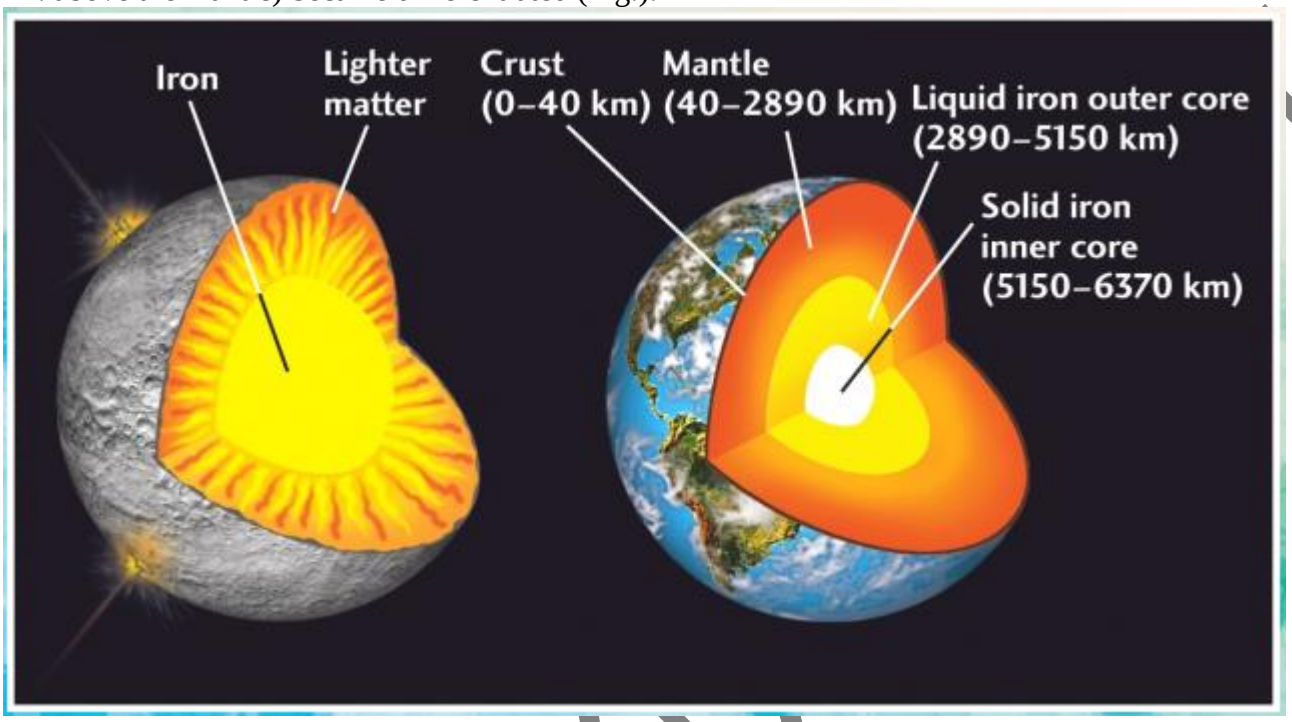
Once the universe's first stars ignited, the light they unleashed packed enough punch to once again strip electrons from neutral atoms, a key chapter of the universe called reionization. In February 2018, an Australian team announced that they may have detected signs of this "cosmic dawn." By 400 million years after the big bang, the first galaxies were born. In the billions of years since, stars, galaxies, and clusters of galaxies have formed and re-formed—eventually yielding our home galaxy, the Milky Way, and our cosmic home, the solar system.

Even now the universe is expanding, and to astronomers' surprise, the pace of expansion is accelerating. It's thought that this acceleration is driven by a force that repels gravity called dark energy. We still don't know what dark energy is, but it's thought that it makes up 68 percent of the universe's total matter and energy. Dark matter makes up another 27 percent. In essence, all the matter you've ever seen—from your first love to the stars overhead—makes up less than five percent of the universe.

ORIGIN OF EARTH

The earth originated sometime about 4.5 billion years ago either as a part shot out from the sun or in the form of interstellar gas cloud.

Most authorities believe that the earth originally was a very hot and homogeneous gaseous ball. That in due course of time cooled down due to cosmic cooling effect and as a result of the cooling three zones viz.. Core (radius 2500 km. from the centre), mantle (about 2900 km. above the core surface) and solid crust (about 9 to 40 km. above the mantle) became differentiated (Fig.).



The crust is the superficial solid zone of earth sphere, the mantle is the middle zone which is still in molten condition and earth core is the central part that is most possibly in vaporized state. The conditions on the primitive earth were not suitable for life as free oxygen was not available in the earth's atmosphere.

All the oxygen was in combined state, either in the form of water or as oxides of other elements. It is perhaps about 2.5 billion years ago that conditions consistent with life developed.

In the cooling process several physical changes took place in the earth and as a result of these changes, oceans and mountains were formed. Side by side, innumerable continuous chemical reactions were going on. Water which was formed first was not in the liquid state but remained in vaporized state for very long time. The water vapour accumulated and became condensed to form dense cloud around the earth.

The water vapour on cooling precipitated in the form of rain drops. Rain drops on reaching the hot surface of earth became instantaneously vaporized and the vapour again accumulated to form clouds. Continuous and prolonged raining made the surface of earth colder. Then the rain water accumulated in the low areas and formed oceans on the earth.

Countless volcanoes and fissures continuously gushed methane, steam, ammonia and perhaps carbon dioxide which accumulated around the earth and formed earth's first atmosphere. That air contained four main elements of life, carbon, hydrogen, oxygen and nitrogen which were in the form of gases deadly to present day life.

Moreover the atmosphere was flooded with short wave cosmic radiations or ultraviolet radiations and was stabbed by incessant lightning. This is the brief history of origin of earth and its atmosphere.

ORIGIN OF LIFE

Life is the part and parcel of the universe and both are very intimately associated with each other. We know that "Life is the most unique, complex organisation of molecules, expressing itself through chemical reactions which lead to growth, development, responsiveness, adaptation and reproduction" that matter has achieved in our universe. Origin of life is a unique event in the history of universe.

The modern concept or the naturalistic theory suggests that there was no trace of life on this planet about two billion years ago. Life on earth appeared 500 million years after earth's formation. Different theories were given by different thinkers and scientists.

(i) Theory of Special Creation:

It states that God created life by his divine act off creation, i.e., the earth; light; plants and animals are all being created by the supernatural power.

Religions preach that life was created by God whether it is Hinduism, Islam or Christianity. Religions teach that all species of plants and animals were individually created by God for specific purpose and that they do not have any relationships among them. According to Hindu mythology, life was the creation of Brahma, Islam says Allah created living beings and According to a Spanish Priest Father **Suarez** (1548 – 1617 B.C.), the whole universe was created in six days by the God. First day Earth and heaven, second day sky, third day dry land and vegetation, fourth day Sun, Moon and other planets, fifth day fishes and birds, and sixth day human beings other animals were created by God. This theory was based on some supernatural power. However, science cannot accept theories that have no concrete evidence.

This theory has following connotations:

(a) All living organisms (species or their types) that we see today were created as such.

(b) Diversity of life form will not change in future.

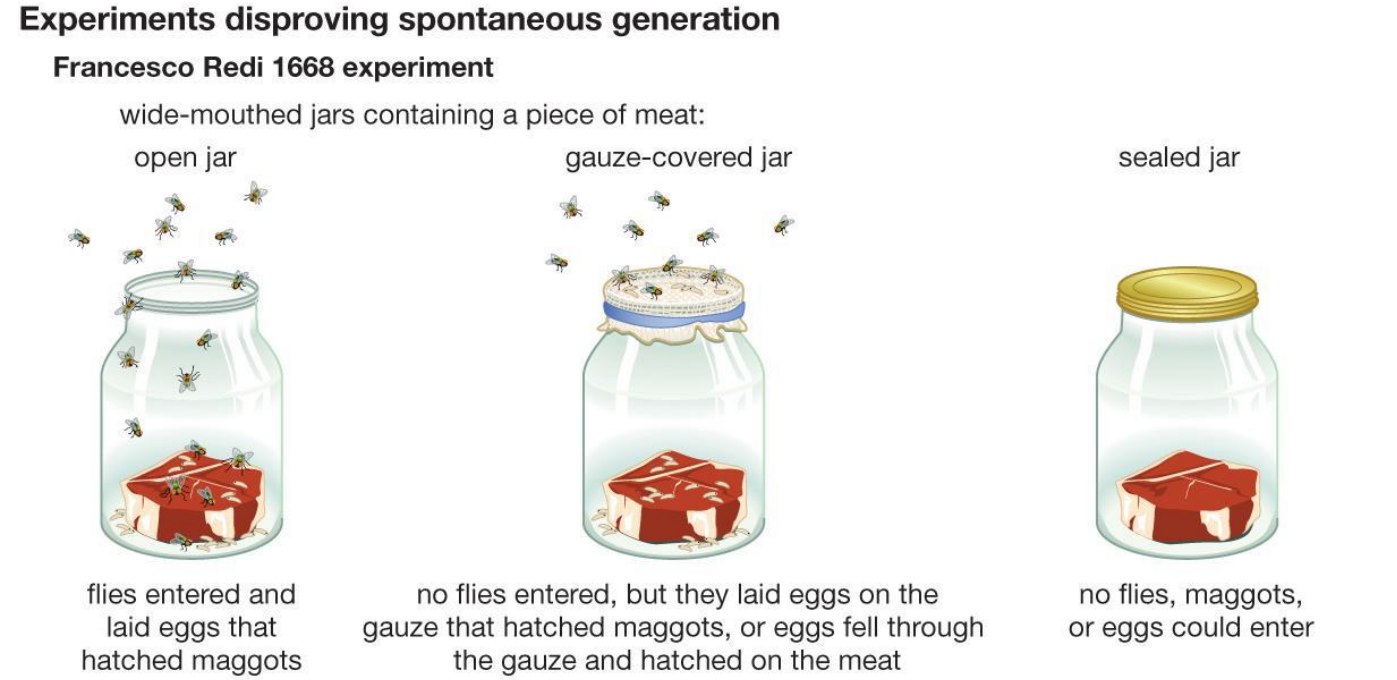
(ii) Cosmozoic or Extraterrestrial or Interplanetary or Panspermiatic Theory : Richeter

(1865), **Preyer** (1880), **Arrhenius** (1908), **Hoyle** (1950) and **Bondi** (1952) believed in eternity of life. According to Arrhenius life was transferred from "cosmozoa" (life of outer space) to different planets small units called 'spores'. The spores were covered by a thick protective covering. When the spores got favourable conditions and temperature, the spore coat was dissolved and gave birth to initial living organisms. This theory does not explain as to how the life originated in space and how the life originated in spores remain impenetrable by ultraviolet and gamma rays.

(iii) Theory of Spontaneous Generation (=Abiogenesis):

Abiogenesis means origin of life from non-living organisms. It states that life originated from decaying and rotting matter like straw, mud, etc. **Plato, Aristotle, Thales, Anaximander John Ray, Needham, Von Helmont**, *etc.*, believed that life originated from the non-living things and still continues to evolve by this method. For example, worms and frogs can appear spontaneously from mud and fly larvae can appear in rotten meat. Francisco Redi (1690) was the first scientist to refute this theory by experimentally demonstrating that if you cook fish and meat then no organisms appear in it in a sealed container. But people still believed that microorganisms developed by spontaneous generation.

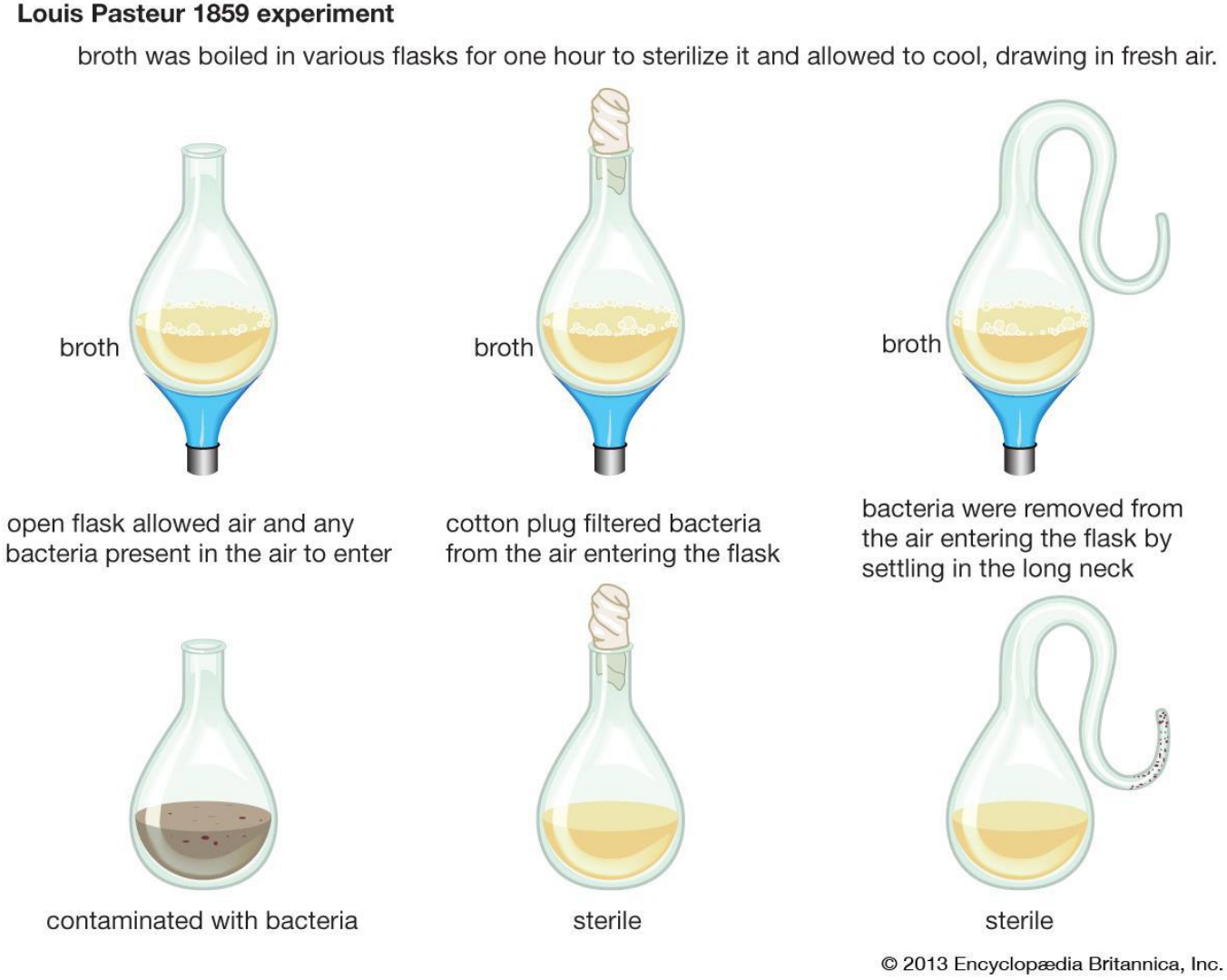
Another scientist, Spallanzani (1780) boiled and sealed broth and found that no micro-organisms appeared spontaneously. Later, Louis Pasteur (1860) conducted a more sophisticated experiment in which flasks whose neck was drawn like swan's neck were used to trap micro-organisms and dust. The boiled broth was kept in these flasks did not rot, suggesting that micro-organisms did not appear spontaneously.



Huxley (1870) criticized this theory and propounded the theory "life originated from preexisting life only."

(iv) Theory of Biogenesis

Redi, Richter, Spallanzani and **Louis Pasteur** rejected the theory of abiogenesis and proposed a new theory called Biogenesis. The theory postulates that life can emerge only from a form of life such as spores, eggs or hibernating animals and not from inorganic substances. But still this theory does not explain how life originated in the beginning.



(v) Theory of Vitalism:

This concept attributes the distinctive properties of the living things to a supernatural life force.

(vi) Theory Of Catastrophism or Theory of sudden creation from inorganic material:

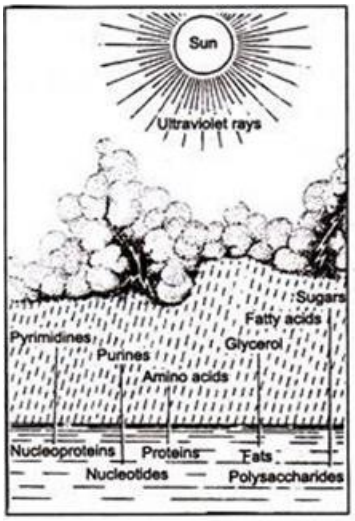
Cuvier (1769-1832) believed in catastrophism. According to him, the catastrophy destroys the whole life on earth, and after that, new life originates called it as Mechanistic theory. This is the extension of the theory of special creation. This theory assumes that life is originated by the creation and it is followed by catastrophe due to geographical disturbances. Each catastrophe destroyed the life completely whereas each creation forms life different from the previous one. Hence, each round of catastrophe/creation is responsible for evolvement of different types of organisms on earth. The criticism of the current theory is same as previous one, No scientific experiment to support the hypothesis and mostly be based on imaginary concepts.

(vii) Theory of Eternity of Life:

This theory assumes that life had no beginning or end. It believes that life has ever been in existence and it will continue to be so ever. It further believes that there is no question of origin of life as it has no beginning or end. The theory is also known as steady state theory. The main objection against the proposed theory that it could not be able to explain; evidences support that initially earth forms and then life appeared on it. Where life exist before the formation of earth?

(viii) Modern Theory / Oparin Haldane Theory / Chemical Theory / Naturalistic Theory / Materialistic Theory:

Oparin (1924) proposed that "life could have originated from non-living organic molecules." He believed in Biochemical origin of life. **Haldane** (1929) also stated similar views. Oparin greatly expended his ideas and presented them as a book "The origin of life" in 1936.



.3 Formation of organic chemicals in primitive ocean.

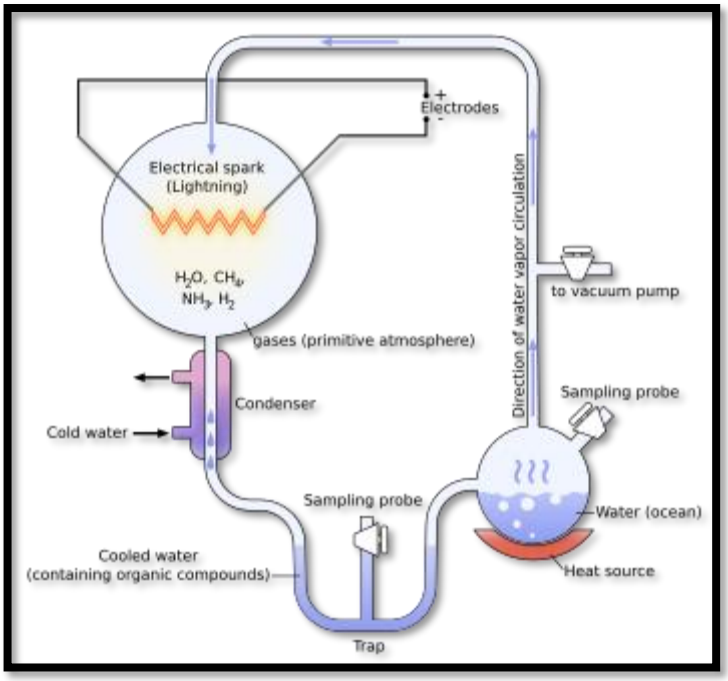
According to this theory, the Earth originated about 4,500 million years ago. When the earth was cooling down, it had a reduced atmosphere. In this primitive atmosphere nitrogen, hydrogen, ammonia, methane, carbon mono-oxide and water were present. Energy was available in the form of electric discharges by lightening and ultraviolet rays. As soon as the earth crust was formed, it was very much folded. Torrential rains poured over the earth for centuries and were deposited in deep places.

The atmospheric compounds, inorganic salts and minerals also came in deep places oceans, these molecules gave rise to a variety of compounds and finally to the self-duplicating molecules. Ultimately these molecules were enclosed in membranes derived from lipids and proteins, along with water and chemical compounds, giving rise to cell like units. Again random combinations may have led to the formation of chlorophyll– containing organisms which could produce their own food (autotrophs) by a process called photosynthesis. These organisms had a better chance to live because they synthesize starch from carbon dioxide and water in presence of sunlight. Starch could be used as further source of energy. During photosynthesis, oxygen was produced. The oxygen was used by other organisms for respiration. Also oxygen, when acted upon by ultraviolet rays, formed ozone layer through which ultraviolet rays cannot pass. This layer is formed about 25km. from earth's surface. After the formation of ozone layer, organisms could come to the surface of the ocean and could survive even on land, if thrown out of oceans. The Oparin's and Haldane's theory of origin of life is most accepted these days as it is supported by Miller's experiment duly supported by David Buhal, Melvin Kelvin's experiment *etc.* O_2 is absent in the primordial atmospheres at the time of origin of life.

Miller's Experiment: Urey (1952) postulated that the earth cloud originally contained chiefly hydrogen, methane, inert gases, a large number of iron compounds, ammonia, silicates etc.

Dr. Harold Urey, atomic scientist, in those days at the University of Chicago reasoned that if hydrogen, ammonia and methane were probable constituents of primitive atmosphere of earth, what would happen if these gases were placed in a flask and intermittent electric discharge was passed into it.

In 1953, Stanley Miller, a student of Dr. Urey performed one such experiment under support Oparin's theory of origin of life. He believed that basic compounds which are essential for life can be synthesised in the laboratory by creation in the laboratory, on a small scale, the conditions which must have existed at the time of origin of life on earth.



Miller took a flask and filled it with methane, ammonia and hydrogen in proportion of 2:1:2 respectively at 0°C. This proportion of gases probably existed in the environment at time of origin of life. This flask was connected with a smaller flask, that was filled with water, with the help of glass tubes. In the bigger flask, two electrodes of tungsten were fitted. Then a current of 60,000 volts was passes, through gases containing bigger flask for seven days. At the end of seven days, when the vapours condensed, a red substance was found in the U-tube. When this red substance was analyzed, it was found to contain amino acids, Glycine and nitrogenous bases which are found in the nucleus of a cell.

• An experiment to prove that organic compounds were the basis of life, was performed by miller.

From the above theory we conclude that life first originated in water. Therefore, water still continues to be an essential constituent of life.

The entire process of the origin of life, as proposed by Oparin, can be summarized as under:

(a) **The Chemical Evolution**

(1) **Step 1 : Formation of simple molecules** : The reactions between simple atoms like carbon, hydrogen oxygen and nitrogen in the primitive atmosphere led to the formation of simple compounds like water, ammonia and methane. But since the earth was very hot, all these substances remained in the form of vapours in the atmosphere. Gradually, as the earth started cooling down, the dense clouds began raining on the earth. But the liquid was still very hot. Therefore, as the liquid water touched the earth's surface, it again got vaporized to be returned to the atmosphere. This process continued for millions of years. As a result of these heavy downpours, the earth's surface got filled with water to form rivers and oceans. Ammonia and methane got dissolved in the oceanic water. The mineral elements, which were dissolved in rivers, were also carried into the oceans when rivers joined it.

The scientists have found that simple unicellular organisms (resembling modern cyanobacteria) were present on this earth about 3600 million years ago. It is believed, therefore, that life must have originated on this earth about 4600 to 3600 million years ago.

It must be clear that the earth's atmosphere at that time was quite different from as it exists today. The earth's atmosphere at that time was reducing**,** not oxidizing (as it is today). The primitive atmosphere of earth had hydrogen, nitrogen, water vapours, carbon dioxide, methane and ammonia abundance. Oxygen was not available in free state in sufficient quantities.

(2) **Step 2 : Formation of simple organic compounds :** Continuous rains provided opportunities for different types of molecules to collide with each other and react. Methane is an active compound, and it reacted with other compounds to form organic compounds like ethane, butane, propane, ethyl alcohol. From such organic compounds which were formed in the ocean and which played a role in the origin of life include –

(i) **Sugars, glycerol and fatty acids:** These were formed by the combination of carbon, hydrogen and oxygen.

(ii) **Amino acids:** These were formed by the combination of carbon, hydrogen, oxygen and nitrogen.

(iii) **Pyrimidines and Purines:** These were formed by the combination of carbon, hydrogen and nitrogen.

These compounds were formed at the time when sunlight could not reach earth because of dense clouds in the sky. Under such circumstances, the energy required for the synthesis of above–mentioned chemical substances must have been obtained from the cosmic rays and lightening in the sky. Haldane proposed that these simple organic compounds gradually accumulated in the water bodies and finally a **'hot thin soup'** or **'prebiotic soup'** or broth was formed. This set the stage for the chemical reactions.

(3) **Step 3 : Formation of complex organic compounds :** The simple organic compounds combined in different ratios to form complex organic compounds like polysaccharides, fats and proteins.

(i) Simple sugars combined in different ratios to form polysaccharides like starch, cellulose, glycogen *etc.* The formation of such compounds had been very important for the origin of life because cell walls are made up of cellulose and energy–giving molecules are stored in the form of starch and glycogen.

(ii) The reaction between glycerol and fatty acids yielded fats.

(iii) Different types of combinations between a variety of amino acids yielded different types of proteins. The formation of protein was a very important step in the origin of life because proteins are not only structural components of cell organelles, but many proteins, called enzymes, work as catalysts for biochemical reactions.

In fact, the present-day organisms synthesise their complex molecules from simple organic substances with the help of enzymes only. However, enzymes themselves are proteins. Therefore, first of all protein must have been synthesized without the help of any enzyme.

(4) **Step 4: Formation of nucleic acids and nucleoproteins:** The reaction between methane, ammonia and water resulted in the formation of purines and pyrimidines. Some of the purines and pyrimidines combined with sugar and phosphorus to form nucleotides. Many molecules of nucleotides combined to form nucleic acids–DNA and RNA. The formation of nucleic acid in the oceanic water was a big step in the direction of origin of life. Nucleic acids combined with the proteins to form nucleoproteins**.** Some of the nucleoproteins developed the capability to synthesize molecules similar to themselves, from organic and inorganic substance present in the ocean**.** In other words, the capability to reproduce had evolved. As a result of continuous

reproduction, the number of nucleoproteins went on increasing. Since, organic substance were required for this, the organic substances started being depleted resulting in competition between the nucleoproteins. Physical and chemical changes sometimes led to the changes in the competition of nucleoproteins, and new types of nucleoproteins came into existence by mutations. Those new nucleoproteins which were successful in the competition, increased in number.

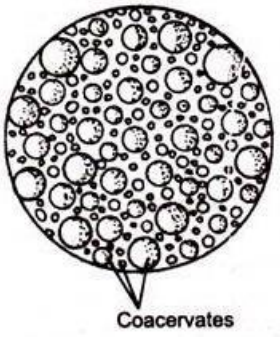


Fig. 26.4 Small uniform microscopic spherules like present-day bacteria or blue green algae.

(b) **Organic Evolution (Biological Evolution)**

(1) **Step 5: Formation of Coacervates:** Oparin believed that the formation of protein was a very important step in towards the origin of life. The **Zwitterionic** nature of the protein molecules enabled these colloidal structures to maintain their identify in spite of being surrounded by water molecules–forming a type of emulsion. The coalescence of these colloidal structures led to the formation of structures called **coacervates**. These coacervates had the ability to exchange substances with the surrounding water and accumulating required substances within them. Under certain conditions scientists have obtained cell like structures. These are known as **coacervates**.

Sydney F. Fox's experiment: Sydney F. Fox of Florida University, obtained some complex molecules by heating upto 90°C a dry mixture of many amino acids found in living organisms. The molecules so obtained very much resembled the proteins. He heated these molecules in water and allowed the mixture to cool down. In the fluid so obtained, he could observe minute structures resembling the cells. He called them **microspheres.** The microspheres are surrounded by membranes, and these also reproduce vegetatively just like yeast. Surprisingly the biochemical processes like breakdown of glucose also occur there. However, electron microscopic examination of these does not reveal any cellular structure. On keeping in distilled water, these become turgid**,** but these get shrunk if immersed in salt solution. Oparin's coacervates and Fox's microspheres are infact, similar structures or **Protocells or Protobionts or Eubionts**.

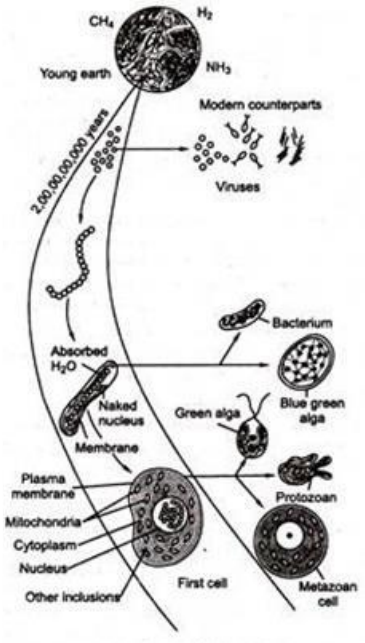


Fig. 26.5 Origin of first organisms.

(2) **Step 6: Formation of Primitive living system:** A primitive cell membrane was formed by the arrangement of lipid molecules between the surface of coacervates and external watery medium. This provided stability to the coacervates. It is believed that a primitive cell was formed when–

(A) **Nucleic acids** having the property of self-duplication entered the coacervates.

(B) **Rearrangement** of molecules occurred inside the coacervate surrounded by lipid molecules.

(3) **Step 7: Formation of first cell:** Evidences available so far indicate that the cells of the earliest organisms did not contain either nucleus or cell organelles. The molecules of nucleic acid were surrounded by a colloidal mixture (may be called protoplasm) of proteins and organic compounds. This, in turn, was surrounded by a thin protein-lipid membrane. Water and soluble substances would pass through this membrane. Some proteins achieved the ability to act as enzyme. Such cells which lacked nuclei were called prokaryotic cells**.** In 1966, some fossils have been discovered from 300 million years old rocks. These fossils are of prokaryotic organisms. Earliest organism is chemoheterotrophs.

(4) **Step 8: Origin of autotropism:** In the primitive organisms, the process of metabolism began because all the substances required for reduction were available in water. Of course, oxygen was not available which was required for oxidation of substances to yield energy. Thus, the first living organisms were anaerobes and heterotrophs. The primitive cells respired anaerobically i.e., these used to obtain energy by fermenting the organic compounds obtained from the water, with the help of enzymes due to fast nutrition, growth and multiplication, their number in the ocean increased greatly. As a result, scarcity of organic substances developed in the ocean. A struggle started between the cells for obtaining nutrition.

At such a time, some of these organisms developed the capability of synthesizing organic substances. Such organisms began synthesizing energy– giving substances (carbohydrates) from simple inorganic substances abundantly available in the environment. In this way, evolution of autotrophs from heterotrophs took place.

This was the beginning of autotrophic nutrition**.** However, it was quite different from the photosynthesis which is carried out by green plants, because it utilized energy obtained by anaerobic respiration (not solar energy). Therefore, such type of nutrition is also called **chemoautotrophic nutrition.** Such type of nutrition is observed even today in the sulphur bacteria.

$$6CO_2+12H_2S-\rightarrow-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\!\!-\text{FermentationChemicalenergy}C_6H_{12}O_{11}+6H_2O+12S$$

- At the same time, from different chemicals present in the oceanic water evolved porphyrins which where like modern chlorophyll led to the evolution of present chlorophyll, so that these cells started utilizing H_2O instead of H_2S for photosynthesis. Thus they performed anoxygenic photosynthesis.

$$6CO_2+12H_2S-\rightarrow--\text{SolarEnergy}C_6H_{12}O_6+6H_2O+12S$$

Till then, oxygen was not freely available in the atmosphere. However, gradually molecular changes in the bacteriocholorophyll led to the evolution of present chlorophyll, so that these cells started utilizing H_2O instead of H_2S for photosynthesis. Thus they performed oxygenic photosynthesis using water as hydrogen donar.

$$6CO_2+12H_2O-\rightarrow--\text{SolarEnergy}C_6H_{12}O_6+6H_2O+6O_2$$

In this way, the prokaryotic cells which were chemoautotrophs, became photo autotrophic. These cells resembled modern cyanobacteria. In 1968, the forms of such types of cells have been recovered from 320 million years old rocks. These have been given the name *Archaeospheroides barbertonensis.* Due to the absence of well-defined nuclei in them, these have been included under the kingdom 'Monera'. Thus, release of O_2 in the atmosphere and its free availability was the result of photosynthesis. This was a revolutionary change which greatly affected the course of organic evolution.

(5) **Step 9 : Origin of Eukaryotic cells :** As a result of photosynthesis, oxygen was released in the atmosphere which started reacting with methane and ammonia in the atmosphere. Its reaction with methane yielded CO_2 and H_2O. On the other hand, reaction between oxygen and ammonia resulted in the formation of CO_2 and nitrogen. In the course of these changes, Ozone (O_3) gas was formed from oxygen; the ozone spread in the form of an envelope surrounding the earth, the distance between the ozone layer and the earth's surface being approximately 15 miles. Thus free oxygen changed the reducing atmosphere into oxidizing atmosphere. As free oxygen became available on the earth, gradual changes took place in cell structure also. Membrane bound organelles *i.e.*, mitochondria, chloroplasts, Golgi bodies, lysosomes evolved. Thus, prokaryotic cells. Most of the organisms on the earth today are eukaryotic. Gradual changes in the earth's atmosphere led to gradual changes in the eukaryotic cells also. Instead of living separately, the cells started living together in the form of colonies**.** Simultaneously, multinucleation of multicellular structures forming tissues**.** Different types of tissue combined to form special organs. From the organs, organ systems and ultimately complex bodies of organisms were formed.

- Organic evolution would have not been taken place if individuals in a population did not show genetic variation.
- Synthetic theory is the most accepted theory of organic evolution.
- The greatest evolutionary change enabling the land vertebrates to be completely free from water, was the development of shelled eggs and internal fertilization.
- The material for organic evolution is mutation.

EVOLUTION:

The term evolution is derived from two Latin words – e = from; volvere = to roll and means the act of unrolling or unfolding, i.e., the doctrine according to which higher forms of life have gradually arisen out of lower. This term was first used by **Charles Bonnet** (1720–1793), extrapolating from progressive embryogenesis (in individuals) to the development of species (see Calow, 1983). However, according to **Savage** (1969), the term evolution was first used by English philosopher **Herbert Spencer.**

The four main theories in mechanism of evolution are:

1. Lamarck's Theory
2. Darwin's Theory of Evolution
3. De Vries' Theory
4. Modern Theory of Evolution.

Fig. 195. JEAN BAPTISTE LAMARCK (1744–1829).

1. Lamarck's Theory:

Jean Baptiste Lamarck (1744-1829), a French naturalist, made several valuable contributions to biological science, including the coining of the term 'biology' and using the same in its true sense. He studied comparative anatomy and planned a tree of life for explaining the phylogenetic relationship among organisms.

He believed in the fundamental unity of living things and in a progressive development of forms and functions in all organisms. But the most important contribution of Lamarck —his **theory of evolution**—was framed in **1801** and published in the '**Philosophic Zoologique'** in **1809**, that is the year in which Charles Darwin was born.

Lamarckism:

The essence of the Lamarckian theory or Lamarckism may be summarised as follows:

(1) Necessity in the organism may give rise to new structures or may lead to the disappearance of certain parts. Lamarck expressed this as the **law of use and disuse**. According to Lamarck an organ which is used extensively by the organism would enlarge and become more efficient, while disuse or lack of use of a particular organ would lead to its degeneration and ultimate disappearance.

For example, the **webbed toes of aquatic birds** such as swans developed due to constant stretching of the skin at the bases of the toes in some ancestral form which lived on land. The necessity of the web of skin arose when the ancestors migrated into the water in search of food. This led to constant use and stretching, thereby a change was induced and a paddle-like foot evolved.

Similarly, the ancestors of the snakes were lizard-like creatures with 1 two pairs of limbs and the modern snakes lost their limbs by constant disuse while passing through narrow crevices. Thus by differential use and disuse of various parts, an organism could change a good deal; that is, the organism acquires certain new characteristics.

(2) The second part of Lamarck's theory postulated that acquired traits induced by use or disuse of organs were transmitted to the offspring; this is the **law of inheritance of acquired characters**. Lamarckism explains evolution of the **modern giraffe** in the following way.

There was a short-necked ancestral stock which used to feed on tree leaves. It stretched its neck further up, to reach higher levels, when the leaves lower down were finished. Due to constant stretching the neck length increased a little and his new trait was inherited by the offspring.

The latter in turn kept on stretching their necks and this was continued for many generations. Each successive generation would acquire the gains of the previous generation by inheritance, and would itself add a bit to the neck length. In the course of time, the long-necked modern giraffe evolved out of the short-necked ancestral form.

Criticism of Lamarckism:

The first part of Lamarck's theory, that is, the law of use and disuse is acceptable. For example, moderate exercise taken regularly builds big muscles, or a limb put up on splints and not used for a long time undergoes atrophy. But the second part of Lamarckism, that is, inheritance of acquired characters, is not acceptable.

It implies that a man who has developed large muscles by lifelong exercise will beget children with big muscles. Lamarckism was chiefly **opposed** by **Weismann** (1834- 1914) who postulated that germ cells are not affected materially by changes in the body cells.

In spite of the laborious research of **neo-Lamarckists** such as **Guyer, Smith** and **Cope**, Lamarckism is untenable. Acquired characters are phenotypic variations. They cannot affect the genes. As such they cannot be transmitted to the offspring.

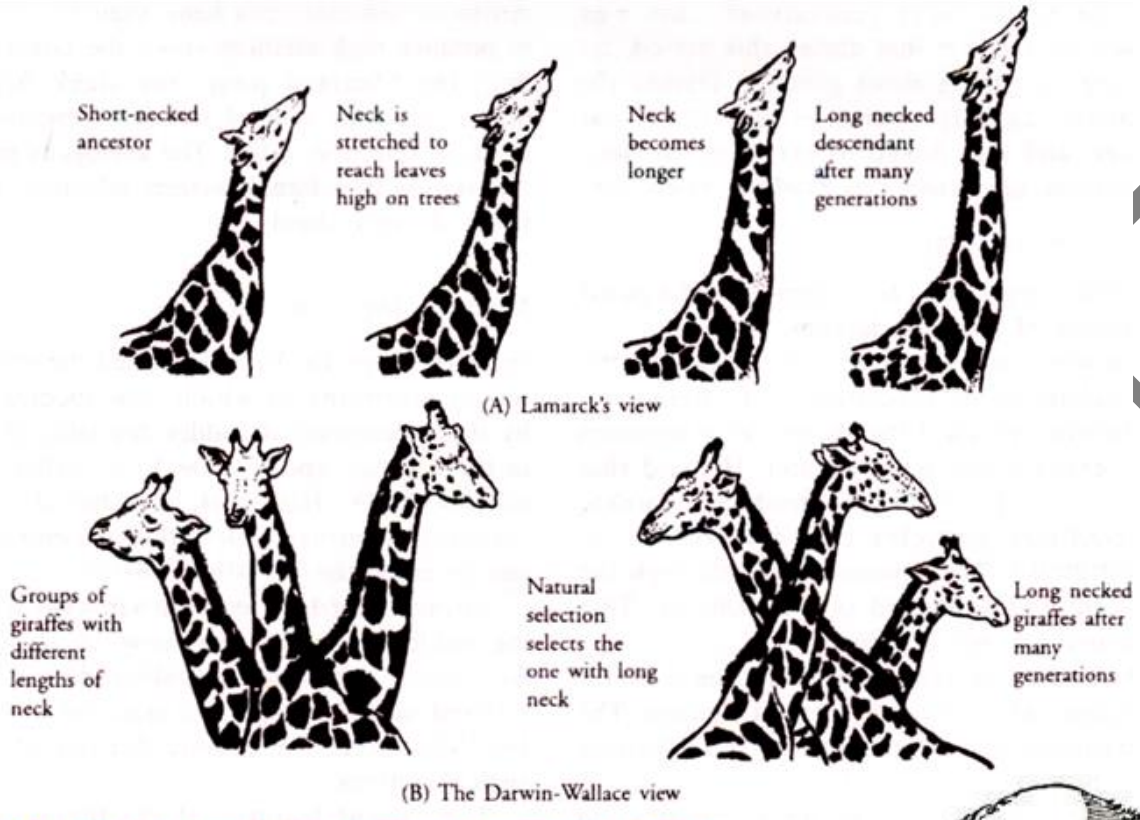


Fig. 8 The length of the giraffe's neck according to Lamarck and Darwin.

2. Darwin's Theory of Evolution:

The name of **Charles Robert Darwin** (1809-1882) is grandson of Erasmus Darwin was born in 1809 and his date of birth coincided with that of Abraham Lincoln. In his early life Darwin, like all other scientists of his time, believed in Lamarckism.

As a young man he joined the **naval expeditionary** ship '**H.M.S. Beagle**' and undertook a circumglobal voyage for **five long years**. He spent his time in **collecting numerous specimens of plants** and **animals** from different parts of the world. After returning home Darwin **spent 20 years** in **studying** his collections.

Fig. 196. Charles Robert Darwin (1809–1882).

At this time he was greatly **influenced** by the publications of **Lyell** and **Malthus**. By studying Lyell's '**Principles of Geology'** Darwin learnt about the changing **Jonas of the earth**, and about the **fossils** which were known at that time. The famous **essay** on population published by **Malthus** taught Darwin about **overpopulation** and consequent **competition for food** and **shelter**.

Having completed his study, Darwin was preparing his theory of natural selection for explaining the mechanism of organic evolution when he received an **essay** from a **younger scientist, Alfred Russell Wallace** (1823-1913), who was working independently on the **flora and fauna of Malayan archipelago**. To his amazement Darwin found that **Wallace's views** on the origin of species **coincided** with **his own theory**.

The **natural selection theory** was first published as a paper under **joint authorship** in **1858**. Two renowned scientists of that time, Lyell and Hooker, presented the paper at the meeting of the Linnean Society and Darwin was conspicuous by his absence. In the following year that is in 1859 Darwin published his classical work in the form of a book—**"On the origin of species by means of natural selection."**

Essence of Darwinism:

Darwin's theory is based on intrinsic analysis of facts in a scientific spirit by induction and deduction.

The following is the essence of Darwinism:

(1) Prodigality of Production:

The **plants and animals** have a tendency to **increase in geometric progression**, but the **habitable space** and the **food supply** remain **constant**. **Darwin** calculated that starting from a **pair of elephants**, the **herd** will increase to about 20,000,000 in 1000 years, and elephants are the slowest breeders producing **4 to 6 calves in their life-time**. Such enormous prodigality in production **results in struggle for existence**.

(2) Struggle for Existence:

This means a keen **competition amongst** the **living forms for food and shelter**.

It operates in a threefold way:

(a) **Interspecific**, that is, struggle in between different species of organisms,
(b) **Intraspecific**, that is, struggle between members of the same species, and
(c) **Environmental**, that is, struggle against the changes of the environment.

(3) Variation:

Darwin observed that no **two living forms** were exactly **alike**. **Diversity** tends to **appear** even among members belonging to the **same species**. Darwin paid particular attention to **small, fluctuating** and **continuous variations** which appeared randomly.

According to him these **continuous variations** help the organism to **win the struggle for existence**. **Large**, **discontinuous variations**, which appeared suddenly, were considered by Darwin as mere '**sports of nature'**, and **therefore ignored**.

(4) Survival of the Fittest:

The **organisms possessing suitable variations** which helped them to **win the struggle for existence** were **better adapted** to their **environment**. They **survived** and **propagated** their **variations** to the **next generation**. The others with **unsuitable variations perished**.

(5) Natural Selection:

This is the most important deduction of Darwin. **Natural selection** is the process by which **individuals** possessing **favourable variations enjoy** a **competitive advantage over** the **others**.

They are **better adapted** to their **environment**, and therefore they **survive** in **proportionately greater numbers** and **produce** more **offspring**. The rest with **disadvantageous variations fail** to **adapt properly** to their **environment** and therefore **eliminated by natural selection**.

The **favourable variations** which are the **cause of success** are **handed down to** the **offspring by inheritance**. Thus the **number of the favoured individuals increase rapidly**, and if **natural selection operates** for a **long time**, those **favourable variations** which have **attained** the **survival value** are **intensified successively** from **generation to generation**, until the **original ancestral forms** are **thoroughly changed** into a **new species**.

For example, Darwinism explains the evolution of the **modern giraffe** in the following manner. The original **ancestral forms** were **short-necked, leaf-eating animals**. Darwin assumed that as a result of **individual variation**, some of them had **slightly longer** or **shorter necks** in **comparison** with the **population's average neck-length**.

The **longer-necked forms** were **better adapted** to get at **foliage's** situated a **bit higher up**. Consequently they were **better fed than** the **shorter-necked fellows**, and they **produced proportionately** greater numbers of **offspring**.

As a **result of natural selection** the proportion of the **longer-necked population** would be **doubled** in the **next generation**. This is **repeated** in **successive generations** until the **entire population** would be **transformed** into **individuals** with **slightly longer necks**.

Individual variation would occur in the **new population** and **actual neck-lengths** would **vary more** or **less** on **either side** of an **average**. **Long necks** would again be **favoured** in a **second round** of **natural selection** and then in **successive rounds**, **until** the **modern giraffe** with **very long neck evolved out of** the **short-necked ancestral stock**.

Criticism of Darwin's Theory:

In spite of strong evidences and critical scanning of facts, Darwinism suffers from certain serious drawbacks.

A few objections to Darwinism are briefly discussed as follows:

(1) Variations were accepted by Darwin to be the chief tool in the process of evolution of new species, and he believed that small continuous variations of fluctuating type were inherited by the offspring. **Unfortunately Darwin had no knowledge about the real cause of variation.**

At this time the science of **genetics was unknown**, and the **laws of inheritance** were **unexplored**. Most of the fluctuating variations considered by Darwin to be important factors in his theory of natural selection are not genotypic and as such they are not inherited.

(2) **Darwin**, like Lamarck, **believed in the inheritance of acquired characters**—a fact which is **not proved by genetics**.
(3) Darwin's **natural selection** mainly **operates** in **one direction**, and often **leads to over specialisation** and **ultimate extinction**. The **canine teeth of the sabre-toothed tiger** and the **antlers of the Irish elk increased progressively** in **size** because the **characteristics in both** the cases were **favoured by natural selection**.
But **ultimately**, the **structures** became so **large** that **instead of being helpful** they **became hindrance** in the **struggle for existence**, and **led to the extinction of the species**.
(4) **Natural selection theory fails** to **account for** the **degeneracy** which is very **often observed** in the **parasitic forms**.
(5) The essence of Darwinian natural selection is the **elimination of the unsuitable forms**. Hence it is better to name it as the '**theory of natural rejection'**.
(6) **Darwin** actually **observed large**, **discontinuous variations or mutations to occur in nature**. He **rejected** them as they **occurred less frequently**. **But mutations** are **genotypic variations** and they have **now** been **recognised** as **important** factors in the **origin of new species**.
In spite of its weakness Darwinism is still accepted as one of the important factors in evolution. Thanks to the untiring efforts of Thomas Henry Huxley (1825-1895), the great champion of natural selection, and others, such as August Weismann, the theory has been firmly established.

III. Mutation Theory of Evolution:

The mutation theory of evolution was proposed by a Dutch botanist, **Hugo de Vries** (1848-1935 A.D.) (Fig. 7.38) in 1901 A.D. in his **book** entitled "**Species and Varieties**, Their **Origin by Mutation"**. He **worked** on **evening primrose** (***Oenothera lamarckiana***).


Fig. 7.38. Hugo de Vries (1848—1935 A.D.)

A. Experiment:
Hugo de Vries **cultured *O. lamarckiana*** in **botanical gardens** at Amsterdam. The plants were, allowed to **self-pollinate** and **next generation** was **obtained**. The plants of **next generation** were again **subjected** to **self-pollination** to obtain **second generation**. **Process** was **repeated** for a **number of generations**.
B. Observations:
Majority of plants of **first generation** were found to be **like the parental type** and showed only minor variations **but 837** out of **54,343 members** were found to be **very different** in **characters** like **flower size**, **shape** and **arrangement of buds, size of seeds** etc. These **markedly different plants** were called **primary** or **elementary species**.
A **few plants** of **second generation** were found to be **still more different**. **Finally**, **a new type**, **much longer than the original type,** called ***O. gigas***, was **produced**. He also found the **numerical chromosomal changes** in the **variants** (e.g. with **chromosome numbers 16, 20, 22, 24, 28** and **30**) upto 30 (**Normal diploid number** is **14**).
C. Conclusion:
1. The **evolution** is a **discontinuous process** and **occurs by mutations** (L. **mutate** = **to change**; **sudden** and **inheritable large differences** from the **normal** and are **not connected to normal** by **intermediate forms**). **Individuals with mutations** are called **mutants**.
2. **Elementary species** are **produced** in **large** number to **increase chances** of **selection** by **nature**.
3. **Mutations** are **recurring** so that the **same mutants appear again and again**. This **increases** the **chances of** their **selection** by **nature**.
4. **Mutations occur** in **all directions** so may cause **gain** or **loss** of **any character**.
5. **Mutability** is **fundamentally different** from **fluctuations** (**small** and **directional changes**).
So **according** to **mutation theory**, **evolution** is a **discontinuous** and **jerky process** in **which** there is a **jump from one species** to **another** so that new species arises from pre-existing species in a single generation (macrogenesis or saltation) and not a gradual process as proposed by Lamarck and Darwin.
D. Evidences in favour of Mutation theory:

1. Appearance of a **short-legged sheep variety**, **Ancon sheep** (Fig. 7.39), **from long-legged parents** in a **single generation** in 1791 A.D. It was first noticed in a ram (male sheep) **by** an **American farmer, Seth Wright.**

Fig. 7.39. Appearance of short-legged Ancon sheep mutant.

2. Appearance of **polled Hereford cattle from horned parents in a single generation** in 1889.
3. **De Vries observations** have been **experimentally confirmed by McDougal** and **Shull** in America and Gates in England.
4. **Mutation theory** can **explain** the **origin of new varieties or species by a single gene mutation e.g.** ***Cicer gigas***, **Novel orange. Red sunflower, hairless cats, double- toed cats**, etc.
5. It can **explain** the **inheritance of vestigial and over-specialized organs**.
6. It can **explain progressive** as well as **retrogressive evolution**.

E. Evidences against Mutation theory:

1. It is **not able** to **explain** the **phenomena** of **mimicry** and **protective colouration**.
2**. Rate of mutation is very low**, i.e. one per million or one per several million genes.
3. **Oenothera lamarckiana** is **a hybrid plant** and **contains anamolous type** of **chromosome behaviour**.
4. **Chromosomal numerical changes** as reported by de Vries are **unstable**.
5. **Mutations** are **incapable** of **introducing new genes** and **alleles into** a **gene pool**.

IV. Neo-Darwinism or Modern Concept or Synthetic Theory of Evolution:

The **detailed studies of Lamarckism, Darwinism and Mutation theory of evolution showed that no single theory is fully satisfactory**. **Neo-Darwinism** is a **modified** version of **theory of Natural Selection** and is a sort of **reconciliation between Darwin's and de Vries theories**.

Modern or synthetic theory of evolution was **designated by Huxley** (1942). It **emphasises** the **importance of populations as** the **units of evolution** and the **central role of natural selection** as the most **important mechanism of evolution**.

The **scientists** who **contributed to** the outcome of **Neo-Darwinism** were: **J.S. Huxley, R.A. Fischer** and **J.B.S. Haldane** of England; and **S. Wright, Ford, H.J. Muller** and **T. Dobzhansky** of America.

A. Postulates of Neo-Darwinism:

1. Genetic Variability:

Variability is an **opposing force to heredity** and **is essential for evolution** as the variations **form the raw material** for **evolution**. The **studies showed** that the **units of both heredity** and **mutations are genes** which are **located** in a **linear manner on the chromosomes**.

Various sources of genetic variability in a gene pool are:

(i) Mutations:

These are **sudden**, **large** and **inheritable changes in** the **genetic material**. On the **basis** of **amount** of **genetic material** involved, **mutations** are of **three types**:

(a) Chromosomal aberrations:

These include the **morphological changes in** the **chromosomes** without affecting the number of chromosomes. These result **changes either** in the **number of genes** (**deletion and duplication**) or in the **position of genes (inversion)**.

These are of four types:

1. **Deletion (Deficiency**) involves the **loss of a gene** block **from** the **chromosome** and may be **terminal** or **intercalary**.
2. **Duplication** involves the **presence of some genes more than once**, called the **repeat**. It may be **tandem** or **reverse duplication**.
3. **Translocation** involves **transfer** of a **gene** block from **one chromosome to** a **non-homologous chromosome** and may be **simple** or **reciprocal** type.
4. **Inversion** involves the **rotation of an intercalary gene** block through **180°** and may be **paracentric** or **pericentric**.

(b) Numerical chromosomal mutations:

These include **changes in the number of chromosomes**. These may be **euploidy** (**gain or loss of one or more genomes**) or **aneuploidy** (**gain or loss of one or two chromosomes**). **Euploidy** may be **haploidy** or **polyploidy**.

Among polyploidy, **tetraploidy** is most **common**. **Polyploidy** provides **greater genetic material for mutations** and **variability**. In **haploids**, **recessive genes express in the same generation**.

Aneuploidy may be hypoploidy or hyperploidy. **Hypoploidy may be monosomy** (**loss of one chromosome**) **or nullisomy (loss of two chromosomes**). **Hyperploidy may be trisomy (gain of one chromosome) or tetrasomy (gain of two chromosomes).**

(c) Gene mutations (Point mutations):

These are **invisible changes in chemical nature (DNA) of a gene** and are of **three types:**

1. **Deletion** involves **loss of one** or **more nucleotide pairs**.
2. **Addition** involves **gain of one** or **more nucleotide pairs**.
3. **Substitution** involves **replacement of one or more nucleotide pairs by other base pairs**. These may be **transition** or **transversion type**.

These changes in DNA cause the changes in the sequence of amino acids so changing the nature of proteins and the phenotype.

(ii) Recombination of genes:

Thousands of new combinations of genes are **produced due** to **crossing over**, **chance arrangement of bivalents at the equator during metaphase – I** and **chance fusion of gametes during fertilization**.

(iii) Hybridization:

It involves the interbreeding of two genetically different individuals to produce 'hybrids'.

(iv) Physical mutagens (e.g. radiations, temperature etc.) and chemical mutagens (e.g. nitrous acid, colchicine, nitrogen mustard etc.).

(v) Genetic drift:

It is the **elimination of the genes of some original characteristics of a species by extreme reduction in a population due to epidemics or migration or Sewell Wright effect**.

The **chances of variations are also increased by non-random mating**.

2. Natural Selection:

Natural selection of Neo- Darwinism differs from that of Darwinism that **it does not operate through "survival of the fittest" but operates through differential reproduction** and **comparative reproductive success**.

Differential reproduction states that those members, which are best adapted to the environment, **reproduce at a higher rate and produce more offsprings than those which are less adapted**. So **these contribute proportionately greater percentage of genes to the gene pool of next generation while less adapted individuals produce fewer offsprings.**

If the differential reproduction continues for a number of generations, then the genes of those individuals which produce more offsprings will become predominant in the gene pool of the population as shown in **Fig. 7.40.**

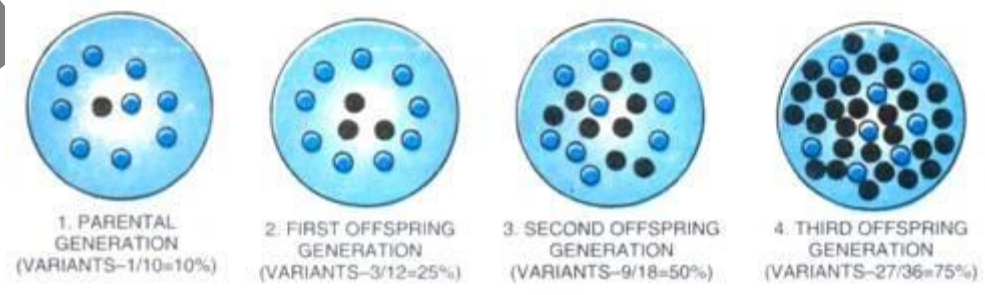


Fig. 7.40. Spread of genetic variability by differential reproduction.

Due to sexual communication, there is free flow of genes so that the genetic variability which appears in certain individuals, gradually spreads from one deme to another deme, from deme to population and then on neighboring sister populations and finally on most of the members of a species. So natural selection causes progressive changes in gene frequencies, 'i.e. the frequency of some genes increases while the frequency of some other genes decreases.

Which individuals produce more offsprings?

(i) Mostly those individuals which are best adapted to the environment.

(ii) Whose sum of the positive selection pressure due to useful genetic variability is more than the sum of negative selection pressure due to harmful genetic variability?

(iii) Which have better chances of sexual selection due to development of some bright coloured spots on their body e.g. in many male birds and fish.
(iv) Those who are able to overcome the physical and biological environmental factors to successfully reach the sexual maturity.
So natural selection of Neo-Darwinism acts as a creative force and operates through comparative reproductive success. Accumulation of a number of such variations leads to the origin of a new species.

3. Reproductive isolation:

Any factor which reduces the chances of interbreeding between the related groups of living organisms is called an isolating mechanism. **Reproductive isolation is must** so as **to allow the accumulation of variations leading to speciation by preventing hybridization**.
In the **absence of reproductive isolation**, these **variants freely interbreed which lead to intermixing of their genotypes**, **dilution of their peculiarities and disappearance of differences between them**. So, **reproductive isolation helps in evolutionary divergence.**

ROLE OF MUTATIONS IN EVOLUTION

Evolution is the process by which populations of organisms change over generations. Genetic variations underlie these changes. Genetic variations can arise from gene mutations or from genetic recombination (a normal process in which genetic material is rearranged as a cell is getting ready to divide).

A mutation is a change in the sequence of an organism's DNA. Mutations can be caused by high-energy sources such as radiation or by chemicals in the environment. They can also appear spontaneously during the replication of DNA.

Mutations generally fall into two types: point mutations and chromosomal aberrations. In point mutations, one base pair is changed. The human genome, for example, contains over 3.1 billion bases of DNA, and each base must be faithfully replicated for cell division to occur. Mistakes, although surprisingly rare, do happen. About one in every 10^{10} (10,000,000,000) base pair is changed. The most common type of mistake is a point substitution. More uncommon is the failure to copy one of the bases (deletion), the making of two copies for a single base (point duplication) or the addition of a new base or even several bases (insertion). Chromosomal aberrations are larger-scale mutations that can occur during meiosis in unequal crossing over events, slippage during DNA recombination or due to the activities of transposable events. Genes and even whole chromosomes can be substituted, duplicated, or deleted due to these errors. These variations often alter gene activity or protein function, which can introduce different traits in an organism. If a trait is advantageous and helps the individual survive and reproduce, the genetic variation is more likely to be passed to the next generation (a process known as natural selection). Over time, as generations of individuals with the trait continue to reproduce, the advantageous trait becomes increasingly common in a population, making the population different than an ancestral one. Sometimes the population becomes so different that it is considered a new species.

A mutation that occurs in body cells that are not passed along to subsequent generations is a somatic mutation. A mutation that occurs in a gamete or in a cell that gives rise to gametes are special because they impact the next generation and may not affect the adult at all. Such changes are called germ-line mutations because they occur in a cell used in reproduction (germ cell), giving the change a chance to become more numerous over time. If the mutation has a deleterious affect on the phenotype of the offspring, the mutation is referred to as a genetic disorder. Alternately, if the mutation has a positive affect on the fitness of the offspring, it is called an adaptation. Also, many genetic changes have no impact on the function of a gene or protein and are not helpful or harmful. In addition, the environment in which a population of organisms lives is integral to the selection of traits. Some differences introduced by mutations may help an organism survive in one setting but not in another—for example, resistance to a certain bacteria is only advantageous if that bacteria is found in a particular location and harms those who live there. Thus, all mutations that affect the fitness of future generations are agents of evolution.

Mutations are essential to evolution. Every genetic feature in every organism was, initially, the result of a mutation. The new genetic variant (allele) spreads via reproduction, and differential reproduction is a defining aspect of evolution. It is easy to understand how a mutation that allows an organism to feed, grow or reproduce more effectively could cause the mutant allele to become more abundant over time. Soon the population may

be quite ecologically and/or physiologically different from the original population that lacked the adaptation. Even deleterious mutations can cause evolutionary change, especially in small populations, by removing individuals that might be carrying adaptive alleles at other genes.

Most mutations occur at single points in a gene, changing perhaps a single protein, and thus could appear unimportant. For instance, genes control the structure and effectiveness of digestive enzymes in your (and all other vertebrate) salivary glands. At first glance, mutations to salivary enzymes might appear to have little potential for impacting survival. Yet it is precisely the accumulation of slight mutations to saliva that is responsible for snake venom and therefore much of snake evolution. Natural selection in some ancestral snakes has favored enzymes with increasingly more aggressive properties, but the mutations themselves have been random, creating different venoms in different groups of snakes. Snake venoms are actually a cocktail of different proteins with different effects, so genetically related species have a different mixture from other venomous snake families. The ancestors of sea snakes, coral snakes, and cobras (family Elapidae) evolved venom that attacks the nervous system while the venom of vipers (family Viperidae; including rattlesnakes and the bushmaster) acts upon the cardiovascular system. Both families have many different species that inherited a slight advantage in venom power from their ancestors, and as mutations accumulate the diversity of venoms and diversity of species increased over time.

Although the history of many species have been affected by the gradual accumulation of tiny point mutations, sometimes evolution works much more quickly. Several types of organisms have an ancestor that failed to undergo meiosis correctly prior to sexual reproduction, resulting in a total duplication of every chromosome pair. Such a process created an "instant speciation" event in the gray treefrog of North America (Figure 2).

The consequence of doubling the genome size in plants is often abnormally large seeds or fruits, a trait that can be of distinct advantage if you are a flowering plant! Most cereals that humans eat have enormous seeds compared to other grasses, and this is often due to the genomic duplications that occurred in the ancestors of modern rice and wheat and, because the mistake occurred in reproductive organs, was successfully passed on to future generations. Humans themselves have mimicked this process by interbreeding individual plants with the largest fruits and seeds in the process of artificial selection, creating many of our modern agricultural crop strains. The idea of evolution by natural selection, first described by Charles Darwin and Alfred Russell Wallace, requires differential survival due to some individuals having greater evolutionary fitness. Whether that fitness is affected by genetic disorders, venomous saliva or enlarged offspring, heritable variation can only arise by mutation. Evolution is simply not possible without random genetic change for its raw material.

So why do some harmful traits, like genetic diseases, persist in populations instead of being removed by natural selection? There are several possible explanations, but in many cases, the answer is not clear. For some conditions, such as the neurological condition Huntington disease, signs and symptoms do not occur until after a person has children, so the gene mutation can be passed on despite being harmful. For other harmful traits, a phenomenon called reduced penetrance, in which some individuals with a disease-associated mutation do not show signs and symptoms of the condition, can also allow harmful genetic variations to be passed to future generations. For some conditions, having one mutated copy of a gene in each cell is advantageous, while having two mutated copies causes disease. The best-studied example of this phenomenon is sickle cell disease: Having two mutated copies of the HBB gene in each cell results in the disease, but having only one copy provides some resistance to malaria. This disease resistance helps explain why the mutations that cause sickle cell disease are still found in many populations, especially in areas where malaria is prevalent.

GENE DUPLICATION (2R HYPOTHESIS)

Understanding Gene Duplication Through Keratin

Hair and nails are both comprised of a protein called keratin. It is also the protein used to make horns, feathers, and wool. Even your skin and eyes have a thin layer of keratin. How does a protein like this have such diverse roles in different animals or in even different parts of the body?

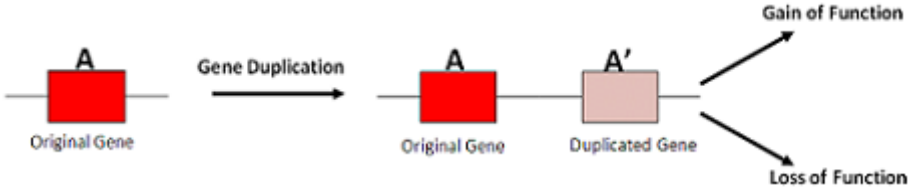


Figure 1: Gene duplication results in a gene being copied in an organism's genome.

Each of the different types of keratin found throughout the body are expressed by different genes that are

duplicates of each other. **Gene duplication** happens when an extra copy of a gene is made in an organism's genome. In some cases, the duplication leads to the gain of a new function, but in other cases, protein function is lost, as shown in Figure 1.

Sometimes, gene duplication is beneficial to the organism and may eventually lead to the development of a new species. The various types of keratin in the body are the result of duplications of a single gene. Over time, as species diversified, new genes for keratin with different functions arose in different species. Other times, gene duplication can have negative effects on individuals, like those seen with Down syndrome and other chromosomal disorders.

Gene Structure

Figure 2: The basic structure of a gene.

The essential components of a gene (Figure 2). The instructions to make the protein are found in the **coding region** of the gene. **Promoters** are sequences of DNA that ensure the gene gets copied into RNA in order to be converted into a protein. If a sequence does not have a promoter, the DNA sequence will not be expressed as a protein (trait) even if it codes for one.

How Do Genes Get Duplicated?

Gene duplication can be DNA- or RNA-based. Gene duplication that is DNA-based happens during any process in which DNA is replicated. Here, we will focus on DNA replication during meiosis (formation of egg and sperm cells). When a gene is duplicated in the genome of an egg or sperm cell, the organism's offspring will inherit the duplicated gene. RNA-based gene duplication happens when an mRNA is turned back into DNA and then reinserted into the genome.

DNA-Based Gene Duplication

Unequal Crossing-Over

Crossing-over takes place during the first half of meiosis when all of the chromosomes are lined up at the center of the cell during metaphase I.

As shown in Figure 3, when homologous chromosomes are joined together, they may exchange genes with one another in a process called **homologous recombination**, or crossing-over. In Figure 3, the homologous chromosomes shown in orange (maternal) and yellow (paternal) exchange the genes labeled y and z.

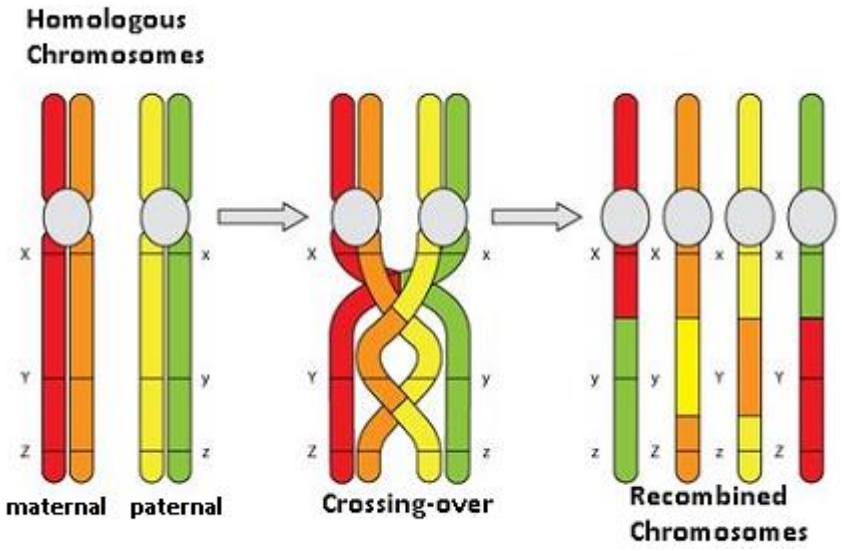


Figure 3: Crossing-over recombines genes on chromosomes.

Normally, crossing-over results in each chromosome ending up with all the genes that are supposed to be found on that chromosome. In order for this to happen, the chromosomes have to be aligned correctly, as shown in Figure 4a. Ideally, this alignment would happen in coding regions of genes to ensure that each cell will end up with a normal chromosome. Unfortunately, chromosomes can align at any DNA sequence with enough similarity, and homologous recombination can occur anywhere. As shown in Figure 4b and 4c, this results in the generation of chromosomes with an abnormal number of genes. The misalignment can result in complete (4b) or partial (4c) gene duplication.

Chromosome Duplication

Chromosomal duplication is another way genes become duplicated. This process is exactly what it sounds like: It results in the duplication of a partial or full chromosome. Chromosome duplication can happen during both stages of meiosis as chromosomes are being separated.

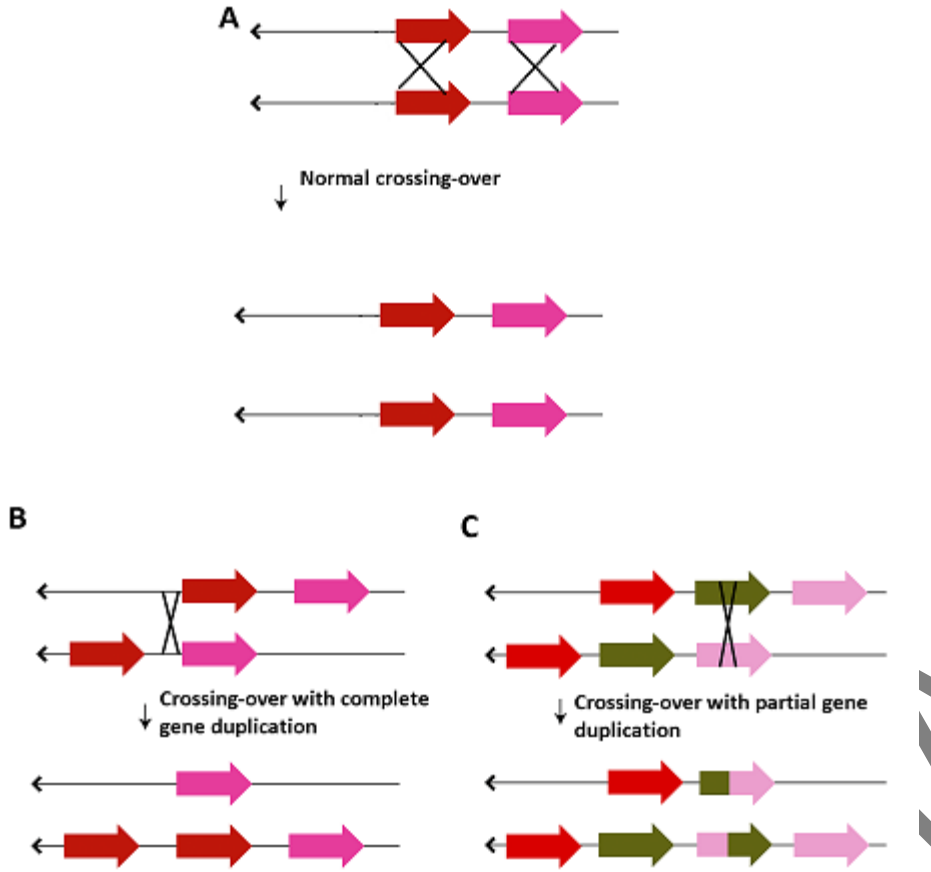


Figure 4: Uneven crossing-over results in chromosomes with a disproportionate number of genes.

2R HYPOTHESIS

Susumu Ohno (1970) has presented a treatise on evolution by gene duplication. He emphasized his view of evolution by gene duplication in a broader context and tried to explain major events of vertebrate evolution by gene duplication. One of his main arguments was that genome duplication has an advantage over tandem gene duplication in the formation of new genes because in genome duplication both protein-coding and regulatory regions of genes are duplicated at the same time whereas tandem duplication may disrupt the coordination of regulatory elements and protein- coding regions of genes. On the basis of genome sizes, he then proposed that the vertebrate genome experienced about two rounds of genome duplications before the evolution of the X and Y or the Z and W chromosomes in reptiles. Later, several authors called this the 2-round (or 2R) hypothesis of genome duplication (e.g. Kasahara, Hughes). However, this hypothesis has been controversial.

Recent studies have shown that a large portion of duplicate genes are lost from the genome after genome duplication and most polyploidy genomes are quickly diploidized. It is also known that a large number of tandem or segmental duplications have occurred during the past several hundred million years and the duplicate genes have often been transferred to different chromosomes or different chromosomal segments as in the case of olfactory receptor genes. Therefore, even if the 2R hypothesis is correct, it would be very difficult to prove it now because the history of genome duplication has been largely erased. Note also that unlike Ohno's original argument tandem duplication has no disadvantage in creating new genes compared with genome duplication because tandem duplication usually includes the regulatory region at the same time. However, whatever the mechanism is, we should note that gene duplication is a form of mutation that generates various evolutionary innovations. In plants, there is no dispute about the importance of genome duplication in evolution. The 2R hypothesis has important implications for understanding the evolution of the immune system, including the origin of the major histocompatibility complex and natural killer receptors.

NUMBER OF CHROMOSOMES:

There are three types of chromosome number, viz., haploid, diploid and basic number as given below:

i. Haploid: It represents half of the somatic chromosome number of a species and is denoted by n. Since haploid chromosome number is usually found in the gametes, it is also known as gametic number.

ii. Diploid: It refers to somatic chromosome number of a species and is represented by 2n. Since diploid chromosome number is found in zygotic or somatic cells it is also referred to as zygotic or somatic number.

iii. Basic Number: The gametic chromosome number of a true diploid species is called basic number. It is the minimum haploid chromosome number of any species which is denoted by x. For example, in wheat, the basic number is 7, whereas the haploid number is 7, 14 and 21 for diploid, tetraploid and hexaploid species, respectively.

Thus haploid chromosome number differs from basic number. Both are same in case of true diploid species but differ in case of polyploid species. Thus, basic number can be a haploid number but all haploid numbers cannot be basic number. Chromosome number differs from species to species. In plant kingdom, chromosome number usually is higher in dicots than in monocots.

Variations in Chromosome Number (Numerical Changes):

The organisms are usually diploid (2n), i.e., they possess two sets of chromosomes. Variation in the normal diploid chromosome number is termed ploidy. Numerical change in chromosome or variations in chromosome number (heteroploidy), can be mainly of two types, namely (i) aneuploidy and (ii) euploidy.

(a) Aneuploidy:

It involves addition or deletion of one or few chromosomes to the usual diploid set of chromosomes. The aneuploids arise due to failure of the separation of homologous chromosomes of particular pair during meiosis. It is known as non-disjunction. As a result two types of gametes are produced; one type contain more chromosomes than the normal number and the other type of gamete contain less chromosomes.

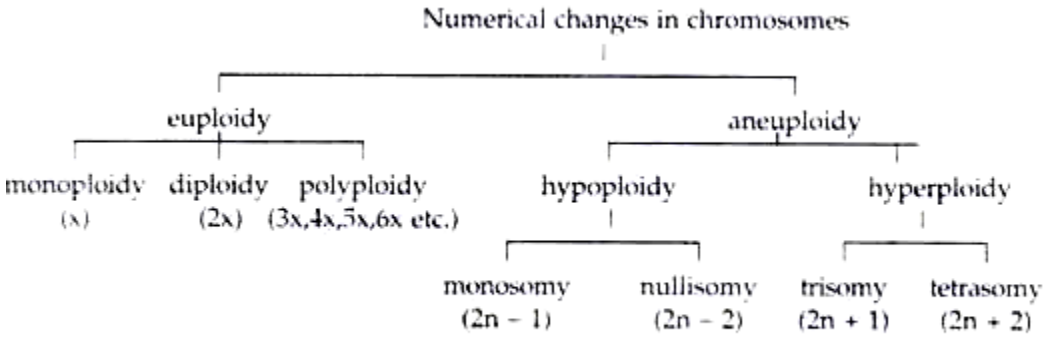


Fig. 5.60. Differnt kinds of numerical changes in chromosomes (X = basic chromosome number, 2n=somatic chromosome number

Aneuploids are of following types:

(i) Monosomics:

They arise by the loss of one chromosome from the diploid set i.e., 2n-l. They can form two types of gametes, (n) and (n-1).

(ii) Nullisomics:

These arise by the loss of a particular pair of chromosomes i.e., 2n-2. They arise by the fusion of two (n-1) type of gametes.

(iii) Trisomics:

These arise by addition of an extra chromosome to the normal diploid set with the genetic formula, 2n + 1. Such individuals are formed by the union of a (n + 1) gamete with a normal gamete (n).

(iv) Tetrasomics:

These arise by the addition of an extra pair of chromosome to the diploid set with a chromosomal formula 2n + 2. By this a particular chromosome is represented in four doses instead of normal two.

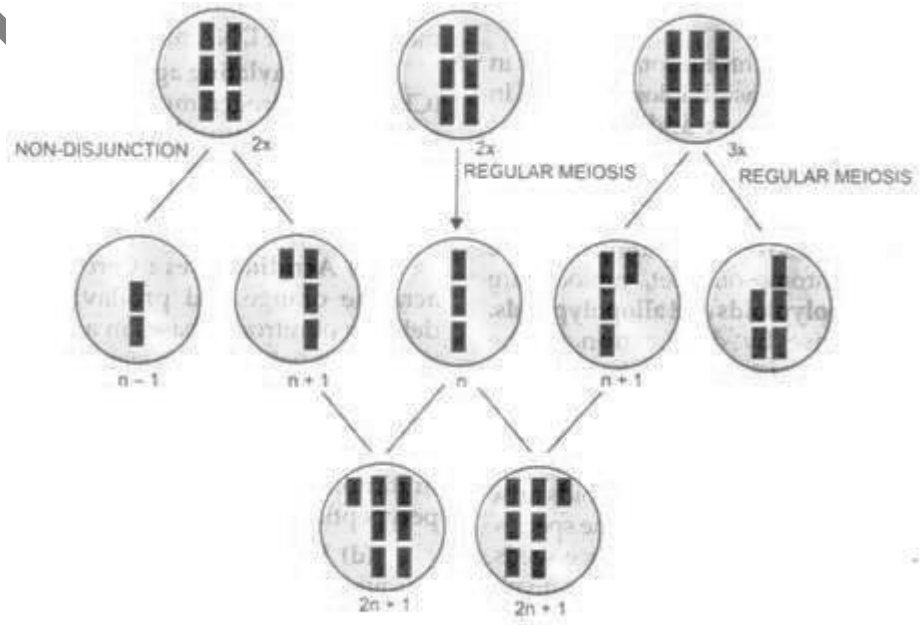


Fig. 5.59. Origin of trisomics.

(b) Euploidy:

Normally organism possesses two sets of chromosomes i.e., they are diploid (2n). At times there is addition or loss of complete one set (n) or more than one set of chromosomes is observed. It is called as euploidy.

Euploidy is of following types:

(i) Hapioidy or Monoploidy:

Out of two sets of chromosomes of a normal organism when one set is lost, the resulting offspring's have just one set of chromosomes (n).

(ii) Polyploidy:

Organisms having more than two normal sets of chromosomes (2n) are called polyploids. Organisms with three sets of chromosomes (2n + n) = 3n are triploids; those with four sets of chromosomes (2n + 2n) = 4n are tetraploids and those with five sets (2n + 3n) = 5n and six sets (2n + 4n) = 6n are known as pentaploids and hexaploids respectively.

Depending upon the source of the additional chromosome set, euploids are further classified into autopolyploids and allopolyploids.

Autopolyploids may arise by duplication of the chromosome sets of single species i.e., chromosome duplication from a single diploid species gives a tetraploid. Suppose a diploid species with two similar genomes called (AA), and then autotriploids are called (AAA). Similarly autotetraploids represent (AAAA). Autopolyploids arise when the additional sets originate from the same species. Autotriploids remain sterile and cannot produce seeds. However, such plants can be propagated vegetatively because they have normal mitosis.

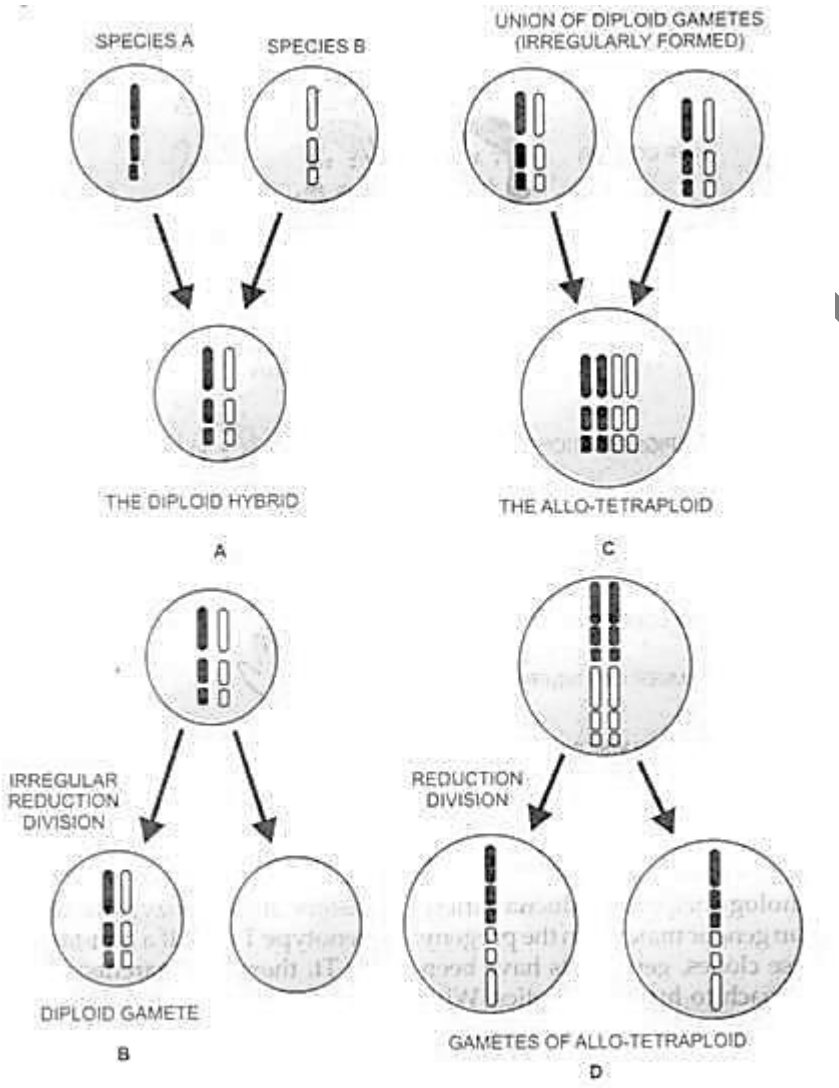


Fig. 5.61. Formation of Allotetraploids by irregularly formed diploid gametes.

Allopolyploids are produced from hybridization of two closely related species having chromosomal sets AA and BB. The hybrid (AB) will be sterile because of its inability to produce viable gametes. In such organisms, the chromosomes will fail to develop pairing. If new AB genetic combination undergoes a natural or induced chromosomal doubling, a fertile AABB tetraploid is produced.

The origin of allotetraploid is schematically represented below:

Parents AA x BB
Hybrid AB
Doubling of chromosomes
AABB (Meiosis)
Gametes AB x AB
Fertilization AABB
(An allopolyploid)

Allotetraploids are also formed due to fusion of irregularly formed diploid gametes (Fig 5.61).

The common examples are autopolyploidy is the common 'doob' grass (*Cynodon dactylon*) and those of allopolyploidy is *Raphanobrassica* and common wheat.

Polyploidy is generally found among the plants but rarely found among animals. About one third of all the grasses are polyploids, common bread wheat is hexaploid (6n), some strawberries are octaploid (8n).

Many commercial fruits and ornamental plants are polyploids. The primary cause for relatively low frequency of polyploids among animals is attributed to their sex balance, which is more delicate than that in plants. However, few animals such as the brine shrimp, some annelids, the axolotl larva and the golden hamster show evidence of polyploidy. Sometimes certain specialized tissues (e.g., liver) within a diploid organism may be polyploid. Polyploidy can be induced by treating the living tissues with a chemical (an alkaloid) called colchicine. Temperature treatment in maize and decapitation in tomato induced polyploidy.

Polyploidy originates in several ways. It may arise due to abnormal mitosis. Sometimes diploid spores and gametes are produced due to defective meiotic division in which there is no reduction of chromosome number. Such diploid gametes fuse to form tetraploid individuals. Participation of more than two nuclei in fertilization in endosperm tissue of seed plants results in polyploidy.

CHROMOSOMAL ABERRATIONS

Chromosomes are the vehicle of hereditary material or genes. Any alteration, addition or deletion of chromosomal part leads to alteration of number, position or sequence of genes in the chromosome. Such change of structure is referred to as chromosomal aberrations or chromosomal mutations.

Aberration alters the chromosome structure but do not involve a change in chromosome number. The mechanics signify chiefly a rearrangement through loss, gain or reallocation of chromosomal segments.

In normal course of cell-division cycle, the chromosomes duplicate and segregate in an orderly manner. The sequence of gene loci in the chromosome also maintains an orderly arrangement. But recent genetic research reveals that, in rare cases, genetic variation (i.e. variation in the structure and arrangement of the chromosomes) do occur and bring about certain major clinical disorders through phenotypic changes in organisms. Each chromosomal abnormalities are usually associated with different congenital malformations and diseases.

These aberrations may be of two kinds – **Spontaneous aberration and Induced aberration**.

(i) The naturally occurring structural rearrangements of the chromosomes are called Spontaneous aberration. The reason behind such aberrations is not clearly understood. Factors like cosmic radiation, nutritional insufficiencies, and several other environmental factors may hamper the original chromosomal structure or number.

(ii) The architectural changes deliberately produced by the use of a physical or chemical agents are called Induced aberrations. A variety of agents are able to induce mutations. They also cause breakage in chromosomes and all these result in chromosomal aberrations. Spontaneous aberrations occur very rarely whereas scope of induced aberrations is several times higher than that of spontaneous aberrations.

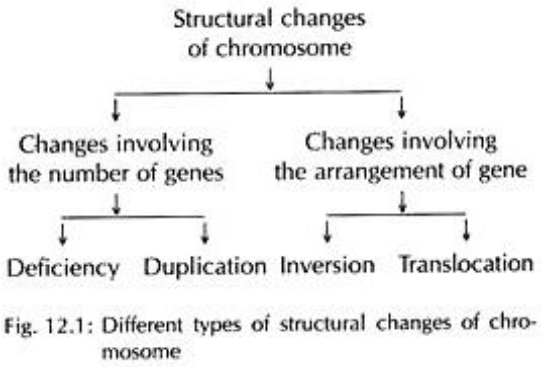


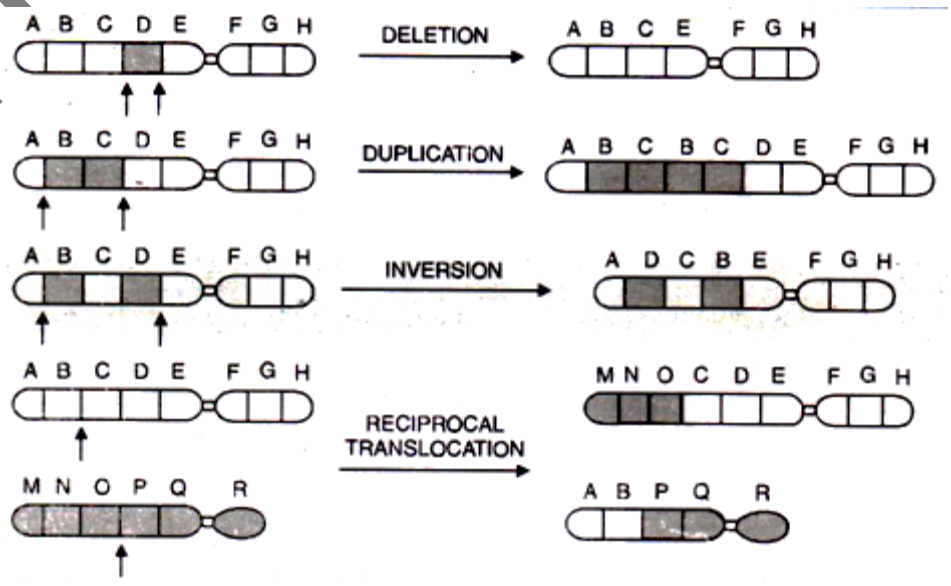
Fig. 12.1: Different types of structural changes of chromosome

The geneticists are more concerned with the spontaneous aberrations because these phenomena occur naturally, without any known causal factor.

Spontaneous Chromosomal aberrations involve two types of changes:

(i) Changes in number of genes in a chromosome **1. Deficiency or Deletion 2. Duplication.**

(ii) Changes involving arrangement of genes. **3. Inversion 4. Translocation.**



Forms of chromosomal aberration showing deletion, duplication, inversion & reciprocal translocation.

(a) Changes in the number of genes in a chromosome:

I. Deficiency or Deletion:

- When a chromosome is broken into several pieces, the healing (reunion) of the segments takes place and it is possible that the two ends of the fragments unite together leaving one or more acentric parts free (Fig. 22.3).
- The acentric pieces of chromosomes as mentioned earlier disappear. The healed segments will form a chromosome that will be deficient for some of the genes, particularly those found on the lost part. Depending upon the length of chromosome segment lost in this way, the loss involves one gene or a block of genes.
- The loss of a section of genetic material and genetic information from a chromosome or linkage structure is termed deficiency or deletion. The deletion of a portion of chromosome is a very rare event. It produces some striking genetic and morphological / physiological consequences. Each deletion gives rise to a distinct set of symptoms which characterizes an abnormality and called as a syndrome.

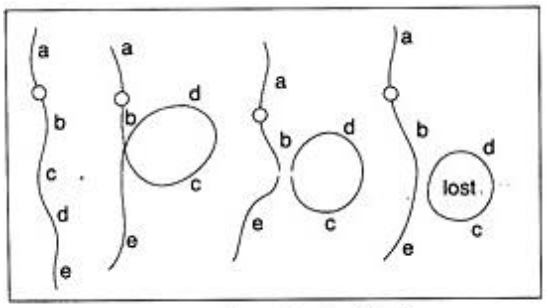


Fig. 12.4: The origin of a deletion

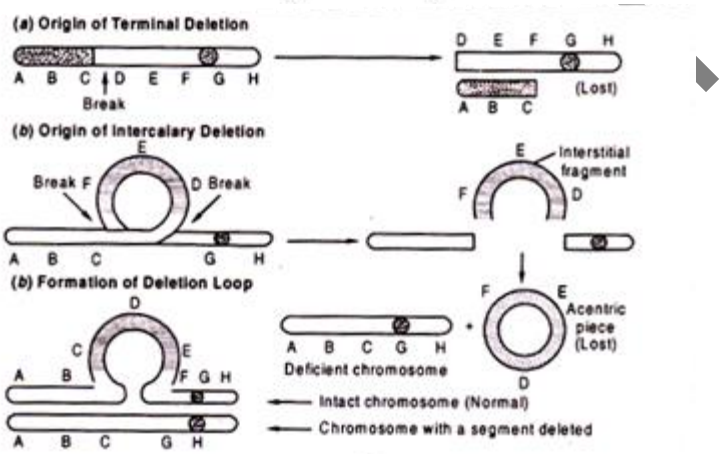


Fig. 22.3 Terminal (*a*), and interstitial (*b*) deletions, (*c*) Formation of loop in deletion heterozygote.

- **Deficiency or deletion are of two types:**

(i) Terminal deletion:

A single break near the end of a chromosome would be expected to result in a terminal deficiency;

(ii) Intercalary deletion:

If two breaks occur, a section may be deleted and an intercalary deficiency is created.

A specific deletion in chromosome no. 22 produces a condition, called '**Philadelphia 22'**; this is associated with chronic **myelogenous leukemia**. Again, another deficiency in chromosome no. 5 creates '**Cri-du-chat' (cry-of-cat) syndrome** where the individuals produce a characteristic mewing cry like cat during childhood. They also possess some unique facial features and exhibit severe physical as well as mental retardations. In the lower organisms, for example, *Chlamydomonas*, yeasts and some fungi, deficiencies are totally lethal, i.e., they result in the death of the individuals.

II. Duplication:

- The presence of an additional chromosome segment (as compared to that of normal number) in a nucleus is known as duplication. In this process, a segment of a chromosome is added to another chromosome; the extra part of the chromosome constitute duplication when this extra-chromosome segment is located immediately after the normal segment following the same orientation (i.e. the same gene sequence is maintained), it is called **Tandem duplication**.
- When the gene sequence in the extra-chromosome occurs in a reverse order, it is known as **Reverse Tandem duplication**. The Reverse duplication is almost same as Tandem duplication, but here the additional segment is inverted in order.
- Sometimes, the additional segment is found to be located in the same chromosome but away from the normal segment: such cases are termed as **displaced duplication.**

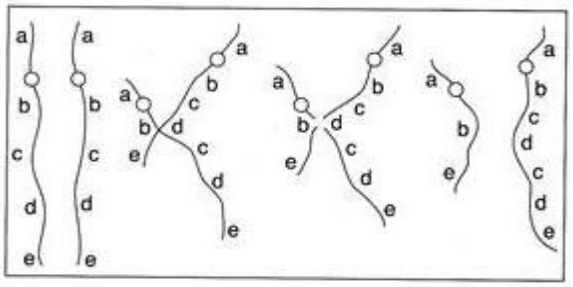
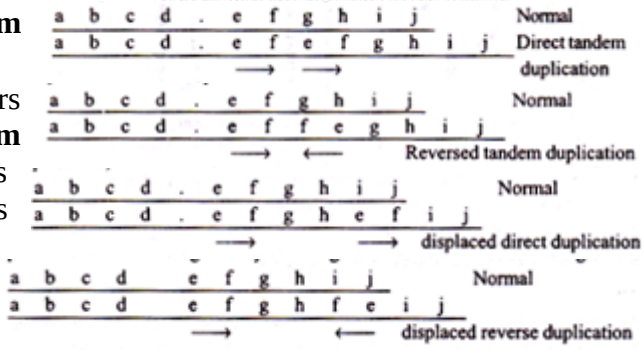


Fig. 12.6: The origin of a duplication



- Another case is the **Translocation duplication**, when the additional chromosome segment is found to be translocated into a non-homologous chromosome.

- In general, duplications do not produce any drastic consequences as like deletion in terms of phenotype and survival. It has been postulated that the increase in DNA content per cell accompanied the process of evolution; the origin of new genes with distinct functions was possible only for the event of duplication.

III. Inversion:

- When a segment of a chromosome is found to be oriented in reverse direction, it is called inversion. Two breaks are required within a chromosome to get this situation. The segment rotates in 180°.angle and reinserted between the breaks. As a result, the linear order of the genes becomes exactly opposite, in comparison to its normal homologous segment.

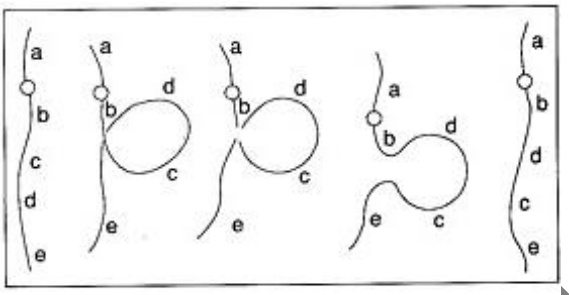


Fig. 12.12: The origin of an inversion

- Suppose, the normal order of a few genes in a segment of the chromosome is abcde (in the original chromosome). If an inversion takes place between c and d (i.e. cd segment), the order of the genes in the inverted segment will be abdce.
- However, inversion may be of two types – **Paracentric inversion and Pericentric inversion.** If the inverted segment does not contain a centromere, it is termed as **Paracentric inversion.** But if it contains a centromere, it is called **Pericentric inversion.**

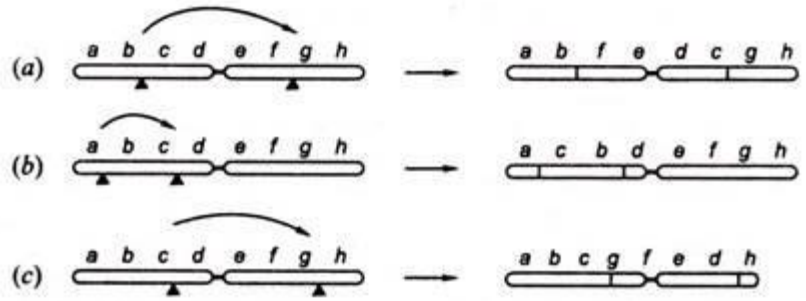


Fig. 22.17 Diagram showing various types of inversions. (*a*) pericentric; (*b*) paracentric; (*c*) pericentric which results in a change of chromosome morphology.

- As the two chromatids resulting from crossing over have deficiencies and duplications, the gametes having these chromosomes do not function and lead to considerable gametic or zygotic lethality. The plants show pollen sterility. The only crossovers which can be recovered are double crossovers, and the observed frequency of recombination between any two genes is considerably reduced.
- Thus inversions are called crossover suppressors. This property of inversion has been utilized in the production of CIB stock, used by Muller for detection of sex linked lethal mutations.

IV. Translocation:

- Integration of chromosome segment into a non-homologous chromosome is known as translocation. It involves the transfer of a segment of chromosome to a different part of the same chromosome or a different chromosome. There are three basic types of translocation—**simple, reciprocal and shift.**

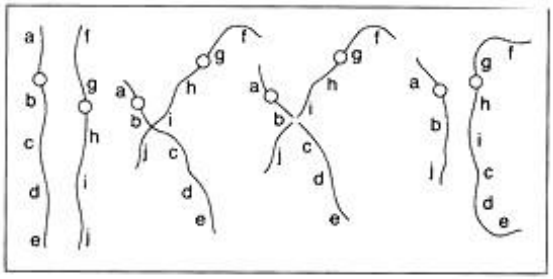


Fig. 12.17: The origin of a translocation

- **Simple translocation** shows an attachment of a small terminal segment of a chromosome to the end of a homologous chromosome or to a non-homologous chromosome. This attachment is not a fusion at all.

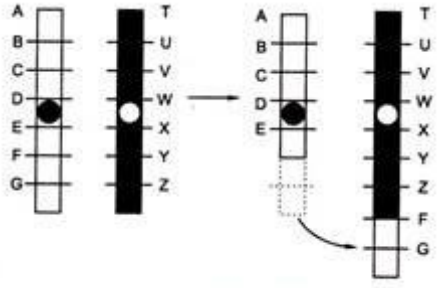


Fig. 22.8 Simple translocation.

- In **Reciprocal translocation**, the breakage takes place in two non--homologous chromosomes, and is followed by the reunion of broken segment to the wrong partners. Shift translocation requires at least three breaks in the chromosome. Among these, Reciprocal, and shift are the most common types.

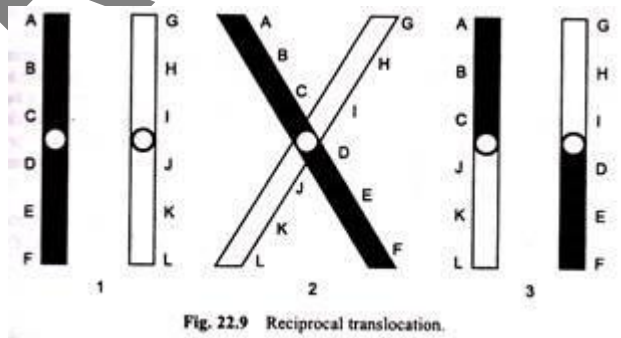


Fig. 22.9 Reciprocal translocation.

The main genetic effects of translocations are as follows:

(i) It brings about a qualitative change in the chromosomes structure or linkage group,

(ii) It brings about change in the sequences of genes in chromosomes which may eventually produce several abnormalities in body characters. This is position effect.
(iii) Semi-sterility. The translocation heterozygotes are generally semi-sterile because they produce gametes containing duplicated and deficient chromosomes as a result of typical pairing behaviours, crossing over and segregation patterns of chromosomes.
The translocation is of great importance for the individuals and the species. The most harmful effect of a reciprocal translocation is the semi-sterility it causes. It also causes severe modifications of the normal developmental pattern.
In the evening primrose (*Oenothera*) a number of variations are associated with translocations. This was the plant whose variability led De Vries to propose his popular mutation theory.

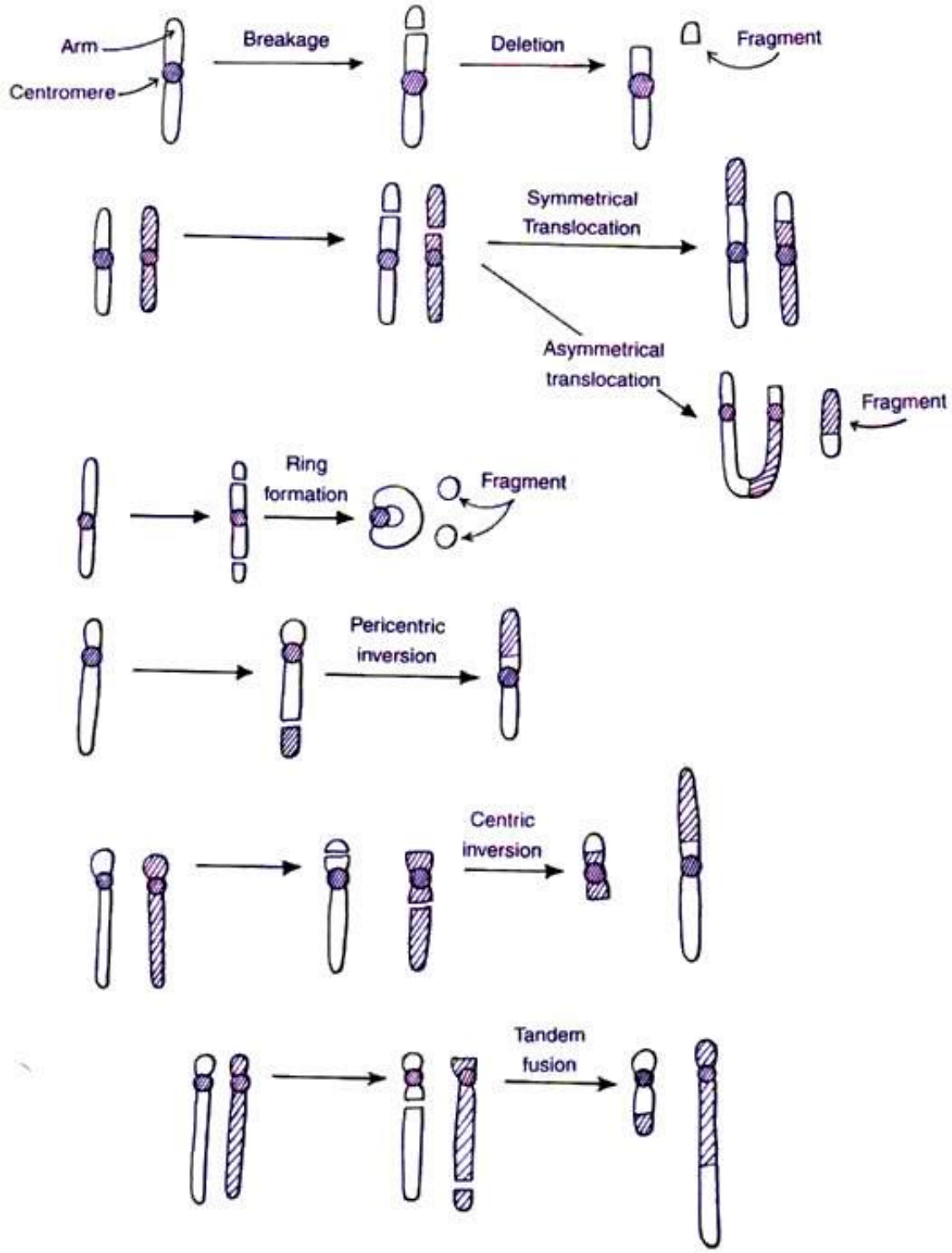


Fig. 6.9. Schematic Representation of Aberration (Chromosome Structure)

Structural Chromosome Aberration

Table 12.1: Common types of Chromosomal Aberrations

Type	Change in Chromosome Structure
Deficiency	Loss of a segment of chromosome.
1. Terminal deletion	Lost segment includes telomere.
2. Interstitial deletion	A segment between telomere and centromere is lost.
Duplication	A chromosome segment present in more than two copies in the same nucleus.
1. Tandem duplication	The additional chromosome segment located just after the normal segment, gene sequence being the same.
2. Reverse tandem duplication	Same as above but the gene sequence of the additional segment is inverted.
3. Displaced duplication	The additional segment located in the same chromosome but away from the normal segment.
4. Transposed duplication	The additional segment located in a nonhomologous chromosome.
Inversion	A chromosome segment contains genes in a sequence which is reverse of the normal.
1. Paracentric inversion	The inverted segment does not contain centromere.
2. Pericentric inversion	The inverted segment contains centromere.
Translocation	Chromosome segment integrated into nonhomologous chromosomes.
1. Simple translocation	A segment of a chromosome integrated into a nonhomologus chromosome.
2. Reciprocal translocation	A segment of chromosome integrated into a nonhomologous chromosome from which a segment is integrated into the first one.

DEFINITION, AIM, OBJECTIVES AND SCOPE OF PLANT BREEDING

DEFINITION:

Plant breeding can be defined "as an art and science" and technology of improving the genetic makeup of plants in relation to their economic use for the man kind.

Or

Plant breeding is the art and science of improving the heredity of plants for the benefit of mankind.

Or

Plant breeding deals with the genetic improvement of crop plants also known as science of crop improvement.

Or

Science of changing and improving the heredity of plants.

AIM:

Plant breeding aims to improve the characteristics of plants so that they become more desirable agronomically and economically. The specific objectives may vary greatly depending on the crop under consideration.

OBJECTIVES OF PLANT BREEDING:

1. Higher yield: The ultimate aim of plant breeding is to improve the yield of "**economic produce on economic part".** It may be grain yield, fodder yield, fiber yield, tuber yield, cane yield or oil yield depending upon the crop species. Improvement in yield can be achieved either by evolving high yielding varieties or hybrids.

2. Improved quality: Quality of produce is another important objective in plant breeding. The quality characters vary from crop to crop. Eg. Grain size, colour, milling and baking quality in wheat. Cooking quality in rice, malting quality in barley, colour and size of fruits, nutritive and keeping quality in vegetables, protein content in pulses, oil content in oilseeds, fibre length, strength and fineness in cotton.

3. Abiotic resistance: Crop plants also suffer from abiotic factors such as drought, soil salinity, extreme temperatures, heat, wind, cold and frost, breeder has to develop resistant varieties for such environmental conditions.

4. Biotic resistance: Crop plants are attacked by various diseases and insects, resulting in considerable yield losses. Genetic resistance is the cheapest and the best method of minimizing such losses. Resistant varieties are developed through the use of resistant donor parents available in the gene pool.

5. Change in maturity Duration / Earliness: Earliness is the most desirable character which has several advantages. It requires less crop management period, less insecticidal sprays, permits new crop rotations and often extends the crop area. Development of wheat varieties suitable for late planting has permitted rice-wheat rotation. Thus breeding for early maturing crop varieties, or varieties suitable for different dates of planting may be an important objective. Maturity has been reduced from 270 days to 170 days in cotton, from 270 days to 120 days in pigeon pea, from 360 days to 270 days in sugarcane.

6. Determinate Growth: Development of varieties with determinate growth is desirable in crops like mung, pigeon pea (*Cajanus cajan*), cotton (*Gossypium sp.*), etc.

7. Dormancy : In some crops, seeds germinate even before harvesting in the standing crop if there are rains at the time of maturity, e.g., greengram, blackgram, Barley and Pea, etc. A period of dormancy has to be introduced in these crops to check loss due to germination. In some other cases, however, it may be desirable to remove dormancy.

8. Desirable Agronomic Characteristics : It includes plant height, branching, tillering capacity, growth habit, erect or trailing habit etc., is often desirable. For example, dwarfness in cereals is generally associated with lodging resistance and better fertilizer response. Tallness, high tillering and profuse branching are desirable characters in fodder crops.

9. Elimination of Toxic Substances : It is essential to develop varieties free from toxic compounds in some crops to make them safe for human consumption. For example, removal of neurotoxin in Khesari – lentil (*Lathyruys sativus*) which leads to paralysis of lower limbs, erucic acid from *Brassica* which is harmful for human health, and gossypol from the seed of cotton is necessary to make them fit for human consumption. Removal of such toxic substances would increase the nutritional value of these crops.

10.Non-shattering characteristics: The shattering of pods is serious problem in green gram. Hence resistance to shattering is an important objective in green gram.

11.Synchronous Maturity : It refers to maturity of a crop species at one time. The character is highly desirable in crops like greengram, cowpea, castor and cotton where several pickings are required for crop harvest.

12.Photo and Thermo insensitivity: Development of varieties insensitive to light and temperature helps in crossing the cultivation boundaries of crop plants. Photo and thermo-insensitive varieties of wheat and rice has permitted their cultivation in new areas. Rice is now cultivated in Punjab, while wheat is a major *rabi* crop in West Bengal.

13.Wider adaptability: Adaptability refers to suitability of a variety for general cultivation over a wide range of environmental conditions. Adaptability is an important objective in plant breeding because it helps in stabilizing the crop production over regions and seasons.

14.Varieties for New Seasons : Traditionally maize is a *kharif* crop. But scientists are now able to grow maize as *rabi* and *zaid* crops. Similarly, mung is grown as a summer crop in addition to the main *kharif* crop.

SCOPE OF PLANT BREEDING (FUTURE PROSPECTS)

From times immemorial, the plant breeding has been helping the mankind. With knowledge of classical genetics, number of varieties have been evolved in different crop plants.

Since the population is increasing at an alarming rate, there is need to strengthen the food production which is serious challenge to those scientists concerned with agriculture. Advances in molecular biology have sharpened the tools of the breeders, and brighten the prospects of confidence to serve the humanity. The application of biotechnology to field crop has already led to the field testing of genetically modified crop plants. Genetically engineered rice, maize, soybean, cotton, oilseeds rape, sugar beet and alfalfa cultivars are expected to be commercialized before the close of 20th century. Genes from varied organisms may be expected to boost the performance of crops especially with regard to their resistance to biotic and abiotic stresses. In addition, crop plants are likely to be cultivated for recovery of valuable compounds like pharmaceuticals produced by genes introduced into them through genetic engineering. It may be pointed out that in Europe hirudin, an anti-thrombin protein is already being produced from transgenic *Brassica napus.*

Undesirable effects

Plant breeding has several useful applications in the improvement of crop plants. However, it has five main undesirable effects on crop plants.

1. Reduction in Diversity: Modern improved varieties are more uniform than land races. Thus plant breeding leads to reduction in diversity. The uniform varieties are more prone to the new races of pathogen than land races which have high genetic diversity.

2. Narrow genetic base: Uniform varieties have narrow genetic base. Such varieties generally have poor adaptability.

3. Danger of Uniformity: Most of the improved varieties have some common parents in the pedigree which may cause danger of uniformity.

4. Undesirable combinations: Sometimes, plant breeding leads to undesirable combinations. The examples of manmade crops having undesirable combination of characters are *Raphanobrassica* and Pomato.

5. Increased susceptibility to minor diseases and pests : Due to emphasis on breeding for resistance to major diseases and insect pests often resulted in an increased susceptibility to minor diseases and pests. These have gained importance and, in some cases, produced severe epidemics. The epidemic caused by *Botrytis cinerea* (grey mold) in chickpea during 1980-82 in Punjab and Haryana. The severe infection by Karnal bunt (*Tilletia sp.*) on some wheat varieties, infestation of mealy bugs in Bt cotton.

HISTORY AND DEVELOPMENT OF PLANT BREEDING

- About 10,000 years ago when man is believed to have started agriculture.
- Plant breeding began when man first choose certain plants for cultivation.
- The process of bringing a wild species under human management is referred to as **domestication**
- Domestication may be the most basic method of plant breeding
- All other breeding method become applicable to a plant species only after it has been successfully domesticated.
- Domestication continuous today and is likely to continue for some time in future
- Ex : In case of timber trees medicinal plants, microbes
- During the long period of historic cultivation natural selection has definitely acted on the domesticated species.
- Movement of man from one place to another brought about the movement of his cultivated plant species
- 700 BC - Babylonians and Assyrians pollinated date palm artificially
- 17th century - several varieties of **heading lettuce** were developed in France
- **1717 - Thomas Fair Child - produced the first artificial hybrid, popularly known as Fair Child's mule, by using carnation with sweet William**

- 1727 - The first plant breeding company was established in France by the vilmorins.
- 1760-1766 - Joseph koelreuter, a German, made extensive crosses in tobacco.
- 1759-1835 – Knight was perhaps the first man to use artificial hybridization to develop several new fruit varieties.
- Le couteur and Shireff used individual plant selections and progeny test to develop some useful cereal varieties
- 1873 - the work of Patrick Shireff was first published.
- He concluded that only the variation heritable nature responded to selections, and that there variation arose through 'natural sports' (= mutation) and by 'natural hybridization' (= recombination during meiosis in the hybrids so produced).
- 1856 - Vilmorin developed the progeny test and used this method successfully in the improvement of sugar beets.
- 1900 - Nilson-Ehle, his associates developed the individual plant selection method in Sweden.
- 1903 - Johannsen proposed the pureline theory that provided the genetic basis for individual plant selection.
- The science of genetics began with the rediscovery of Gregor Johan Mendel's paper in 1900 by Hugo de veris, Tshermark and Correns which was originally published in 1866.
- The modern plant breeding methods ha ve their bases in the genetic and cytogenetic principles.
- Numerous workers who determined the various modes of inheritance have contributed to the development and understanding of plant breeding.
- The discovery of chromosomes as carriers of genes has led to the development of specialized plant breeding methods for chromosome engineering.
- The totipotency of plant somatic and gametic cells allows regeneration of complete plants from single cells. This, coupled with the development of recombinant DNA technology, has enabled the transfer of desirable genes from any organism into plants. Crop varieties developed in this manner are already in cultivation in several countries.

HISTORY OF PLANT BREEDING IN INDIA

- 1871 – The Government of India created the Department of Agriculture
- 1905 – The Imperial Agricultural Research Institute was establish in Pusa, Bihar
- 1934 – The buildings of the institute damaged in earthquake
- 1936 – Shifted to New Delhi
- 1946 – Name was changed Indian Agricultural Research Institute
- 1901-05 – Agricultural Colleges were established at Kanpur, Pune, Sabour, Llyalpur, Coimbatore
- 1929 – Imperial council of Agricultural Research was established
- 1946 – Name was change to Indian Council Agricultural Research
- 1921 – Indian Central Cotton Committee was established – Notable researches on breeding and cultivation of cotton. Eg : 70 improved varieties of cotton
- 1956 – Project for intensification of regional research on cotton, oilseeds and millets (PIRRCOM) was initiated to intensify research on these crops – located at 17 different centers throughout the country
- 1957 – All India Coordinated maize improvement project was started with objective of exploiting heterosis
- 1961 - The first hybrid maize varieties released by the project
- ICAR initiated coordinated projects for improvement of the other crops
- 1960 – First Agricultural University established at Pantnagar, Nainital, U.P.

SOME INDIAN PLANT BREEDERS AND THEIR CONTRIBUTIONS

T.S. Venkatraman - An eminent sugarcane breeder, he transferred thick stem and high sugar contents from tropical noble cane to North Indian Canes. This process is known as noblization of sugarcane.
B.P. Pal - An eminent Wheat breeder, developed superior disease resistant N.P. varieties of wheat.
M.S. Swaminathan - Responsible for green revolution in India, developed high yielding varieties of Wheat and Rice
Pushkarnath - Famous potato breeder
N.G.P. Rao - An eminent sorghum breeder
K. Ramaiah - A renowned rice breeder
Ram Dhan Singh - Famous wheat breeder
D.S. Athwal - Famous pearl millet breeder
Bosisen - An eminent maize breeder
Dharampal Singh - An eminent oil-seed breeder

C.T. Patel - Famous cotton breeder who developed world's first cotton hybrid in 1970
V. Santhanam - Famous cotton breeder

VEGETATIVE PROPAGATION

Vegetative propagation or vegetative reproduction is the process of multiplication in which a portion or fragment of the plant body functions as propagates and develops into a new individual. Some plants are able to multiply by vegetative methods, which involve the production of new plants without the act of fertilization or sexual union.

Asexual or vegetative propagation of plants is that form of plant propagation in which the new individual arises from any vegetative part of the parent. Many plants produce modified stems, roots, and leaves, specially for natural vegetative propagation and possesses exactly the same characteristics of the parent plant from which it was sampled. At the same time, man too has developed various methods of artificial vegetative propagation for many useful plants.

The methods of vegetative propagation may be classified into the following two types:

(1) Natural vegetative propagation
(2) Artificial vegetative propagation

1. Natural Vegetative Reproduction:

Different plant parts are variously modified for vegetative propagation. The common structures that take part in vegetative propagation are root, stem, leaves and buds.

These are briefly described below:

(i) Vegetative Propagation by Roots:

The ordinary roots in many plants, such as *Dalbergia sisso*, *Populus*, *Guava*, *Murraya*, *Albizia lebbek* develop adventitious buds which grow to form new plants. Root tubers with adventitious buds occur in sweet potato (Fig. 10.1), Tapioca, Yam, *Dahlia* (Fig. 10.2) and *Asparagus*.

When placed in the soil, the buds present on the tuberous roots grow in to leafy shoots called slips. Slips develop adventitious roots at their base. Slips are detached and planted to form new plants. Many garden plants, such as *Phlox* and *Dahlia*, are propagated from roots which have been separated from the parent plant and cut into pieces.

(ii) Vegetative Propagation by Stems:

This is one of the most common and prevalent methods of vegetative propagation. Different plant parts, such as bulbs, runners, rhizomes, corms, tubers, offset etc. help the plant to multiply under favourable conditions.

(a) Bulb: It is a modified shoot that has a very short stem and apical and axillary buds. Some of these grow to form shoots, e.g., *Allium* (onion), *Allium sativum* (garlic) (Fig. 10.3 & 10.4), *Tulip* (Tulipa), *Jonquil* (Narcisus) etc.

(b) Runners:

These are creeping stems which produce roots at nodes. Runners break at places and each piece develops into an independent plant, e.g., *Cynodon* (doob grass), *Oxalis* etc. (Fig. 10.5 & 10.6).

(c) Rhizomes:

These are underground, horizontally growing stems. They have prominent nodes, internodes and axillary buds. Aerial branches sprout from the axillary buds which get separated from the rhizome and form new plants, e.g. Zinger (Fig. 10.7).

(d) Corm:

It is, in fact, a condensed rhizome that grows in vertical direction. The axillary buds, present in the axil of scale leaves, produce daughter corms which later on form new independent plants, e.g., *Crocus* (Saffron), *Colocasia* (Taro) *Arisaema* etc. (Figs. 10.8 & 10.9).

(e) Tuber:

It is a modification of underground stem. The "eyes" or buds present on the tuber form new independent plants. Potato (*Solanum tuberosum*) is the most common example (Figs. 10.10 & 10.11).

(J) Offset:

It looks like a modification of runner, in being more or less thickened, prostrate branch with a tuft of leaves at the apex, e.g., *Pistia* (water lettuce), *Eichhornia* (water hyacinth). They develop from the base of an old shoot or crown and after growing horizontally for some distance give rise to new crowns. They may break and form many independent plants (Fig. 10.12).

VEGETATIVE PROPAGATION BY ROOTS

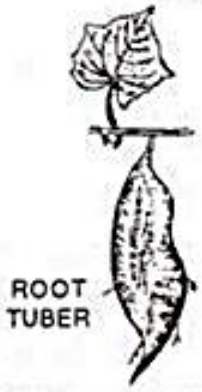


Figure 10.1 Tuberous roots of sweet potato.

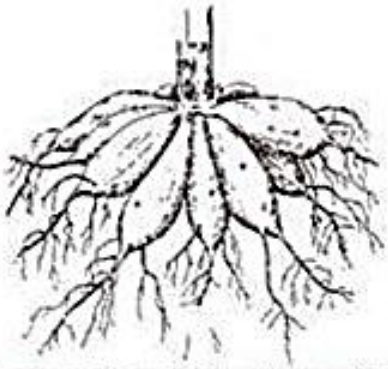


Figure 10.2 Fasciculated roots of Dahlia.

VEGETATIVE PROPAGATION BY STEM

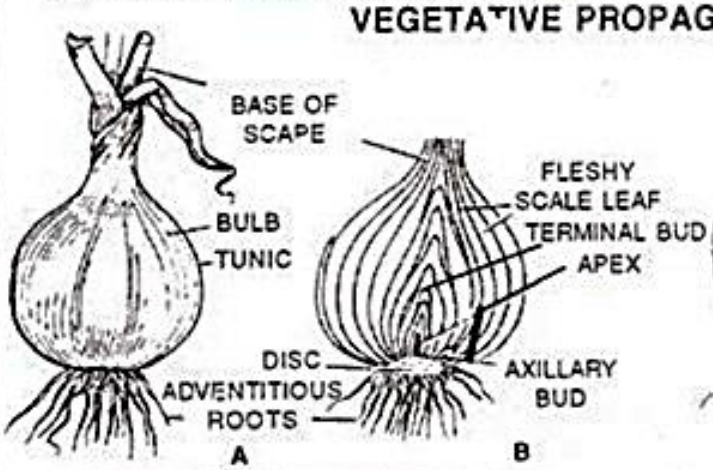


Figure 10.3. A. Tunicated bulb of onion (*Allium cepa*); B. V.S. of the same

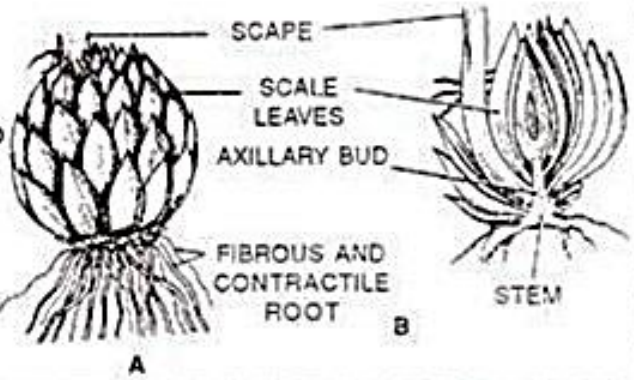


Figure 10.4. A. Scaly bulb of garlic (*Allium sativum*); B. L.S. of the bulb.

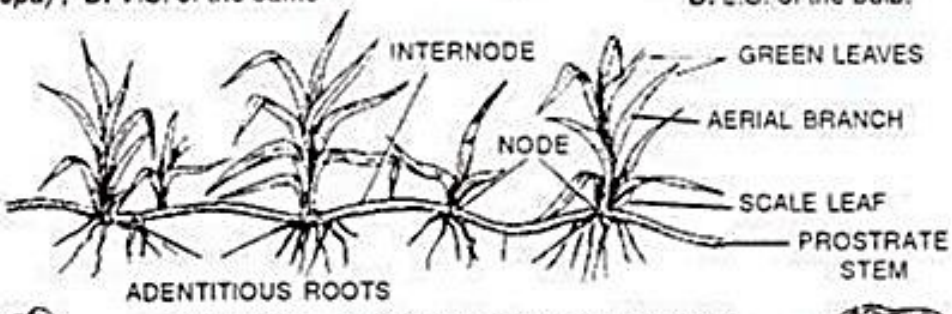


Figure 10.5. Runner of Doob grass (*Cynodon*)

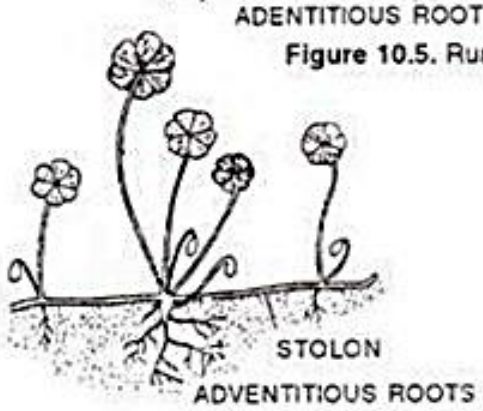


Figure 10.6. Runner (Stolon) of *Oxalis*.

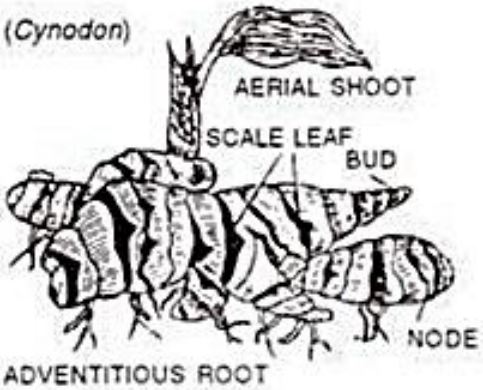


Figure 10.7. Straggling rhizome of ginger showing sympodial axis, and an aerial shoot coming out of the apex of axis.

(g) Aerial Shoots:

A stem segment of *Opuntia* and other cacti develops into a new plant after falling on the soil (Fig. 10.13). A similar segment of sugarcane with at least one node is used in agriculture to produce new plant.

(iii) Vegetative Propagation by Leaves:

Leaves of a number of plants possess adventitious buds for vegetative propagation, e.g., leaf tips of walking fern (*Adiantum caudatum*), marginal notches in Kalanchoe and *Bryophyllum* (Fig. 10.14). In *Bryophyllum*, the marginal buds sprout while the leaf is attached to plant. In some other plants, the buds develop only when the leaf is injured or detached and fall on the moist soil, e.g., *Begonia*, *Saintpaulia*, and *Streptocarpus*.

(iv) Vegetative Propagation by Bulbils:

They are fleshy buds which develop into new plants after falling on the soil, e.g. *Agave*, *Oxalis*, Pineapple (*Ananas camosus*), *Dioscorea*, Lily, *Chlorophytum* etc. Some of them are modified floral buds, e.g., *Agave*. In *Dioscorea*, they develop in the axils of leaves while in *Oxalis* they occur above the base of fleshy root. (Fig. 10.15).

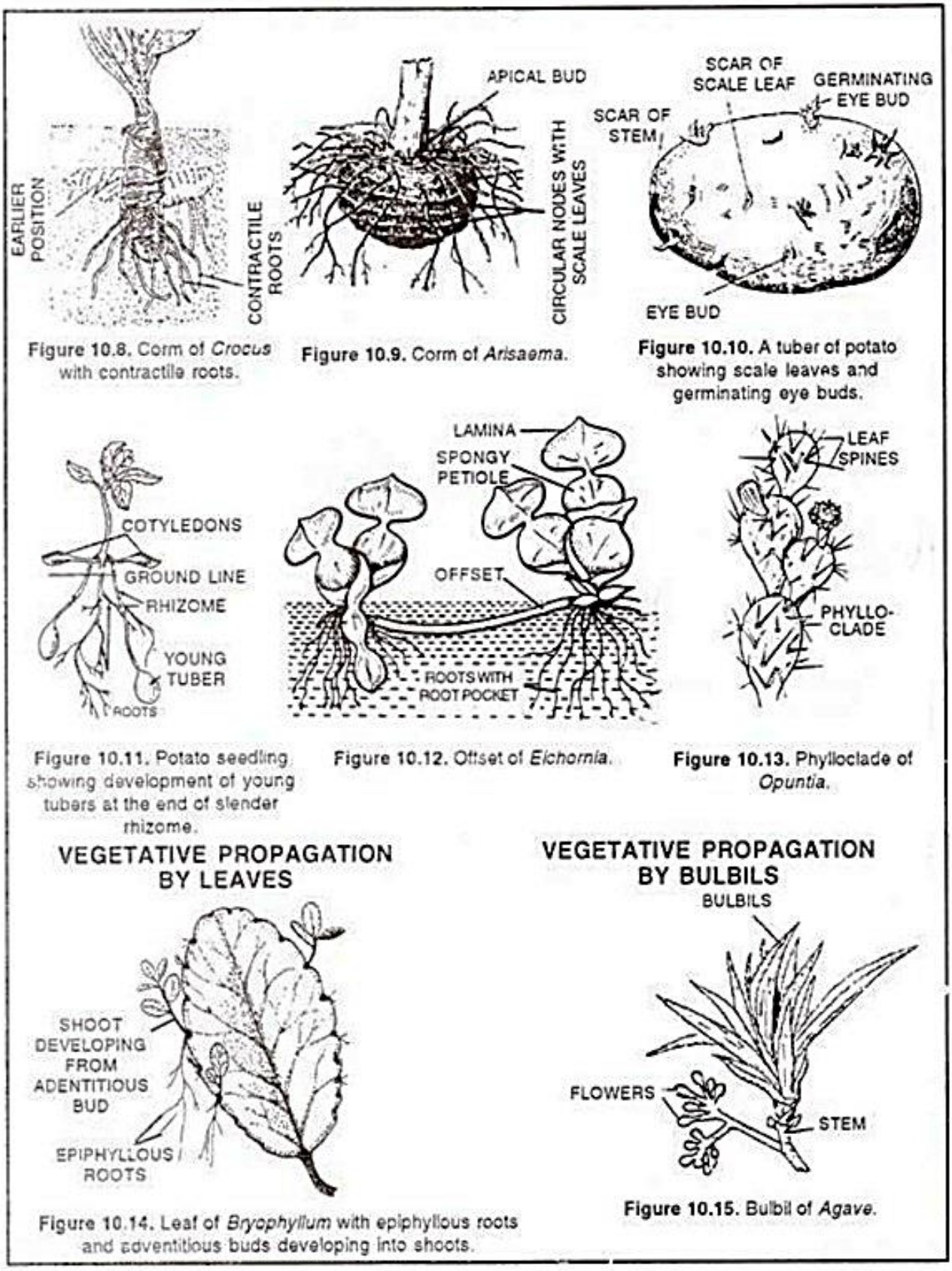


Figure 10.8. Corm of *Crocus* with contractile roots.

Figure 10.9. Corm of *Arisaema*.

Figure 10.10. A tuber of potato showing scale leaves and germinating eye buds.

Figure 10.11. Potato seedling showing development of young tubers at the end of slender rhizome.

Figure 10.12. Offset of *Eichornia*.

Figure 10.13. Phylloclade of *Opuntia*.

Figure 10.14. Leaf of *Bryophyllum* with epiphyllous roots and adventitious buds developing into shoots.

Figure 10.15. Bulbil of *Agave*.

2. Artificial Methods of Vegetative Propagation:

In some plants, where vegetative reproduction by natural means is difficult to occur, special techniques can be used. We know that plant cells are more totipotent than animal cells. So they can be forced to reproduce vegetatively. Thus all the techniques or methods which are carried out by human beings to produce plants vegetatively, are called artificial methods, these include stem cuttings, layering, root cuttings, grafting, gootee & micro-propagation (by tissue culture method)

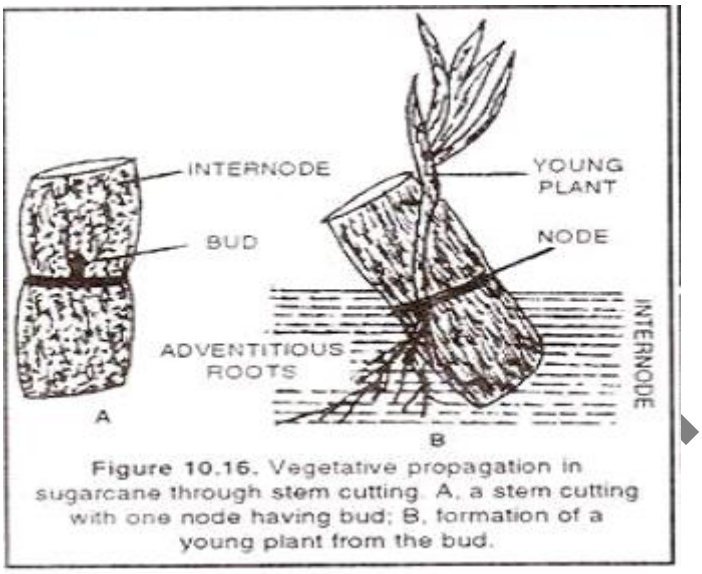


Figure 10.16. Vegetative propagation in sugarcane through stem cutting. A, a stem cutting with one node having bud; B, formation of a young plant from the bud.

1. Cutting:

(a) It is a simple method, in which a suitable part of stem or root (about 20 to 30 cm long) is cut and it is planted in the soil, along with some nutrients. This cut part soon develops new roots and develops into a new plant.

(b) Root cutting are commonly used in plants like lemon, tamarind, Blackberry and raspberry etc. Stem cuttings are very common in plants like Rose, *Croton*, Sugarcane, Tapioca, China rose, *Bougainvillea*, Lemon, Coffee and Grape etc.

(c) Leaf cutting also used to produce new offspring, in plants like *Sansevieria*.

2. Layering:

(a) It is one of the most common methods of artificial vegetative reproduction in plants.

(b) In this method, a twig (branch) of a plant is bent down, below the level of soil. This bent part is called layer.

(c) A small incision is made in this layer (bent portion). Now the portion is covered with soil. Moisture is given at regular intervals.

(d) Soon this covered portion develops new roots and become separated (or can be cut) from main body, giving rise to new plant. This plant then can be shifted to some new location.

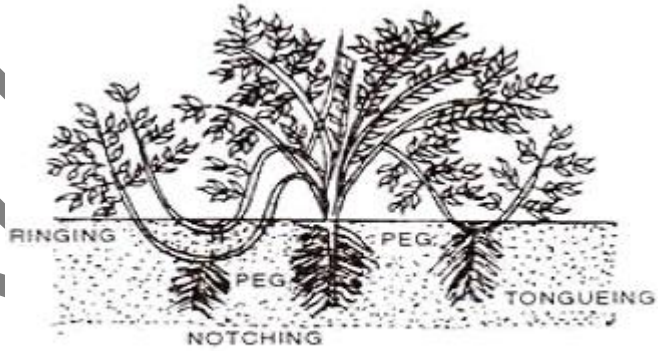


Figure 10.17. Vegetative propagation by layering.

(e) Layering is common in plants like-jasmine, Strawberry, Grapevine and Cherry etc.

In tip layering, the tip of the current season's shoot is bent into the soil by digging a sloping hole. Soon the bent part develops roots. The tip also comes out as vertical shoot. The rooted shoot is separated, e.g., Blackberry, Raspberry.

In serpentine layering, the basal branch is pegged down in the soil at several places to form a number of new plants from a single branch, e.g., *Clematis*. In mound layering, the basal part of a lower branch is bent down and covered with soil. The branch tip is kept outside the soil, e.g., Currant, Gooseberry.

Trench Layering consists of pegging a branch or young plant (e.g., Walnut) in horizontal position in a trench. The horizontal shoot begins to develop vertical shoots. As soon as the shoots come out, their bases are covered to fasten rooting.

3. Grafting:

(a) It is the technique of joining parts of two different plants to form a composite plant.

(b) It can be done efficiently in those plants, which are closely related and have vascular cambium.

(c) One plant, which has a strong root system, is selected as stock or stump (basal part). The branch of other plant (which is to be grafted) is selected as scion. Scion is usually selected from plants which have desired or superior characters.

(d) The shoot of the stock is cut 20-30 cm above the ground. Leaves and buds are removed from this part.

(e) Now, complementary cuts are made in stock and scion, so that scion can be fitted exactly in the grooves of stock.

(f) After this fitting, the area is tied tightly with the help of a tough thread and then it I is covered with grafting wax, to avoid any infection.

(g) Grafting is carried out commonly in plants like Mango, Guava, Apple, Rubber plant, Citrus and Pear etc.

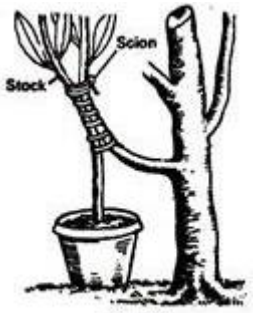


Fig. 47.

Types of grafting:

On the basis of method of uniting two parts, grafting can be of following types:

(a) Inarching or approach grafting:

By this method a branch (scion) of a plant is made to unite with a seedling (stock) by firmly tieing them together by means of a chord (Figure 47).

Before doing this, a small portion of the bark is sliced off from each to ensure a closed contact and quicker union between the two. When proper fusion has taken place (usually within two to three months), the stock is cut-out above the joining and the scion below, thus leaving the scion standing on the stock. Some of the fruit trees like mango, litchi, guava, plum, etc., readily respond to this method.

(b) Bud grafting:

For this method a T-shaped (Figure 48) incision is made in the bark of the stock, and a bud, cut out clean from a selected plant, is inserted into the T-shaped slit and properly bandaged. By this method it has been found possible to grow several varieties of roses on one rose stock good varieties of orange, lemon, etc., on inferior stocks, several varieties of China rose on one, cacti on one and so on.

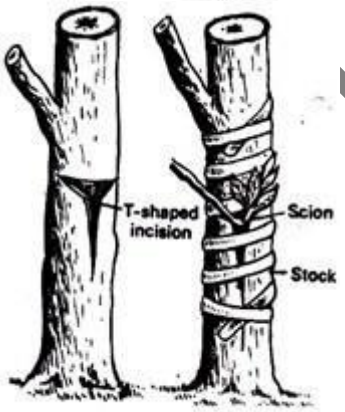


Fig. 48.

(c) Tongue grafting :

In this case the stock and scion have almost same diameter. They are given oblique or sloping cuts. A small notch is given to ensure perfect fixing of scion into stock groove.

(d) Wedge grafting:

In this case also, the stock and scion have same diameter. But a 'V shaped notch is given the stock while scion is cut like a wedge.

(e) Crown grafting:

In this case stock has a lager diameter than scion. Many scions are selected and all of them are grafted on a single stock.

(iv) Side grafting:

In this case, lateral or side cuts are made in stock. One scion is fitted in each lateral cut of stock.

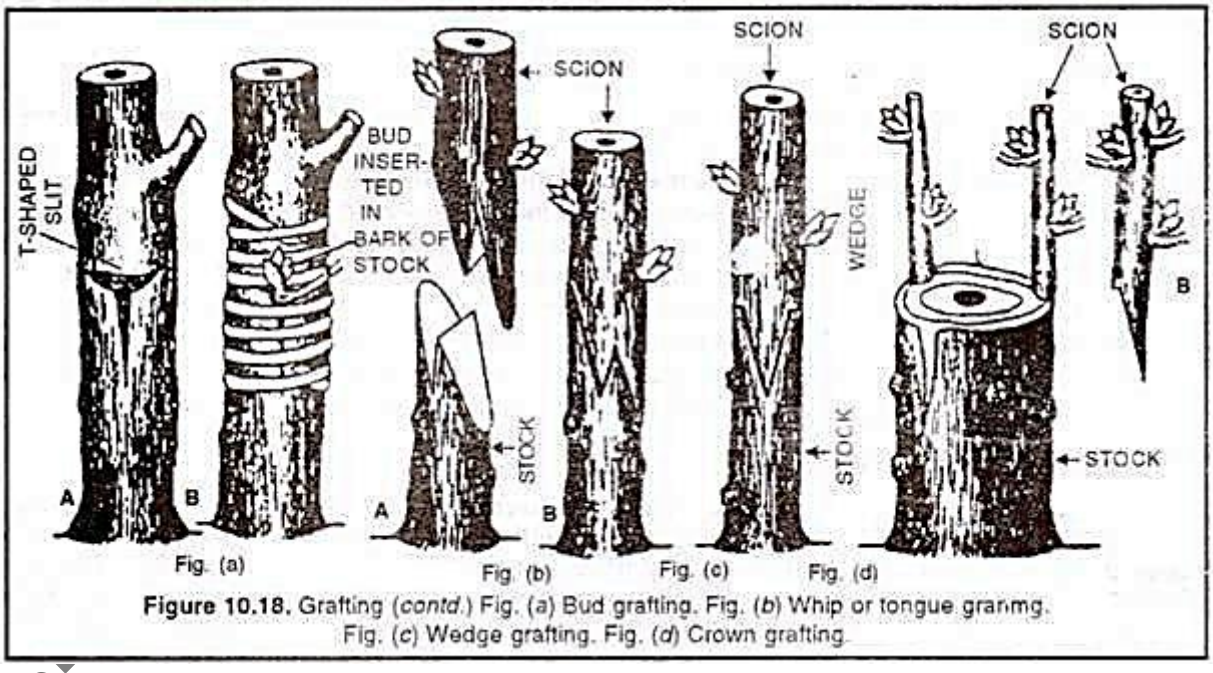


Figure 10.18. Grafting (*contd.*) Fig. (*a*) Bud grafting. Fig. (*b*) Whip or tongue grafting. Fig. (*c*) Wedge grafting. Fig. (*d*) Crown grafting.

4. Gootee:

a. It is also called air layering. It is commonly employed for the propagation of litchi, lemon, guava and orange etc.

b. In this method a healthy, leaf bearing branch of the main plant is selected.

c. A ring of bark is removed (for a distance of 2-5 cm) from the basal part of this branch.

d. The open part is covered with moist grafting clay (2 parts clay, 1 part cow dung, some fine cut way, moss or cotton and water). The graft is enriched with a root-promoting chemical.

e. This area is then wrapped with a polythene paper to prevent desiccation and infection.
f. This area develops small roots 1-3 months.
g. The branch is cut down and is planted to a new location.

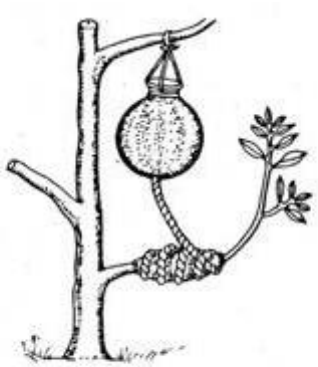


Fig. 46.61. Gootee. Method usually employed for vegetative propagation of lemon, orange, litichi, etc.

Advantages of vegetative reproduction:

(a) It is easiest method of reproduction in plants.
(b) Since the offspring and parent both have the same genotype and same characters, this type of reproduction helps to preserve the useful characters of the parental plant.
(c) It is a quick method of multiplication.
(d) It is a very helpful method of reproduction in those plants, which are sexually weak or have long dormant period of seeds.
(e) Vegetative reproduction helps in cloning and micro propagation of plants. Which in turn helps in standing a uniform population of plants.
(f) This type of reproduction helps to remove common infections, through pruning, micro-grafting, and micro-propagation.
(g) Methods like grafting helps in getting economically important plants, which have useful character of two different individuals.
(h) In seedless plants such as Banana, sugarcane, seedless grapes etc., it is predominant method of reproduction.

Disadvantages of Vegetative Reproduction:

1. Good qualities cannot be introduced nor bad characters eliminated in plants multiplied through vegetative propagation.
2. Disease contacted by a parent spreads to all the daughter plants.
3. Vegetative organs useful for propagation cannot be preserved for long.
4. Vegetative propagates are not so efficiently protected as the seeds are. They get easily decayed and are prone to various viral, fungal and bacterial diseases
5. The plants may show degeneration due to absence of sexual stimulus and variation.
6. Variability is absent. So, adaptability to changed environment decreases.
7. There is no mechanism for dispersal. Vegetative multiplication causes overcrowding and hence severe competition which can damage most of the plants.

CLONES

Clonal propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants. The term clone is used to represent a plant population derived from a single individual by asexual reproduction. The *in vivo* clonal propagation is often difficult, expensive and even unsuccessful. Tissue culture method offers an alternative way of clonal propagation which is popularly known as micro-propagation.

Here in this method a multiple number of miniatures of vegetative shoots are produced from a clone within a short time and space (Fig. 18.8). Use of tissue culture for micro-propagation was initiated by G. Morel (1960) who found this as the only commercially viable approach for orchid propagation.

Stage	Methodology involved
Stage 0	Selection of mother plant and its maintenance
↓	
Stage I	Initiation and establishment of culture
↓	
Stage II	Multiplication of shoots or rapid somatic embryo formation
↓	
Stage III	*In vitro* germination of somatic embryos and/or rooting of shoots
↓	
Stage IV	Transfer of plantlets to sterilized soil for hardening under greenhouse environment

Fig. 47.1 : Major stages involved in micropropagation.

Asexual reproduction through multiplication of vegetative parts is the only method for the *in vivo* propagation of certain plants, as they do not produce viable seeds e.g. banana, grape, fig, and chrysanthemum. Clonal propagation has been successfully applied for the propagation of apple, potato, tuberous and several ornamental plants.

Technique of Micro propagation: Micro propagation is a complicated process and mainly involves 3 stages (I, II and III). Some authors add two more stages (stage 0 and IV) for more comprehensive representation of micro- propagation. All these stages are represented in Fig. 47.1, and briefly described hereunder.

Stage 0:

This is the initial step in micro- propagation, and involves the selection and growth of stock plants for about 3 months under controlled conditions.

Stage I:

In this stage, the initiation and establishment of culture in a suitable medium is achieved. Selection of appropriate explants is important. The most commonly used explants are organs, shoot tips and axillary buds. The chosen explant is surface sterilized and washed before use.

Stage II:

It is in this stage, the major activity of micro propagation occurs in a defined culture medium. Stage II mainly involves multiplication of shoots or rapid embryo formation from the explant.

Stage III:

This stage involves the transfer of shoots to a medium for rapid development into shoots. Sometimes, the shoots are directly planted in soil to develop roots. *In vitro* rooting of shoots is preferred while simultaneously handling a large number of species.

Stage IV:

This stage involves the establishment of plantlets in soil. This is done by transferring the plantlets of stage III from the laboratory to the environment of greenhouse. For some plant species, stage III is skipped, and un-rooted stage II shoots are planted in pots or in suitable compost mixture.

The different stages described above for micro propagation are particularly useful for comparison between two or more plant systems, besides better understanding. It may however, be noted that not all plant species need to be propagated in vitro through all the five stages.

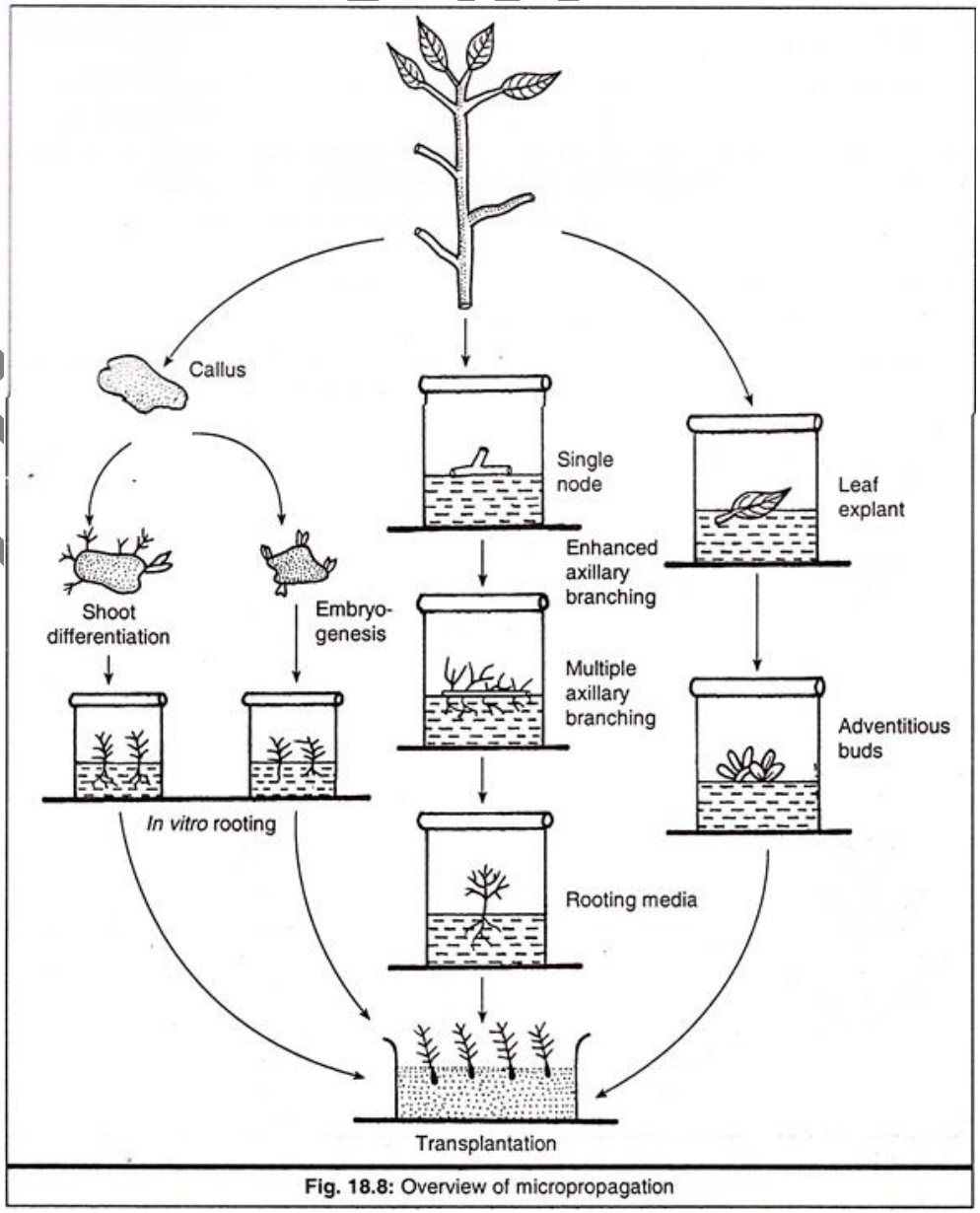


Fig. 18.8: Overview of micropropagation

Micro propagation mostly involves in vitro clonal propagation by two approaches:

1. Multiplication by axillary buds/apical shoots.
2. Multiplication by adventitious shoots.

Besides the above two approaches, the plant regeneration processes namely organogenesis and somatic embryogenesis may also be treated as micro propagation.

3. **Organogenesis:** The formation of individual organs such as shoots, roots, directly from an explant (lacking preformed meristem) or from the callus and cell culture induced from the explant.
4. **Somatic embryogenesis:** The regeneration of embryos from somatic cells, tissues or organs.

HYBRIDIZATION

Individual produced as a result of **cross between two genetically different parents** is known as hybrid. The **natural or artificial process** that results in the **formation of hybrid** is known as **hybridization**.
Or
The **production of a hybrid** by **crossing two individuals** of **unlike genetical** constitution is known as **hybridization**. Hybridization is an important method of combining characters of different plants. Hybridization does not change genetic contents of organisms but it produces new combination of genes.
The **first natural hybridization** was recorded by **Cotton Mather** (1716) in **corn**. The **first artificial interspecific plant hybrid** was **produced** by **Thomas Fairchild** in 1717. It is commonly known as **'Fairchild Mule'**.
Hybridization was **first practically utilized in crop improvement by German botanist Joseph Koerauter in 1760**. Mendel onward, the hybridization had become the key method of crop improvement. Today, it is the most common method of crop improvement, and the vast majority of crop varieties have resulted from hybridization.

Objectives of Hybridization:
I. To artificially create a variable population for the selection of types with desired combination of characters.
II. To combine the desired characters into a single individual, and
III. To exploit and utilize the hybrid varieties.

Types of Hybridization:

(i) Intra-varietal hybridization:
The crosses are made between the plants of the same variety.

(ii) Inter-varietal or Intraspecific hybridization:
The crosses are made between the plants belonging to two different varieties.

(iv) Interspecific hybridization or intrageneric hybridization:
The crosses are made between two different species of the same genus.

(v) Introgressive hybridization:
Transfer of some genes from one species into the genome of the other species is known as introgressive hybridization.
The **crosses between different species of the same genus or different genera of the same family** are also known as **distant hybridization or wide crossing**. Such **crosses** are called **distant crosses**.

Procedure of Hybridization involves the following steps:
(i) Selection of parents.
(ii) Selfing of parents or artificial self-pollination.
(iii) Emasculation.
(iv) Bagging
(v) Tagging
(vi) Crossing
(vii) Harvesting and storing the F_1 seeds

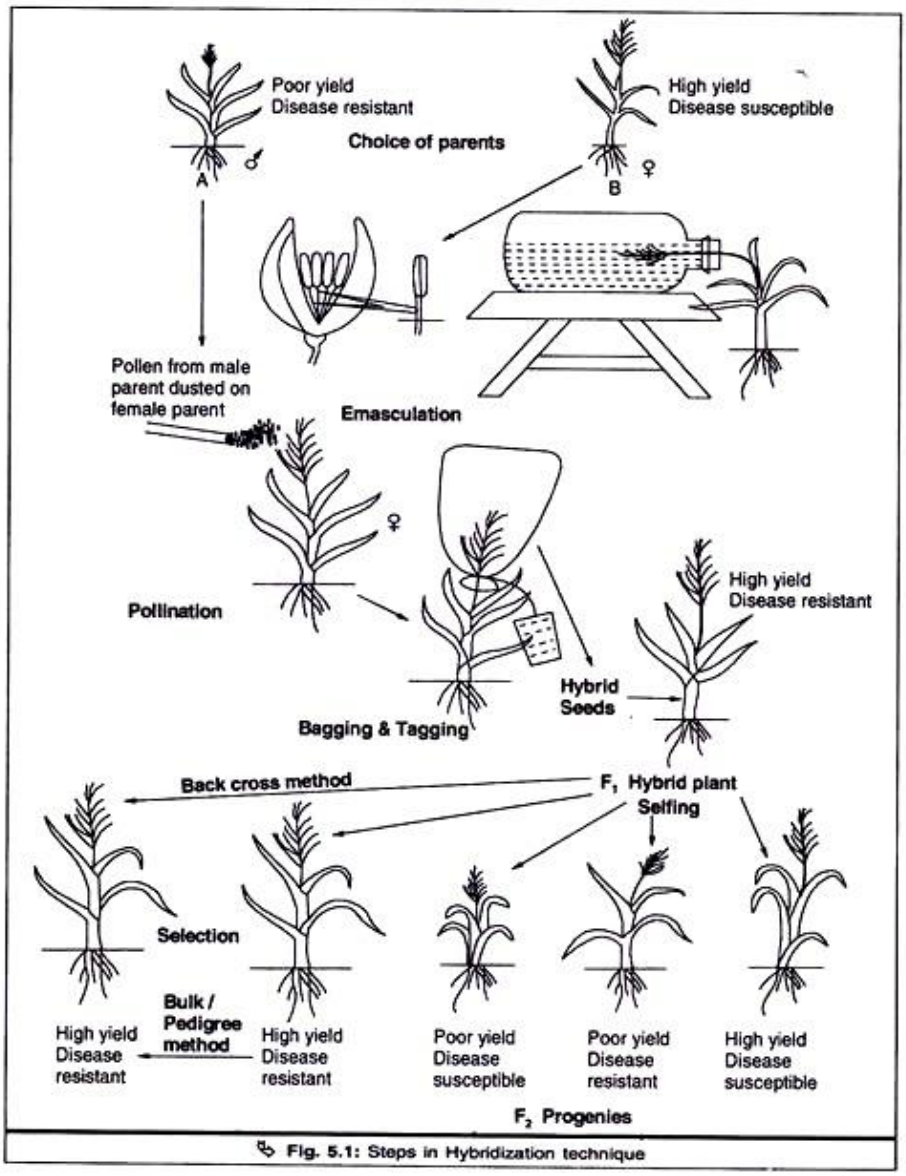


Fig. 5.1: Steps in Hybridization technique

(viii) Raising the F_1 generation.

(i) Selection of parents:

The selection of parents depends upon the aims and objectives of breeding. Parental plants must be selected from the local areas and are supposed to be the best suited to the existing conditions.

(ii) Selfing of parents or artificial self-pollination:

It is essential for inducing homozygosity for eliminating the undesirable characters and obtaining inbreeds.

(iii) Emasculation:

Inbreeds are grown under normal conditions and are emasculated. **Emasculation is the removal of stamens from female parent before they burst and shed their pollens.** It can be defined as the removal of stamens or anthers or the killing of the pollen grains of a flower without affecting in any way the female reproductive organs. Emasculation is not required in unisexual plants but it is essential in bisexual or self-pollinated plants.

Various methods used for emasculation are:

(a) Hand Emasculation or Forceps or Scissor Method:

This method is generally used in those plants which have large flowers. In this method the corolla of the selected flowers is opened and the anthers carefully removed with the help of fine-tip forceps.

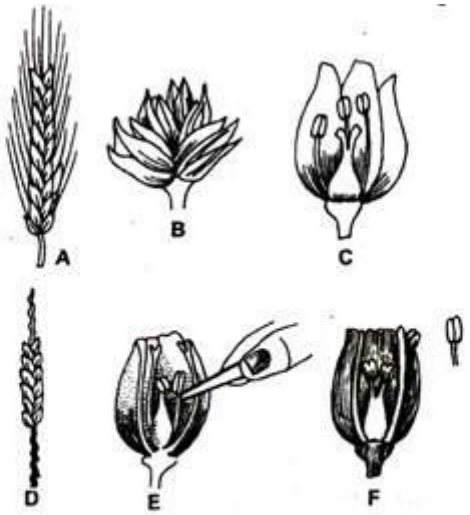


Fig. 6. (A-F) Emasculation in wheat. (A) Spike of spikelets, (B) spikelet, (C) Floret, (D) Upper and lower spikelets removed, awns removed, upper portion of florets cut, (E) Anthers removed with the help of fine-tip forceps. (F) Removed anther.

Following are the important precautions while performing this method:

i. Flowers should be selected at proper stage.

ii. Stigma should be receptive and anthers should not have dehisced.

iii. All the anthers should be removed from the flowers without breaking (Fig. 6).

iv. Stigma and ovary of the flower should not be damaged.

(b) Hot Water Treatment:

Removal of stamens with the help of forceps is very difficult in minute flowers. In such small hermaphrodite flowers (e.g., Bajra, Jowar) emasculation is done by dipping the flowers in hot water for a certain duration (1-10 minutes) of time.

The time varies from species to species. This method is based on the fact that gynoecia can withstand the hot temperature at which the anthers are killed. In this method an equipment is used which is placed on a simple heavy stand.

It consists of a cylindrical metallic container of 60 cm length, with one hole of 5 cm to 16 cm diameter on one end to pass over a bajra or jowar head. After inserting the panicle inside the container a cork is fitted in the hole to close it.

A 35 cm long rubber tube or belt is stretched over the side of the container, and when in use this tube is tied around the peduncle of the head. To measure the temperature, in the upper side of the container a thermometer is placed. In the field water is carried in a thermos jug (Fig. 7). The panicle is inserted in the container prior to blooming for a particular duration of time. It has been observed that pollen

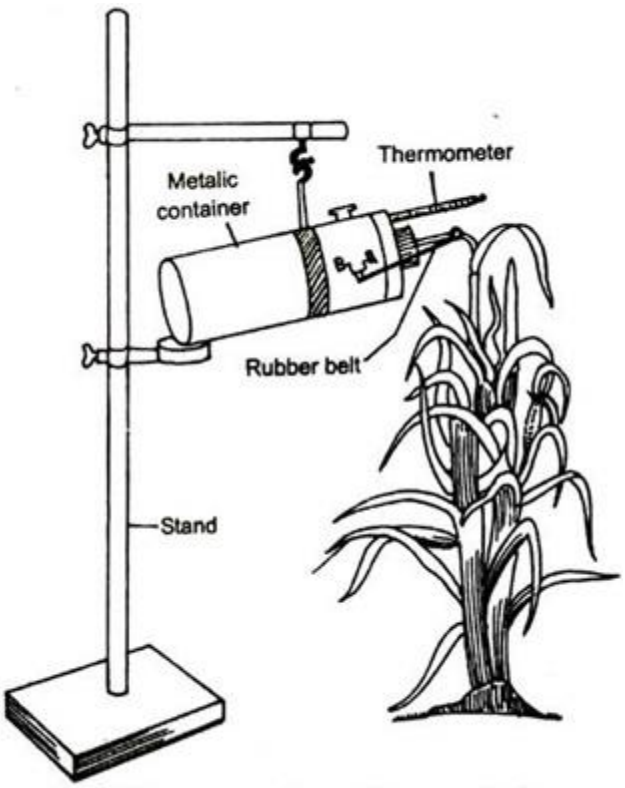


Fig. 7. Hot water equipment for emasculation.

grains of rice are killed by immersing the inflorescence for 5 to 10 minutes in the hot water maintained at 40-44°C in a thermos flask.

(c) Cold Water Treatment:

Like hot water cold water also kills pollen grains without damaging the gynoecium. In rice 0-6°C temperature is maintained to kill the pollen grains. This method is less effective than hot water treatment.

(d) Alcohol Treatment Method:

This method is not commonly used for emasculation because duration of treatment is an important factor since a very short duration is required failing which even the gynoecium may be damaged. Flowers or inflorescences are immersed in alcohol of a suitable concentration for a brief period. In alfa-alfa, a treatment of even 10 seconds with 57 % alcohol is sufficient to kill the pollen grains.

(e) Suction Method:

It is a mechanical method and is suitable for the crops having minute flowers. In this method the amount of pressure is applied in such a way that only anthers are sucked out and other parts of the flower like gynoecium remain intact. However, in this method 10-15% self pollination takes place. It is one of the major drawback of this method.

(f) Male Sterility or Self-incompatibility Method:

Emasculation option can be eliminated by the use of male-sterile plants, In some self-pollinated plants for example, Sorghum, Onion, Barley etc. anthers are sterile and do not produce any viable pollens! Similarly self-incompatibility may also be used to avoid emasculation.

(g) Chemical Gametocides:

Certain chemicals are capable of causing male sterility, when sprayed before flowering e.g., 2, 4-D, naphthalene acetic acid (NAA), maleic-hydrazide (MA), tribenzoic acid etc. FW450 in cotton may be used for bringing about emasculation.

(iv) Bagging:

It is the fourth step and completed with emasculation. The emasculated flower or inflorescence is immediately bagged to avoid pollination by any foreign pollen. The bags may be made of paper, butter paper, glassine or fine cloth. Butter paper or vegetable parchment bags are most commonly used.

Fig. 8. Different methods of Bagging.

The bags are tied to the base of the inflorescence or to the stalk of the flower with the help of thread, wire or pins. The bagging is done with the emasculation in bisexual plants and before the stigma receptivity and dehiscence of the anthers in unisexual plants. Both male and female flowers are bagged separately to prevent contamination in male flowers and cross-pollination in female flowers (Fig. 8).

(v) Tagging:

The emasculated flowers are tagged just after bagging. Generally circular tags of about 3 cm or rectangular tags of about 3 x 2 cm are used. The tags are attached to the base of flower or inflorescence with the help of thread.

The information on tag must be as brief as possible but complete bearing the following information:

(i) Number referring to the field record

(ii) Date of emasculation

(iii) Date of crossing

(iv) Name of the female parent is written first followed by a cross sign (x) and then the male parent, e.g., C x D denotes that C is the female parent and D is the male parent.

(vi) Crossing: It can be defined as the artificial cross-pollination between the genetically unlike plants. In this method mature, fertile and viable pollens from the male parent are placed on the receptive stigma of emasculated flowers to bring about fertilization.

Pollen grains are collected in petridishs (e.g., Wheat, cotton etc.) or in paper bags {e.g., maize) and applied to the receptive stigmas with the help of a camel hair brush, piece of paper, tooth pick or forceps. In some crops (e.g., Jowar, Bajra) the inflorescences of both the parents are enclosed in the same bag.

(vii) Harvesting and Storing the F_2 Seeds:

Seeds are stored properly with original tags.

(viii) Raising the F_1 generation:

In the coming season, the stored seeds are sown separately to raise the F_1 generation. The plants of F_1 generation are progenies of cross seeds and therefore are hybrids.

Based on the taxonomic relationships of the two parents, hybridization may be classified into two broad groups:

1. Intervarietal Hybridization:

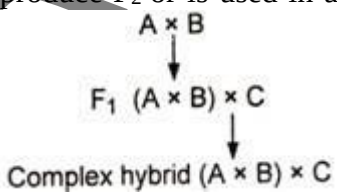
The parents involved in hybridization belong to the same species; they may be two strains, varieties or races of the same species. It is also known as intraspecific hybridization. In crop improvement programmes, intervarietal hybridization is the most commonly used. An example would be crossing of two varieties of wheat (*T. aestivum*), rice (*O. Sativa*) or some other crop. The intervarietal crosses may be simple or complex depending upon the number of parents involved.

Simple Cross:

In a simple cross, two parents are crossed to produce the F_1. The F_1 is selfed to produce F_2 or is used in a backcross programme, e.g., A x B → F_1 (A x B).

Complex Cross:

More than two parents are crossed to produce the hybrid, which is then used to produce F_2 or is used in a backcross. Such a cross is also known as convergent cross because this crossing programme aims at converging genes from several parents into a single hybrid.



2. Distant Hybridization:

This includes crosses between different species of the same genus or of different genera. When two species of the same genus are crossed, it is known as **inter-specific hybridization**; but when they belong to two different genera it is termed as **intergeneric hybridization**. Generally, the objective of such crosses is to transfer one or few simply inherited characters like disease resistance to a crop species.

Interspecific hybrid was **first developed** by **Thomas Fairchild** in 1717 between **sweet William** and **carnation** species of ***Dianthus*** (***Dianthus barbatus* x *D. caryophyllus***). Interspecific hybridization is used when the desirable character is not found within the species of a crop.

Interspecific crosses are of following three types:

(i) Fully fertile crosses – Such crosses are obtained between those species which have complete chromosome homology.

(ii) Partially fertile crosses – Such crosses are obtained between those species which differ in ploidy level but have some chromosomes in common.

(iii) Fully sterile crosses – Such crosses are obtained between those species which do not have chromosome homology.

Sometimes, **interspecific hybridization** may be used for developing a new variety, e.g., Clinton oat variety was developed from a cross between *Avena sativa* x *A. byzantina* (both haploid oat species), and CO 31 rice variety was developed from the cross *Oryza sativa var. indica* x *O. perennis*. *Annona squamosa* × *A. cherimola* is a cross between the sugar apple and the cherimoya called the atemoya.

These are the primary reasons for creating interspecific hybrid plants:

Improved Yield

Crop yields increase dramatically when hybridization is used to exceed one or more of the parents in size and reproductive potential. For example, boysenberries (*Rubus ursinus x idaeus*) were developed at Knott's Berry Farm in California. They are a result of a set of crosses between blackberries (*Rubus fruticosus*), European raspberries (*Rubus idaeus*) and loganberries (*Rubus* × *loganobaccus*). Hybrids can yield up to 100 percent more crops than to their resistance to disease and increased vigor.

Longer Growing Season

Many hybrid plant varieties are bred to extend the growing season and mature faster than non-hybrid varieties. Hybrids may also be developed to turn annual plants into perennials; for farmers, this can increase profitability reduce some of the environmental impacts of agriculture.

Higher Quality

Interspecific hybrids may surpass traditional varieties in taste, shelf-life, size, texture, nutrition, etc. Stone fruit, in particular, has a seemingly unending number of interspecific hybrid varieties developed for their flavor and novel appearance: pluots, plumcots, and apriums are all common examples of this phenomenon at work, but there are also nectaplums, peacharines, and pluerries now being grown in fruit orchards.

The first **intergeneric cross** between radish and cabbage was made by Karpechenko in 1928 in Russia. *Triticale* and *Raphanobrassica* are outcome of intergeneric crosses. *Triticale* is a crop species resulting from a plant breeder's cross between wheat (*Triticum*) and rye (*Secale*). The parents of Raphanobrassica are radish (*Raphanus stativus*) and cabbage (*Brassica oleracea*).

TABLE 21.1 Differences between Interspecific and Intergeneric hybridization

Particulars	Interspecific Hybridization	Intergeneric Hybridization
1. Parents involved	Involves two different species of the same genes.	Involves two different genera of the same family.
2. Fertility	Such hybrids vary from completely fertile to completely sterile.	Hybrids are always sterile.
3. Seed setting	More than intergeneric crosses.	Low
4. Use in crop improvement	More than intergeneric crosses.	Less than interspecific crosses.
5. Release of Hybrid varieties	Possible in some crops.	Not possible.
6. Evolution of new crops	Not possible, but evolution of new species is sometimes possible.	Sometimes possible, example is *Triticale*.

MAINTENANCE OF GERMPLASM

Germplasm refers to the hereditary material (total content of genes) transmitted to the offspring through germ cells. Germplasm provides the raw material for the breeder to develop various crops. Thus, conservation of germplasm assumes significance in all breeding programmes.

As the primitive man learnt about the utility of plants for food and shelter, he cultivated the habit of saving selected seeds or vegetative propagules from one season to the next one. In other words, this may be regarded as primitive but conventional germplasm preservation and management, which is highly valuable in breeding programmes.

The very objective of germplasm conservation (or storage) is to preserve the genetic diversity of a particular plant or genetic stock for its use at any time in future. In recent years, many new plant species with desired and improved characteristics have started replacing the primitive and conventionally used agricultural plants. It is important to conserve the endangered plants or else some of the valuable genetic traits present in the primitive plants may be lost.

A global body namely International Board of Plant Genetic Resources (IBPGR) has been established for germplasm conservation. Its main objective is to provide necessary support for collection, conservation and utilization of plant genetic resources throughout the world.

There are two approaches for germplasm conservation of plant genetic materials:

1. In-situ conservation
2. Ex-situ conservation

1. In-Situ Conservation:

The conservation of germplasm in their natural environment by establishing biosphere reserves (or national parks/gene sanctuaries) is regarded as in-situ conservation. This approach is particularly useful for preservation of land plants in a near natural habitat along with several wild relatives with genetic diversity. The in-situ conservation is considered as a high priority germplasm preservation programme.

The major limitations of in-situ conservation are listed below:

i. The risk of losing germplasm due to environmental hazards

ii. The cost of maintenance of a large number of genotypes is very high.

2. Ex-Situ Conservation:

Ex-situ conservation is the chief method for the preservation of germplasm obtained from cultivated and wild plant materials. The genetic materials in the form of seeds or from in vitro cultures (plant cells, tissues or organs) can be preserved as gene banks for long term storage under suitable conditions. For successful establishment of gene banks, adequate knowledge of genetic structure of plant populations, and the techniques involved in sampling, regeneration, maintenance of gene pools etc. are essential.

Germplasm conservation in the form of seeds:

Usually, seeds are the most common and convenient materials to conserve plant germplasm. This is because many plants are propagated through seeds, and seeds occupy relatively small space. Further, seeds can be easily transported to various places.

There are however, certain limitations in the conservation of seeds:

i. Viability of seeds is reduced or lost with passage of time.

ii. Seeds are susceptible to insect or pathogen attack, often leading to their destruction.

iii. This approach is exclusively confined to seed propagating plants, and therefore it is of no use for vegetatively propagated plants e.g. Potato, Ipomoea, Dioscorea.

iv. It is difficult to maintain clones through seed conservation.

Certain seeds are heterogeneous and therefore, are not suitable for true genotype maintenance.

In vitro methods for germplasm conservation:

In vitro methods employing shoots, meristems and embryos are ideally suited for the conservation of germplasm of vegetatively propagated plants. The plants with recalcitrant seeds and genetically engineered materials can also be preserved by this in vitro approach.

There are several advantages associated with in vitro germplasm conservation:

i. Large quantities of materials can be preserved in small space.

ii. The germplasm preserved can be maintained in an environment, free from pathogens.

iii. It can be protected against the nature's hazards.

iv. From the germplasm stock, large number of plants can be obtained whenever needed.

v. Obstacles for their transport through national and international borders are minimal (since the germplasm is maintained under aspectic conditions).

There are mainly three approaches for the in vitro conservation of germplasm:

1. Cryopreservation (freeze-preservation)
2. Cold storage
3. Low-pressure and low-oxygen storage

Cryopreservation:

Cryopreservation (Greek, krayos-frost) literally means preservation in the frozen state. The principle involved in cryopreservation is to bring the plant cell and tissue cultures to a zero metabolism or non-dividing state by reducing the temperature in the presence of cryoprotectants.

Cryopreservation broadly means the storage of germplasm at very low temperatures:

i. Over solid carbon dioxide (at -79°C)

ii. Low temperature deep freezers (at -80°C)

iii. In vapour phase nitrogen (at -150°C)

iv. In liquid nitrogen (at -196°C)

Among these, the most commonly used cryopreservation is by employing liquid nitrogen. At the temperature of liquid nitrogen (-196°C), the cells stay in a completely inactive state and thus can be conserved for long periods.

In fact, cryopreservation has been successfully applied for germplasm conservation of a wide range of plant species e.g. rice, wheat, peanut, cassava, sugarcane, strawberry, coconut. Several plants can be regenerated from cells, meristems and embryos stored in cryopreservation.

Mechanism of Cryopreservation:

The technique of freeze preservation is based on the transfer of water present in the cells from a liquid to a solid state. Due to the presence of salts and organic molecules in the cells, the cell water requires much more lower temperature to freeze (even up to -68°C) compared to the freezing point of pure water (around 0°C).

When stored at low temperature, the metabolic processes and biological deteriorations in the cells/tissues almost come to a standstill.

Precautions/Limitations for Successful Cryopreservation:

Good technical and theoretical knowledge of living plant cells and as well as cryopreservation technique are essential.

Other precautions for successful cryopreservation are listed below:

i. Formation ice crystals inside the cells should be prevented as they cause injury to the organelles and the cell.
ii. High intracellular concentration of solutes may also damage cells.
iii. Sometimes, certain solutes from the cell may leak out during freezing.
iv. Cryoprotectants also affect the viability of cells.
v. The physiological status of the plant material is also important.

Technique of Cryopreservation:

An outline of the protocol for cryopreservation of shoot tip is depicted in Fig. 48.1. The cryopreservation of plant cell culture followed by the regeneration of plants broadly involves the following stages

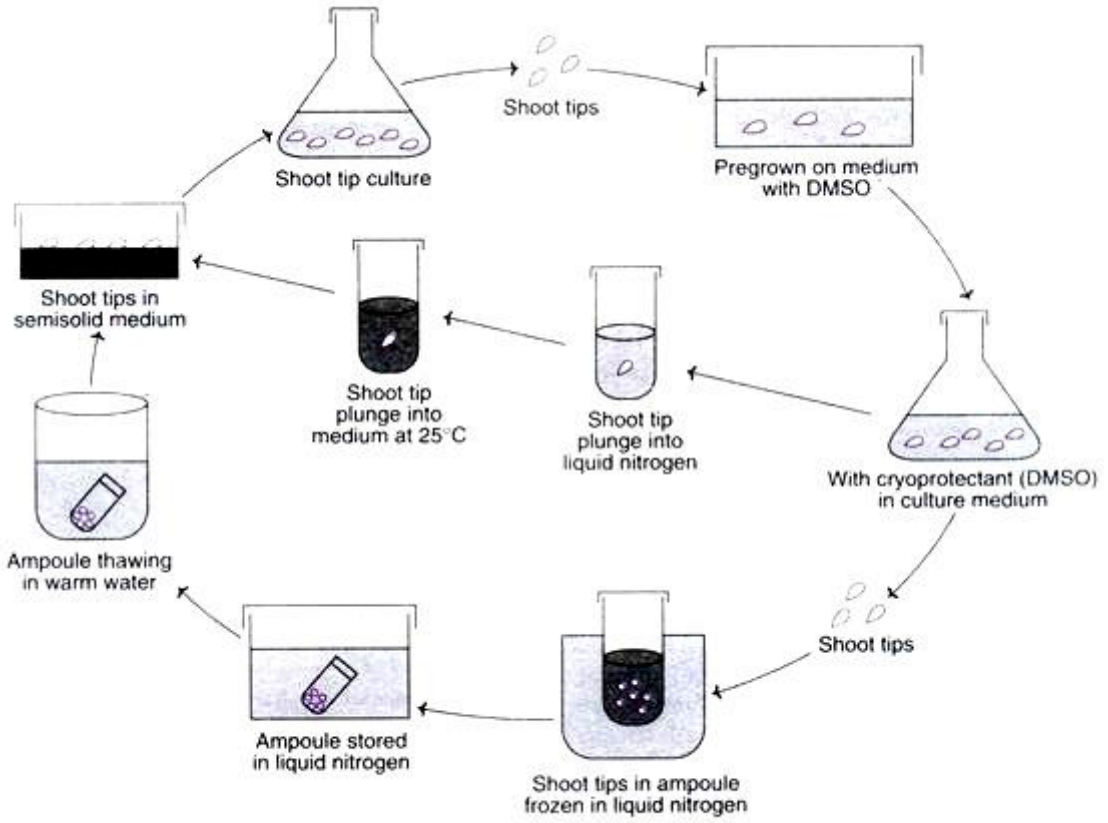


Fig. 48.1 : An outline of the protocol for cryopreservation of shoot tip (DMSO–Dimethyl sulfoxide).

1. Development of sterile tissue cultures
2. Addition of cryoprotectants and pretreatment
3. Freezing
4. Storage
5. Thawing
6. Re-culture
7. Measurement of survival/viability
8. Plant regeneration.

The salient features of the above stages are briefly described.

Development of sterile tissue culture:

The selection of plant species and the tissues with particular reference to the morphological and physiological characters largely influence the ability of the explant to survive in cryopreservation. Any tissue from a plant can be used for cryopreservation e.g. meristems, embryos, endosperms, ovules, seeds, cultured plant cells,

protoplasts, calluses. Among these, meristematic cells and suspension cell cultures, in the late lag phase or log phase are most suitable.

Addition of cryoprotectants and pretreatment:

Cryoprotectants are the compounds that can prevent the damage caused to cells by freezing or thawing. The freezing point and super-cooling point of water are reduced by the presence of cryoprotectants. As a result, the ice crystal formation is retarded during the process of cryopreservation.

There are several cryoprotectants which include dimethyl sulfoxide (DMSO), glycerol, ethylene, propylene, sucrose, mannose, glucose, proline and acetamide. Among these, DMSO, sucrose and glycerol are most widely used. Generally, a mixture of cryoprotectants instead of a single one is used for more effective cryopreservation without damage to cells/tissues.

Freezing:

The sensitivity of the cells to low temperature is variable and largely depends on the plant species.

Four different types of freezing methods are used:

1. Slow-freezing method:

The tissue or the requisite plant material is slowly frozen at a slow cooling rates of 0.5-5°C/min from 0°C to -100°C, and then transferred to liquid nitrogen. The advantage of slow-freezing method is that some amount of water flows from the cells to the outside. This promotes extracellular ice formation rather than intracellular freezing. As a result of this, the plant cells are partially dehydrated and survive better. The slow-freezing procedure is successfully used for the cryopreservation of suspension cultures.

2. Rapid freezing method:

This technique is quite simple and involves plunging of the vial containing plant material into liquid nitrogen. During rapid freezing, a decrease in temperature -300° to -1000°C/min occurs. The freezing process is carried out so quickly that small ice crystals are formed within the cells. Further, the growth of intracellular ice crystals is also minimal. Rapid freezing technique is used for the cryopreservation of shoot tips and somatic embryos.

3. Stepwise freezing method:

This is a combination of slow and rapid freezing procedures (with the advantages of both), and is carried out in a stepwise manner. The plant material is first cooled to an intermediate temperature and maintained there for about 30 minutes and then rapidly cooled by plunging it into liquid nitrogen. Stepwise freezing method has been successfully used for cryopreservation of suspension cultures, shoot apices and buds.

4. Dry freezing method:

Some workers have reported that the non-germinated dry seeds can survive freezing at very low temperature in contrast to water-imbibing seeds which are susceptible to cryogenic injuries. In a similar fashion, dehydrated cells are found to have a better survival rate after cryopreservation.

Storage:

Maintenance of the frozen cultures at the specific temperature is as important as freezing. In general, the frozen cells/tissues are kept for storage at temperatures in the range of -70 to -196°C. However, with temperatures above -130°C, ice crystal growth may occur inside the cells which reduces viability of cells. Storage is ideally done in liquid nitrogen refrigerator at —150°C in the vapour phase, or at -196°C in the liquid phase.

The ultimate objective of storage is to stop all the cellular metabolic activities and maintain their viability. For long term storage, temperature at -196°C in liquid nitrogen is ideal. A regular and constant supply of liquid nitrogen to the liquid nitrogen refrigerator is essential. It is necessary to check the viability of the germplasm periodically in some samples. Proper documentation of the germplasm storage has to be done.

The documented information must be comprehensive with the following particulars:

i. Taxonomic classification of the material
ii. History of culture
iii. Morphogenic potential
iv. Genetic manipulations done
v. Somaclonal variations
vi. Culture medium
vii. Growth kinetics

Thawing:

Thawing is usually carried out by plunging the frozen samples in ampoules into a warm water (temperature 37-45°C) bath with vigorous swirling. By this approach, rapid thawing (at the rate of 500- 750°C min^{-1}) occurs, and this protects the cells from the damaging effects ice crystal formation.

As the thawing occurs (ice completely melts) the ampoules are quickly transferred to a water bath at temperature 20-25°C. This transfer is necessary since the cells get damaged if left for long in warm (37-45°C) water bath. For the cryopreserved material (cells/tissues) where the water content has been reduced to an optimal level before freezing, the process of thawing becomes less critical.

Re-culture:

In general, thawed germplasm is washed several times to remove cryoprotectants. This material is then re-cultured in a fresh medium following standard procedures. Some workers prefer to directly culture the thawed material without washing. This is because certain vital substances, released from the cells during freezing, are believed to promote in vitro cultures.

Measurement of survival/viability:

The viability/survival of the frozen cells can be measured at any stage of cryopreservation or after thawing or re-culture.

The techniques employed to determine viability of cryopreserved cells are the same as used for cell cultures. Staining techniques using triphenyl tetrazolium chloride (TTC), Evan's blue and fluorescein diacetate (FDA) are commonly used. The best indicator to measure the viability of cryopreserved cells is their entry into cell division and regrowth in culture. This can be evaluated by the following expression.

$$\frac{No.of\ cells/organs\ growing}{No.of\ cells/organs\ thawed} \times 100$$

Plant regeneration:

The ultimate purpose of cryopreservation of germplasm is to regenerate the desired plant. For appropriate plant growth and regeneration, the cryopreserved cells/tissues have to be carefully nursed, and grown. Addition of certain growth promoting substances, besides maintenance of appropriate environmental conditions is often necessary for successful plant regeneration.

A selected list of plants (in various forms) that have been successfully used for cryopreservation is given in Table 48.1.

Table 48.1 A selected list of plants in various forms that are successfully cryopreserved

Plant material	Plant species
Cell suspensions	*Oryza sativa*
	Glycine max
	Zea mays
	Nicotiana tabacum
	Capsicum annum
Callus	*Oryza sativa*
	Capsicum annum
	Saccharum sp
Protoplast	*Zea mays*
	Nicotiana tabacum
Meristems	*Solanum tuberosum*
	Cicer arietinum
Zygotic embryos	*Zea mays*
	Hordeum vulgare
	Manihot esculenta
Somatic embryos	*Citrus sinensis*
	Daucus carota
	Coffea arabica
Pollen embryos	*Nicotiana tabacum*
	Citrus sp
	Atropa belladona

Cold Storage:

Cold storage basically involves germplasm conservation at a low and non-freezing temperatures (1-9°C) The growth of the plant material is slowed down in cold storage in contrast to complete stoppage in cryopreservation. Hence, cold storage is regarded as a slow growth germplasm conservation method. The major advantage of this approach is that the plant material (cells/tissues) is not subjected to cryogenic injuries.

Long-term cold storage is simple, cost-effective and yields germplasm with good survival rate. Many in vitro developed shoots/plants of fruit tree species have been successfully stored by this approach e.g. grape plants, strawberry plants.

Virus- free strawberry plants could be preserved at 10°C for about 6 years, with the addition of a few drops of medium periodically (once in 2-3 months). Several grape plants have been stored for over 15 years by cold storage (at around 9°C) by transferring them yearly to a fresh medium.

Low-Pressure and Low-Oxygen Storage:

As alternatives to cryopreservation and cold storage, low-pressure storage (LPS) and low-oxygen storage (LOS) have been developed for germplasm conservation.

Low-Pressure Storage (LPS):

In low-pressure storage, the atmospheric pressure surrounding the plant material is reduced. This results in a partial decrease of the pressure exerted by the gases around the germplasm. The lowered partial pressure reduces the in vitro growth of plants (of organized or unorganized tissues). Low-pressure storage systems are useful for short-term and long-term storage of plant materials.

The short-term storage is particularly useful to increase the shelf life of many plant materials e.g. fruits, vegetables, cut flowers, plant cuttings. The germplasm grown in cultures can be stored for long term under low pressure. Besides germplasm preservation, LPS reduces the activity of pathogenic organisms and inhibits spore germination in the plant culture systems.

Low-Oxygen Storage (LOS):

In the low-oxygen storage, the oxygen concentration is reduced, but the atmospheric pressure (260 mm Hg) is maintained by the addition of inert gases (particularly nitrogen). The partial pressure of oxygen below 50 mm Hg reduces plant tissue growth (organized or unorganized tissue). This is due to the fact that with reduced availability of O_2, the production of CO_2 is low. As a consequence, the photosynthetic activity is reduced, thereby inhibiting the plant tissue growth and dimension.

Limitations of LOS:

The long-term conservation of plant materials by low-oxygen storage is likely to inhibit the plant growth after certain dimensions.

Applications of Germplasm Storage:

The germplasm storage has become a boon to plant breeders and biotechnologists.

Some of the applications are briefly described:

1. Maintenance of stock cultures: Plant materials (cell/tissue cultures) of several species can be cryopreserved and maintained for several years, and used as and when needed. This is in contrast to an in vitro cell line maintenance which has to be sub-cultured and transferred periodically to extend viability. Thus, germplasm storage is an ideal method to avoid sub-culturing, and maintain cells/ tissues in a viable state for many years.

2. Cryopreservation is an ideal method for long term conservation of cell cultures which produce secondary metabolites (e.g. medicines).

3. Disease (pathogen)-free plant materials can be frozen, and propagated whenever required.

4. Recalcitrant seeds can be maintained for long.

5. Conservation of somaclonal and gametoclonal variations in cultures.

6. Plant materials from endangered species can be conserved.

7. Conservation of pollen for enhancing longevity.

8. Rare germplasms developed through somatic hybridization and other genetic manipulations can be stored.

9. Cryopreservation is a good method for the selection of cold resistant mutant cell lines which could develop into frost resistant plants.

10. Establishment of germplasm banks for exchange of information at the international level.

Limitations of Germplasm Storage:

The major limitations of germplasm storage are the expensive equipment and the trained personnel. It may, however, be possible in the near future to develop low cost technology for cryopreservation of plant materials.

POLLEN BANK

Systematic research on pollen storage started at the end of the 19th century. There is large number of crop species, including vegetables, fibre and fruit crops, forage and cereals, for which pollen storage strategies are desirable. Genetic conservation through pollen storage is desirable for a variety of horticultural plant species, since pollen is known to transmit important genetically heritable characters.

Pollen is a product of genetic recombination and can provide a reliable source of nuclear genetic diversity at the haploid stage. Although genetic conservation through pollen storage does not accomplish the whole genome conservation, a plant breeder involved in genetic enhancement of a given horticultural crop could have access to a facility called 'Pollen Cryobank', from where he can draw pollen parents of his choice in the process of breeding a new cultivar.

Keeping the viability and vigour intact the pollen grains can be suitably stored in appropriate containers like, glass or plastic vials for an extended period of time. Such containers are stored in desiccators with dehydrating agents to control humidity. Saturated solutions of different salts are used to obtain the required humidity.

Lycopodium spores are used as diluents before storage to increase the bulk of pollen and prevent wastage of pollen sample during artificial pollination. This diluent has all the property of a good diluent, like non sticky and non-hydrating and in addition it keeps the viability rate quite high. It also provides its own growth factors, which leads to higher percentage of germination.

Comprehensive studies have been done to assess the different storage conditions that can prolong the viability of pollen grains. This storage can be conveniently grouped as short term and long term storage methods.

Method of Pollen Storage:

I. Short Term Pollen Storage:

It includes the effect of temperature and humidity, and pollen storage in organic solvent.

i. Effects of Temperature and Humidity:

In general low temperature and relative humidities are favourable for most taxa. However, pollen of a large number of taxa can be successfully stored for a limited period of time through the manipulation of storage temperature and humidity.

Tricellular pollen of Gramineae requires sophisticated environmental conditions to preserve viability and fertility even for a short period storage.

ii. Pollen Storage in Organic Solvent:

Iwanami and Nakamura (1972) first demonstrated the use of different organic solvents in pollen storage. The organic solvents include benzene, petroleum, diethyl ether, acetone, chloroform, etc., whose efficiency varies greatly for different plant species.

The Citrus pollen maintained viability in different organic solvents for three months. Investigation of Liu et al. (1985) on plants like, *Armenica vulgaris, Camellia japonica, Ginkgo biloba, Juglans regia, Malus pumila, Prunus triloba, Prunus percia, Salix babylonica,* and *Zea mays* shows that the insect pollinated species stored in a suitable organic solvent at 4°C for 35-40 days exhibited the needed viability.

Chrysanthemum pacificum pollen loses viability (12%) in dry conditions (25° C) within 60 minutes. When pollen grains stored in such dry conditions for 30 minutes are then transferred to diethyl ether, the viability remained at the level of 12% even after 20 days of storage.

Removable from the organic solvent thereafter, lead to complete loss of viability within 30 minutes. Literally while storage in organic solvent there was no loss of viability which has been referred as absolute dormancy. Inspite of the fact that the efficacy of individual organic solvents varies greatly for different plant solvent has proved to be better for the storage of any pollen than low temperature and humidity.

II. Long Term Pollen Storage:

Storage at temperatures above 0°C slows down the metabolic activity of the pollen, resulting in gradual decrease and finally total loss of pollen viability. Thus for a long term preservation cryogenic technique seems to be more promising. Some of the methods of long term preservation are stated below.

i. Storage at Sub-Zero Temperatures:

Using a storage temperature of -10° C and – 34° C the longevity of bicellular pollen (desiccation tolerant) and pollen with original low content of moisture has been successfully extended between 1 and 3 years.

ii. Freeze or Vacuum Drying (lyophilization):

Pollen of different taxa especially the desiccation-tolerant pollen can be successfully preserved for a long period of time by freeze or vacuum dying method. Freeze-drying involves the rapid freezing of pollen to sub-zero temperature of -60° C or -80° C using inert gas helium or nitrogen, and then the gradual removable of water under vacuum sublimation.

In vacuum drying the pollens are directly exposed to a vacuum and simultaneous cooling. The moisture is later withdrawn by evaporative cooling. In number of taxa when freeze drying is combined with lyophilization then storage and viability of pollen has been found to be very effective.

Table 7.3 : Cryostorage of pollen from few crop species

Taxa	Storage temperature (°C)	Duration of storage
Beta vulgaris	-196	1 year
Brassica oleracea	-196	16 months
Capsicum annuum	-196	42 months
Carica papaya	-196	485 days
Glycine max	-192	21 days
Helianthus annuus	-76,-196	4 years
Lycopersicon esculentum	-196	1062 days
Prunus persica	-196	1 year
Pyrus communis	-196	7 months
Pyrus malus	-196	673 days
Solanum tuberosum	-196	24 months
Vicia faba	-196	1 month
Zea mays	-196	10 years

iii. Cryopreservation by Deep-Freezing:

Long term preservation can also be done by ultra low temperature, ranging between -70° C and -196° C. A list of few important crop species whose pollen has been successfully stored at cryogenic temperature is presented below in Table 7.3.

A reduction in the pollen water content below a threshold level before low temperature exposure seems to be important for achieving viability. Thus partially dehydrated pollen possesses less freezable water and can survive deep freezing.

However, further studies are needed to determine the critical moisture level of the pollen grains for a successful long-term cryostorage. Studies on the molecular structure of the membrane using Fourier transform infrared spectroscopy (FTIR) indicate that lipid transitions in membranes may cause major damage during freezing or warming after freeze-thaw has been completed.

The Graminaceous pollen contains nearly 35 to 60% water when shed, thus immediate freezing would cause irreversible structural damage as a result of ice formation. Thus the water content of the pollen needs to be reduced before cryostorage, which is however, problematic, as there is rapid loss of viability with decreasing water content.

Water loss without any detrimental effects in pollen viability ranges between 50 % and 80%, this however, again depends on the species and its genotype. *Secale cereale* and *Zea mays* can tolerate higher degrees of desiccation than the grains of *Triticale*. *Triticum aestivum* is however, intolerant to any degree of dehydration. Some of the agronomically important Gramineae species shown in the Table 7.4 have been stored successfully at cryogenic temperatures or in deep freezer for long periods.

Table 7.4 : Gramineae pollen in cryostorage

Species	Storage temperature (°C)	Storage time	Comments
Avena sativa	-192	1 day	TTB+
Saccharum spontaneum	-80	30 to 140 days	Seed set
Secale cereale	-192	7 days	Seed set
Triticum aestivum	-196	Few weeks	Seed set
	-196	10 years	Germination
	-192	1 day	TTB+
	-196	few weeks	Germination
Zea mays	-196	180 days	Germination
	-76	363 days	Seed set
	-196	10 years	Seed set

Significance of Storage:

Pollen storage has both its application in basic and applied sciences. Some of the applications include:

i. The spatial and temporal isolation of parental species that enforce cross pollination barriers can be overcome.

ii. In order to continue productivity supplementary pollinations like pollen sprays can be implemented.

iii. In breeding programmes there is no need to grow the pollen parent continuously.

iv. Genetic property can be conserved and can be a source for germplasm in international exchange programmes.

v. In the study of pollen allergy and pollen biology it can serve as a continuous source of pollen.

vi. Exotic nuclear genetic diversity can be easily received and exchanged through pollen, thereby eliminating the need to go through a long juvenile phase, common in most fruit trees to produce pollen for hybridization at a desired location. Thus, stored pollen can be used to improve breeding efficiency.

vii. Fruit tree pollen is generally required to be stored for controlled crossings, either to achieve a desired breeding objective, or to overcome a constraint involved in commercial fruit production.

viii. Pollen storage has come to the rescue, where stored pollen indexed as viable can be used in crossing with the desired female clone so as to accomplish the breeding objective.

PLANT QUARANTINE METHODS :

Plant quarantine can be defined as a legal restriction on the movement of agricultural commodities for the purpose of exclusion, prevention or delay in the establishment of plant pests and diseases in areas where they are not known to occur. From time to time, the introduced pests/pathogens have devastated crops and even created famine conditions in different parts of the world. The Ireland famine of 1845 was the result of an almost total failure of the potato crop due to the introduction of the late blight pathogen (*Phytophthora infestans*) from Central America. Introduction of powdery mildew (*Uncinula necator*), *Phylloxera* and the downy mildew (*Plasmopara viticola*) in quick succession about the middle of 19th century from America, virtually annihilated the grape vine industry of France. The chestnut blight (*Endothia parasitica*) was introduced into the US on the nursery stocks imported from the Orient about 1906. Within 25 years, the American chestnut was almost exterminated as a forest tree causing an estimated loss of 1000 million US dollars. In Sri Lanka, coffee was replaced by tea as a plantation crop because of the widespread epiphytotics of coffee leaf rust (*Hemileia vastatrix*) in 1868. Also, about 20,000 hectares of coconut plantation was devastated by the introduced coconut leaf minor (*Promecotheca cumingi*) during the late 1960s.

In India also, several pests and diseases got introduced from time to time, some of which, like late blight of potato, banana bunchy top, bacterial blight and streak diseases of paddy, have since become widespread. Some

others like golden nematode and wart disease of potato and downy mildew of onion are still localized in certain parts of the country.

The above examples only highlight the risks involved in inadvertent introduction of serious pests/diseases alongwith the planting material imported without adequate safeguards. Plant quarantine can provide such safeguards. Plant quarantine measures aim at providing protection to the agriculture of a country or region against the likely ravages of alien pests/pathogens should they get introduced and established. These measures are of particular importance and relevance to countries like India whose economy is largely based on agriculture. Quarantine not only helps to ward off the threats of exotic pests, but also aim to eliminate and prevent further spread of pests/pathogens (both indigenous and introduced) with restricted distribution within the country (domestic quarantine). Thus, plant quarantine, in real sense, serves as a national service by preventing the introduction of exotic pests/pathogens/weeds and their further spread. However, such endeavours could succeed only with the active support of all-the administrators, general public, farmers, scientists, communication media, customs and others.

PEST/PATHOGEN DETECTION TECHNIQUES

Success or failure of plant quarantine measures would depend, to a great extent, on the ability of plant quarantine officials to detect pests and pathogens that may be associated with the introduced planting material. For quarantine purposes, techniques should be sensitive enough to detect even trace infections. This is particularly important in case of pests/pathogens with very high multiplication rate like certain pycnidial fungi, downy mildews, bacteria and also viruses when the insect vectors are efficient.

A wide variety of pests and pathogens (insects, mites, nematodes, fungi, bacteria, viruses, viroids/MLOs, spiroplasma, etc.) and weeds are the objects for quarantine consideration. Similarly, planting material also may be introduced in a variety of forms, i.e., true seed, corms, bulbs, rhizomes, suckers, runners, budwood, scions, cuttings and rooted plants. Therefore, detection techniques would vary depending on the type of material, the host species and the type of pests/pathogens involved. Many a times, more than one technique would have to be used. Detection techniques may broadly be classified into two groups: (a) generalized tests which would reveal a wide range of pests/pathogens; and (b) specialized or specific tests which are used to detect specific pests/pathogens .

Generalized tests

A very widely used method is the inspection of dry seed with the naked eye or under the low power of microscope. This method would reveal a wide range of free moving insects, their eggs and larval stages, mites on or with the seed, weeds, soil, infected/infested plant debris, fungal fructifications like sclerotia, smut and bunt balls, nematode galls, discoloured or deformed seeds mixed with seed; oospore or bacterial crusts, acervuli, pycnidia, sclerotia and even free spores of rusts, smuts and many other fungi on the seed surface. Examination of dry seed under UV or NUV light may reveal infections of certain fungi and bacteria through emission of fluorescence of different colours. Examination of seed washings may reveal surface contamination by rusts, smuts, downy mildews and a large number of other fungi.

Most commonly used incubation methods for the detection of fungi are the common moist blotter and agar tests wherein seeds are incubated on these media for a specific length of time (generally about a week) at a suitable temperature under alternating light and dark cycles. These two media reveal a wide range of internally seed-borne fungal and some bacterial pathogens in a wide variety of crops. Seedling symptom test and the growout test are quite versatile and reveal the symptoms produced by any category of plant pathogens including fungi, bacteria and viruses. Growout test is the simplest of the tests extensively used for the detection of viruses. However, some viruses may be carried symptomlessly in the plant and, therefore, it should be used in combination with other tests like indexing on indicator test plants and serology.

Specialized tests

Insects

X-ray radiography has been used very successfully all over the world for the detection of hidden infestation of insects, particularly seed infesting chalcids and bruchids. Seed transparency test (boiling the seeds in lactophenol to make them transparent) may also be used for the detection of hidden infestation and extraction of the insects for identification. X-ray radiography is also very effective in salvaging infested seed lots.

Nematodes

For the detection of seed-borne nematodes, seeds are soaked in water for about 24 hours. This makes the nematodes active, which then come out of the seed into the water, or the seeds may be teased out with the help

of forceps and a needle and examined for detection of nematodes under a stereo microscope. In rooted plants, the accompanying soil and plant debris may similarly be soaked in water and nematodes may be extracted for identification using nematological sieves or tissue paper.

Fungi, bacteria and viruses

Serological tests are very effective for the detection and identification of viruses and bacterial pathogens and are being used in various plant quarantine stations with great success. Phage-plague technique is still more sensitive for bacterial pathogens as even strains of bacteria can be identified. Indicator test plants are also very helpful as they may reveal pathogenic races within a species of a fungus, bacterium and specific strains within a virus.

Modifications of the generalized incubation tests (agar and blotter tests) have also been used for the detection of specific plant pathogens. Deep-freezing blotter test and 2,4-D blotter test are very efficient for detection of black-leg pathogen (*Phoma lingam*) in crucifer crops. Potato-dextrose-oxgall agar is useful for the detection of *Septoria nodorum* in wheat while PCNB agar is a selective medium for detection of *Fusarium* species in cereals.

In the case of vegetative propagules, laboratory methods may suffice for the detection of insects and mites, nematodes, majority of fungi and certain bacteria. However, for the detection of systemic fungal pathogens, bacteria, viruses, viroids and MLOs, isolation growing for a season or a year or more in quarantine glass-houses/net-houses is required. Availability of glass-houses/net-houses in large number is an expensive proposition, but the quarantine safeguards afforded by them to any country are worth that expenditure.

SALVAGING OF INFESTED/INFECTED MATERIAL

Once a pest, pathogen or a weed has been detected in the introduced planting material, quarantine officials must make all efforts to disinfect/decontaminate the material and make it available for further exploitation in the country without undue delay. However, it may be kept in mind that treatments, which only reduce the inoculum, may be acceptable for general agricultural practices, but they are not acceptable in plant quarantine. For quarantine purposes, tolerances are zero and, therefore, no residual inoculum of exotic pests/pathogens must remain. Fool-proof eradicative treatments are required to be employed before release of the planting material from quarantine.

Fumigation

Fumigation of the material under atmospheric or under reduced pressure has been found acceptable as a quarantine treatment against insects and mites. Fumigants like methyl bromide, HCN, phosphine and EDCT (ethylene dichloride + carbon tetrachloride mixture) are commonly used.

Heat treatment

Hot water treatment or hot air treatment are also used in quarantine for eradication of insects, mites, nematodes, fungi, bacteria and viruses. The basic principle involved is that treatment temperature should be sufficiently high to kill the associated pest/pathogen but not the host. However, in most cases, margin of safety is very narrow and, therefore, the temperature should be very accurately controlled. Some recommended hot water treatments are:

1. *Against nematodes:* Flower bulbs, 44° C for 240 min; *Chrysanthemum*, 48° C for 25 min; potato tubers, 45° C for 5 min;
2. *Against insects and mites: Narcissus* bulbs, 44° C for 180 min; strawberry runners, 46° C for 10 min;
3. *Against viruses:* Grape vine, 45° C for 120-180 min; sugarcane setts, 50° C for 120 min.; potato tubers, 50° C for 17 min;
4. *Against fungi:* Celery seed, 50° C for 25 min; wheat seed, 52-54° C for 10 min;

Eradication of *Phoma betae* in sugarbeet seed by hot water treatment at 50° C for 30 min. Hot water seed treatment to eradicate certain bacterial pathogens like black-rot pathogen (*Xanthomonas campestris* pv. *campestris*) in crucifer seeds at 50° C for 30 min; bacterial blight of cluster bean (*X. campestris* pv. *cyamopsidis*) at 56° C for 10 min and bacterial blight of sesame (*X. campestris* pv. *sesami*) at 52° C for 10 min.

Chemical treatments

Chemicals may be applied as dust, slurry, spray or as dip. It should be ensured that dosage of chemical should be enough to eradicate the inoculum but should not kill the host and the chemical should not be hazardous to personnel handling the treated seed. Treatment should be given on arrival and only after ascertaining the health status of the material. The choice of the chemical and dosage to be used should be made depending upon the

pest/pathogen involved. Seeds treated at origin are not only difficult to examine but are hazardous to inspect also. Heavily treated seed, which makes inspection difficult, should be denied entry.

Tissue culture

Tissue culture as a safeguard in quarantine has been advocated by Kahn (1979). This method reduces the pest/pathogen introduction risk in two ways: (i) the size of the consignment is very much reduced since the introductions are represented by meristem tips, excised buds or embryos, and (ii) the aseptic plantlet system has built-in pest/pathogen detection capability. All insects, mites, nematodes and most fungi can be eliminated. Symptoms on young seedlings, and growth of the organisms on the agar medium, if any, may be visible through the transparent culture tubes, and these could be discarded. Tissue culture in combination with thermotherapy and chemotherapy is an excellent safeguard from quarantine angle. However, certain systemically infecting pathogens like rusts, downy mildews, bacteria, viruses, viroids and MLOs, may still get transported. Therefore, as an additional safeguard, the tissue culture material could be passed through post-entry quarantine isolation growing and indexed/tested for the suspected pathogens. Indeed, tissue culture technology provides an exciting prospect for large scale exchange of genetic stocks with very little pest/pathogen introduction risk.

Examination of pest/pathogen risk in plant introduction

Analysis of pest risk in plant introduction is essential to decide as to whether a particular planting material could be permitted entry or not. Such risk analysis provides sound biological basis to decide quarantine policies. The attitude towards 'entry status' of a material may be liberal or conservative depending on the risks involved in its introduction. If risks are low, quarantine would be liberal in permitting the entry. However, if risks are very high, the material may be denied entry. Whether an introduced pest could establish, spread and become serious, depends on three factors viz. (i) availability of susceptible host in abundance; (ii) ability of the introduced pest/pathogen to multiply and spread rapidly; and (iii) availability of favourable environmental conditions. Agricultural practices and the pest/pathogen management strategies in the country of introduction are also important. However, the host-pathogen-environment interactions are very complex and it is not always easy to understand them. As such, many a times, our predictions about risks involved and quarantine importance of a pest may go wrong.

Quarantine regulations

Plant quarantine regulations are promulgated by the national and the state governments to prevent the introduction and spread of harmful pests and pathogens. Plant quarantine will be justified only when the pest has no natural means of spread and when they are based on biological considerations only, i.e., pest/pathogen introduction risks and the available safeguards.

Based on risk factors, plant quarantine regulates the introductions as follows:

1. *Complete embargo/prohibition:* When the pest risk is very high, the safeguards available in the country are not adequate and, therefore, import is prohibited.
2. *Post-entry quarantine:* The risk is very high but adequate safeguards in the form of post-entry isolation growing facilities are available.
3. *Restricted:* Pest risk is not high and import permit is required stipulating conditions for entry, inspection and treatment.
4. *Unrestricted:* Import permit is not required, and material may enter without restriction.

Plant quarantine system in India

Plant quarantine activities in India are carried out under the *Destructive Insects and Pests Act* (*DIP Act*) of 1914, and the rules and regulations framed from time to time thereunder by the Govt. of India. Seed was not covered under the *DIP Act* until 1984, when the Govt. of India brought forward a comprehensive *'Plants, Fruits and Seeds* (*Regulation of Import into India*) *Order, 1984'* which came into force in June 1985.

With a view to provide the farmers the best planting materials available in the world for maximising productivity per unit area and to encourage the private seed industry in India not only to meet the internal requirements but also to develop export potential for high quality planting materials, the Government of India announced a 'New Policy on Seed Development' in September 1988. The new policy covers the import of seeds/planting materials of wheat, paddy, coarse cereals, oilseeds, pulses, vegetables, flowers, ornamentals and fruit crops; procedures for their import and the related plant quarantine procedures/requirements.

This policy also states that absolutely no compromise shall be made with the requirements of plant quarantine procedures to prevent entry into the country of exotic pests, diseases and weeds detrimental to Indian agriculture. Therefore, to meet the plant quarantine requirements in respect to the provisions of the new seed

policy, the Govt. of India brought forward, through Gazette Notification, the updated *Plants, Fruits and Seeds* (*Regulation of Import into India*) *Order*, *1989* (Anonymous, 1989).

The main features of the existing plant quarantine regulations in India are as follows:

1. No consignment of seeds/planting materials shall be imported into India without a valid 'Import Permit', which is to be issued by a competent authority, to be notified by the Central Government from time to time in the Official Gazette.
2. No consignment of seeds/planting materials shall be imported into India unless accompanied by a 'Phytosanitary Certificate', issued by the official Plant Quarantine Service of the source country.
3. All consignments of plants and seeds for sowing/propagation/planting purposes shall be imported into India through land customs station, seaport, airport at Amritsar, Bombay, Calcutta, Delhi and Madras, and such other entry points as may be specifically notified by the Central Government from time to time, where these shall be inspected and, if necessary, fumigated, disinfested/disinfected by authorised plant quarantine officials, before quarantine clearance.
4. Seeds/planting materials requiring isolation growing under detention, shall be grown in post-entry quarantine facility approved and certified by the Designated Inspection Authority (DIA) to conform to the conditions laid down by the Plant Protection Adviser to the Govt. of India.
5. Hay, straw or any other materials of plant origin shall not be used as packing material.
6. Import of soil, earth, sand, compost, and plant debris accompanying seeds/planting materials shall not be permitted. However, soil can be imported for research purposes under a special permit issued by the Plant Protection Adviser to the Govt. of India.