CYTOLOGY, GENETICS AND PLANT BREEDING
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CYTOLOGY

LESSON-I

COMPARATIVE STUDY OF PROKARYOTIC AND EUKARYOTIC CELLS

QBJECTIVES

1. Introduction
2. Cell organisation in Prokaryotes
   i. Bacterial cell organization
   ii. Cyanobacteria
   iii. The mycoplasmas or PPLO
3. Cell organization in Eukaryotes
4. Summary

1. Introduction

The cells of all living organisms are subdivided into two major classes – Eukaryotes (i.e. true nucleus) and Prokaryotes (before nucleus). Essentially all animal and plant cells are eukaryotic whereas prokaryotic cells include, bacteria, blue green algae (cyanobacteria) and the so called PPLO (pleuro pneumonia like organisms) or mocoplasmas. The main difference between these two cell types is that prokaryotic cells lack a nuclear envelope. The prokaryotic chromosome occupies a space in the cell called nucleoid and is in direct contact with the rest of the protoplasm. Eukaryotic cells have a true nucleus with an elaborate nuclear envelope through which nucleocytoplasmic exchange takes place. The presence or absence of true nucleus is the most obvious and main distinction between Eukaryotic and Prokaryotic cells. However, as there is progress in scientific knowledge many more differences came to light between these two groups (Table 1).

2. Cell organization in prokaryotes

From an evolutionary stand point prokaryotes are considered to be ancestors of eukaryotes. In prokaryotes both autotrophs and heterotrophs are present. Photosynthetic bacteria and blue green algae are autotrophs while the other bacteria are heterotrophs. Essentially the cell organisation is similar in all prokaryotes in major aspects.

Table 1

Differences between Prokaryotic and Eukaryotic cells

Character	**Prokaryotic cells**	**Eukaryotic cells**
Nuclear Envelope	absent	present
DNA	naked	combined with proteins called histones
Chromosomes	single	multiple
Nucleolus	absent	present
Division	amitosis	mitosis and/or meiosis
Ribosomes	70s (30s + 50s)	80s (60s + 40s)
Endomembrane	absent	present
Mitochondria	Absent. Respiratory chain located on the plasma membrane	present
Chloroplast	absent	present
Cell weall	non-cellulosic	Cellulosic – present only in plant cells
Exocytosis & Endocytosis	absent	present
Locomotion	Single fibril called flagellum	Cilia and flagella
Protein synthesis inhibited by	Chloramphinicol	Cyclohexamide
Transcription and translation	Occurs in the same place i.e. in protoplasm	Takes place in separate sites viz., nucleus and cytoplasm

i) Bacterial cell organisation:

E. coli cell is taken as a representative of bacterial cell structure. Electron micrographic studies revealed that each bacterium is surrounded by two definite membranes separated by periplasmic space. The outer layer is rigid, serves for mechanical protection and is designated as cell wall. It differs from the plant cell wall in that cellulose is absent. Its chemical composition is rather complex consisting of polysaccharide, lipid and protein molecules. One of the most abundant polypeptide porin forms channels that allow for diffusion of solutes. The porin channels are made up of 6-8 subunits that span the full thickness of the membrane as shown in Fig. 1.1. At the bottom, some proteins appear attached to the peptidoglycon grid (a protein carbohydrate complex) present in the periplasmic space. The outer layer is rigid, serves for mechanical protection and is associated with proteins (i.e. proteoglycons and mucoproteins) forming a gel. This peptidoglycon is the basis of histochemical classification of bacteria, being high in gram positive bacteria (such as *Bacillus subtlis*) and low in gram negative bacteria such as *E. coli* and *Simonsiella*. In some bacteria, the cell wall is surrounded by an additional structure called capsule (Fig. 1.2). The cell wall and capsule confer shape and form to the bacterium and also act as a physical barrier between the cell and its environment.

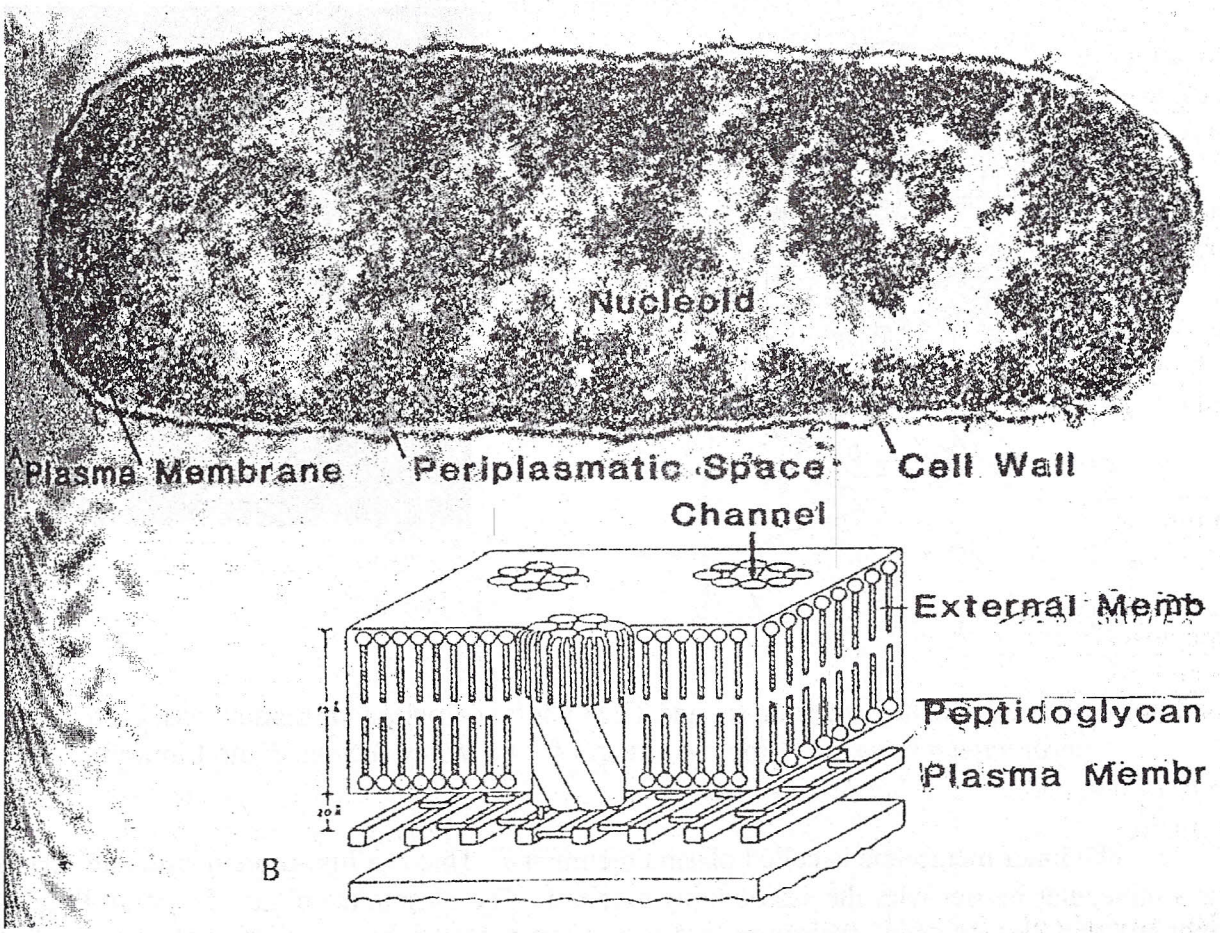

Fig. 1.1. **A**, electron micrograph of *E. coli* showing in its surface: the cellwall, the periplasmatic space, and the plasma membrane. The nucleoid appears as an irregular-shaped region of low electron density in which DNA filaments are observed. The rest of the protoplasm contains dense granules representing the ribosomes. (Courtesy of B. Menge, M. Wurtz, and E. Kellenberger). **B**, diagram of the cell wall of a gram-negative bacterium. Observe the porin channels formed by 6 to 8 subunits traversing the lipid bilayer. Each subunit has 3 suspended hydrocarbon chains. The periplasmatic space contains a reticulum of peptidoglycans. A partical view of the plasma membrane is shown at the bottom.

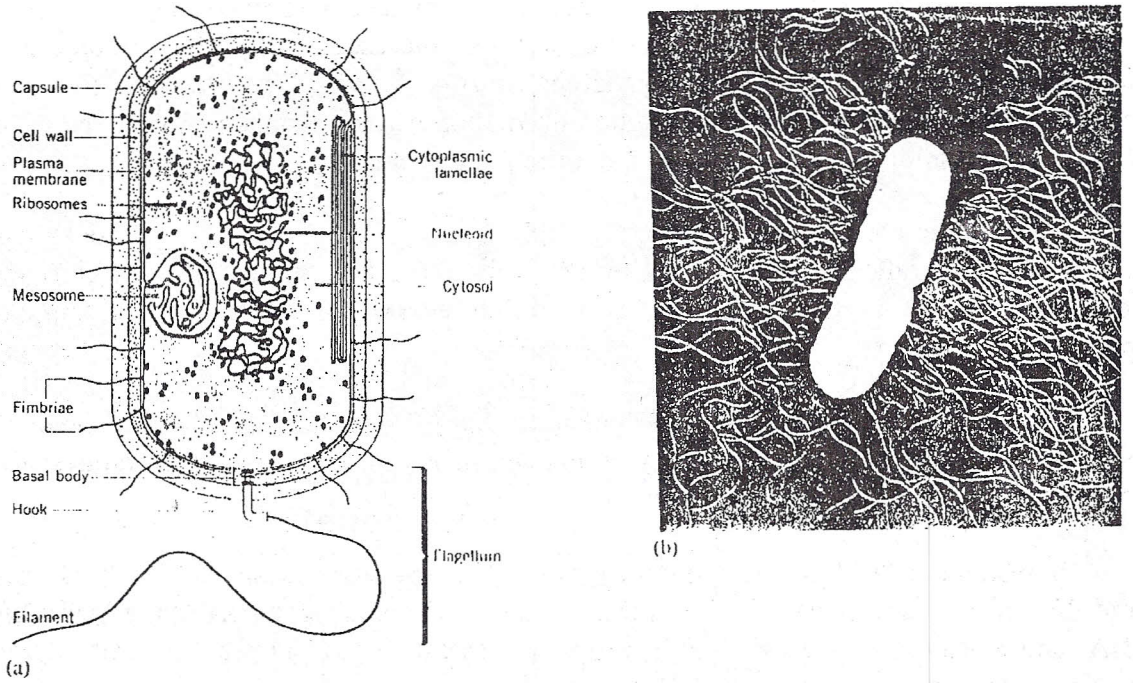

Fig.1.2 The composite bacterium (a) and TEM photomicrograph of shadow-cast bacterium *Caryophanum latum*, (b) [Photo courtesy of John Wiley & Sons Photo Library].

The inner membrane is called plasma membrane. This is a lipo-protein structure serving as a molecular barrier with the surrounding medium. The plasma membrane by controlling the entrance and exit of small molecules and ions contributes to the establishment of a carefully regulated internal milieu for the protoplasm of the bacterium. It is interesting that the enzymes involved in the oxidation of the metabolites (i.e. the respiratory chain) as well the photosystems used in photosynthesis are present in the plasma membrane of the prokaryotes. The foldings of the gram positive bacteria give rise to structures called mesosomes which are believed by many scientists to play a role in the division of the cell. In some bacteria there is lamellar arrangement of membranes within the cytoplasm. However, there are no structures comparable to the endoplasmic reticulam of animal and plant cells. Between the outer and inner membranes there are localized regions called adhesions or junctions as can be demonstrated by the following experiment.

If gram negative bacteria such as *E. coli* are exposed to hyperosmotic sucrose solution, the inner membrane pulls away from the cell wall by a process called plasmolysis and zones of attachment become apparent. These adhesion zones contain receptors (i.e. recognition sites) for bacteriophase and for the attachment of flagella and pili. These regions are probably also the sites through which the lipid and proteins are exported across the inner membrane to the periplasmitic space and the surrounding medium.

The bacterial chromosome is a single circular molecule of naked DNA tightly coiled within the nucleoid which appears in the electron microscope as a lighter region of the protoplasm. It is important to remember that the DNA of *E. coli* which is about 1 mm long (10^6 nm) when uncoiled contains all the genetic information of the organism. In this case, there is sufficient information to code for 2000-3000 different proteins.

The single chromosome is circular and at one point it is attached to the plasma membrane. It is thought that this anchoring may help in the separation of the two chromosomes after DNA replication. In fact, the growth of the intervening membrane may accomplish this separation.

In addition to a chromosome certain bacteria contain a small extra chromosomal circular DNA called a plasmid. A plasmid may confer resistance to one or more antibiotics upon the bacterial cell. These plasmids play an important role in genetic engineering.

Surrounding the DNA in the dark region of the protoplasm are 20000-30000 particles about 25 nm in diameter called ribosomes. These particles are composed of ribonucleic acid (RNA) and proteins and are the sites of protein synthesis. Ribosomes consists of a larger and smaller subunit and exist in groups called polyribosomes or polysomes. The remainder of the cell is filled with water, various RNAs, protein molecules (including enzymes) and various smaller molecules.

Certain motile bacteria have hair like processes of variable length called flagella, which are used for locomotion. In contrast with cilia and flagella of Eukaryotes which contain several microtubules each flagellum in bacteria is made up of a single fibril. There is evidence that flagella rotate in a kind of socket located in the cell membrane which consists of the basal body and the so called hook which leads into the flagellum. The mechanism by which rotations occur at the basal body is completely unknown. Some bacteria such as *E. coli*, *Proteins mirabilis* and *B. subtilis*, occur as separate individual cells. However, in a number of groups, the daughter cells remain attached to each other following division so that chains (eg. *Strepto cocci*) or filaments are formed. An example of filamentous genus is *Somansiella.*

ii) Cyanobacteria

The cyanobacteria also called blue green algae are photosynthetic prokaryotes and occur as individual cells or small clusters or colonies of cells or as long filamentous chains. Cyanobacteria lack flagella but are able to glide over a gelatinous layer, secreted through the cell surface. The photosynthetic apparatus consists of lamella called thylakoids. the thylakoids are lined with pigment granules referred to as phycobilosomes. Cyanobacteria also contain several kinds of membrane bound inclusions such as gas vacuoles which provide buoyancy in aquatic cyanobacteria and carboxysomes which contain enzymes involved in carbondioxide fixation (Fig. 1.3)

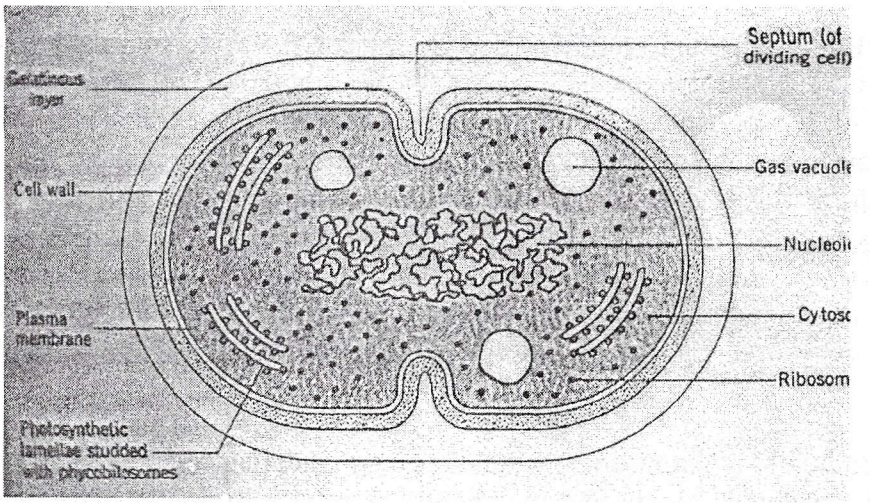

Fig. 1.3 Cyanobacteria

iii) The Mycoplasmas or PPLO (Pleuro Pneumonia like organisms)

Mycoplasmas which cause a number of diseases in humans and other animals are the smallest (i.e. about 0.1 μm in diameter) and simplest of all cells capable of autonomous growth. They are smaller than some of the larger viruses. A mycoplasma is bounded at its surface by a membrane composed of proteins and lipids but there is no cell wall. Internally, the cell's composition is more or less diffuse. Within the cell, the genetic complement which consists of a double helical strand of circular DNA and a number of ribosomes are discernable. Mycoplasmas appear to contain the bear minimum of structural organisation required for a viable, free living cell and may represent a form intermediate between viruses and bacteria (Fig. 1.4).

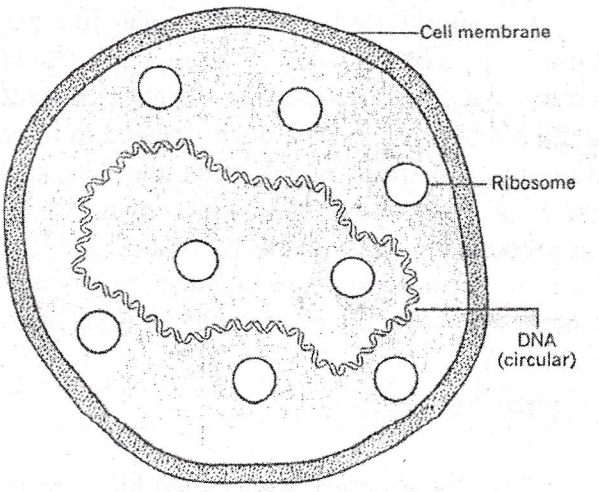

Fig. 1.4 Structure of mycoplasma

3. Cell organization in Eukaryotes

The cells of algae, fungi, protozoa, higher plants and higher animals are known as Eukaryotic cells. These cells differ from prokaryotes in having a distinct membrane bound nucleus and some membrane bound cytoplasmic structures called organelles with some specialized functions. Again plant and animal cells differ in certain characteristics. Plant cells have a cell wall and plastids which are not found in animal cells (Fig. 1.5).

Plant and animal cells

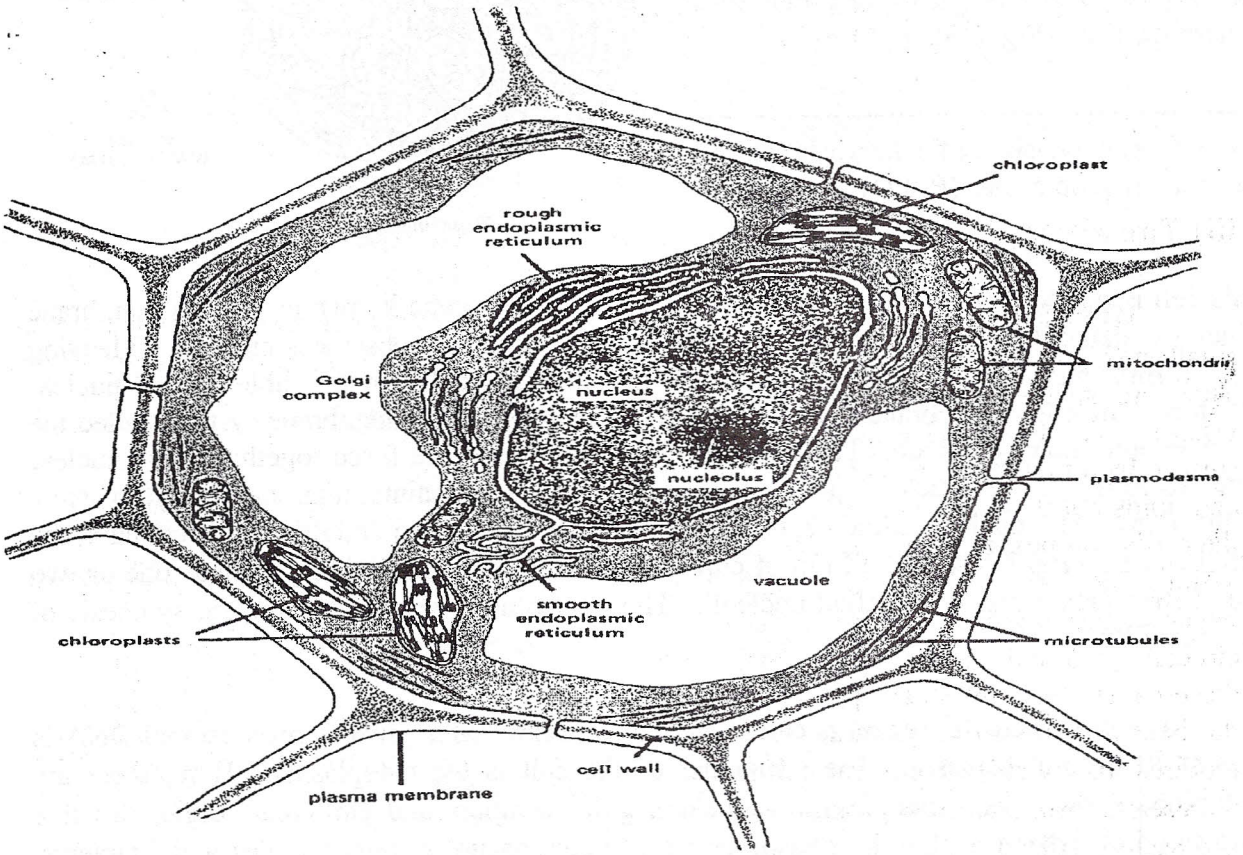

Fig. 1.5 Diagram of plant cell showing the following cell components: the cell wall, the plasmodesmata, chlorophyta and vacuoles.

The Eukaryotic cell is a complex structure with self controlled systems of generating energy, transportation of necessary materials for its metabolic activities and also with an autolytic (self destructing) process.

A cell is a unit of biological activity delimited by a selectly permeable membrane and capable of self reproduction in a medium free of other living systems. In unicellular organisms this cell is a general purpose factory performing all the services and manufacturing all the products to continue life. But in multicellular organisms, it is a speciality shop doing only a single kind of job, for **e.g.** nerve cells for communication or muscle cells for movement. There is no typical plant or animal cell that contains all the features we wish to examine. However, for purpose of discussion we can think of a generalized cell (Fig. 1.6).

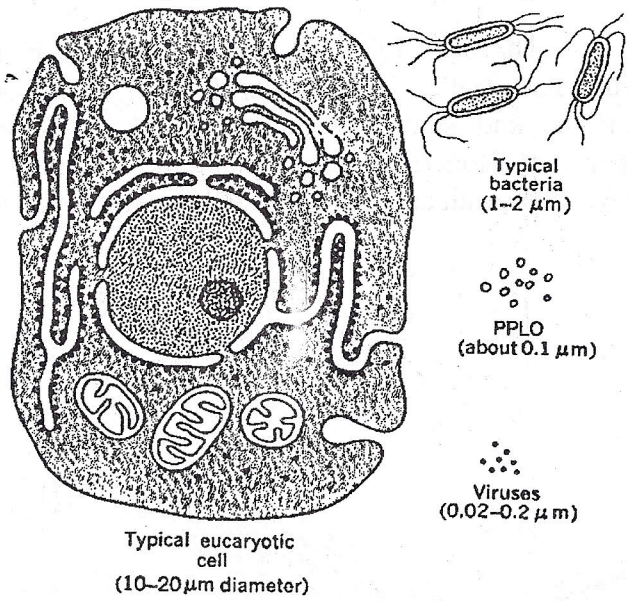

Fig. 1.6 Relative sizes of eukaryotic cells, bacteria, mycoplasmas (PPLO) and viruses

This cell is bounded at its outer living limits by a unit membrane known as plasma membrane which separates it from the environment and through which the substances entering or leaving the cell must pass. Our general cell contains a nucleus surrounded by a double layered nuclear envelope. The outer membrane is continuous with the cytoplasmic membrane system, called the rough endoplasmic reticulam. The outer and inner membrane are fused together at the nuclear pore regions. The nuclear pores are responsible for maintaining nucleo-cytoplasmic connections. The nucleus is the centre of control of all metabolic activities as it contains the hereditary material DNA in the form of chromosomes. In the non-dividing nucleus one or two round structures are present called nucleoli. The nucleolus is responsible for the synthesis of ribosomal RNA.

The non-nucleolar region is called the nucleoplasm. Most of the chromosomal DNA is present in the nucleoplasm. The remainder of the cell is the cytoplasms. Here, there are membrane systems like endoplasmic reticulam, golgi complex and particulate organelles like mitochondria, lysomes, plastids, ribosomes / centrioles, vacuoles, microtubules and filaments, secretory products like glycogen, mucous, starch etc., and soluble components that through chemical reactions control the problems of synthesis, energy transfer and conversion necessary for the tasks that cells perform.

4. Summary

The cells of all living organisms are either prokaryotic or eukaryotic. Prokaryotic cells include, bacteria, blue green algae and mycoplasmas whereas all animal and plant cells except

the above mentioned ones are eukaryotic. Prokaryotic cells lack a nuclear membrane and evolutionarily primitive. Definite nucleus is absent instead a lightly stained nucleoid is present. Chromosome single, DNA naked, cell division amitosis, ribosomes 70s. Cell organelles absent, transcription and translation occurs in the same site i.e. in protoplasm. Protein synthesis inhibited by chloramphinical, cell wall non-cellulosic.

In eukaryotic cells a definite nucleus with double membraned nuclear envelope with nuclear pores and nucleolus is present. Chromosomes are many. DNA is associated with basic proteins called histones. Cell division are mitosis and meiosis. Ribosomes 80s. Cell organelles present. Cell wall if present is cellulosic. The sites of transcription and translation are different, nucleus and cytoplasm respectively. Protein synthesis inhibited by cyclohexamide.

Questions

1. Give an account of structure and organization of prokaryotic cells.
2. Compare and contrast the cellular organization of prokaryotes and eukaryotes.
3. Write short notes on:
   (a) Bacterial Cell Wall
   (b) Peptidoglycon grid
   (c) Cyanobacteria
   (d) Mycoplasma
   (e) Cell organisation in eukaryotes

Reference Books

1. De Robertis E.D.P. & De Robertis (Jr.) E.M.F. 1995. **Cell and Molecular Biology**, Eighth Edition, B.I. Waverly Ltd.
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2)

CYTOLOGY

LESSON-II

CELL CYCLE

OBJECTIVES

1. Introduction
2. Interphase
   - i. G_1 phase
   - ii. S phase
   - iii. G_2 phase
3. Regulatory activities of Cell cycle
4. Enzymes that control cell cycle
5. Mode of action of M-phase Kinase
6. Summary

1. Introduction

Each cell has two phases in its life cycle, the dividing phase or mitosis and non-dividing phase, the interphase. The duration of the cell cycle varies greatly from one cell to another. The shortest cell cycle occurs in early embryo and can last for a short period of 8 minutes. The cell cycle of an eukaryotic cell lasts from 90 minutes to more than 24 h, its duration varying considerable in different types of cells.

The cell cycle of the eukaryotic cell is divided into two fundamental parts. Interphase and mitosis (including cytokinesis) (Fig. 2.1). Interphase is the period of non-apparent division where as mitosis is the period of division. Until recent years, interphase was mistakenly considered as resting stage as no visible changes can be seen under the microscope. Actually interphase is the most critical stage during which the cell prepares itself for division. It is a period of intense biosynthetic activity in which the cell doubles in size and duplicates precisely its chromosome complement. So this phase is also called metabolic phase and the nucleus, the metabolic nucleus.

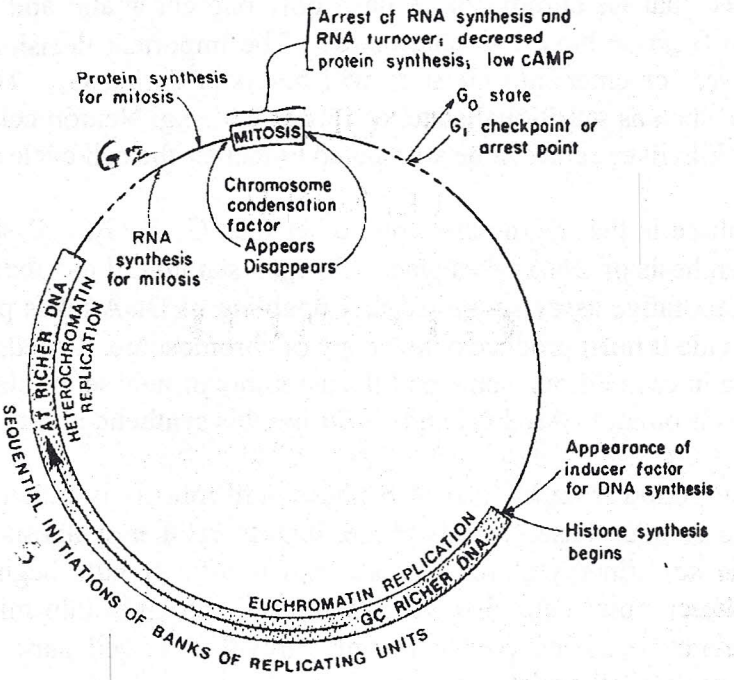

Fig. 2.1. Molecular events during the cell cycle. (From Prescott, D.M.: Reproduction of Eukaryotic Cells, New York, Academic Press, Inc., 1976).

2. Interphase

The time from the end of one mitosis to the start of the next mitosis is called interphase. This is the longest period in cell cycle. The interphase can be divided into three periods G1, S & G2 and it normally comprises 90% or more of the total cell cycle.

i) G_1 phase: The period between the end of telophase and just before the entry of S phase is called the G_1 phase. For a mammalian cell with a generation time of 16 h the different periods for example could be G_1 = 5 h, S = 7h, G_2 = 3h and mitosis = 1 h. G_1 is the most variable period. Depending on the physiological conditions of the cell, it may last days, months or years. Cells that stop proliferating become arrested at a certain point of G_1 and remain withdrawn from the cell cycle and enter into what is called G_0 state. Arrested cells can be induced to divide again. For example, liver cells normally neither grow or divide but liver damage rapidly induces them to divide.

Intensive cellular synthesis takes place during G_1 phase. Mitochondria, chloroplasts, endoplasmic reticulam lysosomes, golgi apparatus, vacuoles and vesicles are produced. There is marked synthesis of mRNA, tRNA and proteins during G_1 but there is no DNA synthesis. The enzymes and substrates necessary for DNA synthesis during S phase are also synthesized during G_1. Cellular metaboli rate will be very high in G_1 as a result cell growth occurs. Cell fusion

experiments revealed that G_1 chromosomes have only one chromatid and G_2 chromosomes after DNA replication in S phase have two chromatids. The important decision whether the cell will continue the cell cycle or enter into G_0 state will be taken during G_1. This will depend on the commitment factors such as nutritional state, cell mass etc. e.g. Neuron cells enter into non-cyclic state permanently while liver cells can be stimulated to reenter the cell cycle again.

ii) S phase:- 'S' phase is the intermediate phase between G_1 & G_2. 'S' stands for synthesis and during this phase synthesis of DNA takes place. Fuelgen staining of metabolic nucleus followed by cytophotometric quantitative assay suggested that doubling of DNA takes place during interphase. Before a cell can divide it must produce a new copy of chromosome, it needs both the replication of long DNA molecule in each chromosome and the assembly of new set of chromosomal proteins on to the DNA to form chromatid. All this happens during this synthetic phase.

iii) G_2 phase:- The period from the end of S phase until mitosis is called G_2 phase. This is the most shortest period of interphase. In this phase, intense cellular synthesis occurs. Mitochondria and chloroplasts divide. Energy stores increase and mitotic spindle begins to form during this phase. There is a check point here also whether the cell can enter into mitosis or not. Thus the control points in cell cycle are coupled with time, growth rate, cell mass and completion of the DNA replication.

3. Regulatory activities of Cell Cycle

Cell fusion experiments involving chemical agents or Sendai virus suggested that the cytoplasm of S phase cell contains an activator or regulator for DNA replication. The regulator identified is called 'S' phase activator whose nature is unknown. Further it was revealed that DNA that was once replicated will become recalcitrant to the effects of the S phase activator. It means that the S-phase activator fails to induce DNA replication in G_2 cell where DNA replication has already been completed once.

Blocking of DNA replication with inducers such as hydroxy urea prevents somatic cells from entering into G_2 & M phase. This result suggests that completion of DNA replication is a prerequisite for cell division in the cell cycle of all eukaryotes.

4. Enzymes that control cell cycle

The active component which induces cells to enter into mitosis is called MPF (Maturation Promoting Factor). Since MPF has enzymatic activity and has the ability to phosphorylate target protein, it is also called M-phase, kinase. M-phase kinase consists of two protein subunits P^{34} and P^{45}, the numbers indicating molecular weight. The two sub-units have different functions. P^{34} is a catalystic sub-unit which phosphorylates serine and threonine residues of the target protein. P^{45} is a regulatory subunit which has kinase activity with appropriate substrate. This sub-unit is also named as **Cyclin**. Cyclins can be classified into A & B types.

The cell in G_2 phase does not enter into mitosis unless and until metaphase kinase is activated. Phosphorylation and dephosphorylation of P^{34} and cyclin respectively are the major activities that induce the cell to mitosis.

5. Mode of action of M-phase Kinase

M-phase Kinase can act directly or indirectly. If the phosphorylatin of crucial substrates necessary for M-phase is directly by M-phase kinase itself it is direct mode of action. On the other hand if M-phase kinase phosphorylates target proteins which in turn regulate other necessary functions is an indirect mode of action.

The action of M-phase kinase is always reversible. This directly or indirectly triggers several activities that causes the on set of mitosis.

The activities are:

(i) Condensation of chromatin
(ii) Dissolution of nuclear lamina and break down of nuclear envelope except yeast where the NE does not break down.
(iii) Reconstruction of microtubules into a spindle.
(iv) Reconstruction of actin filaments for cytokinesis.

The genes responsible for encoding M-phase kinase have been identified in yeast. P^{34} is encoded by CDC_2 in *S. pombe* while it is CDC_{28} in *S. cerevisiae*.

6. Summary

Each cell undergoes a cycle of two phases, the dividing phase and a non-dividing phase, the interphase. The duration of cell cycle varies from species to species. The period of division is mitosis. Interphase is the most critical period during which the cell prepares itself for division. It is a period of intense biosynthetic activity, where there is the duplication of DNA content. Interphase is the longest period in cell cycle. It is divided into three substages, G_1, S & G_2. G_1 is the period between the end of telophase and just before the entry of S phase. This is the most variable period. Under unfavourable conditions cells may stop proliferating and withdraw into G_0 state. Intensive cellular synthesis takes place during G_1. G_1 chromosomes have only one chromatid. 'S' period is the intermediate phase between G_1 & G_2. DNA replication occurs in this phase. G_2 phase is the period from the end of 'S' phase until mitosis. This is the shortest period of interphase. It is a period of intense cellular activity. Spindle fibers are synthesized in this phase. Cell cycle is regulated by certain activators and unless and until DNA replicates there won't be any entry of the cell into mitosis. M-phase kinase is the promoting factor which induces cells to enter into mitosis. It has the ability to phosphorylate target protein. M-phase kinase consists of two submits P^{34} & P^{45}. P^{45} is also called cyclin which is of two types A & B. M-phase kinase has both direct and indirect action. Cell cycle is under genetic control and the genes responsible for cell cycle have been recently identified in yeast.

Questions

1. Give an account of the cell cycle of an eukaryotic cell.
2. Describe the molecular events that happen during the cell cycle.
3. Write short notes on:
   (a) Interphase
   (b) Regulatory activities of cell cycle
   (c) Enzymes that control cell cycle
   (d) Mode of action of M-phase kinase.

Reference Books

1. De Robertis, E.D.P. & De Robertis (Jr.) E.M.F. 1995. **Cell and Molecular Biology** (Eighth Edition), B.I. Waverly Ltd.
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CYTOLOGY

LESSON-III

CELL DIVISIONS IN EUKARYOTIC CELLS

OBJECTIVES

I. MITOSIS

1. Introduction
2. Interphase
3. Prophase
4. Metaphase
5. Anaphase
6. Telophase
7. Cytokinesis
8. Significance of mitosis

II. MEIOSIS

1. Significance
2. The meiotic cycle
3. Premeiotic interphase
4. Prophase-I
5. Metaphase-I
6. Anaphase-I
7. Telophase-I
8. Second Meiotic division

III. SUMMARY

I. MITOSIS

1. Introduction

The body of many multicellular organisms is composed of two main types of cells – the somatic and germ cells. The somatic cells make up the soma (the different parts of the plant body) which perform all the life functions except reproduction. Multiplication of somatic cell involves the formation of daughter cells of equal size with mathematical precision necessary to maintain the genetic uniformity. This type of cell division goes by the name somatic mitosis. This division

leads to the formation of new cells and responsible for the continuation of chromosome type and number. Meiotic mitosis is the modified form which gives rise directly or indirectly to sexual gametes. The third form of mitosis is known as endomitosis and involves progressive duplication of the chromosomes without cell division or any of the usual mitotic events as seen in the case of polytene chromosomes of *Drosophila*. Mitosis in the general sense means somatic mitosis. This was first studied in detail by Walter Flemming (1878-1882) and Beneden (1883). In this, the nuclear division (Karyokinesis) is followed by the division of the cytoplasm. The process of mitosis is divided into five stages. Interphase, prophase, metaphase, anaphase and telophase (Fig. 3.1). Each stage of cell division is identified by certain landmark features but however the cell division is a dynamic and continuous process each step passing imperceptibly into the next.

2. Interphase

This is considered as the most important crucial period during which the cell prepares itself for division. It is a time of important metabolic reactions both in the nucleus and in the cytoplasm marked by DNA replication as well as the effective duplication of chromosomes in mitotic cells. Interphase occupies the time between the end of telophase and the beginning of the next prophase and its duration varies from organism to organism. It is divided into three substages G_1, S & G_2. It is during the 'S' period DNA synthesis takes place (For details of these periods refer to the lesson on cell cycle). The nucleus of the interphase is characterised by the following features:

(i) A nuclear envelope surrounding a non-staining or slightly chromophilic mass, the nucleoplasm.

(ii) The chromosomes which are considered to be nucleo-protein structures.

(iii) The chromocentres which are fuelgen positive.

(iv) One or more basophilic bodies called nucleoli. In this stage, the chromosomes may or may not take stain which depends on the distribution of nuclear proteins. The significant events relating to the mitosis being the division of the centriole, replication of chromosomes and the synthesis of spindle proteins.

3. Prophase

Prophase is the longest stage of mitosis and lasts from one to several hours depending upon the tissue type. For example in onion it lasts for 71 minutes, in grass-hopper neuroblast for about 102 hours and in pea endosperm it takes nearly 40 h. During this stage, the chromosomes become progressively dehydrated, increasingly condensed and more stainable. Consequently, the chromosomes get shortened and become larger in diameter. At this stage, the chromosomes appear as a ball of yarn carelessly interwoven together inside the nuclear cavity. The chromosomes are longitudinally doubled and each half is the chromatid. The two chromatids are closely appressed

together and undergo a regular cycle of coiling. Such twisting of paired chromatids is known as relational coiling. As prophase progresses the chromosomes become more and more shorter, thickened and distinct. The shortening of the chromosomes is as a result of establishment of a coiled structure within each chromatid. Two levels of coils are established within each chromatid larger somatic coils and smaller minor coils. The number of gyres in the chromatid decrease progressively with the advance in the stage and their diameter increases. consequently the chromosome undergoes a process of despiralisation. The period of initial coiling is called the period of spiralization. If the two chromatids are tightly twisted and not easy to separate them it is called plectonemic coiling. This differs from paranemic coiling where the chromatids can be separated laterally and this is the feature in meiosis.

Associated with these changes in the chromosomes, the nucleoli become diminished in size and gradually disappear with the on set of metaphase. The mitosis in which the achromatic figure and spindle are formed by the centrioles and asters is called astral or amphiastral mitosis. This is common in animal cells and lower plants. Mitosis in which these centres are absent is called the anastral and is characteristic of good number of plants.

The final step of prophase is the breakdown of the nuclear membrane. The membrane disappears into cytoplasm and endoplasmic reticulam.

4. Metaphase

This is the stage of alignment of the chromosomes on the equatorial plate which is the mid portion of the spindle apparatus. In this stage, the chromosomes reach their maximum condensation and acquire a smooth out line. The spindle serves to bring the chromosomes onto the equatorial plate. This is the first step in the separation of the daughter chromatids. This movement of chromosomes has been distinguished into three components, the congression, orientation and distribution. The interaction of spindle and the centromere region of the chromosome brings the chromosomes to a position of equilibrium midway between the poles. The chromosomes are oriented on the plate in such a way that the centromeres are oriented on the longitudinal axis of the spindle while chromatid arms project at either side. Thus the centromere is the only portion that is in association with the spindle. In most plant mitosis, the chromosomes lie dispersed in an haphazard arrangement while in animals they are arranged in a straight line. The spindle fibers at metaphase are of two types, chromosomal spindle fibers extending from centromere to the pole and the continuous spindle fibers extending from pole to pole.

5. Anaphase

Metaphase passes into the anaphase at the time when centromere becomes functionally double and the chromatid begins to move towards the respective poles. It is the shortest of all stages in the mitotic cycle. The daughter centromeres separate apart and move towards the poles. The spindle that is present between the two groups of chromosomes is called the interzonal fibers.

There are mechanisms that are involved in the movement of the chromosomes. There may be continuous coiling of the chromosomes so they are more and more condensed.

6. Telophase

The chromosomes are at the poles in telophase and become less distinguishable as individual strands owing to their uncoiling, which increases their length and their entanglement within the restricted polar region. Several events of importance occur during telophase and it may last as long as prophase. As the chromosomes uncoil, the nucleolus begins to reappear. The nuclear envelope figured around each group of daughter chromosomes, this new envelope originates from the elements of the endoplasmic reticulam. Although the chromosomes are uncoiled, the remaining relic coil persists through the succeeding interphase.

7. Cytokinesis

The end of telophase coincides with the division or separation of the cytoplasms of the daughter cells. In plant cells, a cell plate is formed in this region of the equatorial plate to form two daughter cells. In animal cells, the daughter cells are separated by a process of furrowing.

8. Significance of mitosis

This is a process by which the cell mass is increased. All the cells produced are identical in genetic constitution. The mathematic precision with which the division occurs ensures, the daughter cells formed are qualitatively and quantitatively similar to the cell from which they are formed. Hence, this type of cell division is a **conservative** process both in morphogenetic and evolutionary sense.

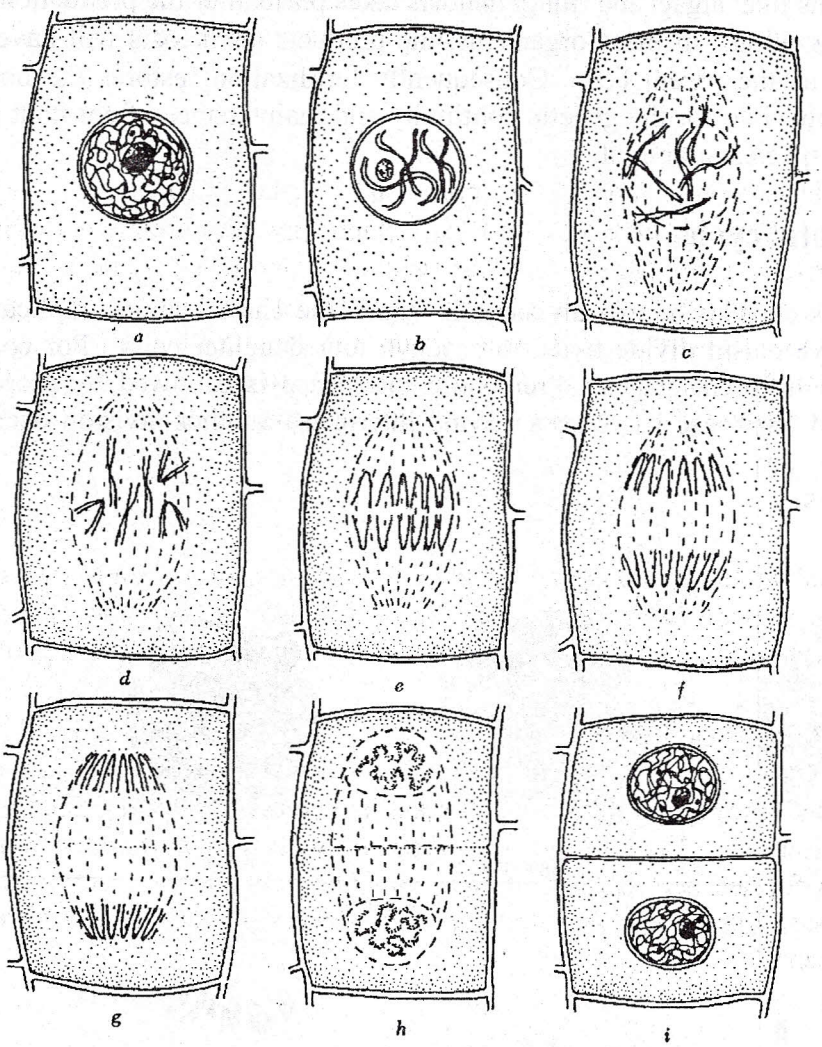


Fig.3.1. The progress of cell division, outlined in schematic form. As the cell prepares to divide, the chromosomes appear as distinct bodies in the nucleus, with a split along their length. The spindle appears at metaphase and separates the two chromatids of each chromosome at anaphase, after which the cell plate cuts the cell into two new cells. Karyokinesis, or mitosis, refers to the nuclear events of cell division; cytokinesis refers to the division of the cytoplasm by the cell plate.

II. MEIOSIS

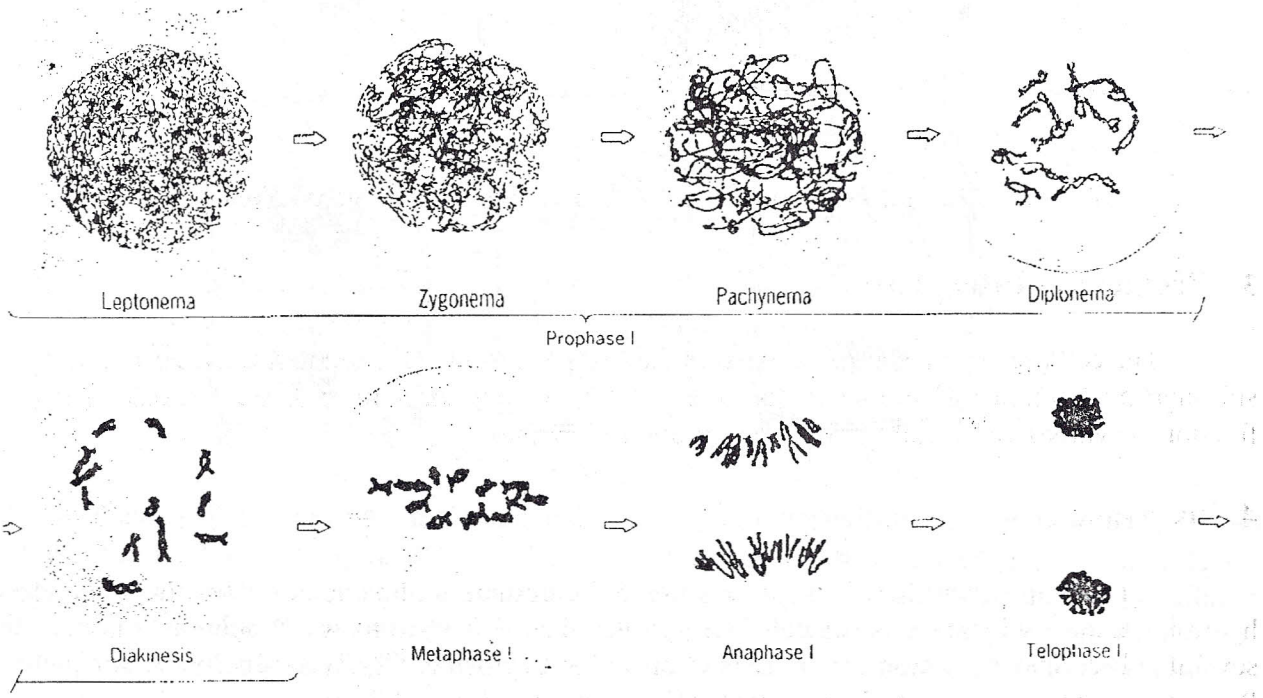
1. Significance

Meiosis is of utmost significance in plants and animals where there is sexual reproduction, this kind of cell division provides a mechanism that reduces the chromosome number to half. The meiotic cycle facilitates orderly distribution of genetic material and permits greater genetic diversity by reorganization of genetic material thus increasing the evolutionary potential in a population. In

higher organisms meiotic division produces the sexual gametes (eggs and sperm) but in certain lower organisms like algae and fungi meiosis takes place after the production of gametes and their fusion in fertilization. In these organisms, the products of meiosis will have as many number of chromosomes as the parent cell. Consequently fertilization restores the normal number. Thus meiosis is responsible for the genetic continuity and maintenance of constant chromosome number in a particular species or animal.

2. The meiotic cycle

Meiosis consists of two divisions in which the chromosomes duplicate only once, but the nucleus and cytoplasm divide twice to produce four daughter cells. For convenience meiosis is organised into different stages. Premeiotic interphase is followed by prophase I, metaphase I, anaphase I and telophase I. After a second interphase another division occurs similar to that of mitosis. (Fig. 3.2)



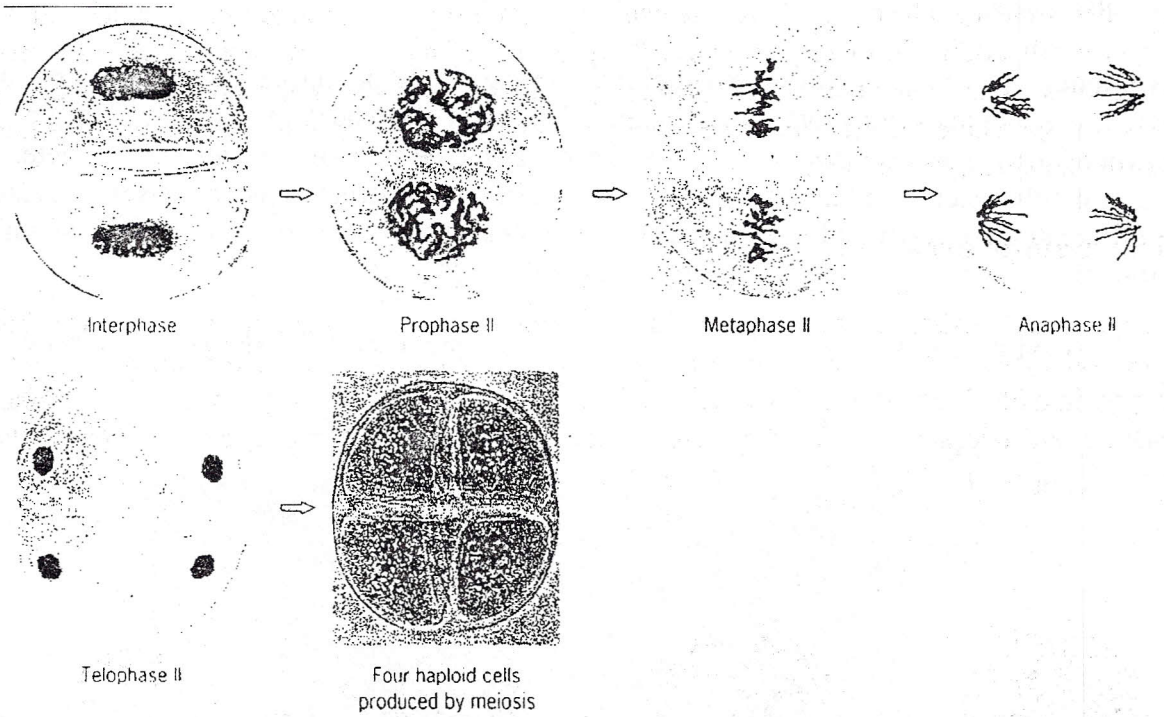


Fig. 3.2 Photographs showing the stages of meiosis in the plant *Lilium regale*.

3. Premeiotic Interphase

The cell prepares for meiotic division during this period. DNA replication occurs during 'S' substage but meiotic 'S' is longer than the mitotic 'S'. In addition to DNA, an unique histone fraction is synthesized which is absent in mitotic interphase.

4. Prophase I

In this stage, there is a decided increase in the volume of the nucleus. This is partly due to hydration and this increase is several times greater than that of mitosis. Prophase I divided into several sub-divisions. These being (I) Leptonema or Leptotene, (ii) Zygonema or Zygotene, (iii) Pachynema or Pachytene, (iv) Diplonema or Diplotene, and (v) Diakinesis.

i) **Leptotene:-** In this substage, the chromosomes are longer and more slender than mitotic prophase chromosomes. As a result of contraction and coiling chromomeres are seen which are constant in number, size and position. These are used as land marks for identification of individual chromosomes. A prominent nucleolus is present. An increased synthesis of RNA is associated with this. In majority of the cases, the chromosomes are randomly distributed but in certain cases, the chromosomes are polarised their ends being drawn together at the side of the nucleus where the

centrosome is located. Such flower like polarisation which may persist until pachynema is known as 'Bouquet' stage.

ii) Zygotene:- The most significant event of this substage is the pairing or synapsis of homologous chromosomes. It may be proterminal (pairing begins at the ends) or procentric (begins near kinetochore) or it may be random. By the end of Zygotene the pairing will be complete. The pairing is highly specific and involves the formation of a special structure which arises as a result of axial differentiation of paired homologous chromosomes. This structure can be seen only with the electron microscope and is named synoptonemal complex. (Fg. 3.3)

It is composed of two lateral dense lines twisted about each other flanking on a central line or medial ribbon.

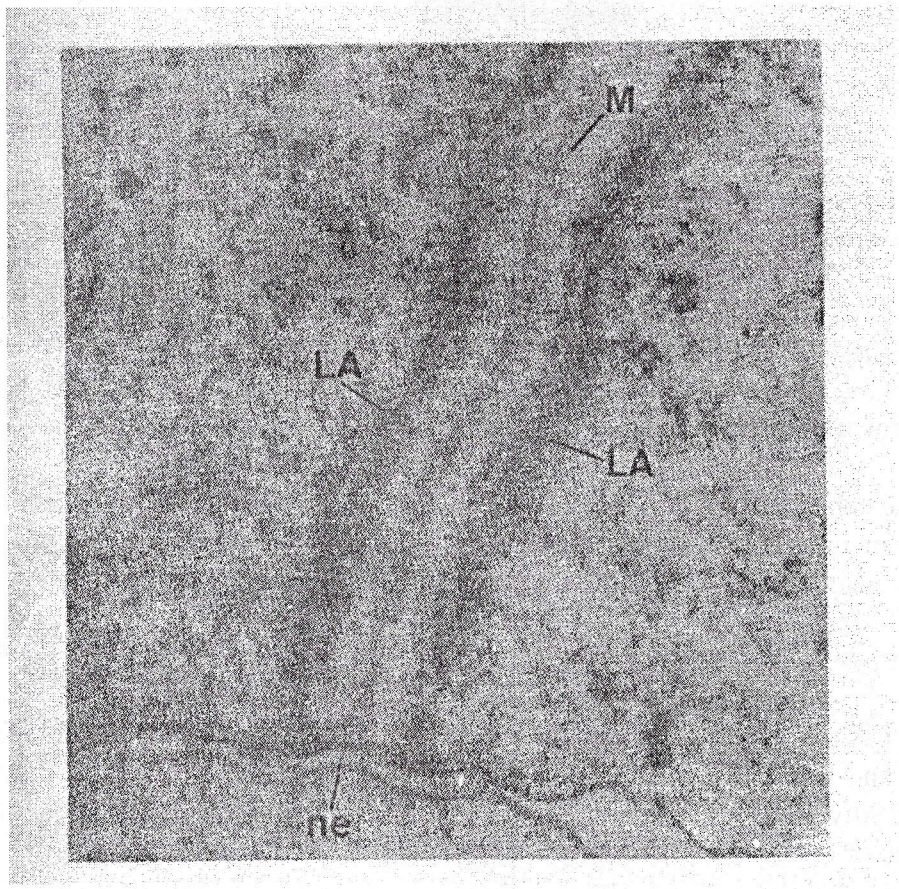


Fig. 3.3. Electron micrograph of the synaptonemal complex in a dog spermatocyte. The two lateral arms (LA), corresponding to the homologous chromosomes, are shown paralled to each other and extending into the nuclear envelope (ne). The medical element (M) is simpler than in certain invertebrates. x125,000 (Courtesy of J.R.Sotelo).

The lateral arms of the complex corresponds to each of the chromosome. The lateral arms are joined to adjacent chromosome by fibrils of 100 A^{o} in length. The medial ribbon has a trilayered

structure and there are bridges joining the lateral arms. The medial ribbon and the microfibrillar material all around the synaptinemal complex forms the bulk of the chromosome. This structure is observed only in regions of pairing. When there is no pairing, the lateral arms of the complex diverge. The synaptinemal complex is responsible for maintaining pairing long enough to allow crossingover to occur. Both DNA and protein including histones are principle components of the axial filaments and are necessary for the integrity of the complex.

iii) Pachytene:- Pairing is complete in this stage. This is the most stable stage of meiosis. Minor coils appear. Bivalents are readily identifiable. Heterochromatic and euchromatic regions can be seen. Genetic crossing over occurs during this stage. Crossing over is facilitated through chiasmata. The number of chiasmata in a bivalent depends on the length of the chromosome. 'P' DNA synthesized during this stage is used in the repair of the broken ends.

iv) Diplonema or Diplotene: Longitudinal separation of paired homologous chromosomes initiates diplonema. The paired homologues move apart from each other as a result of repulsion forces. However, this separation is not complete because the paired homologues are held together at the regions of chiasmata. The bivalents take on the appearance of a cross if there is only one point of contact, a loop if there are two points of contact or a series of loops if there are three or more points of contact. Each point of contact is a chiasma. These may be terminal (at the end) or interstitial (anywhere along the arms of chromosomes). The apparent movement of chromosomes from interstial position to terminal region of bivalent is known as terminalization and is present upto metaphase I stage. Terminalization can be expressed as a coefficient.

$$Tc = \frac{\text{Average number of chiasmata terminalized}}{\text{Average number of chiasmata present}}$$

The chromosomes continue to shorten by coiling. Nucleolus is also present at this stage.

v) Diakinesis:- The bivalent chromosomes are much more condensed and shortened by much more tight coiling. The bivalents are more round in shape.

The last event of prophase-I is disruption of nuclear membrane. By this time spindle fibers are organized and poles are established determining the orientation of the chromosomes.

5. Metaphase I

Bivalents will be moved to the centre of the cell i.e. aligned on the equatorial plate. The nuclear envelope has disappeared and the spindle has been fully formed. The meiotic metaphase I differs from the mitotic metaphase in two important aspects. They are:

(i) Single chromosomes are oriented on metaphase plate in mitosis where as bivalents are oriented in meiotic metaphase I.

(ii) In mitosis chromosome arms are oriented towards the poles and centromeres towards the equatorial plate while meiotic metaphase I the reverse is the case. Centromeres are oriented towards the poles while chromosome arms are towards the equatorial plate.

6. Anaphase I

The movement of chromosomes from the metaphase plate to the poles constitutes anaphase. The centromeres of the homologues in a bivalent remain functionally undivided as they are moved poleward with the result that whole chromosomes instead of chromatids segregate. So each anaphase group is made up of a complete haploid set of chromosomes instead of diploid set as in mitotic anaphase. In this way reduction in chromosome number has been achieved. As the chromosomes move towards the poles, the chiasmata loose their retentive influence and free the separating homologues.

7. Telophase I

The nuclear membrane is formed and nucleolus reappears. The spindle disappears and the chromosomes go into a diffuse interphase.

8. Second Meiotic division

This is just a mitotic division, the two nuclei of telophase I divide mitotically and produce four haploid nuclei. However, four differences are there between second meiotic division and the typical mitotic division.

(i) The chromosomes in each nucleus are haploid instead of a diploid number.

(ii) No period of DNA synthesis precedes this division.

(iii) The chromatids of each chromosome are widely separated instead of closely appressed as in mitosis.

(iv) The chromatids at the end of meiosis are genetically different from the initial chromatids as a result of genetic crossing over by the establishment of chiasmata in meiosis, where as in mitosis the chromatids are qualitatively and quantitatively similar to the original ones from which they are derived.

III. SUMMARY

Mitosis and meiosis are the type of cell divisions that occur in eukaryotic organisms. Mitosis takes places in somatic cells as well as in germ cells because the second meiotic division is nothing but a mitosis whereas meiosis occurs only in germ cells.

Both the cells divisions are dynamic where one stage passes imperceptabily into another. In mitosis one nuclear division is followed by one division of cytoplasm as a result, the end products are two and they are similar qualitatively and quantitatively to the one from which they are derived. Mitosis is divided into 5 stages they being interphase, prophase, metaphase, anaphase and telophase each characaterised by certain land marking features. This division is responsible for increasing the cell mass.

Meiosis is a very significant cell division where there is reduction in chromosome number. This is because one division of nucleus is followed by two divisions of cytoplasm hence the end products are 4. This division is responsible for maintaining the constant chromosome number of a species since in higher plants and animals this occurs before the formation of gametes. The gametes will have reduced number. The fusion of two gametes restores the normal number of a species.

Meiosis is also characaterised by pairing of homologous chromosomes, establishment of chiasmata and exchange of genetic material by the establishment of synaptinemal complexes. Due to genetic recombination, the end products are genetically different from the original. Hence, this division is responsible for creation of variability which is a key factor in speciation. Meiosis consists of two divisions, first and second meiotic divisions. The first division consists of prophase I, metaphase I, Anaphase I and telophase I. The prophase I is again subdivided into, leptotene, zygotene, pachytene, diplotene and diakinesis. The second meiotic division is nothing but a mitotic division which takes place after a brief interphase after the first meiotic division.

Questions:

1. Give an account of mitosis.
2. Describe in detail the meiotic division.
3. Give a comparative account of mitosis and meiosis.
4. Explain the process of meiosis and its significance.
5. Write short notes on:
   (a) Prophase of meiosis
   (b) Synaptinemal complex
   (c) Metaphase of mitosis and meiosis
   (d) Significance of mitosis and meiosis.

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1. De Robertis, E.D.P. & De Robertis (Jr.) E.M.F. 1995. **Cell and Molecular Biology**. B.I. Weaverly Ltd.
2. Swanson, C.P., Timothy Merz and Young, W.J. 1982. **Cytogenetics** (Second Edition), Prentice Hall of India Pvt. Ltd.

CYTOLOGY

LESSON-IV

CHROMOSOME STRUCTURE AND PACKING OF DNA, ORGANIZATION OF CENTROMERE, TELOMERE, NUCLEOLUS AND RIBOSOMAL RNA GENES, EUCHROMATIN AND HETEROCHROMATIN

OBJECTIVES

I. CHROMOSOME STRUCTURE

1. Introduction
2. General Morphology
   - (i) Chromosome shape
     - (a) Acrocentric
     - (b) Submetacentric
     - (c) Metacentra
     - (d) Telocentric
     - (e) Diffuse centromeric activity
     - (f) Neo-centric activity
   - (ii) Chromosome size
   - (iii) Chromosome number
   - (iv) Primary and secondary constrictions
   - (v) Satellited chromosomes
   - (vi) Nucleolar oranizers
   - (vii) Ribosomal RNA genes
3. Detailed Morphology
   - (i) Chromatids
   - (ii) Chromomeres
   - (iii) Centromere
   - (iv) Telomeres

II. PACKING OF DNA IN THE CHROMOSOME

1. Introduction
2. Folded fiber model of chromosome structure
3. Multistranded model

III. EUCHROMATIN AND HETEROCHROMATIN

1. Euchromatin
2. Heterochromatin

(i) Definition
(ii) Types
(iii) Properties
(iv) Conclusion

IV. SUMMARY

I. CHROMOSOME STRUCTURE AND PACKING OF DNA

1. Introduction

Chromosomes are of paramount importance in heredity and they play a vital and conspicuous role in cell division, variation, mutation, evolution and in their control of morphogenesis, multiplication and equilibrium.

A chromosome can be visualized as an individualized protoplasmic nuclear component having special organization and function. Waldeyer 1988 for the first time designated them as chromosomes. In eukaryotic chromosome there is ample increase in DNA content followed by an increase in the size of the chromosome. They are characterised by having a centromere and are associated with basic proteins called histones.

2. General Morphology

The chromosome under light microscope consists of two rod like structures called chromatids joined with each other by centromere. The portions of chromatids on either side of the centromere are called chromosome arms and the ends of chromosomes are called telomeres.

The shape, size and number of chromosomes are species specific and are remarkably constant under normal environmental conditions.

i). Chromosome shape: The shape of the chromosome is largely governed by the type and position of the centromere. According to the position of the centromere, the chromosomes are classified into four types (Fig. 4.1).

a) **Acrocentric:** Rod like chromosomes having a very small arm and other very long. The centromere occupies a nearly terminal position.

b) **Submetacentric:** Chromosomes have unequal arms, resembling 'L' in shape at anaphase.

c) **Metacentric:** Metacentric chromosomes have equal or almost equal arms and are thus resembling V-shape.

d) Telocentric or terminal: Centromere at the extreme end region of chromosome so there is only one arm. These are extremely unstable and formed by the transverse fracturing of the centromere (mis-division).

eg: *Oxalis dispara, Alstroemeria japonica.*

Mis-division leads to the formation of *isochromosomes* in which the two arms are of equal size and identical in genetic constitution. (Fig 4.2)

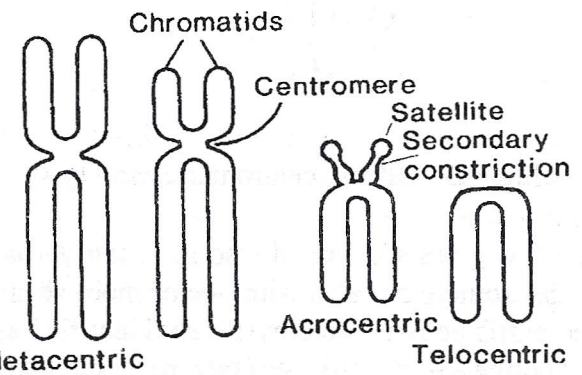


Fig. 4.1 Chromosomes are classified according to the position of the centromere

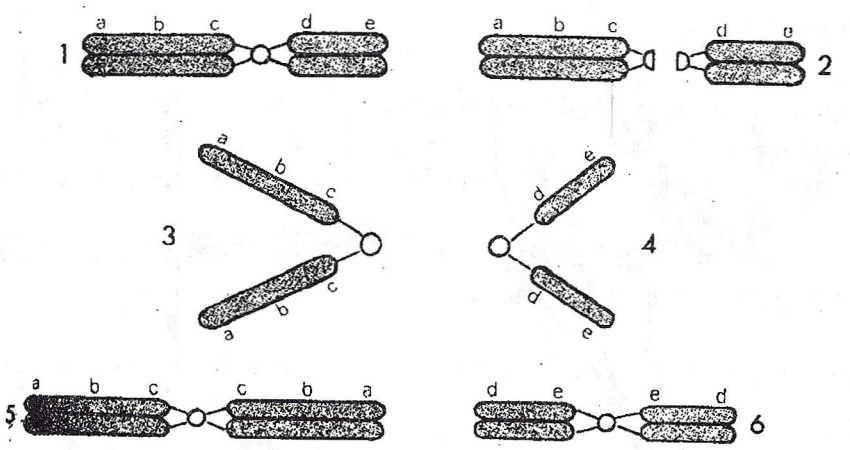


Fig. 4.2 Formation of isochromosomes, 1. Original chromosome, 2.misdivision of the kinetochore at the beginning of mitotic anaphase; 3 and 4.the chromatids unfold into two isochromosomes; 5 and 6.in the next division, two complete isochromosomes are present. Note that in each isochromosome, the arms exhibit similar genetic constitution.

e) Diffuse centromeric activity: Not all centromeres are pin-point structures sharply localized on the chromosomes. In plants like Luzula and in Hemipteran insects the centromeric activity is spread over the entire surface of the chromosome. Each chromosome has rod-shape and go to

the pole with its long axis oriented parallel to plate. The centromere here does not serve to distinguish the two arms of the chromosome (Fig. 4.3).

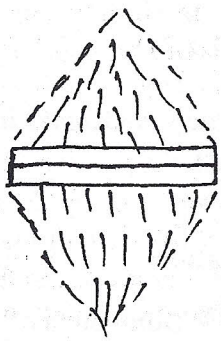


Fig. 4.3. Diffuse centromeric activity

f) **Neocentric activity:** In a few grass speciesand also in *Lilium formosanum*, the association of spindle is not only at the centromere but also with one or more sites on the same chromosome. In maize, the sites of Neo-entric activity were positively identified as the knobs. Lakshmi and Vishnuvardhan (1983) reported Neo-centric activity in a new triploid race of *Pennisetum orientale* and traced neo-entric activity to the heterochromatic knobs of the chromosomes (Fig. 4.4).

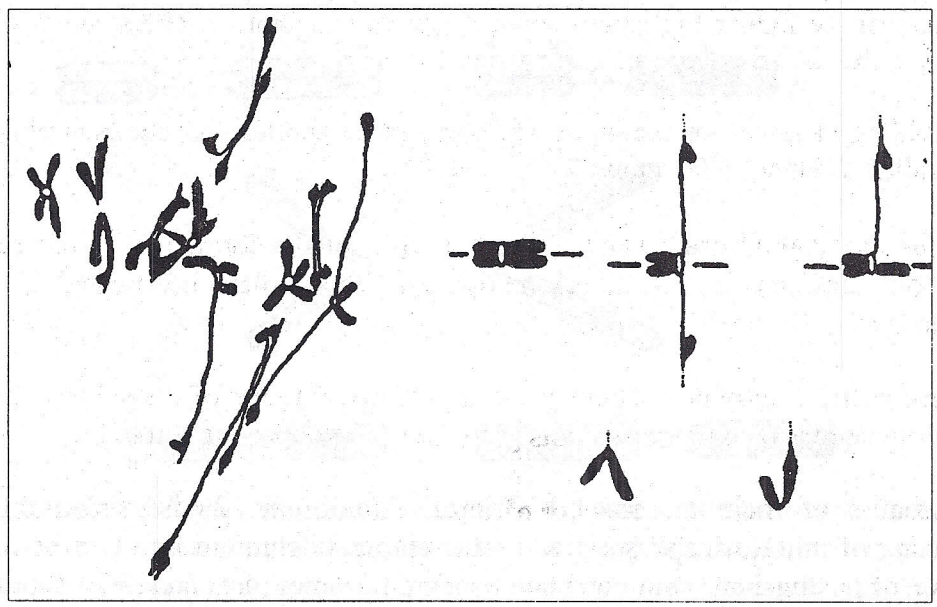


Fig. 4.4. Neocentric activity in maize as the result of the action of abnormal chromosome 10. A number of chromosomes have begun a precocious movement toward the poles, with the result that those regions of the chromosomes possessing knobs lead the way in anaphase. Right, three metaphase II chromosomes, two of which show neocentric activity while the other does not, and below, two chromosomes in anaphase II, one of which is normal in behavior (on the left) while the other shows neocentric activity in one arm (Rhoades, 1942).

ii). Chromosome size: The size of the chromosomes is normally determined by measuring mitotic metaphase chromosomes since they are thick, short rod-shaped and reach maximum condensation during that period. Under normal environmental conditions size characteristics are constant in all the tissues but exceptionally size may vary in chromosomes of different cells. For example, the polytene chromosomes in salivary gland cells of Dipteran insects are several times longer and larger than the normal somatic chromosomes of the same organism.

Within any particular species, the size range of the chromosomes of a haploid group is not great, the smallest usually being no more than $^1/_3$ or ½ of that of the largest. There are numerous exceptions to this case. Chromosome IV of *Drosophila melanogaster* is many times smaller than the others and in plants *Yucca, Agave* and *Polianthus*, the haploid complement consists of 5 large and 25 or numerous quite small chromosomes called micro-chromosomes. It has also been visualized that the length of the chromosomes may be governed by the number of genes it contains. Genetic evidence from *Drosophila melanogaster* indicates that the number of genes in each of the major chromosomes the X, II & III is roughly proportional to the length. On the other hand the Y-chromosome is almost totally made up of a kind of chromatin (hetero chromatin) that generally lacks genes so that size does not provide a reliable guide to genic content.

Generally plants chromosomes are longer than animal ones. In angiosperms monocots generally possess larger chromosomes than dicots. However, the genus *Paeonia* of the family Ranunculaceae provides striking exception with large dicot chromosomes. In animal kingdom only Orthoptera and Amphibians are characterised by their large chromosomes. Variation in size of the chromosomes can be induced by some factors such as temperature, chemicals and the amount of phosphorous in the nutrient medium.

a) Cells dividing at lower temperatures will have more shorter and compact chromosomes than cells dividing at higher temperatures.

b) Colchicine an alkaloid drug which interferes with spindle formation during cell division can bring about condensation of the chromosomes hence colchicine treated chromosomes are shorter in size.

c) Plants grown in a nutrient medium with high concentrations of phosphorus give plants with large chromosomes than those in plants growing in low concentrations or tap water.

iii). Chromosome number: The number of chromosomes may vary from species to species but it remains constant for a particular species. The number of chromosomes serves as an aid in the determination of phylogenetic status and taxonomic position of plant and animal species.

In higher organisms each somatic cell contains one set of chromosomes inherited from the maternal parent and a comparable set of chromosomes (homologous) from the paternal parent. The number of chromosomes in the somatic cell is called diploid (2n) number. Sex cells or gametes which contain half the number of chromosomes are of somatic set referred to as haploid cells and

ıe chromosome number is denoted as haploid (n) number. A single set of chromosomes inherited , called a genome. In diploid there are two genomes. The original number from which all other ımbers are derived is called base number and is denoted by 'x'. For example, in polyploid series f wheat *Tritium aestivum* 2n=42, n=21 and x=7 showing that wheat is a hexaploid.

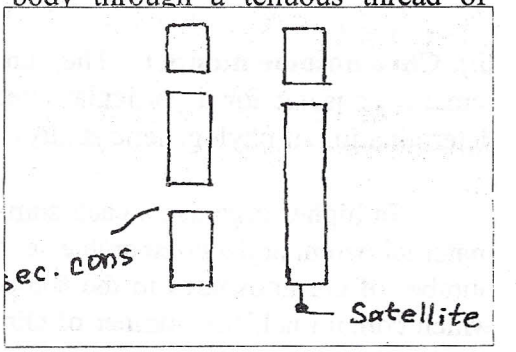
The somatic chromosome number generally remains constant among individuals of the ıme species. But in certain cases such as mammalian liver cells, the somatic cells of the same ndividual may exhibit different (2n, 4n, 8n) chromosome numbers. This is due to endomitosis i.e., luplication of chromosomes without the division of the nucleus resulting in increased chromosome ıumber within cells leading to the production of endopolyploid cells.

The chromosome number varies very widely in plant and animal species. In animal kingdom the lowest (2n=2), chromosome number of n=1 is seen in *Ascaris megalocephala* and in plant kingdom *Haplopappus gracilis* of Asteraceae has the lowest chromosome number of n=2 (2n=4). In animal kingdom the highest chromosome number of 1600 chromosomes is recorded in *Aulacantha*, a radiolarian. In plant kingdom *Ophioglossum reticulatum*, a member of Eusporangiate, primitive fern of pteridiophytes exhibited the highest chromosome number of 2n = 1260 + 10 fragments (Abraham and Ninan, 1956).

The size, shape and number of the metaphase chromosomes constitute the karyotype which is distinctive for each species. The karyotypes are either symmetrical or asymmetrical. (For details see the lesson on karyotype). Karyotype studies are helpful in knowing the interrelationships of the species and in the determination of phylogenetic status of an organism.

iv. Primary and secondary constrictions: The localized centromere produces the primary constriction. In certain chromosomes secondary constrictions do occur and these are the constrictions other than the primary and these provide the characteristic land marking features, for identification of individual chromosomes, as their size, position and number are constant. The can be distinguished from primary constrictions in absence of centromere and absence of marked angular deviations of the chromosomal segments.

v. Satellited chromsomes: Another morphological structure present in certain chromosomes is the satellite. When the secondary constriction is present in a distal portion of an arm they pinch off a portion of the arm which remains attached to the main body through a tenuous thread of euchromatin. These terminal bodies have been termed trabants or satellites and such chromosomes bearing them were often termed SAT-chromosomes. These are also constant in size and position and are hence useful in the identification of individual chromosomes.



vi. Nucleolar organizers: These are certain secondary constrictions which contain the genes coding for 18S and 28S ribosomal RNA and which induces the formation of

nucleoli. The secondary constrictions arise because the rRNA genes transcribed very actively interfering with chromosomal condensation.

vii. Ribosomal RNA genes: RNA Polymerase I (RNA Pol.I) is responsible for the continuous synthesis of rRNAs during interphase. Human cells contain five clusters of around 40 copies of the rRNA gene situated on different chromosomes (Fig.4.5). Each rRNA gene produces a 45S rRNA transcript which is about 13,000 nucleotides long. This transcript is cleaved to give one copy each of the 28S RNA (500 nt), 18S (200 nt) and 5.8S (160 nt) rRNAs. The continuous transcription of multiple gene copies of the RNA's is essential for sufficient production of the processed rRNAs which are packed in ribosomes. Inverted repeated sequences called palindromes and gene amplification are the special features of rRNA genes. Example for palindromic dimers is polyploid macronucleus of *Tetrahymena* and some slim molds. Example for gene amplification is some

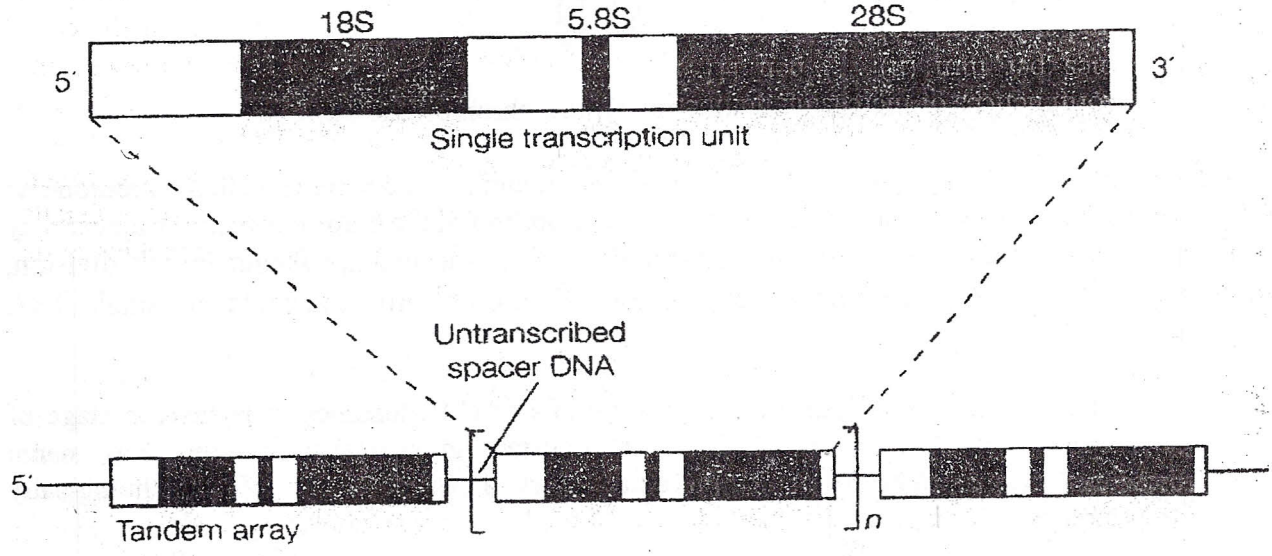


Fig. 4.5 Ribosomal RNA transcription units

3. Detailed Morphology

The chromosome in mainly made up of two chromatids or genomena, a centromere, chromomeres, telomeres (ends of chromosome), primary and secondary constrictions (Fig. 4.6).

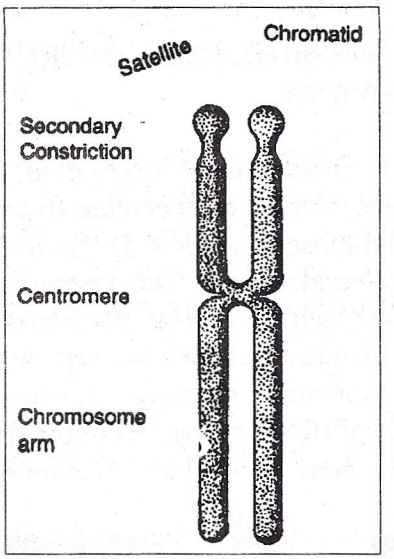


Fig. 4.6 Structure of a typical chromosome

i) Chromatids: The chromatid is made up of longitudinal subdivisions called chromonema (pleural chromonemata) that constitute the gene bearing portion of the chromosome. Whatever may be the number of subdivisions, it is the chromatid which is the functional unit in cell division, crossing over and coiling. According to recent investigations each chromatid contains a single DNA molecule.

ii) Chromomeres: The morphologically distinct regions of chromosomes in pachytene stage of meiosis are referred as chromomeres. These are constant in size and position. The name chromomere has been coined by Balbioni and covers a wide variety of morphologically recognizable structures namely:

(a) The dark staining bands of salivery gland chromosomes.
(b) The chromioles and larger chromomeres of tremendously long lamp brush chromosomes present in the amphibian oocyte.
(c) The telochromomeres seen in meiotic chromosomes of tomato, and
(d) The usual type found for example in *Lilium*.

Recent studies involving micromanipulation and treatment with uncoiling agents such as KCN have indicated that the chromomeres simply represent regions of tight coiling. The chromomeres also represent genetically significant portion of the chromosome.

iii) Centromere: This is the region of the chromosome that becomes attached to the spindle. It lies within a thinner segment of the chromosome called primary constriction.

a) Organization and fine structure of the centromere: Electron micrographic studies revealed that fibrils similar to those of chromonema each one 23 nm in diameter are found to be passing through the centromere. In addition, there is a dense kinetochore granule and this helps in the attachment of the spindle to the kinetochore.

b) Kinetochore: This component of centromere is a disc-shaped protein structure that is attached to the centromeric chromatin. The kinetochore reveals a trilaminar structure. It has a dense outer layer, a middle layer of low density and a dense inner layer tightly attached to the centromere. Between 4-40 microtubules become attached to the kinetochore and provide the force for chromosomal movement during cell divisions. The function of kinetochore is to provide centre of assembly for microtubules.

The two functions of the centromere, holding the two sister chromatids together and binding of the spindle fibers are thus assigned to the two components of the centromere.

Recent studies revealed that yeast centromere is very small which binds only a single microtubule. Centromeric DNA in yeast is organised into 3 regions DNA elements (CDE) I, II & III. Out of these three CDE II & CDE III appear to mediate interaction's with microtubule through centromeric binding factor (CBF) 2 and 3 respectively and through the kinesin related protein kar 3p (Fig. 4.7).

Generally all chromosomes are monocentric. But dicentric and polycentric chromosomes are also present. Dicentric chromosomes are formed due to structural abnormalities. Polycentric chromosomes are seen in *Ascaris megalocephala*.

iv) Telomeres:

(a) Definition: Muller designated the two ends of the chromosomes as telomeres.

(b) Properties: Telomeres have certain properties. Telomere prevents the fusion of intact end of the chromosome with other chromosome segments. Loss of telomere imparts instability to the chromosome and this instability is due to the unsaturated state of the freshly broken end. Telomeres have a polarity which prevents other segments joining with them. In meiotic prophase, the telomeres are often attracted to the centriole and seen to migrate to the nuclear membrane near the centriole. This behaviour often results in that is called a bouquet stage.

Recent experiments revealed that certain regions of centromeres and telomeres are required for replication and stable inheritance of chromosome.

(c) Structure: The telomere differs in structure and composition from the rest of the chromosome. It consists of a basic repeat unit having the general pattern 5 'T 1-4 A0-1 G1-8 3'. In some species the telomere terminates with a single stranded G Rich region of the DNA strand with the 3' end (G so called overhang). The enzyme telomerase conditions the RNA component which provides the

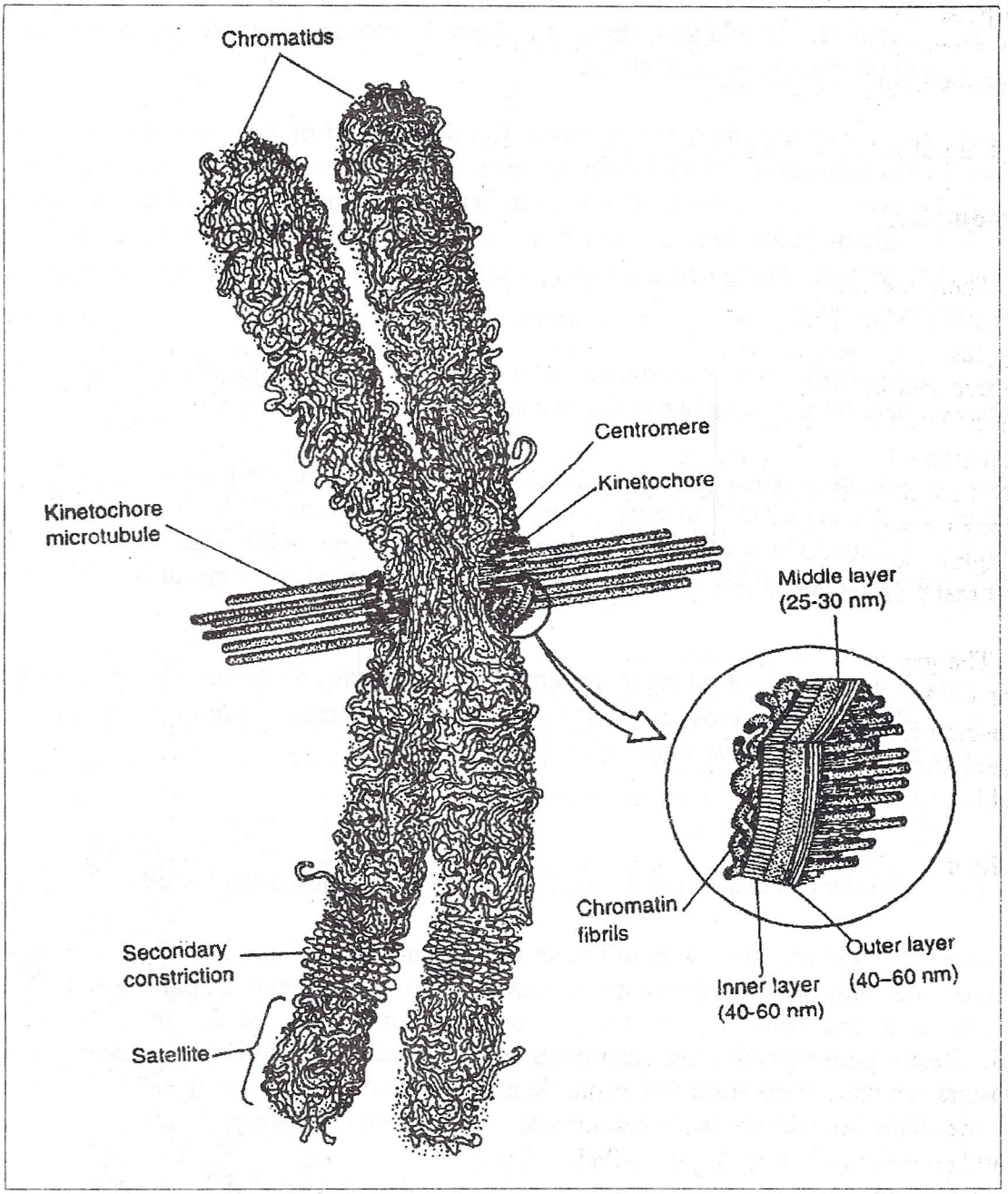


Fig. 4.7 Schematic diagram of metaphase chromosome showing the kinetochore.

template for synthesizing the G-Rich strand. This enzyme acts as a specialized reverse transcriptase. The terminal DNA is folded and forms either a duplex hair pin by G-G pairing or a G quartet when one G5 contributed by each of 4 repeating units. DNA polymerases are not able to replicate the terminal segment.

II. Packing of DNA in the chromosome

1. Introduction

There are three major structural differences between the chromosomes of Eukaryotes and Prokaryotes. Size, presence of centromere and presence of histones. In addition many of the Eukaryotes have several chromosomes whereas the well known prokaryotes have only one. Of these three factors, size of the chromosome is most striking. It is possible to estimate the total amount of DNA in a normal diploid Human nucleus and calculate the length of the double helix that this amount of DNA represents. It turns out to be almost two yards! On this basis, there is roughly 4 cm of double helix per chromatid. It is obvious that no chromosome of that length is ever seen, thus we must envisage that the chromosome is either being composed of multiple strands of DNA, tightly packed much like the cable or composed of a single double helix that has been folded on itself many times so that it is greatly compacted.

The gap between the molecular organisation of chromosomal nucleo-protein DNA and the appearance of the chromosome at the microscopic level is a major obstacle in resolving chromosome fine structure. There are atleast two possible models of chromosome fine structu One views the chromosome as multistranded body and the other interprets it as an enormously lon but highly coiled arrangement of nucleoprotein fibrils.

2. A folded fiber model of chromosome structure

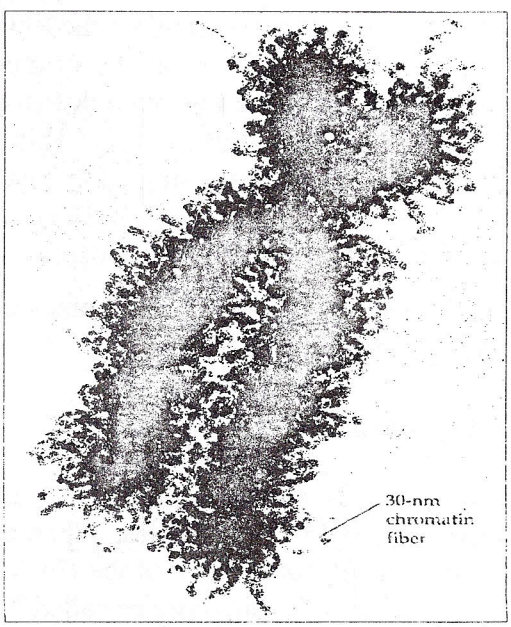


Fig. 4.8. Electron micrograph of a human metaphase chromosome showing the presence of 30-nm chromatin fibers. The available evidence indicates that each chromatid contains one large, highly coiled or folded 30-nm fiber.

Taylor, J.H. (1967) suggested that the body of the classical chromatid is represented by a single DNA protein fiber, first coiled to form the 25-30 nm fiber and then folded back longitudinally and transversely (Fig. 4.8). This model applies to both interphase chromatin and to the anaphase chromosome (unineme theory). This concept is supported by several lines of evidence.

(1) Replication of chromatid is consistent with the replication f a single DNA molecule. When cells labelled with ^3H thymidine with bromodeoxy uridine and then grown for 2 cycles in an unlabelled medium only one of the grand daughter chromatids is labelled as expected for a single DNA molecule.

(2) Exchange between sister chromatids is possible and in this case every change in a chromatid is accompanied by a reciprocal change in the other, a fact which is also consistent with the idea that there is only one DNA molecule per chromatid.

(3) Additional evidence comes with the findings that G_1 chromosomes (which have not yet replicated their DNA) have single chromatid and G_2 chromosomes have two. The uninerve concept of organization is supported by isolation of DNA molecules that are long enough to contain all the DNA from a single chromatid. The length of the giant molecules can be measured by visco-elastic measurements.

3. Multistranded Model

According to this model, several DNA molecules are present in a chromatid. The number of DNA helixes in each unit about the fibril level varies according to the species. The chromosome of a higher organism may contain 8 or more double helixes depending upon its degree of subdivision. The evidence for this model comes from the fact that DNA replication starts in several regions of the chromosome simultaneously. This model is of historical importance and at present scientists believe that there is only single DNA molecule per chromatid.

III. Euchromatin & Heterochromatin

The Eukaryotic chromosomes contain two different kinds of chromatin. Euchromatin and heterochromatin.

1. Euchromatin

Chemistry & Organisation: The cells of Eukaryotic organisms have a DNA content that is characteristic for each species. When packed in a metaphase chromosome, the DNA is compacted to 5000-10000 fold. Most of the DNA is present in chromatin which is a complex of DNA with an equal amount of basic proteins called histones. Histones are small proteins which are basic because they have a high content of arginine or lysine. There are only five histones of which four are fundamental. They are H_2A, H_2B, H_3 & H_4. These fundamental histones are present twice for

every 200 base pairs of DNA. Histones H_3 & H_4 were almost conserved during evolution. Histone H_3 from rat liver differs in only two amino acids from that of pea embryos. The fifth histone H_1 is present only once for every 200 base pairs of DNA and it varies considerably between species and even within the tissues of the same species. Chromatin is formed by a series of repeating units called nucleosomes. Under the electron microscope, the nucleosomes can be viewed as beads about 10 nm in diameter (Fig. 4.9). During spreading of the preparation for electron microscopy, chromatin frequently becomes stretched showing a beads on a striving configuration, however in the living cell, the nucleosomes touch each other forming a 10 nm fiber. Each nucleosome contains a histone octomer consisting of two of each of the 4 histones H_2A, H_2B, H_3 & H_4 with about 200 base pairs of DNA coiled on the outside of the nucleosome. The whole structure has the shape of a flattened disc 11nm in diameter and 5.7 nm in height. Histone H_1 is bound to the DNA linker region which extends between neighbouring nucleosomes and is involved in the production of higher orders of structure. The chains of nucleosomes are folded into a 20-30 nm fiber probably by forming a solenoidal structure having 6 nucleosomes per turn. This structure is stabolized by interaction between different H_1 molecules. The 20-30 nm fiber can be observed in metaphase chromosomes and in interphase nuclei and it probably represents the natural conformation of transcriptionally inactive chromatin (Figs. 4.10 & 4.11).

The packing of DNA in a chain of nucleosome is about 5-7 times more compact than free DNA and in 20-30 nm solenoid, it is packed 40 fold. The euchromatin contains genes and is responsible for the heredity of a species.

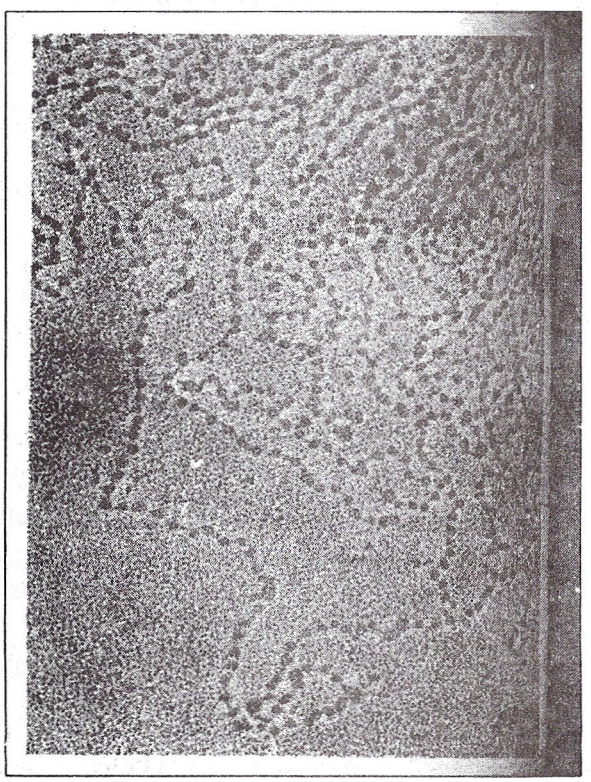


Fig. 4.9. A *Drosophila melanogaster* embryonic nucleus spread for electron microscopy. Most of the DNA, if not all, has a beaded structure. This beads-on-a-string appearance is seen when histone H1 is lost during preparation x50,000 (Courtesy of O.L. Miller, Jr.)

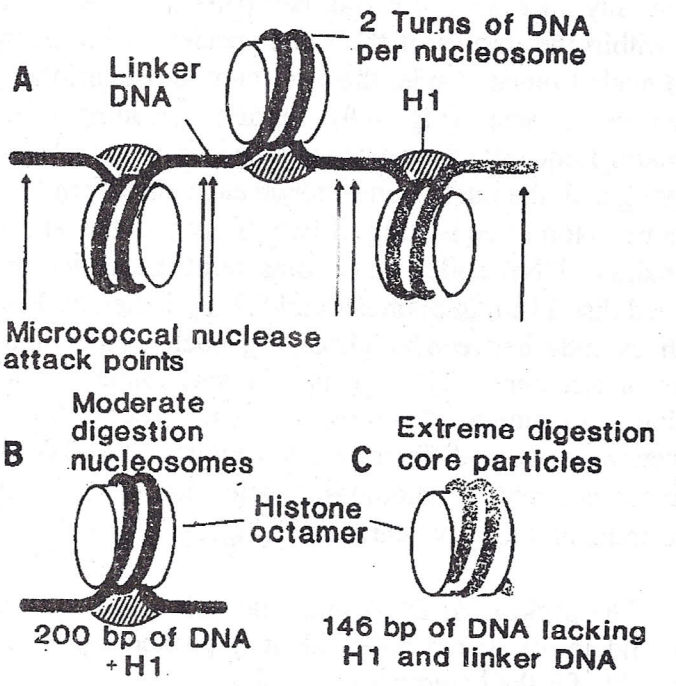


Fig. 4.10 Diagram illustrating the effect of nucleases on chromatin. A,intact chromatin, note that the nucleosome is flat and that each histone octamer has two turns of DNA sealed off by histone H1. In living cells, the nucleosomes would be touching each other forming a 10 nm fiber and not stretched out as shown here. B,nucleosomes released by moderate digestion, containing 200 base pairs of DNA C,core particles obtained by extensive digestion with only 146 base pairs of DNA.

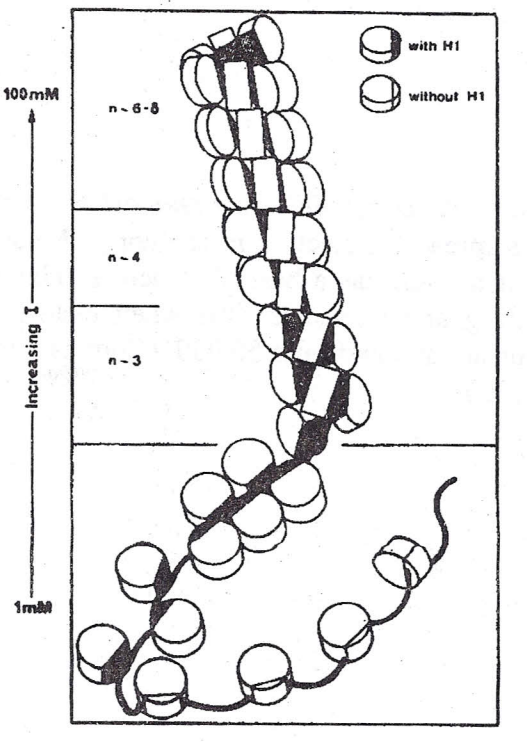


Fig. 4.11 Idealized drawing of the solenoid superstructures found by chromatin containing H1 when exposed to increasing ionic strength. The open zigzag of nucleosomes (bottom left) closes up to form helices of increasing numbers of nucleosomes per turn (n). When H1 is absent (pictured at bottom right), no zigzags or definite higher-order structures are found.

2. Heterochromatin

The term 'heterochromatin' was introduced by Heitz in 1928 to denote those chromosome regions which remained in a highly condensed state throughout the division cycle and did not unravel in telophase like the rest of the chromosome, the parts that underwent normal uncoiling in telophase were termed Euchromatin. He adopted the term from heterochromosome or sex chromosome which is heterochromatic.

i) **Definition:-** The modern definition of heterochromatin is 'it is a state showing properties different from euchromatic regions exhibiting the regular staining and condensation cycle of the chromsome'.

ii) **Types:** Heterochromatin is broadly classified into two types:

(i) Constitutive, (ii) Facultative

Constitutive heterochromatin is present in the same position on the two homologous chromosomes and is inherited. Facultative heterochromatin develops later during the course of development of the organism. Within a chromosome constitutive heterochromatin has been located at specific regions such as centromere, telomere, nucleolus organizing regions etc. Intercalary heterochromatin may be brought out in the form of bands. Entire chromosomes may be heterochromatic including sex chromosomes in some plants and super numerary or accessory chromosomes. **eg:** B-chs of rye.

Facultative heterochromatin is seen to develop during the course of development of an organism. An example where an entire chromosome becomes heterochromatic during the course of development is provided by one of the 'x' chs of human female. In a normal human male, the sex chromosomes are XY while in female XX. The X ch of male, and one of the X chs of female remains euchromatic through out. However, the other X in the female becomes heterochromatic during the course of development from zygote to the embryo. The function of heterochromatin in the hot mammalian female is to equate the number of functional X in the male (X + inert Y) with that in the female (X + hot X). Another example in which the entire genome becomes heterochromatic during development is shown by the male mealy bug. During the development of the male insect, the entire chromosome complement that it has inherited from the paternal parent becomes heterochromatic while the complement inherited from the maternal parent remains euchromatic in nature.

iii) Properties of heterochromatin: Heterochromatin has the following properties.

Genetic inertness: Heterochromain is genetically inert since it does not produce any phenotypic alteration nor it involves in crossing over or mutations. It is normally devoid of genes but however the concept of comple inertness was modified when Muller demonstrated 'bobbed eye gene' on the Y-ch of *Drosophila* which is entirely heterochromatic. Similarly Gates demonstrated the presence

of gene for hairy ear lobes in man on the supposedly inert 'Y' chromosome. In tomato, a gene has been localized on a heterochromatic segment.

Other properties of heterochromatin include position effect, temporary genetic inactivity, absence of template activity in vitro, late replication in synthetic phase, presence of high content of repetitive DNA, breakage of different agents including maleic hydrazide, abnormal mitotic coiling after cold treatment, specific staining with certain flurochrome dyes bringing out bands in the chromosomes and allocycly or heteropycnosity, i.e. maintenance of a condensed state through out the division cycle including the interphase.

iv) Conclusion: Heterochromatin represents the repressed portions of the genome. It offers direct cytological evidence of the degree of nuclear differentiation and of the regulation of gene expression in higher organisms. The amount of histones and the chromatin fibers are identical in both eu and heterochromatin but the fibers in heterochromatin are more tightly coiled which may account for the metabolic peculiarities of heterochromatin.

IV. SUMMARY

Chromosomes are of paramount importance in heredity and they play a vital role in cell division, variation and mutation. A chromosome can be defined as an individualized protoplasmic nuclear component having special organization and function. Waldeyer (1988) first reported chromosomes in the cell. The chromosome size, shape and number are constant for a species under normal environmental conditions. The chromosome structure is studied normally from somatic metaphase chromosomes as they reach maximum condensation during that period. The chromosome is generally a rod-shaped structure with two chromatids and a centromere which serves to separate the two arms. The two ends of the chromosome are called telomeres. In addition to the primary constriction where centromere is located there are other constrictions called secondary constrictions which lack marked angular deviations of the chromosome segments. If secondary constriction is demarkated at the end region and if it forms a satellite or trabant such chromosomes are called satellited chromosomes. Some of the satellited and secondarily constricted chromosomes contain genes which code for 28S and 18S ribosomal RNA are nucleolar organizing in nature. The chromosomes may be sub-metacentric, metacentric, aerocentric or telocentric depending upon the position of the centromere. The centromere in certain plants and animal species may exhibit diffuse centromeric activity. Neo-centric activity has been observed in certain grasses and *Lilium formosanum*. The chromatids of the chromosome constitute the gene bearing portion and contain a single DNA fibril inside which folds back longitudinally and transversely. The morphologically distinct regions on the chromosome are called chromomeres. The centromere is made up of two components, the 23 nm fibrils and a kinetochore granules. The two functions of the centromeres viz., holding the two chromatids and attachment of spindle fibers are performed by these two components respectively. Telomeres consist of a basic repeating unit having the general pattern 5' T1-4 A0-1 G1-8 3". Telomerase is the enzyme which conditions the RNA component which acts as a template for synthesizing G-rich DNA strand. Telomeres impart stability and polarity to the chromosome. There are two models to explain how DNA is packed inside the chromosome, the folded fibre model or unineme theory and the multistranded model or multineme

theory which envisage the presence of a single DNA molecule per chromatid and several DNA molecules per chromatid respectively. At present, all biologists believe that there is only a single DNA molecule per chromatid. Chromatin is a complex of DNA and an equal amount of basic proteins called histones. H_2A, H_2B, H_3 & H_4 are fundamental histones and H1 varies considerably among the species. Chromatin forms nucleosomes and nucleosomes form a 20-30 nm fiber solenoid and it is packed 40 fold in a chromatid. Chromatin is of two different types. The genetically active euchromatin and heterochromatin with special properies and performance. Heterochromatin is of two different types, constitutive and facultative. Heterochromatin is generally considered as genetically inactive and exhibits what is called allocycly. The peculiarities of heterochromatin can be attributed to the tight coiling of the chromatin.

V. QUESTIONS

1. Describe and discuss the detailed morphology of the eukaryotic chromosome.
2. What do you know about DNA packing? Discuss the two theories with evidences.
3. Distinguish between eu- and hetero-chromatin.
4. Compare and contrast between the following:
   (a) Telomere and centromere.
   (b) Primary and secondary constrictions.
   (c) Euchromatin and hetero-chromatin.
5. Short notes Questions:
   (a) Diffuse centromeres
   (b) Neo-centric activity
   (c) Mis-division
   (d) Iso-chromosomes
   (e) Facultative heterochromatin
   (f) Constitutine heterochromatin
   (g) Unineme theory
   (h) Multineme theory
   (i) Chemistry and organisation of chromatin.
   (j) Nucleosome
   (k) Ribosomal RNA genes
   (l) Nucleolar organizers.

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CYTOLOGY

LESSON-V

KARYOTYPE & EVOLUTION AND BANDING PATTERNS

OBJECTIVES

I. KARYOTYPE AND EVOLUTION

1. Introduction
2. Variation in basic chromosome number
3. Variation in form and relative size of the chromosomes
4. Number and size of satellites and secondary constrictions
5. Variation in absolute chromosome size
6. Distribution of Eu & heterochromatin and repetitive DNA
7. Summary

II. BANDING PATTERNS

1. Introduction
2. Types
3. Causes for chromosome banding
4. Importance of banding patterns
5. Conclusion
6. Summary

I. KARYOTYPE AND EVOLUTION

1. Introduction

The concept of karyotype has been formulated by the pioneering work of Russian school of cytologists headed by S. Navaschin. The study of karyotype is important since closely related species have similar karyotypes while distantly related ones have different karyotypes. Livitzky defined karyotype as the phenotypic appearance of the somatic chromosomes in contrast to their genic contents and the term idiogram is applied to the diagrammatic representation of the karyotype.(Fig. 5.1)

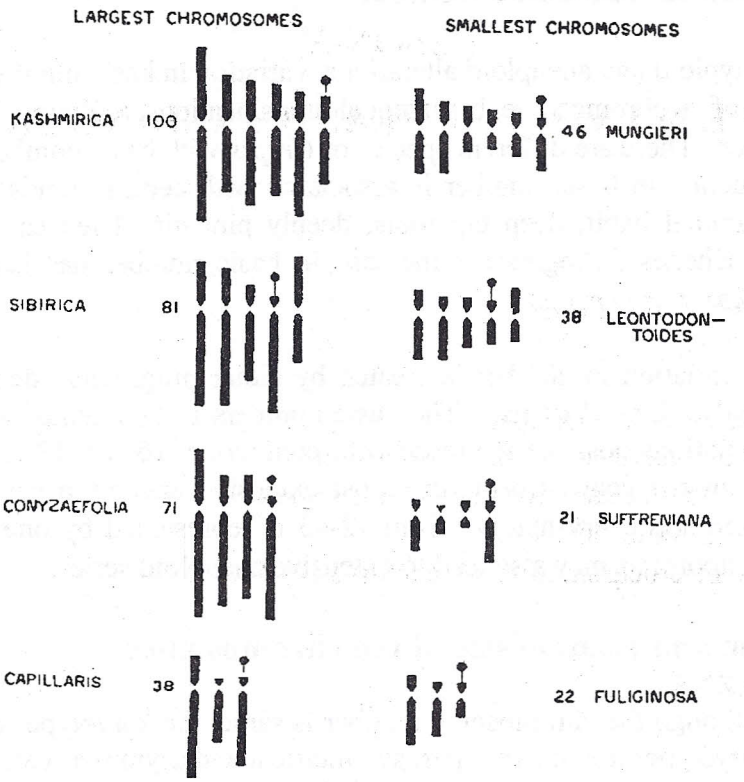

Fig. 5.1. Idiograms of the haploid chromosomes of eight species of Crepis showing reduction in number total length and symmetry. Using 100 as the arbitrary length of the chromosomes of *C. kashmirica*, the relative lengths of the other species are as indicated. *Kashmirica, sibirica, conyzaefolia, mungieri* are perennials with woody rhizomes; *leontodontoides* is a perennial or a biennial with a woody stem; *capillaries* is a biennial or an annual; *suffreniana* and *fuliginosa* are annual herbs (Babcock, Stebbins, and Jenkins, 1942).

The somatic chromosomes of different plant and animal species possess definite individuality in their number, size, shape, position of primary and secondary constrictions or satellites. Karyotypes are usually studied from metaphase stages of root tip cells in plants or from pachytene stages of meiosis.

We can distinguish different karyotypes based on

(i) Variation in basic chromosome number,
(ii) Variation in form and relative size of the chromosomes,
(iii) Variation in absolute chromosome size,
(iv) Variation in number and size of satellites, and
(v) Variation in degree and distribution of heterochromatic regions and repeated DNA segments.

2. Variations in basic chromosome number

In addition to polyploid and aneuploid alterations, variation in basic number can be achieved either by loss or gain of a chromosome by unequal translocations. *Crepis* is an example of reduction in basic number. There are different species of *Crepis* with basic numbers x=7, x=6, x=5, x=4 and x=3. The reduction in basic number is associated with certain trends of morphological specialization such as annual habit, deep tap roots, deeply pinnatified leaves, small involucres, beaked and dimorphic achenes. Progressive increase in basic number has been seen in much smaller number of species.

Another type of variation in number is created by either progressive decrease or increase followed by amphidiploidy. Eg:- *Brassica*. The basic numbers in this genus are x=8, 9 and 10. The amphidiploid combinations possible are those with numbers n=16, 17, 18, 19, 24, 25, 26, 27, 28, 29, 30 and so on. In the genus *Carex*, the most extensive aneuploid series were reported ranging from x=6 to n=56 and every number from 12-43 is represented by one or more species. Polyploidy coupled with apomixis may also lead to extensive aneuploid series.

3. Variations in form and relative size of the chromosomes

In many genera, though the chromosome number is same, the karyotypes differ. Basing on form and relative size karyotypes are of two types, symmetrical and asymmetrical.

i) Symmetrical: In this karyotype, the chromosomes are more or less graded with many median and submedian chromosomes.

ii) Asymmetrical: This karyotype possess many chromosomes with subterminal chromosomes. The chromosomes exhibit great differences in size between the largest and smallest chromosomes or both. The evolution of karyotype is from symmetric to asymmetric types. Centric fusion and centric fission play an important role in karyotype evolution. A unique, bimodal type of karyotype is seen is *Yucca* and *Agave*. The haploid karyotype consists of 5 long and 25 dot like microchromosomes. The karyotype study is helpful in solving taxonomic disputes.

4. Number and size of satellites and secondary constrictions

The presence of satellites and secondary constrictions through out the plant kingdom showed that they are a valuable if not an essential part of chromosome complement. The satellited or secondarily constricted chromosomes which contain gene which code for 18S and 28S RNA are nucleolar organizing in nature. There is no correlation between satellite number and ploidy level of the organism although at one time, it is believed to be so.

5. Variation in Absolute chromosome size

There are certain generalizations with regard to absolute chromosome size.

i) The heterosporous pteridophytian taxa such as *Selaginella* and Marsileaceae tend to have smaller chromosomes than the homosporous ones.

ii) Gymnosperms usually have largest mean and modal size of chromosomes. Among angiosperms monocots generally have larger chromosomes than dicots although, the genus *Paeonia* of Ranunculaceae is a striking exception with large dicot chromosomes. Phylogenetic reduction and phylogenetic increase in chromosome size have been found to be equally common in higher plants. **Eg.** for phylogenetic reduction is Leptosporansiate fern families.

In primitive leptosporangiate fern families like Osmundaceae and Hymenophyllaceae have relatively large chromosomes, those of Cyatheaceae and Polypodiaceae are of intermediate size while the smallest chromosomes are seen in the vegetatively reduced and reproductively specialized heterosporous fern family Salviniaceae. The best example of phylogenetic increase in absolute chromosome size is in the family Gramineae. In primitive grasses of the tribe Bambuseae and the series Phragmitiformis have relatively small chromosomes but grasses predominant in temperate regions contained in his series Festuceaeformis have the base number x=7 and much larger chromosomes. Avdulov considered phylogenetic increase in size has occurred as an adaptation to cool climate and he has suggested that it took place during the period of pleistocene glaciation.

6. Distribution of Eu & heterochromatin and repetitive DNA

Depending on the distribution of Eu and heterochromatin, different types of interphase nuclei have been recognized. These being I) diffuse staining type, ii) Cap nucleus, iii) Multiple chromocentre nucleus, and iv) Prochromosome type. Evolution from diffuse to multiple chromocentre type can proceed in either direction. Evolution from pro-chromosome type to cap nucleus as well as reverse trends also takes place. But trends diffuse to cap nucleus and multiple chromocentre to the prochromosome type are irreversible.

Crytopolyploidy refers to an increase in chromosome size as a result of series of doublings. Seventy per cent of DNA in plants is repetitive and is responsible for speciation to some extent. **Egs**: Most of the conifers differ from each other in the amount of repetitive DNA. In gymnosperms, the chromosome number remains the same but the intra-chromosomal repetitive DNA changes are responsible for speciation.

7. Summary

The phenotypic appearance of somatic chromosomes is called karyotype and the diagrammatic representation is called idiogram. They can be studied at the metaphase stage of mitosis. The somatic chromosomes have several identifying features such as chromosome number,

size, shape, presence of satellites and secondary constrictions etc. The karyotypes can be distinguished from one another on the basis of: i) Chromosome number, (ii) Shape and size, (iii) Absolute chromosome size, (iv) Satellites, and sec. cons and v) Distribution of eu & heterochromatin and repetitive DNA segments. They may be symmetrical or asymmetrical. Karyotype study is helpful in determining inter-relationships (tracing phylogeny) and in solving taxonomic disputes.

II. BANDING PATTERNS

1. Introduction

Differential banding patterns of chromosomes were initially developed for the analysis of human chromosome segments. The bands are made visible through low and high intensity areas under the fluorescence microscope or as differentially stained areas under the light microscope. These methods were first extended to different animals and later to plant chromosomes. After 1968, a new era was initiated with the demonstration of Caspersson and co-workers, of chromosome banding techniques using a fluorescent dye quinocrine mustard. This was followed by the development of a number of banding techniques which by demonstrating a sub-structure in chromosomes have permitted a more precise identification of not only individual chromosomes but also of their parts. These methods have increased the precision of cytogenetic diagnosis by allowing the study of finer chromosomal aberrations such as deletions, duplications, translocations and inversions and so forth of individual chromosomes. The impact of these technical advances has been so great that from the point of view of clinical application, the field of human genetics can be divided historically into two major periods, one before the discovery of the banding techniques and the other after. These advances have permitted the identification of new chromosomal defects involving almost every chromosome of the human karyotype.

2. Types

There are different types of banding techniques such as Q, G, C etc.

C-banding:- This was first developed by Hsu (1973) and Arrighi (1974). In this repetitive DNA sequences were revealed by denaturation-renaturation followed by Giemsa staining.

Q-banding:- Caspersson and his collegues had recorded differential fluorescence of different chromosome segments following staining with various flurochromes. This banding can be observed under ultraviolet light only. Successful analysis of human karyotype was carried with this technique using the flurochrome quinacrine mustard by Caspersson, Lomakki and Zech (1971) and Zech (1973). Such bands were referred to as Q-bands. (Fig. 5.2)

G-bands:- This pattern does not need any denaturation-renaturation. Mere saline treatment followed by Giemsa staining give a set of patterns similar in relative intensities to that of Q-bands.

Such bands are known as G-bands. G-banding can also be obtained by treating with a variety of chemicals like standard saline, NaOH/HCl before incubation, NaOH-trypsin, etc., and even mere heating.

R-banding:- The R-banding or reverse banding is a pattern opposite to that of Q and G bands and was obtained by Duttrilaux (1973) following controlled heating.

N-banding:- This banding is for nucleolar region employing acid extraction.

CT-banding:- This banding produces bands in telomeric and centromeric regions alone (Scheres, 1976).

O-banding:- This banding pattern is similar to the G-band pattern. Orcein staining has to be employed following incubation in X SSC.

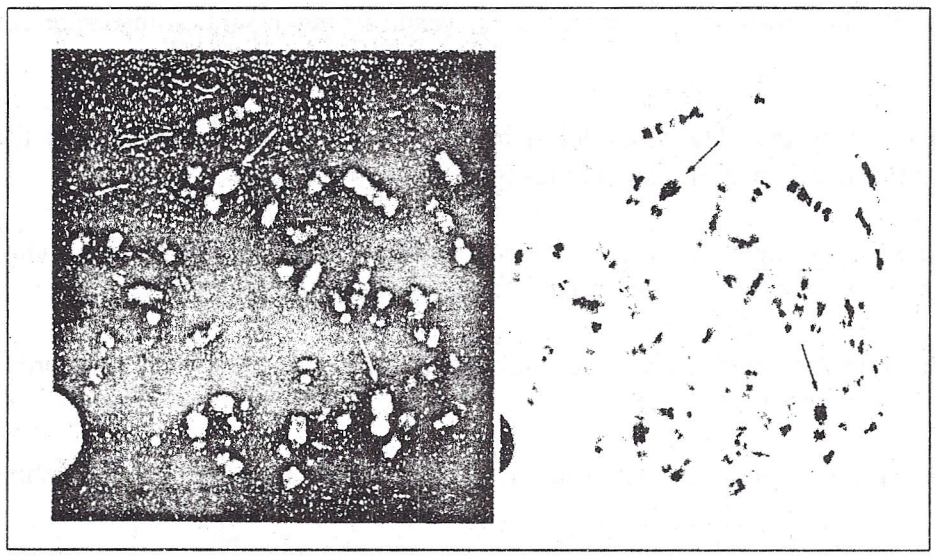

Fig. 5.2. Late replication in a hymphocyte with a karyotype 44 + XXX (i.e., with an extra X chromosome). This cell initiated replication (5 phase) in 5-bromodeoxyuridine and then completed it in deoxythymidine. The late replicating X chromosomes have strong fluorescence (left) and an intense stain (right). The early-replicating X chromosome is indicated by a short arrow, and the two late replicating X chromosomes by long arrows. (Courtesy of S.A. Latt, H.F., Willard, and P.S. Gerald, 1978).

3. Causes for chromosome banding

Various causes have been ascribed to the occurrence of chromosome bands. Four factors appear to be of particular importance namely: (i) the occurrence of repetitive DNA, and (ii)

differences in the base composition of DNA, (iii) in the protein components, and (iv) in the degree of packing of DNA or DNP complex (Pardue and Gall, 1972).

4. Importance of banding patterns

i) In knowing structural details of the chromosomes:- The banding techniques reveal structural details of chromosomes. The chromosome arms can further be subdivided into regions on the basis of certain land marking bands. As a consequence of this many of the structural aberrations like duplications, deletions, inversions and translocations in human chromosomes are detected with ease leading to the identification of many of the chromosome segments responsible for new syndromes.

Examples:-

a) detection of a balanced reciprocal translocation between long arm of Ch.2 and a segment of short arm of Ch. 6.

b) detection of 30 chromosomal syndromes which result from partial trisomy or deletion.

ii) Detection of diseases:- The association between chromosomal changes and the cancers was revealed only after use of banding techniques (Fig. 1).

(a) In chronic myeloid leukemia (a cancer of myeloid cells), the Philadelphia chromosome was acquired by a balanced translocation between 9 and 22 chromosomes.

(b) In Burkitts lymphoma (a cancer of lymphatic cells) a piece of chromosome number 8 is translocated to number 14.

(c) In retinablastoma (a cancer of the retina) there is a deletion in the long arm of chromosome 13.

(d) In some lung cancers a deletion occurs in chromosome number 30.

(e) In cri-du-cat syndrome there is partial deletion of short arm of Ch.5. The affected child shows characteristic mewing cry along with mental retardation.

(f) Deletion of long arm of Ch.18 results in facial alteration, and skeletal and ophthalmalogic abnormalities. Hence, the role of chromosomal alteration in causing certain types of cancers, mal-development, mental retardations, behavioural disorders has been proved with certainty after the advent of banding techniques.

iii) In the study of evolution:- The importance of banding patterns in studying evolutionary aspects of both plant and animal species is obvious. Since the banding techniques provide more precise and finer parameters for comparison of karyotypes homologies can be demonstrated on the

basis of specific patterns such as presence of certain land marking bands, presence of satellite DNA etc. In the case of cereals a comparative analysis of repetitive DNA showed the changes involved in their evolution, using in situ nybridization technique, the evolution of B-genome in wheat and higher primates was studied. Variations in Q-stained regions and C-bands in correlation with their phenotype revealed that Gorilla exhibits striking polymorphism in atleast half of their chromosomes. The bands can be used effectively in pedigree analysis, population studies and also for gene localizations. The reverse banding technique is a powerful tool for gene localizations.

iv) Somatic cell hybridization:- Genetic maps have been constructed in humans and rodents using rodent-human somatic cell hybrids. Using somatic cell hybrids linkage relationships have been studied in many eukaryotic species.

v) In genetic counselling:- Banding techniques have significantly contributed to prenatal diagnosis of several genetic abnormalities in the foetus at the 14^{th}-16^{th} week of pregnancy and has greatly aided in genetic counselling especially in cases of high risk.

5. Conclusion

In conclusion, it can be said that the banding techniques helped in understanding the evolutionary aspects of chromosomes in general and human karyotype in particular, has thrown light on the correlation of chromosome structure and diseases, helpful in obtaining genetic counselling and contributed significantly to the development of human molecular genetics by making feasible the identification of gene locations and construction of genetic maps.

6. Summary

This technique made feasible the identification of chromosomes by demonstrating the sub-structure in chromosomes. Any small alteration in chromosome morphology in the form of structural changes can be detected. The bands are made visible through high and low intensity areas under the fluorescence microscope and as differentially stained areas under the light microscope. There are different types of banding techniques such as Q, G, C, R, N, O, CT etc., depending on the stain used and the type of banding. These bands appear due to differences in base composition DNA, differences in protein components, differences in degree of packing etc. Banding techniques contributed much to the development of human cytogenetics and identification of several types of cancers and several other syndromes which appear as a result of structural changes in the chromosomes. These techniques throw light on evolutionary aspects of chromosome sin general and human karyotype in particular, elucidated the correlation between chromosome structure and diseases, helpful in obtaining genetic counselling and contributed significantly to the development of human molecular genetics by making feasible gene locations and construction of genetic maps.

Questions

1. Give an account of karyotype and evolution.
2. Define karyotype and idiogram. Write about the significance of karyotype study and evolution.
3. Write about the different types and significance of banding patterns.
4. Short notes questions:
   (a) Symmetric and asymmetric karyotypes.
   (b) G and Q bands
   (c) R and C bands
   (d) Significance of karyotype and banding pattern study.

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CYTOLOGY

LESSON-VI

SPECIAL TYPES OF CHROMOSOMES

OBJECTIVES

I. GENERAL INTRODUCTION

II. LAMP-BRUSH CHROMOSOMES

1. Introduction
2. Occurrence and size
3. Morphology
4. Cytogenetic significance

III. SALIVERY GLAND OR POLYTENE CHROMOSOMES

1. Introduction
2. Morphology, structure and function
3. Synaptic behaviour i.e. somatic pairing and banding pattern
4. Puffs in polytenes are the sites of gene transcription

IV. SUPERNUMERARY OR B-CHROMOSOMES

1. Introduction
2. Morphology and Behaviour
3. Origin of B-chromosomes
4. Chemical Nature
5. Adaptive significance

V. SUMMARY

I. GENERAL INTRODUCTION

Besides the normal mitotic and meiotic chromosomes, there are other forms of chromosomes of unknown origin. Among these, the lamp brush chromosomes of vertebrate oocyte, the giant chromosomes of the salivary gland cells of the Dipteran insects and the accessory chromosomes found in both plant and animal kingdom merit detailed consideration.

II. LAMP BRUSH CHROMOSOMES

1. Introduction

Lamp brush chromosomes were first observed by Flemning in 1882 and were described in detail in Shark oocytes by Ruckerts in 1897. He coined this particular name because they looked like the brushes used in those times to clean the chimneys of oil lamps. However, it is in 1977that the structure of these chromosomes in functional terms has been interpreted by the work of H.G. Callan.

2. Occurrence and size

Lamp brush chromosomes occur at the diplotene stage of meiotic prophase in vertebrate oocytes, spermatocytes of some invertebrates and in the giant nucleus of the unicellular green alga *Acetabularia*. Lamp brush chromosomes are not always giant and their size is correlated with the DNA content. Salamandar oocytes have high DNA content and the chromosomes are very large. In Newts the total length of the lamp brush chromosome set may be 5900 μm which is 3 times larger than the polytene chromosomes of *Drosophila*. Species with lower DNA content have small lamp brushes.

3. Morphology

These arise during the diplotene stage of meiosis. The chromosomes undergo a remarkable change characterized by an increase in length and the appearance of radiating hairs or side loops which appear to originate from the chromomeres (Fig. 6.1).

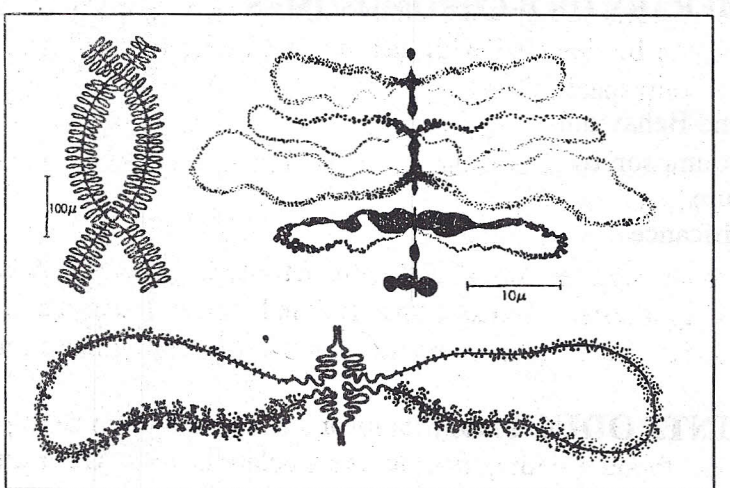

Fig. 6.1. An interpretation of the loops of a lampbrush chromosome. Top right, the chromomeres are of varying sizes and shapes, as are the loops that spin out from them. Below, as interpretation, with RNA synthesis proceeding as the loop spins out from the chromomere at the top and is then compacted back into the chromomere at the bottom; each loop is a portion of a single chromatic (courtesy of Dr. J.Gall).

The chromosomes are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata. On the chromosomes at specific loci are embedded chromatin granules, the smaller chromioles and the larger chromomeres. These granules range from 150-200 in number and are generally paired. It is from the latter that the lateral loops arise giving the characteristic lamp-brush appearance. The side loops grow in number to a maximum in diplotene and then decrease and disappear as meiosis proceeds towards metaphase. Each bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or chromomeres, each one of which has two loop-like lateral extensions, one for each chromatid. Each loop contains a DNA molecule and a matrix of ribo-nucleo protein fibrils inserted perpendicular to the DNA. Within the loop, the matrix is thick at one end and thin at the other end. This is caused by the synthesis of RNA molecules. At the chromosome axis the chromatids are tightly folded, forming chromomeres that are inactive in transcription (Fig. 6.1).

In *Triturus,* the total length of DNA contained in the lateral loops is about 50 cm and this represents 5% of the genome (10 meters). There are about 10000 loops per chromosome set most of which correspond to a single transcriptional unit. The RNA transcripts increase in size from the initiative site (promoter) and become exceedingly long (10-30 μm). The appearance is similar to that of a Christmas tree. These RNA molecules probably represent heterogenous nuclear (hn) RNA that is then processed to make mRNA. The various loops can be distinguished by size, thickness and other morphological characteristics.

Cytogenetic significance:

(i) As each loop can be distinguished, the presence or absence of a loop can be detected easily hence chromosome aberrations which can not be identified in normal chromosomes can be detected with ease in lamp brush chromosomes because of their larger size and loop specificity.

(ii) As each loop appears at a constant position in the chromosome detailed chromosome maps can be constructed.

(iii) Lamp brush loops represent units of genetic activity in which DNA is uncoiled at sites of intense RNA synthesis. Since transcriptional activity can be seen in lamp brush chromosomes they throw light on the mechanism of gene regulation in Eukaryotes.

Nucleoli formation in lampbrush chromosomes follows an unusual pattern. As many as 1000 nucleoli may be found floating free in the nucleoplasm. According to Duryee, these unusual nucleote become detached, migrate to the nuclear membrane and then pass into the cytoplasm. The significance of this phenomenon is not understood and the homology of these nucleoli with the normal somatic or meiotic nucleoli is not clear. But since the nucleoli are rich in ribose nucleic acid (RNA) and proteins, their addition to the cytoplasm during oogenesis

could provide a source of synthetic materials for growth. The function of lampbrush chromosomes involves the synthesis of RNA and proteins by their loops and probably certain amount of yolk for the egg.

III. SALIVARY GLAND OR POLYTENE CHROMOSOMES

1. Introduction

Balbioni (1881) discovered giant chromosomes in the salivary gland cells of dipteran species like *Drosophila, Chironomus, Sciara* and *Rhynosciara*. These are strikingly different from the typical somatic chromosomes of the same organism in their size as well as in their morphological structure. Though they were discovered in the year 1881, they received very little attention until Kostoff (1930), painter (1933) and Heitz and Bauer (1933) demonstrated their cytological importance. From this time onwards, these chromosomes received enormous amount of attention in respect of their cytogenetic analysis rather than as to their origin, detailed morphology and physiological significance.

2. Morphology, Structure and Function

On examination each of the visible salivary gland chromosome was found to be composed of two intimately synapsed and loosely twisted homologous chromosomes. They are present in their diploid number (Fig. 6.2).

This close association or synapsis of homologous chromosomes is known as somatic pairing and the chromosomes are considered to be in permanent prophase condition.

The salivary gland chromosomes are of enormous size having lengths which may be 100 times more than somatic chromosomes at metaphase. The volumes of these polytene chromosomes is about 1000 times greater than that of the somatic chromosomes. The total length of the four paired set is 200 μm compared to 75 μm in somatic cells. The mechanism of length increase is not known. This may be either by molecular stretching or by means of actual growth. This enormous size is reached by a series of 9-10 duplication cycles of the chromosomes (endo-mitosis) which increases the DNA content 1000 times.

3. SYNAPTIC BEHAVIOUR i.e. SOMATIC PAIRING AND BANDING PATTERN

More important than size is their synaptic behaviour (this is as intimate as that of meiotic chromosomes of pachytene) and their distinctive pattern of transverse banding which consists of alternating dark and light stained areas. These bands differ in thickness and other structural features in a specific manner which permits the accurate mapping of each chromosome. Interbands are fibrillar, do not take dark stain and fuelgen negative and do not absorb ultraviolet light. Dark bands on the other hand take dark stain and fuelgen positive and absorb ultraviolet light and they are considered to be discs of varying size.

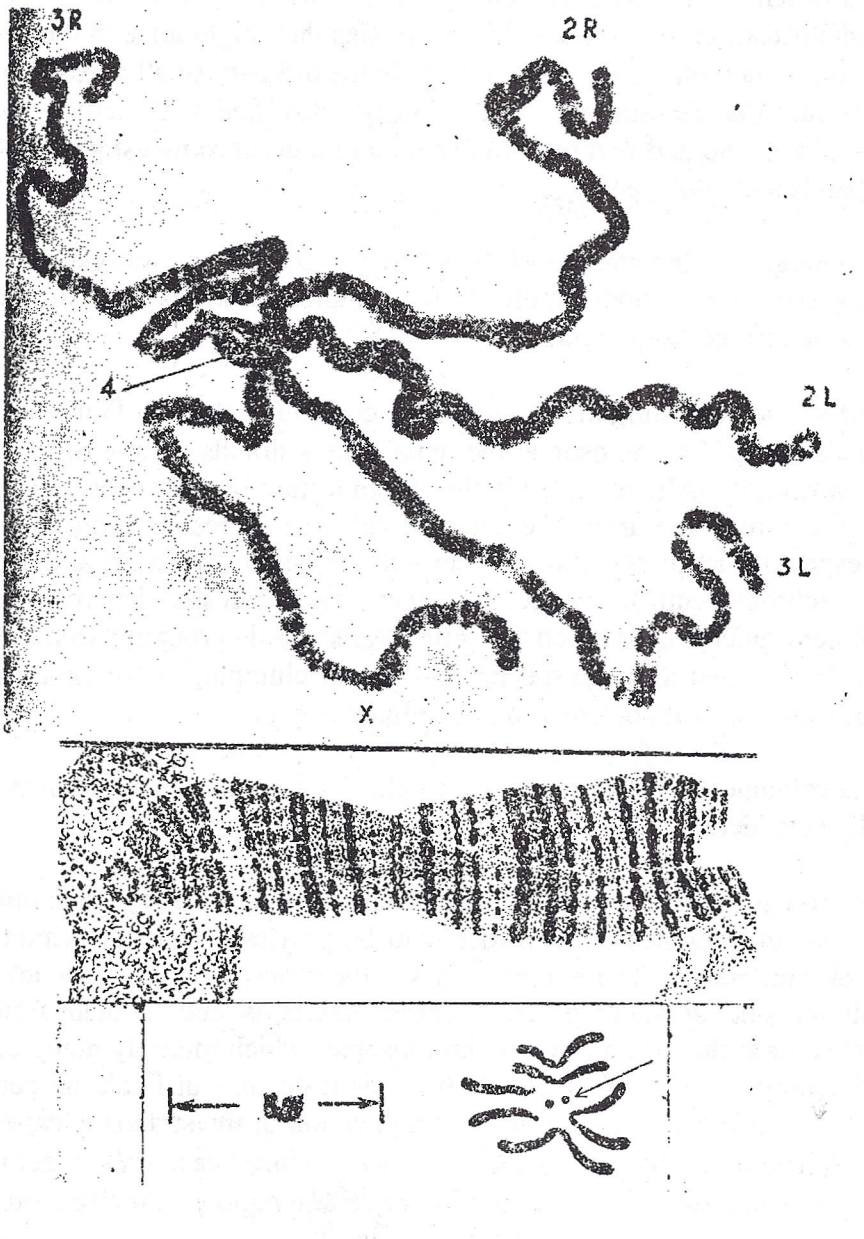

Fig. 6.2. The polytene chromosomes from a sallvary gland cell of D. melanogaster (top), an enlarged drawing of the small chromosome 4 (middle), and, for comparative purposes, a drawing of the metaphase chromosomes from a gangllonic cell (bottom, with arrow pointing to chromosome 4). The top figure shows the X chromosome, the arms of the two autosomes (2L, 2R, 3L and 3R) and chromosome 4, linked together at the chromocenter, the latter formed by an aggregation of the centric heterochromatic regions of all of the chromosomes. The diploid number of chromosomes is present, but the homologues are in intimate synapsis (top, courtesy, B.P. Kaufmann; middle and bottom, from Bridges, 1935).

The significance of these two characters i.e. synaptic behaviour and band specificity is fully recognised when it was realized that synapsis was a band to band phenomenon thus permitting the identification of many small irregularities that might arise in the internal structure of the chromosomes that would pass unrecognised in the ordinary small chromosomes. With the discovery of the fact that certain gene loci could be identified with certain bands the closer relationships between gene and chromosome was more and more investigated. In *Drosophila*, there are 5000 bands and 5000 genes.

In *D. melanogaster*, the nucleus of the salivary gland cell shows one short and five long strands emnating out from a single more or less amorphous mass the chromocentre. These strands have been identified as in Figure.

The short strand consisting of but few bands constitutes the tiny IVth chromosome. One of the long strands is the X chromosome, the remaining 4 strands are the right and left arms of the II & III chromosomes. All are united in the chromocentre by their centromeric regions. The failure of the Y-chromosome from the male larval smears results from the fact that this chromosome despite its large somatic size consists of heterochromatin and is submerged in heterochromatin (chromocentre). Since the other four possesses heterochromatic portions adjacent to their centromeres their union gives the characteristic grouping seen in the cells of the salivary glands. In the other dipteran species this type of clumping is not exhibited though they also possess chromosomes with heterochromatic regions.

Not all developmental features of salivary gland chromosomes are known with certainity but it is generally considered that the following events occur.

There is increase in size and length of the chromosomes. They are exhibiting synaptic behaviour. These chromosomes are considered to be polytenic in nature (multistranded) and bear numerous chromomeres. The evidence to say these are polytenic is conclusive since the peculiar constitution and diameter of these chromosomes is due to their formation from a number of fibers (four at the origin of each chromosome) which multiply many times remaining together like the threads of a rope. Each fiber delicate and difficult to perceive may be considered as a chromonema. The process of reduplication of the strands is called endomitosis. About nine reduplication of the strands are probably produced resulting in about 1000 fibers. Morphologically also we can observe the splitting of certain regions into numerous sub units.

The salivary chromosomes of many species show local variations in the degree of condensation of bands, some bands being discrete and sharply defined while others appear as diffuse or "puff". Such modification in chromosome structure occurs in different cell types and at specific times during larval growth (Fig. 6.3).

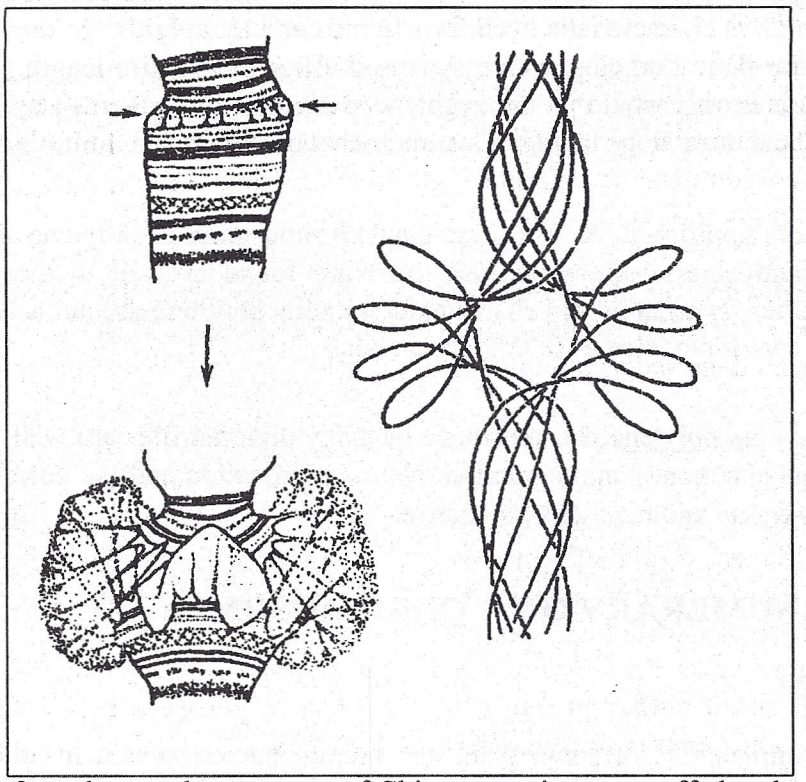

Fig. 6.3. A portion of a polytene chromosome of Chiranomus in an unpuffed and a puffed state, together with Beermann's (1952) interpretation of such a puffed region.

4. PUFFS IN POLYTENES ARE THE SITES OF GENE TRANSCRIPTION

One of the most remarkable characteristics of polytene chromosomes is that, it is possible to visualize in them, the genetic activity at specific chromosomal sites. The morphological expression of such sites is represented by local enlargements of certain regions called 'puffs'. A 'puff' can be considered as a band in which the DNA unfolds into open loops that actively synthesize RNA. The larger puffs are called Balbioni rings and have maximally extended DNA fibers which form lateral loops. Puffs are the morphologic expression of gene transcription and provided a unique opportunity to study the regulation of Eukaryotic genes. Polytene chromosomes have provided the strongest evidence that in Eukaryotes gene activity is regulated at the level of RNA synthesis. Beerman (1952) correctly interpreted the puffing of polytene chromosomes as an expression of intense gene transcription. In salivary glands, the appearance of some puffs has been correlated with the production of specific proteins.

A very special type puff has been described in *Rhyncosciara angelae* by Pavan & Breyer. The so called DNA puffs are able to incorporate H^3 thymidine and they represent regions of extra replication of DNA i.e. a case of gene amplification. Puffs can be induced by the steroid hormone Ecdysone and also by heat shock.

In meiotic zygotene stages a two by two association of homologues at any single point along their length is characteristic even for triploids and tetraploids. In the salivary gland nuclei of a triploid, the three homologues are synapsed along their entire length. The meaning is not clear although it is suggested that the zygotene attractions are governed by bilateral symmetrics and salivary gland attractions by radial symmetrics where there are multiple strands.

It is more appropriate to call these giant chromosomes as polytene chromosomes instead of salivary gland chromosomes because these are found as well in the cells of malpighian tubules, fat bodies, ovarian nerse cells and gut epithelia but these are not accessible for study nor they reach the maximum size as in salivary glands.

In short, the polytene chromosomes of many dipteran flies are well suited for studies of nucleic acid, gene action, gene regulation, gene amplification and the correlation of cytological changes with genetic and biochemical changes.

IV. SUPERNUMERARY OR B-CHROMOSOMES

1. Introduction

The complements of some plant and animal nuclei contain in addition to the normal chromosomes one or more accessory or supernumerary chromosomes. Wilson (1905) first discovered these in the hemipteran insect, the *Metapodius*. Since then they have been found in a wide variety of insects and higher plants (Darlington 1937, Fernandes 1946, Melandes 1950, Lakshmi and Bapa Rao, *Galphimia gracilis* 1974, Venkateswarlu and Pantulu 1971 in *Pennisetum*, Lakshmi and Venkateswara Rao 1979 in *Trigonella corniculata*. Lakshmi and Vishnuvardhan (1987) 7 Bchs in *Pennisetum americanum*.

2. Morphology and Behaviour

In many cases, they are smaller than normal, appear to be genetically inert since variation in their number is not accompanied by any marked phenotypic variation and frequently show the staining reaction characteristic of heterochromatin. However, in the limited chromosomes of *Sciara*, the super numeraries are larger than the normal. In *Tradescantia* and *Trillium* they are entirely euchromatic, while the B-chromosomes of maize are partially heterochromatic and partially euchromatic (Fig. 6.4).

Fig. 6.4. Diagram of the B-chromosome of maize in the pachytene stage of meiosis. The centromere is represented as terminal although it may be slightly subterminal; the segment to the left with the six small chromomeres is euchromatic, the remainder largely heterochromatic.

They tend to persist in a population with a constant average frequency per individual hence it is suggested that they perform a rather subtle function which is of value to the species as a whole. In mitotic and meiotic divisions, the B-chromes behave erratically. They fail to segregate properly to the poles. This has been attributed to their centromeres which fail to divide properly at anaphase. In the case of maize the B-chromosomes are preserved in the germ track because there is 100% non-disjunction. Their transfer to the next generation is assured by preferential fertilization i.e. sperm bearing B-chromosomes preferentially fertilize the egg nucleus. Randolph studied the behaviour of B-chromosomes in several Indian strains of maize and he found that there was accumulation of 25-30 B-chs in a single plant. He was of the opinion that these B's although inert in nature bring about some reduction in fertility and vigour when too many B-chs are present in the same nucleus (Randolph, 1941).

In inbred population of *Cimex*, where they are derived from the sex chromosomes they provide a source of variability independent of breeding system and are acting as a buffer against the effects of imposed inbreeding.

An intermediate situation between A & B chromosomes is found in *Nicandra physaloids* where the 10^{th} chromosome pair consists of two isochromosomes which pair irregularly. As a consequence seedlings arise with single isochromosome (9 II x 1 iso) then germination period is enhanced thus a homozygous form can be made to vary by an organized uncertainity like B-chromosomes. B-chromosomes do not possess any region homologous with other regions of the normal chromosomes. Normally B-chromosomes are often telocentric or isochromosomes but cases of submetacentric or metacentric chromosomes are also on record.

3. Origin of B-chromosomes

In plants their origin is not known although it is suggested that they represent kinetochore containing heretrochromatic segments from normal chromosomes while in certain animals they appear to have arisen from the members of the sex chromosome complement. Sybenga (1972) opines that they represent stabilized rements of interspecific hybridization with non-disjunctional system restricted to critical stages where accumulation instead of elimination results.

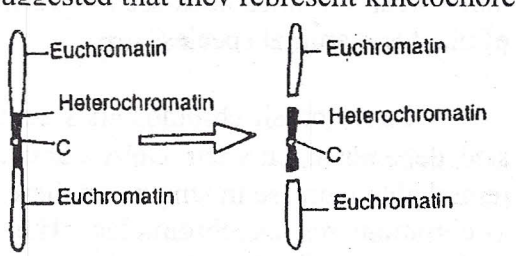

Fig. 6.5 Derivation of B-chromosome (C=centromere)

4. Chemical nature

Van Shaik & Pitout (1966) reported a dramatic difference in the base composition of seed DNA of OB and 4B strains in maize. But this is not supported by later data on base composition of leaf DNA. In grass hopper *Myrmeleotettix maculatus* an appreciable quantity of

satellite DNA is present with lower cytosine and guanine content. This satellite DNA is not present in O-B populations. The DNA of B-chromosomes from the study of maize seedlings with and without Bs through buoyant density distribution of CsCl gradients has been seen to be very closely related to that of A chromosomes (Chilton & Mc Carthy, 1973).

5. Adaptive significance

In majority of the cases, the B-chromosomes are genetically inert and they do not cause any phenotypic changes. But in some cases, they are capable of bringing certain subtle physiological changes and in lower numbers they exert a favourable influence on the genotype. eg:- *Lilium medeoloides*.

In *Pennisetum americanum* there is increase in chiasma frequency i.e. increased variability in the distribution pattern of chiasmata as well as increase in the duration of mitotic cycle in plants having B-chromosomes. In certain other cases, the B-chromosomes exert deleterious effect. For example in *Plantago coronapus* they bring about serious genetic disturbances as a result, all individuals carrying them are male sterile.

Randolph observed severe reduction in vigour and fertility in maize strains carrying B-chromosomes. Decline in yield and vigour was observed in Rye having increased number of B's i.e. upto 6. Mehra and Mann (1976) reported that accessories in *Pterotheca falconeri* had deleterious effect on vegetative growth, pollen and seed fertility.

V. SUMMARY

Besides normal mitotic and meiotic chromosomes, three types of special chromosomes are present. These are salivary gland or polytene chromosomes present in Dipteran insects, lamp-brush chromosomes seen in vertebrate oocytes and B-chromosomes present large number of plant and animal species.

Lamp brush chromosomes occur during diplotene stage of meiotic prophase and their size depends upon their DNA content. During diplotene, the bivalent chromosome attains remarkable increase in length and there is appearance of radiating hairs or loops from the regions of chromomeres and chromioles. They just look like brushes which used to clean the chimneys in those days. Each loop consists of a DNA molecule and a matrix of ribonueleo protein fibrils inserted perpendicular to DNA. These chromosomes are of cytogenetic significance since each loop is a unit of transcriptional activity. They are helpful for identification of structural changes and they throw light on mechanism of gene regulation.

Polytene chromosomes present in Salivary glands of Dipteran insects are characterised by their large size, somatic pairing or synapsis and presence of alternate light and dark bands. The 1000 times volume increase than somatic chromosomes is achieved by 9-10 reduplication cycles (endomitosis) of the chromosomes. The large size, synaptic behaviour and specific banding

pattern gives the chromosomes immense cytogenetic importance for detection of chromosome aberration, gene location and for understanding of gene regulation. Larger puffs of polytene chromosomes are called Balbioni rings and these are the regions where DNA unfolds into open loops which actively synthesize RNA. In short these are the morphological expression of gene transcription. It is more appropriate to call these chromosomes as 'polytene' instead of salivary gland chromosomes since these are present in other cells also occasionally. These chromosome are well suited for studies of nucleic acid, gene action, gene regulation, gene amplification and correlation of cytological changes with genetic and biochemical changes.

B-chromosomes are the supernumerary or additional chromosomes that are present in addition to the normal chromosome complement in plants and animals. There are several reports on B-chromosomes. They are generally smaller than the normal chromosomes and appear to be genetically inert but there are rare cases where they are larger than normal chromosomes and genetically active. Normally they are made up of heterochromatin. In maize, they are partially heterochromatic and partially euchromatic. The number of B-chromosomes vary from cell to cell. In mitotic and meiotic divisions these chromosomes behave erratically and they fail to segregate properly to the poles. The B-chromosomes are preserved in the germ track because of non-disjunction and they are transferred to the next generation by preferential fertilization. The B-chromosomes do not possess any homology with normal 'A' chromosomes, hence they do not pair with them. The B-chs originate from sex chromosomes. Sybenga opined that they are stabilized remnents of interspecific hybridization. Their chemical nature revealed that B-DNA is very closely related to 'A' DNA in some cases and in some other cases, it is made up of satellite DNA with lower cytosine and guanine content. In majority of the cases they do not have any adaptive value but in a few instances they exerted beneficial effect on the genotype. In cases where there is accumulation of Bs in large numbers they bring about deleterious effects such as reduction in vigour and fertility.

QUESTIONS

1. Give an account of the special kinds of chromosomes.
2. Write about the origin, occurrence, meiotic behaviour and adaptive significance of B-chromosomes.
3. Describe the morphology and cytogenetic significance of polytene and lamp-brush chromosomes.
4. Write short notes on:
   (a) Lamp-brush chromosomes
   (b) Balbioni rings
   (c) B-chromosomes
   (d) Morphology of polytene chromosomes
   (e) Puffs of polytenes are the sites of gene transcription.
   (f) Meiotic behaviour and adaptive significance of B-chromosomes.

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CYTOLOGY

LESSON VII

THE CHROMOSOME ABERRATIONS OR STRUCTURAL ALTERATION OF CHROMOSOMES, DEFICIENCIES AND DUPLICATIONS

OBJECTIVES

1. Introduction

2. Deficiencies or deletions
- i) Terminal
- ii) Interstitial

3. Duplications
- i) Tandem
- ii) Reverse tandem
- iii) Displaced

4. Evolutionary significance
5. SUMMARY

1. Introduction

Chromosome aberrations leading to the rearrangements in the linear order of genes may be grouped into four classes: (1) deletions or deficiencies, (2) duplications, (3) inversions, and (4) translocations. The first three as a general rule affect only a single chromosome whereas translocations may involve one, two or more number of chromosomes. Their detection can be made cytologically from the chromosome configurations found at prophase, metaphase and anaphase of meiosis.

2. Deficiencies or deletions

A deficiency involves the detachment and loss of a block of chromatin from the remainder of the chromosome. Deficiencies are of two types viz., terminal and interstitial (Fig. 7.1).

i) Terminal: Deficiency may be terminal when it involves the end of the chromosome. By this a small acentric bit is formed. In this type there will be loss of normal end of a chromosome or telomere will occur and there will be transformation of a bipolar segment of the chromosome into a unipolar structure takes place. These are less frequent than interstitial deficiencies.

ii) Interstitial: Interstitial deficiencies are produced by two breaks in the body of the chromosome in an acentric region. These also give rise to acentric fragments. The deleted portion will not survive in higher organisms, since it lacks a centromere. Due to this, they have no capacity for movement at anaphase. Since a deficiency involves loss of genetic material deficiencies would have deleterious effect on the organisms, the effect depending on the amount of genetic material lost and its quality. A deficiency may vary in size from a minute submicroscopic segment to a major portion of the chromosome. Deficiencies maybe homozygous or heterozygous. Generally homozygous deficiencies will be lethal. However in haploid bacteria and phase there are viable deficiencies. A mutant phase has lost 23% of its chromosome through deletion but still viable. However, special nutrient conditions are required for successful propagation. If the deficiency is sublethal it may cause some functional or morphological change in the organism which is conveyed to future generations as a gene mutation.

CYTOGENETICS

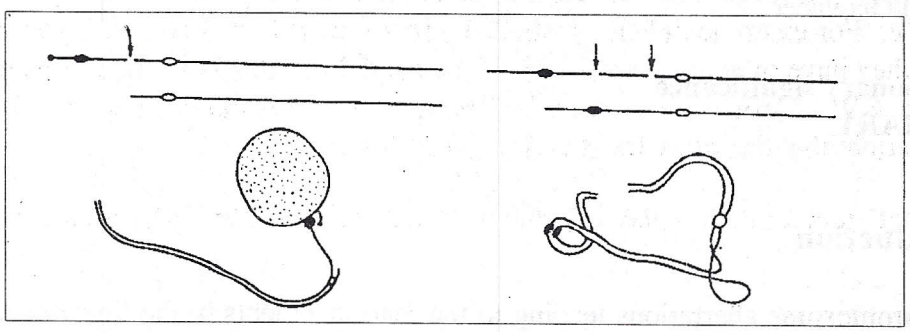

Fig. 7.1 Diagram to illustrate the origin of terminal (left) and interstitial (right) deletions and their appearance at pachynema in the microsporocytes of maize when paired in the heterozygous state. Left, chromosome 6 with one of the homologues missing part of the short arm, including the nucleolar organizer and terminal knob. Right, pairing is relatively complete, with the normal chromosome forming an unpaired loop corresponding in size to the missing piece in the other chromosome. Redrawn from B. McClintock.

Deficiencies can be detected with ease in the SG chromosomes of *Drosophila* by comparing the band structures of the deficient and normal chromosomes and in the lamp brush chromosomes by the absence of specific loops. In normal chromosomes deficiency heterozygosity can be detected at pachytene by the formation of a loop by the normal chromosome. However, if both the chromosomes of a homologous pair lack a particular gene or genes it is difficult to detect them cytologically but the effect is often lethal.

Examples in Human beings

In man a number of congenital abnormalities have been traced to chromosome deficiencies in the heterozygous state. Chronic myeloid lukemia is associated with 22 chromosome minus a substantial portion of its long arm. The cri-du-chat (cat cry) syndrome so named because of the characteristic mewing cry of the affected child but also including severe mental retardation and other physical abnormalities results, from the loss of short arm of a chromosome in the 4-5 group.

Examples from Drosophila:- In Drosophila in some cases there is a clear correlation between the phenotypic effect and the number of bands deleted. For example vestigial wing (wing size reduced) is present in certain flies due to certain deletions. If more bands are missing the wing size is much more reduced.

Deletions for location of genes: Deficiencies have been employed to locate genes on SG chromosomes of *Drosophila*. The principle used is a simple one. A correlation is made between the absence of a particular band in the chromosome and the presence of a particular morphological phenotype. For example deletions labelled 258-14, 264-31 and N-8 Mohr are of particular interest because they have in common, the loss of the band 3C2, all show white phenotype in heterozygous condition + W. Deficiency of band 3C2 removes the + allele leaving the 'W' allele hemizygous. So it is certain that the white locus is near the 3C2 band.

Deficiencies do not play any role in evolution since they diminish the metabolic controls.

3. Duplications

Duplications occur in two ways. One type of duplication results from the addition of a segment of a chromosome to a chromosome. Segments vary in size and therefore in genetic constitution. Another type resu 'ts from the addition of a small centric fragment to a genome.

Duplication by the addition of a segment to a chromosome depends upon breakage in the chromosome since a new segment can not fuse with a normal chromosome. Duplications can be detected with ease cytologically in a polytene chromosome as an extra band or bands but even in plants a study of meiotic pairing in haploids reveals some apparently homologous synapsis indicate the presence of duplications.

Duplications are more frequent and less lethal to the individual than are deficiencies. Swanson (1950) noted three types of duplications, tandem, reverse tandem and displac ed.

i) Tandem:- In tandem, duplications the added segment has the same genetic order as the original segment and is adjacent to the original segment.

ii) Reverse tandem:- In reverse tandem duplications, the duplicated segment is adjacent to the original segment in the reverse order.

iii) Displaced:- In the displaced duplication, a segment is inserted into a different chromosome.

ABC o DEF	Normal chromosome
ABC ABC o DEF	Tandem
ABC CBA o DEF	Reverse tandem
ABC GHI o DEF	Displaced

Examples of Duplications

Drosophila offers an excellent example for the genetic effect of duplication in the chromosome. A gene for eye shape and development has been located on the X-chromosome. The

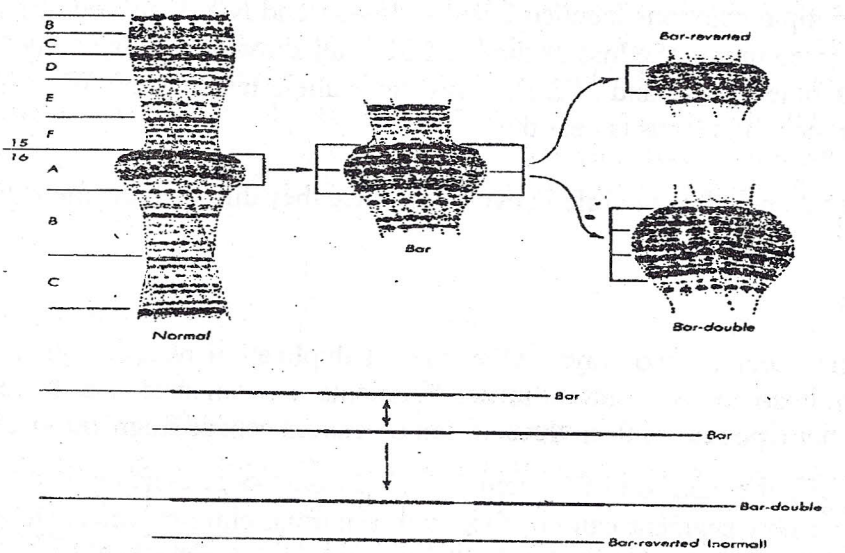

Fig. 7.2. The Bar phenotype in *D. melangaster* is due to a duplication of the 16A region, as indicated above, while Bar-double is due to triplication of the same region of the X chromosome. Below, diagram to illustrate how unequal crossing over in Bar females leads to Bar-double and to normal phenotypes. T.H. Morgan, C.B. Bridges, and J. Schultz, Carnegie Inst. Wash, Yrbk, 31 (1932), 303-07.

normal eye of the fly has 780 facets and is generally round in shape. The other types of eyes are found which were originally thought to be developed from gene mutation but which actually arise from duplication. A bar eye has only 325-358 facets and the shape of the faceted area is irregular. The bar double eye has only 200 facets and the faceted area is quite reduced in size. Bar and bar double eyes are caused by unequal crossing over during meiosis. Bar eye is a duplication of the 16 A region (genetic locus) responsible for the development of normal eye. Bar double eye is a triplication and is observed very well in SG chromosomes of *Drosophila*. This example of duplication of chromosomes also demonstrates a position effect and is evidence of correlation of genetic behaviour with specific cytological events (Fig. 7.2).

Another example of duplications can be seen in natural populations of *Drosophila*. They are what are called adjacent gene repititions called repeats. The two doublets in 89E of the right arm of chromosome 3 include the bithorax series of 5 pseudoalleles that is genes which behave as alleles when tested against each other but which can be separated by crossing over.

The two pseudoalleles of the sex-linked vermilion locus are apparently located in 10 A 1-2 double, one in the left half and other in the right. Such gene repeats have been present not only in *Drosophila* but in the fungus *Gnat*, *Sciara*, maize, *Aspergillus, Neurospora*, Bacteria, bacteriophages etc.

4. Evolutionary significance

Duplications play an important role in evolution because they are less deleterious to the organism. These provide a feasible method for the acquisition of new genes hence new physiological functions. At first, the duplicated genes are identical in origin and function but these can diverge through mutation to such an extent that they at a later stage of evolution may control entirely different functions. Generally mutations lead to loss or impairment of function but if mutation takes place in duplicated genes the chances are more for its retention and consequently likely to be selected.

5. Summary

A deficiency involves loss or detachment of a block of chromatin from the remainder of the chromosome. Deficiencies are of two types, terminal and interstitial. In terminal deficiency, a terminal segment is deleted i.e. at the end region of a chromosome. There is only a single break. Interstitial deficiencies are produced by two breaks in the body of the chromosome in an acentric region. Deficiencies are always deleterious to the organism and in homozygous condition they are often lethal. Cytologically they can be detected with ease as there will be formation of loops during pachytene by the normal chromosome as there is no homologous part to pair. Some examples of deficiencies in humans being chronic myoloid leukemia and cry-du-chat syndrome. They do not play any role in evolution as they deminish the metabolic controls.

Addition of a segment of a chromosome to a chromosome is called duplication. These are of three different types, tandem, reverse tandem and displaced. Duplications can be detected cytologically by the formation of a loop as in deficiencies but here the loop is formed by the duplicated chromosome. *Drosophila* offers an excellent example of duplications with modified phenotypic effect. The 16A region is responsible for normal eye shape and development. If 16A is duplicated once it results in bar eye. If it is present in triplicate, it will lead to a bar double eye. Duplications play an important role in evolution since they provide a feasible method for the acquisition of new genes hence new physiological functions.

Questions

1. Write about the different types, detection and cytogenetics of deficiencies.
2. Give an account of different types, detection and cytogenetics of duplications.
3. Compare and contrast between deficiencies and duplications.
4. Short notes questions.
   (a) Types of deficiencies
   (b) Types and detection of duplications
   (c) Examples of deficiencies in Human beings

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CYTOLOGY
LESSON-VIII

STRUCTURAL ALTERATIONS OF CHROMOSOMES INVERSIONS AND TRANSLOCATION HETEROZYGOTES

OBJECTIVES

I. INVERSIONS

1. Introduction
2. Paracentric inversions
3. Pericentric inversions
4. Evolutionary significance

II. TRANSLOCATIONS

1. Simple
2. Shift
3. Reciprocal
4. Meiotic behaviour
5. Translocation complexes
6. Translocations and evolution
7. Robertsonian centric fusion
8. Practical applicability
9. Summary
   i) Inversions
   ii) Translocations

I. INVERSIONS

1. Introduction

Strulevent first discovered inversions in *Drosophila*. An inverted chromosome is one in which a portion of the gene sequence evidently as a consequence of chromosome breakage has become rearranged in reverse order. Inversions are of two types.

(1) Paracentric:- Centromere is not included in the chromosome segment. It does not produce any change in chromosome morphology.

(2) Pericentric:- Centromere is involved in the inversion and this may markedly alter the chromosome morphology (Fig. 8.1).

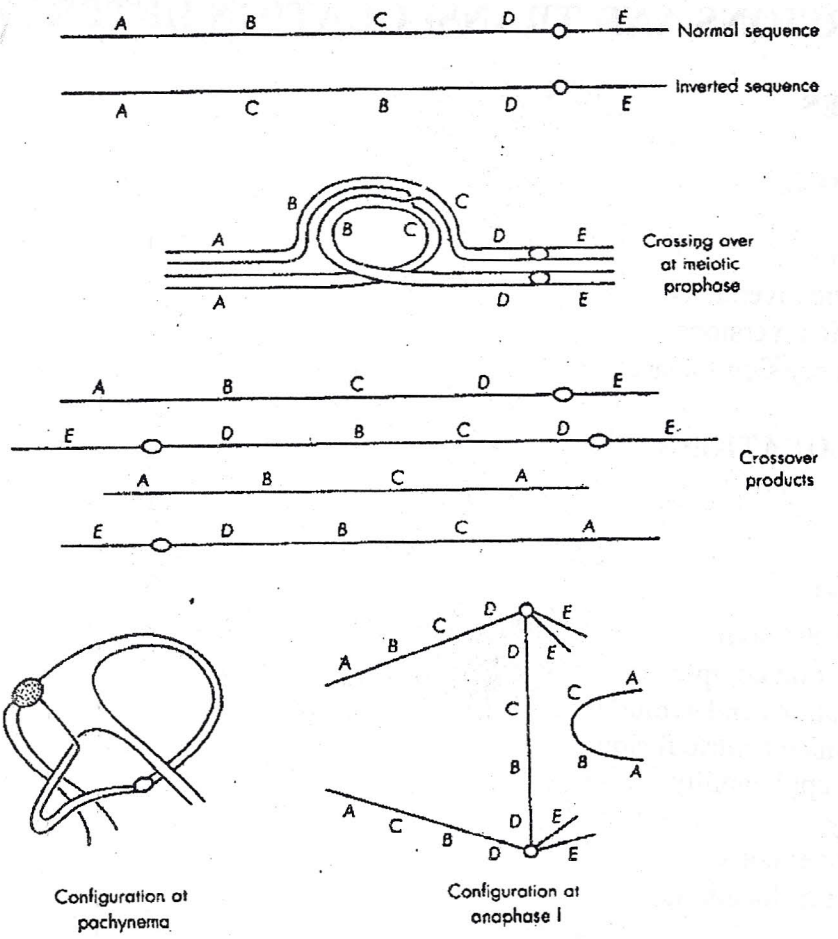

Fig. 8.1. Diagram of a pair of homologues, one having a normal, the other an inverted, sequence of genes, the synaptic configuration at pachynema with a single crossover occurring within the inverted segment, the four chromatid products from the crossover event, configuration as seen in a pachytene stage of maize, and the configuration found at anaphase.

2. Paracentric inversions

If for example, the sequence of a normal chromosome is represented as AB, CDEF, GH an inverted homologue is AB FEDC GH. With the CDEF section being inverted. In pachytene

and in Salivery gland chromosomes inversion in heterozygous condition can be recognised by the formation of an inversion loop which is formed when all portions of the two synapse in a homologous fashion. At Anaphase I, the commonly observed inversion bridge with its accompanying acentric fragment results when a cross over takes place within the inverted segment (Fig. 8.1). The resultant chromatids will be abnormal due to the formation of a decentric and an acentric. The acentric fragment will be lost having no capacity for movement at anapahse and the bridge will be broken either by the strain of the anaphase movement or by the cell wall that will cut across it. Occasionally neither event will happen and the dicentric bridge will be lost by failure to enter one or other of the telophase nuclei. Breakage of dicentric bridge and loss of acentric lead to inviability of haploid cells arising from meiosis or death of the resultant embryos. But in *Drosophia*, no serious reduction in gametic inviability is encountered due to inversion heterozygosity. The situation in males is readily explicable because no crossing over occurs but in females it must be assumed that the failure of inversions to reduce egg viability results either from a lack of cross over within an inversion or from the exclusion of dicentric bridge from the egg nucleus. The latter hypothesis is shown to be correct. the dicentric cromatid resulting from a single cross over within an inversion passes into the polar bodies and the innermost chromatid which is not involved in crossing over remains to be included in the functional egg nucleus (Fig. 8.2).

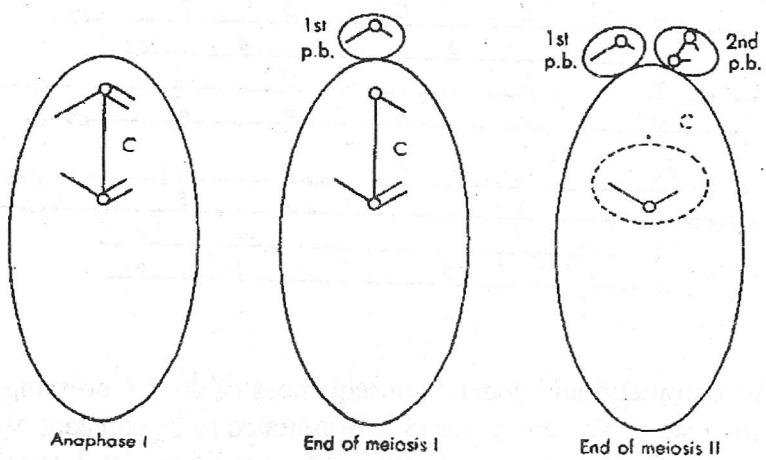

Fig. 8.2. Schematic representation of the fate of an anaphase I bridge resulting from inversion crossing over in the oocyte of *D. melanogaster*. Meiosis occurs near the top of the oocyte, and the chromatid nearest the surface passes into the first polar body. It is presumed that the bridge passes into the second polar body, while the bottom chromatid, which has not been involved in crossing over, passes into the functional egg nucleus. The acentric fragment probably disintegrates in the cytoplasm of the egg.

When two cross overs are formed within an inverted segment, the result will depend upon the number of chromatids involved (Fig. 8.3).

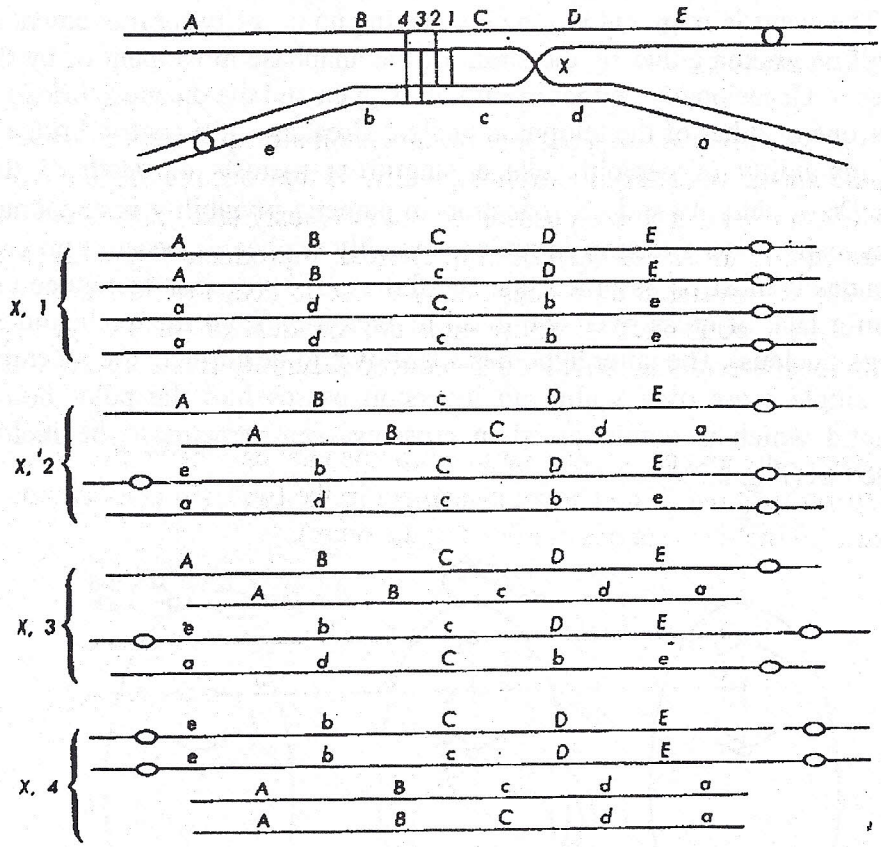

Fig. 8.3. The chromatid and genetic consequences of double crossing over within a paracentric inversion. The crossover at X is assumed to be constant, with the second crossover being at the 1, 2, 3, or 4 position. X,1 would be a two-strand double, X,2 and X,3 would be three-strand doubles, and X,4 would be a four-strand double.

From the diagrams, it can be seen that two strand double crossing over will yield four normal chromatids two of which are non-cross overs and the other two cross overs. Three strand crossing over yields one non-cross over chromatid, one cross over chromatid and a dicentric bridge with its acentric fragment. Four strand crossing over would yield two dicentric and two acentric fragments and duplication deficiency would presumably lead to death of the four products of meiosis or of the zygote if the gametes were involved in fertilization. Clearly therefore inversions seriously interfere with the recovery of chromatids that have been involved in crossing over. The incorporation of a lethal gene within the region of an inversion also serves as a means of indefinitely preserving structural heterozygosity. The famous CIB stock of

Drosophila melanogaster developed by Muller owes its usefulness to the 'C' component which in the form of an inversion acts as a cross over supressor, the 'L' component a recessive lethal which prevents homozygocity for the CLB chromosome and the Bar 'B phenotype which permits identification of individuals carrying the CLB chromosome in a heterozygous state. Thus inversions are useful in the conduct of genetic experiments.

3. Pericentric Inversions

If the two breaks involved in a pericentric inversion are equivalent to the centromere the chromosome would appear unchanged morphologically. If they occur at different distances from the centrome a shift in the centromere position would obviously takes place. So there is reason to believe that pericentric inversion have been influential in producing new karyotypes. Single crossing over within the inversion loop does not give rise to decentric and acentric but instead produces two new chromatids each of whose ends are identical in genetic content duplication and deficiency obviously result and sterility and reduced fecundity ensue because of gene loss or gain (Fig. 8.4).

Two different inversions in the same chromosome can have the following mutual relations (a) Independent (there is no common segment in the two), (b) Overlapping (they have a common segment), (c) Included (one is confined in the other).

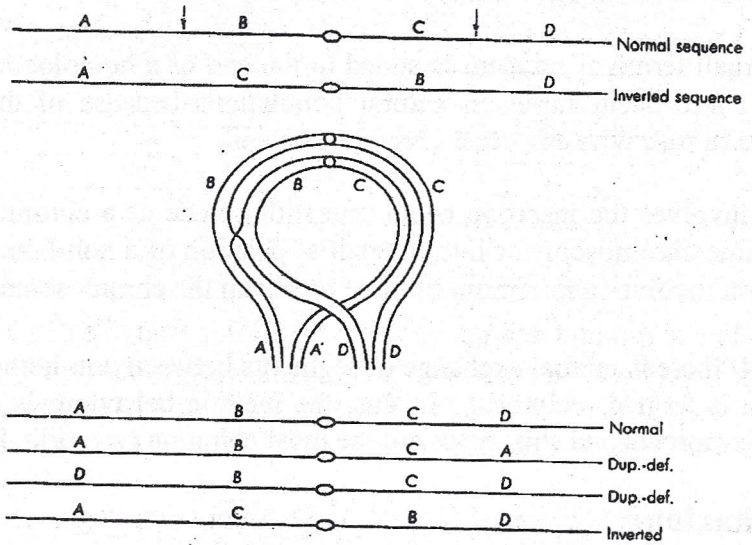

Fig. 8.4. A pericentric inversion, with the break points equidistant from the centromere, the pairing relations at pachynema with a single crossover within the inversion loop, and he chromatid products resulting from such a crossover.

4. Evolutionary significance

Of the two types, paracentric inversions are more significant from the evolutionary point of view. Using overlapping inversions Dobzhousky gathered information in the natural populations of *Drosophila* species such as *D. pseudoobscura, D. persimilis* etc., and found that inversions are not only tolerated but are selected in natural populations because the block of chromatin in the inverted region is allelically different from that where there is no inversion. In inverted regions generally crossing over is suppressed so there won't be any shuffling of genes. Mutations act only on such chromatin where there is no genetic recombination. So these inverted segments due to the action of mutations become allelically different and these are selected in different regions and seasons. Heterozygous inversions may show dominant phenotypic effect. **eg:** curly inversion in *Drosophila.*

II. Translocations

Translocations were first discovered by Bridges (1923) in *Drosophila.* This is a common phenomenon in which there is transfer of a chromosome segment to a different part of the same chromosome or a different chromosome. In the latter case, the transfer may takes place between homologous chromosome or non-homologous chromosome. There are three types of translocations: (1) Simple, (2) Shift, and (3) Reciprocal translocations.

1. Simple:- A small terminal segment is added to the end of a homologous or non-homologous chromosome. These occur rarely in natural populations because of the inability of normal chromosome end to fuse with any other chromosome end.

2. Shift:- Shift involves the insertion of an interstitial piece of a chromosome into a different portion of the same chromosome or into interstitial position of a non-homologous chromosome. Shift translocation involves a minimum of three breaks in the chromosome.

3. Reciprocal:- If there is mutual exchange of segments between non-homologous chromosomes the translocation is termed reciprocal. In this, the meiotic behaviour is altered for a potential genetic effect. Reciprocal and shift types are the most common type (Fig. 8.5).

4. Meiotic behaviour

Translocations may be either homozygous or heterozygous. Homozygous translocations can not be detected cytologically since they behave as normal chromosomes. However new linkage groups become established hence these in natural populations give rise to new chromosomal races. **eg**:- *Oenothera, Datura paeonia, Scorpions* etc. (Fig. 8.6).

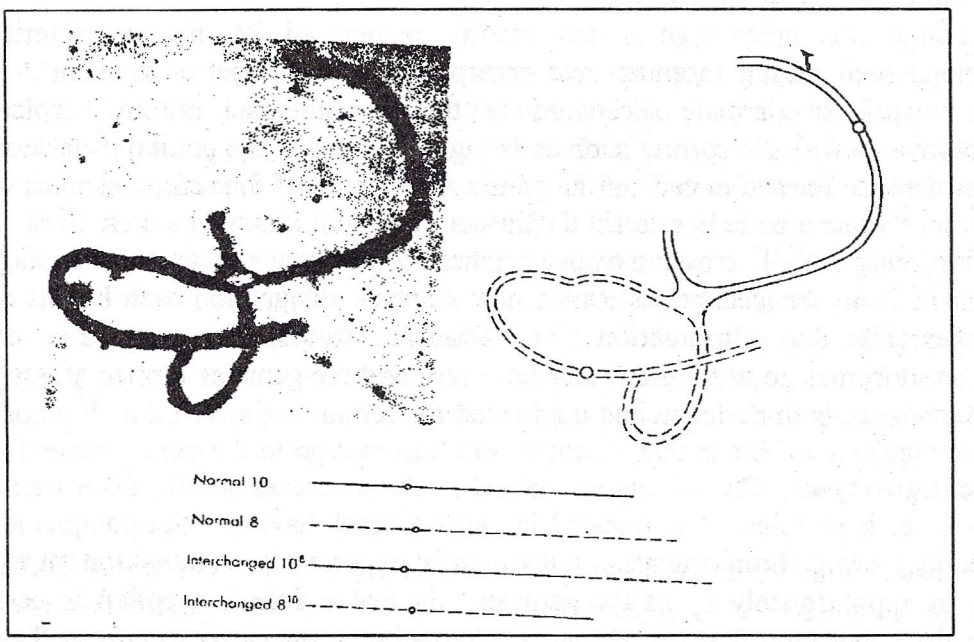

Fig. 8.5. Translocation heterozygote in maize. The normal and translocated chromosomes are indicated below; above, pachytene configuration (photograph and diagram) characteristic of a translocation heterozygote. M.M. Rhoades, in Corn and Corn Improvement (New York: Academic Press, 1955), pp.123-219.

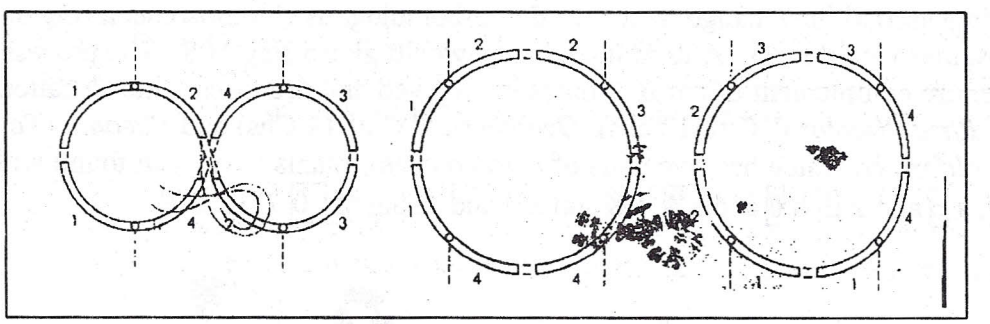

Fig. 8.6. Diagram of alternate (left) adjacent-1 (middle), and adjacent-2 (right) arrangement of a translocation of 4 chromosomes. Segregation from these positions would lead to viable gametes from the alternate position, duplication and deficiency from adjacent-1, with homologous centromeres going to the same pole, and duplication and deficiency from adjacent-2, with nonhomologous centromeres going to the same pole.

Translocation heterozygotes are readily recognised by the characteristic pairing configurations seen during prophase and metaphase of first meiotic division. Homologies determine synapsis, so complete pairing requires that the chromosomes form a typical cross-like figure at pachytene with the pairing partners being exchanged at the point of translocation break. If the chiasmata are formed in each of the paired arms a ring of four chromosomes would result at metaphase. If one arm fails to form a chiasma, the result would be a chain of four when a translocation ring of 4 chromosomes reaches a metaphase plate, three arrangements are possible since each arrangement determine, the anaphase disjunction each has its own genetic consequences (Fig. 8.6). In adjacent I and adjacent 2 types, the rings are so oriented that adjacent chromosomes go to the same anaphase pole and the gametes formed although different from each other are both deficient and duplicated for certain regions of the chromosomes. The alternate arrangement differs in that alternate chromosomes go to the same pole and the gametes formed are of two types. One having normal set of chromosomes and the other translocated set. Neither gametes is deficient or duplicated in any way each having a full complement of genes represented by the two chromosomes. So it would be expected that a translocation would lead to inviability in approximately $^{2}/_{3}$ of the gametes. In maize defective pollen is nearer to 50% leading to the conclusion that the zigzag or alternative arrangement occurs with a frequency approaching 50%. This is called semi-sterility theory.

5. Translocation complexes

In the formation of translocation ring of 4 chromosomes only two non-homologous chromosomes are involved. However, if the arm of one of the translocated chromosomes is involved in a second interchange with a third non-homologous chromosome, a ring or a chain of 6 chromosomes would form. A third interchange would give a ring of 8. The process can go on until the entire complement of chromosomes is involved and produces a translocation complex. Example: *Rheo discolor* (TC of 12 chs), *Oenothera* (TC of 14 Chs) and *Paeonia* (Tc of 10). In *Paeonia californica* which has five pairs of chromosomes, plants have been found with a ring of 4 and 3 II, a 6 and 2 II, a 6 and a 4, a 8 and a II and a ring of 10. (Fig. 8.7).

Fig. 8.7. Meiotic metaphase in Rhoeo discolor. Left, a ring of twelve chromosomes; right, an open chain of twelve chromosome. Courtesy of Dr. K.Sax.

6. Translocations and Evolution

The evolutionary consequences of translocations are intriguing with *Oenothera* and *Datura* providing most of the available information. In a translocated heterozygote what were initially two independent linkage groups are now united into one despite the fact that the chromosome exist as independent entities. As the translocation complex increases in size, the number of linkage groups decreases until as in *Oenothera* species, the seven haploid chromosomes behave as one large linkage group. There is no independent assortment since they are linked. Structural heterozygosity becomes ensured only when lethal genes are included in the rings of chromosomes. Only alternate segregation from the rings leads to the formation of viable gametes. So each group of 7 chromosomes becomes in essence a single large linkage gorup with recombination of genes being confined to the pairing ends of each chromosome (Fig. 8.10). These linkage groups consisting of 7 separate but collectively inherited chromosome are called Renner Complexes (after the name of the discoverer). In *Oenothera lamarkiana*, the two complexes called Gaudens and Velans yield two very different species hybrids when outcrossed to other forms but when self pollinated they breed true (Fig. 8.9). The incorporation of lethal genes prevent the existence of homozygotes and breakdown of the complexes.

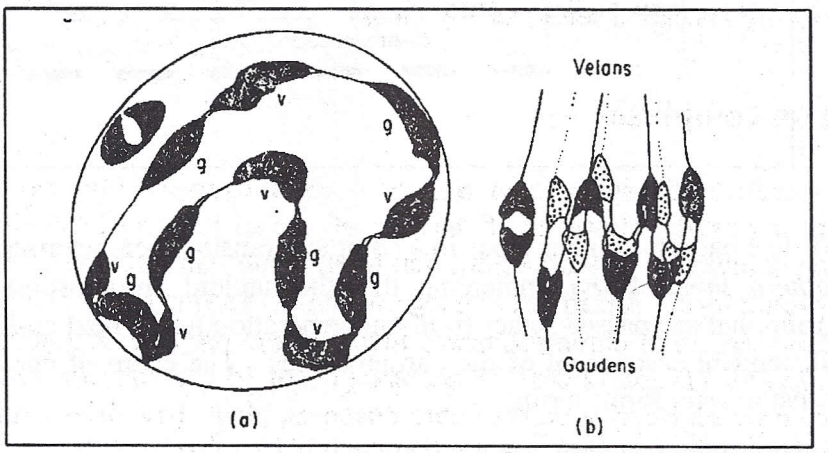

Fig. 8.9. Chromosome pairing at meiotic metaphase in *Oenothera lamarckiana* which possesses a ring of 12 chromosomes and one normal bivalent. The haploid complements contain two different sets of genes, are referred to as the *gaudens* and *velans* sets, and when outcrossed to another form, yield quite different offspring. The ends of the chromosomes in each set have been identified; the end arrangement in the *velans* set is 1-2, 3-4, 5-8, 7-6, 9-10, 11-12, and 13-14. The *gaudens* set is 1-2, 3-12, 5-6, 7-11, 9-4, 8-14, 13-10. Only the chromosomes with ends 1 and 2 can pair to form a bivalent; the others can only pair at their ends. In a ring, therefore, the pairing would be 3-4, 4-9, 9-1, 10-13, 13-14, 14-8, 8-5, 5-6, 6-7, 7-11, 11-12 and 12-3 with the two 3 ends pairing to complete the ring.

The Renner effect is a further refinement of the system. It is achieved when one of the Renner complexes is transmitted only through the sperms. Such a separation can be accomplished by the establishment of either gametic or zygotic lethals. *O. lamarkiana* make use of one method and *O. muricata* the other (Fig. 8.11).

The Oenotheras have utilized a number of genetic devices each deterious to a certain degree and combined them into a system that functions very well. These devices are as follows:

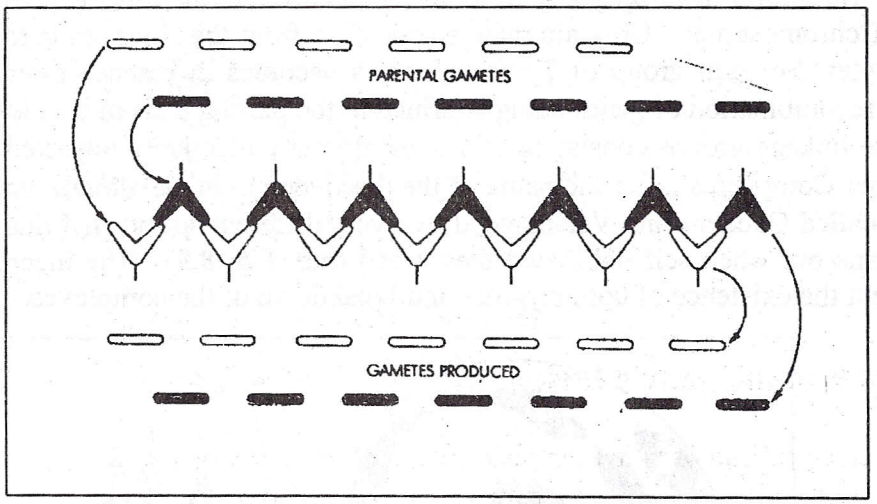

Fig. 8.10. The pattern of inheritance in a complex translocation heterozygote such as *Oenothera lamarckiana*, indicating that the haploid chromosomes form a linked group that are passed intact from one generation to the next and that there is no independent assortment of the chromosomes. The chain of chromosomes (center) customarily forms a ring.

1. Reciprocal translocations which because of formation of linkage system drastically bring reduction in gene recombination.
2. Accumulation of lethal and other deleterious mutations.
3. Self pollination which in itself leads to inbreeding and lack of vigour.

Any one of these three in open pollinated natural species is genetically disadvantageous, yet the translocations lead to the development of diverse linkage groups (complexes), the lethals enforce structural heterozygosity and self-pollination prevents outcrossing which tends to break the complex up. So in the course of evolution of *Oenotheras* formation of ring complexes is followed by the establishment of self pollination. It is only in this order of occurrence that the system makes evolutionary sense when it is viewed in terms of its adaptive success.

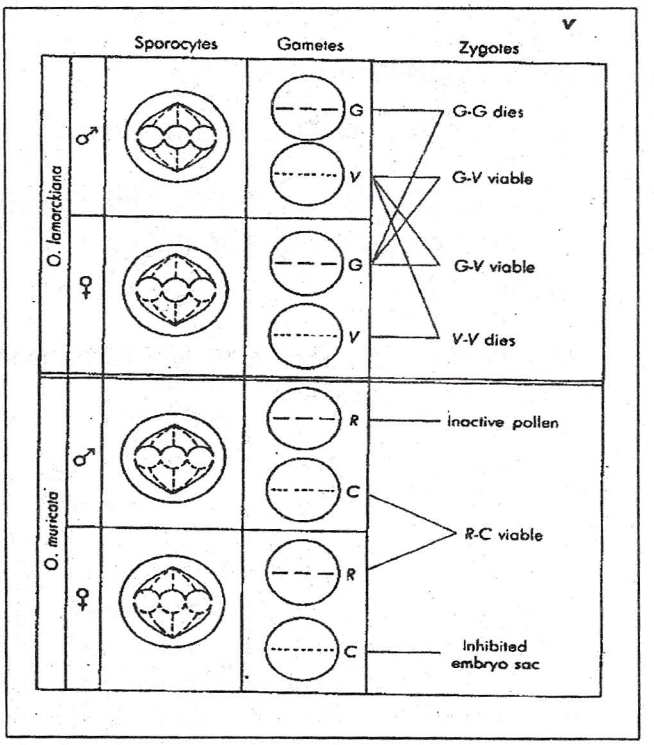

Fig. 8.11. Operation of the Renner effect in two species of *Oenothera*. In *O. lamarckiana*, zygotic lethality removes the homozygotes from the population; in *O. muricata*, homozygosis is prevented through gametic lethality.

7. Robertsonian centric fusion

A special type of translocation between two acrocentric chromosomes can lead to Robertsonian centric fusion. It would be an asymmetric interchange involving the two short arms and resulting in a small acentric fragment that gets lost and a large dicentric with two centromeres so close together that they behave like one. Another origin is a asymmetric translocation between the long arm of one and the short arm of another chromosome. A very small chromosome results that may be dispensable and a large metacentric chromosome that is practically equivalent to the two original chromosomes together. If the small chromosome is lost (translocation monosomy) the remaining three will form a trivalent in meiosis. **eg:-** Man, Cattle (Fig. 8.12).

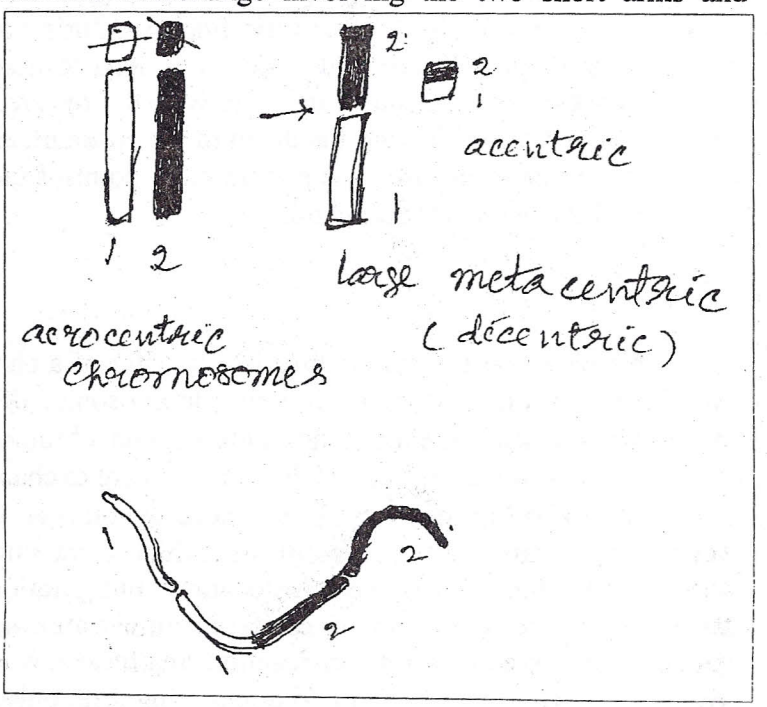

Fig. 8.12. Robertsonian translocation

8. Practical Applicability

1. Production of seed less fruits. **eg:** Melons.

2. Introduction of translocated stocks into the wild populations of insects in large numbers so that their population growth rate can be controlled to a considerable extent. **Eg:** Cockroaches.

3. Translocations can be used as tester stocks for individual identification of extra chromosomes in trisomics.

9. Summary

i) Inversions:

These are quite useful to the genetists in a wide variety of experimental designs. In inversions the gene order is inverted as a consequence of chromosome breakage and rearrangement. Inversions are of two types. Paracentric and pericentric. In paracentric inversions, a centromere is not included in the inverted segment. These do not bring out any alteration in chromosome morphology. In pericentric inversions, a centromere is included in the inverted segment. So if the two breaks are not equidistant from the centromere, it may lead to alteration in chromosome morphology. Inversions can be detected cytologically by the characteristic chromosome configurations during meiosis such as inversion loop during pachytene, dicentric bridge and acentric fragment during anaphase. Occurrence of one or more number of crossovers in inverted region may lead to dicentric, acentric or duplicate deficient chromatids which may seriously interfere with the recovery of chromatids in gamete formation leading to gametic sterility and the death of the resultant embryo. Of the two types, paracentric inversions are important from the evolutionary point of view. Inversions are not only tolerated but also selected in natural populations.

ii) Translocations:

This phenomenon results from the transfer of a chromosome segment to a different part of the same chromosome or a different chromosome. This different chromosome may be a homologous chromosome or a non-homologous chromosome. Translocations are of three different types, simple, shift and reciprocal. Mutual exchange of chromosome segments is called reciprocal. Translocations may be homozygous or heterozygous. Only heterozygous transactions can be detected cytologically which form ring or chain associations depending on the number of chiasmata in the translocation configuration. If there is occurrence of a single translocation it will form a ring or chain of four chromosomes. During metaphase three types of orientations are possible for a translocation ring these are adjacent I, adjacent II & alternate type. Gametes that are formed from alternate ring are only viable. So it is expected that a translocation would lead to inviability in approximately $^2/_3$ of the gametes. In maize alternate or

zigzag arrangement is seen in 50% of pollen grains resulting in 50% fertility. This is called semi-sterility theory. If all the chromosomes of a complement are involved in subsequent reciprocal translocations, it may lead to the formation of a translocation complex as in *Oenothera lamarkiana* where a translocation ring of 14 chromosomes is formed. This is a Renner complex named after the discoverer. The two linkage groups of *O. lamarkiana* each consisting of 7 chromosomes are called Gaudeus and Velans. Translocations will have evolutionary significance in a very intriguing way. Example *Oenothera*. The Oenotheras have utilized a number of genetic devices each deterious to the organism to a certain degree and combined them into a system that functions very well. These devices are reciprocal translocations, incorporation of lethal genes and self-pollination. Translocations lead to the development of diverse linkage groups (complexes), lethals enforce structural heterozygosity and self pollination prevents out crossing and breaking up of complexes. So translocation heterozygosity is maintained and perpetuated in natural populations with an adaptive success.

Questions

1. Define inversion? How can paracentric and pericentric inversions can be distinguished on the basis of meiosis.
2. Write about the different types of translocations and their evolutionary significance.
3. Give an account of reciprocal translocations in *Oenothera*, What do you understand about semi-sterility theory?
4. Compare and contrast between:
   (a) Para and pericentric inversions
   (b) Renner complexes and Renner effect
   (c) Shift and reciprocal translocations.
5. Short notes Questions:
   (a) Included inversions.
   (b) Paracentric inversion
   (c) Pericentric inversions
   (d) Robertsonian translocations
   (e) Co-orientations of translocation heterozygote
   (f) Renner complex
   (g) Renner effect

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CYTOLOGY

LESSON-IX

NUMERICAL ALTERATION OF CHROMOSOMES – ORIGIN, OCCURRENCE, PRODUCTION AND MEIOSIS OF HAPLOIDS, ANEUPLOIDS AND EUPLOIDS

OBJECTIVES

1. Introduction
2. Euploids
3. Haploids
   - (i) Types of haploids
   - (ii) Origin of haploids
   - (iii) Meiosis in haploids
   - (iv) Uses of haploids
   - (v) Limitations of haploid breeding
4. Aneuploids
   - (i) Introduction
   - (ii) Trisomics
     - (a) Origin
     - (b) Types and meiotic behaviour
     - (c) Phenotypic effects
     - (d) Uses of trisomics
   - (iii) Tetrasomics
   - (iv) Monosomics and Nullisomics
     - (a) Origin
     - (b) Cytology
     - (c) Uses
5. Summary

1. Introduction

In general, chromosome number for a group of random individuals of a particular species is similar in all likelihood. The species are reasonably constant biological entites and this stability is determined by constancy in the number and kinds of genes and chromosomes. Just like mutations in genes which bring changes by loss or addition of genes, chromosomes are also capable of changes. Variations do occur and they are sometimes perpetuated to give rise to new chromosomal races.

Variations in chromosome number produce two kinds of individuals.

(i) Those whose somatic complements are exact multiples of the basic number characteristic of the species.

(ii) Those whose somatic complements are irregular multiples of the basic complement.

Individuals of the first type are the euploids and the individual organism may be haploid, diploid, triploid, tetraploid and so on with the higher multiple numbers above, the diploid state are collectively referred to as polyploids. Hence, euploids consist of haploids, diploids and polyploids. Individuals with irregular chromosome numbers are called aneuploids and an organism that has gained an extra chromosome can be designated as (2n+1) trisomic, a loss of one would be indicated as 2n-1 and the individuals are monosomics. A combination of polyploidy and aneuploidy can produce variable number of organisms in any single individual race or species.

2. Euploids: Euploids consist of haploids, diploids and polyploids. A haploid organism contains a single genome or a set of chromosomes. It is consequently homozygous or more properly hemizygous for the genes represented in the genome. A diploid organism however may have its two genomes similar or dissimilar. Members of a pure diploid species would have similar genomes. An organism resulting from a cross between two diploid species would have dissimilar genomes and is generally referred to as a diploid hybrid. Where more than two genomes are involved, however, the terminology becomes increasingly complex as the number of genomes increases so that polyploid organisms possessing similar genomes are known as autopolyploid- those with dissimilar genomes as allopolyploids, the latter must obviously arise through hybridisation. (For details see Lesson X).

3. Haploidy: An individual with gametic chromosome number is known as haploid and such a condition is referred to as haploidy. There is difference between haploids and monoploids. Monoploids have the basic chromosome number (x) of a species. In other words, monoploids contain single set of chromosomes (i.e. single copy of the genome). Haploids have gametic chromosome number (n). In a true diploid species, both monoploid and haploid chromosome number is the same (n=x). Thus a monoploid can be a haploid but all haploids can not be monoploids. The main differences between monoploids and haploids are given in Table 1.

Table 1. Differences between monoploids and haploids

	Monoploids	**Haploids**
1	Represent gametic chromosome number of a diploid species	Represents gametic chromosome number of any species
2	Denoted by 'x'	Denoted by 'n'
3	Monoploids are always haploids	Haploids can not always be monoploids
4	Contain single set of genome	May contain one or more copies of genome

Types of haploids: Haploidy may be a normal or an abnormal state.

Normal haploidy: The gametophytic stage of the lower plants and the males of certain insects such as the bees, wasps and other hymenopteran forms are regularly haploid and the production of gametes proceeds in normal fashion since these organisms are adjusted to this mode of existence.

Haploidy as an abnormality: In plants where haploidy has been observed as an abnormality than in animals, the haploid individuals are characaterised by being smaller than their diploid progenitors. They can be identified by high sterility and small size of the stomatal cell. Direct examination of the chromosome number provides the final identification of the haploidy. Such positive identification of haploids has been made in *Maize, Rye, Nicotiana, Datura* and *Pennisetum*.

Depending upon the origin haploids are of two types viz., euhaploids and aneuhaploids. Euhaploids develop from a euploid species and have complete chromosome set. Euhaploids are of two types viz., monohaploid – which develop from a normal diploid species, and polyhaploids – which develop from polyploid species. The polyhaploids are again of two types namely allohaploids – which arise from allopolyploid species and autohaploids – which develop from autopolyploid species. When a haploid develops from a tetraploid species it is called a dihaploid. Aneuhaploids develop from aneuploid species and have either one additional or missing chromosome. Aneuhaploids include disomic haploids (n+1), nullisomic haploids (n-1), substitution haploids (n-1+1), mis-division haploids etc. The mis-division haploids have an isochromosome which is produced by transverse fracturing of the centromere. Aneuhaploids are generally inviable.

(ii) Origin of haploids: In crop plants haploids may originate in several ways viz., (a) by parthenogenesis, (b) from interspecific crosses, (c) as a member of twin embryos, (d) by semigamy, (e) by alien cytoplasm, (f) by anther culture, and (g) by chromosome elimination

(a) By parthenogenesis: Parthenogenesis refers to development of embryo from the egg cell without fertilization. Haploid embryos give rise to haploid plants. In maize haploids occur spontaneously by pathenogenesis at a very low frequency. In maize both gynogenetic and androgenetic haploids have been observed. Haploids also occur in several fruit crops by parthenogenesis. The frequency of gynogenetic haploids is 1 in 1000 in maize. For androgenetic haploids it is 1 in 80,000. Kostoff has produced a haploid *Nicotiana* with paternal characteristics which suggests that the male sperm penetrated the embryo sac and then developed spontaneously without fertilization.

(b) From interspecific crosses: Interspecific hybridization refers to mating between two different species of the same genus. In some crops, haploids arise from interspecific crosses. In such crosses, the pollen may not effect fertilization, but stimulates the development of the egg cell into haploid embryo. **Eg.** Potato.

(c) From twin embryos: Development of more than one embryo in seed is known as polyembryony. Sometimes twin embryos develop in polyembryony. In twin embryony atleast

one embryo will be a haploid. Development of haploids through twin embryos has been reported in *Linum*, pepper and several horticultural crops.

(d) By semigamy: Semigamy is a form of apomixis in which the sperm nucleus enters the egg cell but fails to fuse with the egg nucleus. In semigamy, the sperm and egg nuclei develop independently resulting in formation of seeds with maternal and paternal chimeras of haploid tissues. Semigamy can be used for production of haploids at will in Egyptian cotton (*Gossypium barbadense*).

(e) By alien cytoplasm: Alien cytoplasm refers to cytoplasm of related species. In wheat haploids have been obtained through the use of alien cytoplasm. About 30% haploids were obtained when nucleus of bread wheat was placed into the cytoplasm of *Aegilops caudata*.

(f) By anther culture: Haploids can be obtained by anther culture i.e. regeneration of plants from anthers in the nutrient medium. Such haploids have been obtained in tobacco, wheat, rice and many other crops. The anther and pollen culture technique was first used for the production of haploids in *Datura* by Guha and Maheswari, 1964. Now this technique is used for the production of haploids in tobacco, rice and several other crops (Fig. 9.2).

(g) By chromosome elimination: The mechanism of chromosome elimination in *Hordeum* hybrids was studied by Subrahmanyam and Kasha (1973) and by Bennett *et al.* (1976). They observed that after fertilization there is a gradual and selective elimination of *H. bulbosum* chromosomes from nuclei of endosperm as well as embryo cells, so that eventually haploid embryos are produced. No evidence of the elimination of *H. vulgare* chromosomes was available.

Differences were observed in the rate of chromosome elimination among different crosses and these were attributed to genotypic differences in the stocks used in hybridization programme. Bennet *et al.* (1976) examined the possible causes of chromosome elimination and concluded that sudden shortage of proteins in the developing embryo and endosperm, and the better ability of *H. vulgare* chromosomes to form spindle attachments relative to *bulbosum* chromosomes may be responsible for the elimination of *bulbosum* chromosomes. It has also been demonstrated that the elimination of *bulbosum* chromosomes is under genetic control (Fig. 9.1).

(iii). Meiosis in Haploids: In monoploids or polyhaploids derived from allopolyploids, the sporophyte has only one chromosome of each type. These chromosomes do not have homologous partners to undergo pairing and regular disjunction. The natural result is that the chromosomes remain as univalents. Since equational division for the separation of chromatids in meiosis takes place in second division, the chromosomes will pass to the poles at the first division without any equational division of the chromosomes. In such a situation, sometimes it becomes difficult to distinguish between the metaphase and anaphase of first meiotic division This is because the univalents are unable to arrange themselves in a definite equatorial plate and are distributed through

out the cell at metaphase I and anaphase I. Person (1955) called such stage as meta-anaphase. Since in the first division, the univalents ordinarily do not divide, these would move to either pole giving unequal number of chromosomes to either pole. Because of incomplete set of chromosomes the spores will abort and lead to complete sterility. Functional pollen grains in haploids may also result due to the formation of restitution nuclei in the first division will be followed by normal equational mitotic division in the second meiotic division, so that two functional spores will be produced.

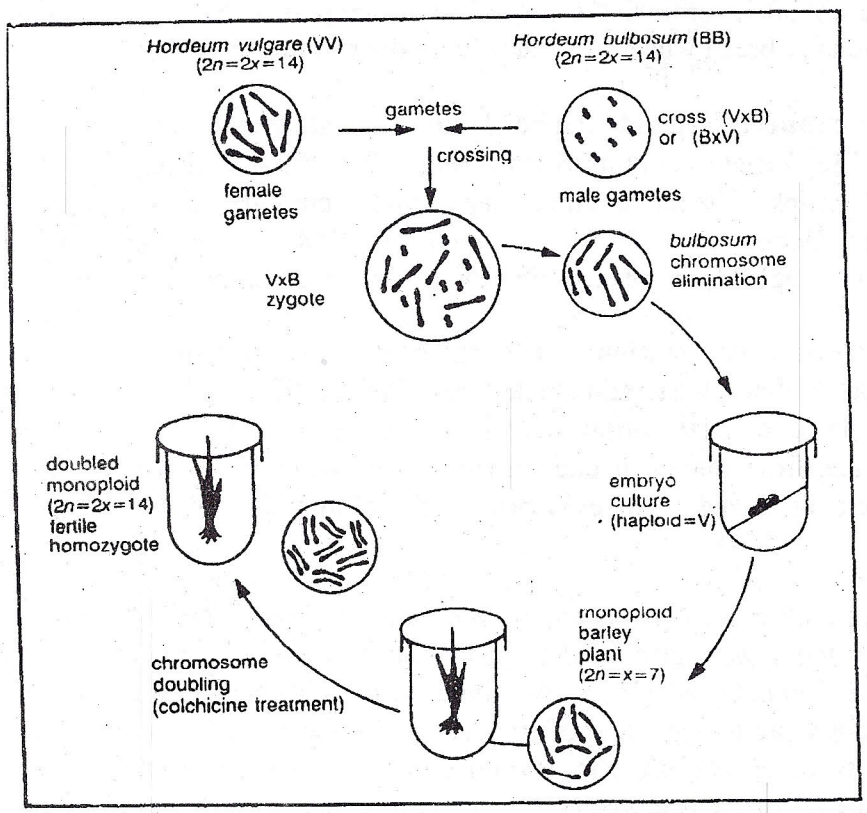


Fig. 9.1. Scheme for production of monoploid and doubled monoploid barley plants using wide hybridization *bulbosum* method involving elimination of *Hordeum bulbosum* chromosomes.

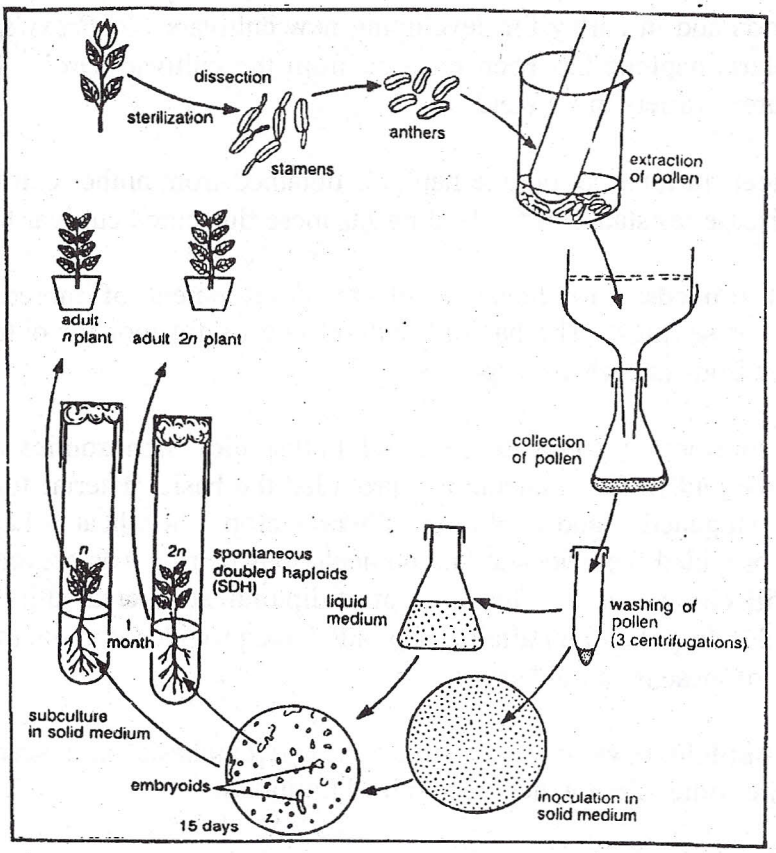


Fig. 9.2. Anther and pollen culture leading to formation of haploid plants

Study of haploid meiosis provides a clue to the nature of the basic chromosome complement. In haploids of *Sorghum* for example which has 10 instead of 20 usual chromosomes, most cells have 10 univalents, some however have an occasional bivalent suggesting either that ten is not the basic number or that duplications are present. A similar situation has been found in pepper (n=12) and as many as 6 bivalents have been found leading to the suggestion that the supposed diploid is a polyploid in which many if not all of the genes are duplicated.

(iii). Uses of haploids: Haploids have several applications in crop improvement as well as in genetic studies. They as used for:

(a) Development of purelines or isogenic lines
(b) Development of disease resistance
(c) Development of inbred lines
(d) Indirect uses

(a) Development of purelines: Purelines or isogenic lines can be obtained by chromosomal doubling of haploids. This is a short cut method for obtaining pure homozygous lines.

Doubled haploids can be used in two important ways viz., as a parent in hybridization and as a cultivar. In maize doubled haploids have been successfully used in the development of commercial hybrids and in barley for developing new cultivars. In *Brassica napus* a doubled haploid called Maris haplona has been released from the cultivar 'Oro' The new variety is superior to the parent variety in oil yield.

(b) Disease resistance: In tobacco, double haploids obtained from anther culture have been used to improve the disease resistance of the leading Japanese flue cured cultivar Mc1610.

(c) Development of inbreds: In dioecious plants, development of inbreds requires several generations (6-7) of selfings. The haploid method is a useful tool for obtaining inbred lines within two crop seasons in such crop species.

(d) Indirect uses: In wheat a complete series of nullisomics monosomics and trisomics was developed from haploids. These aneuploids provided the basic material for extensive studies about breeding, cytogenetics and evolution of wheat crop. In wheat a haploid deficient for chromosome 5B provided the evidence that homoelogous pairing was prevented by the activity of genes on the 5B chromosome. This concept of diploidization created interest about pairing in other polyploids. In potato and alfalfa, haploids have provided convincing evidence for the polysomic nature of these tetraploid crops.

(v) Limitations of haploid breeding: Despite renewed emphasis on research in the area of haploids, following are some of the widely recognized limitations.

(a) High cost of producing haploids.
(b) Difficulty in obtaining haploids in large number in some crops.
(c) The deleterious mutations that sometimes arise in anther or pollen cultures often used for haploid production.
(d) Non-random recovery of haploids leading to uncontrolled selective production of doubled haploids.

In view of the above limitations though, the emphasis on haploid research has declined recently, but it still remains an important component of biotechnology research for crop improvement.

4. Aneuploids

(i) Introduction: Individuals having irregular chromosome numbers are aneuploids. Aneuploids are of different types. A diploid organism that has gained an extra chromosome 2n+1 is a trisomic, 2n+2 tetrasomic or a loss of a single chromosome monosomic (2n-1) or loss of two chromosomes (2n-2) nullisomics. Addition of two different chromosomes will give a double trisomic (2n+1+1) and addition of several different chromosomes will lead to the production of a multiple trisomic. The terms hyperploid and hypoploid have also been used in some text books but these are not

recommended since the same terms have been used for diploids with duplications and deficiencies. A combination of polyploidy and aneuploidy can lead to variable number of chromosomes in any single organism or race of organisms. Non-disjunction described by Bridges is a recognised source of aneuploid gametes in diploid organisms (Fig. 9.3).

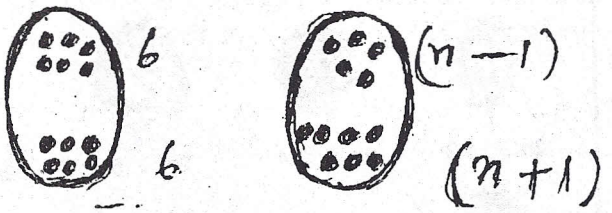


Fig. 9.3. Non-disjunction in disomics.

The complementary gametes that are n+1 and n-1 will on union with a normal gamete (n) produce individuals which are 2n+1 and 2n-1 respectively. These are trisomics and monosomics.

(ii) Trisomics: These were first reported by Belling and Blakeslee in *Datura*. Later in several plants like tomato, chilli, pearl millet, rye, maize, wheat, tobacco, *Drosophila* and humans etc.

(a) Origin: Trisomics are readily obtained in triploid progeny due to the formation of the irregular gametes. From normal disomics they are formed as a result of non-congression and non-disjunction. Asynaptic and desynaptic disomics, translocation heterozygotes may also give rise to trisomics. In addition, ionizing radiations, colchicine and other mutagenic chemicals can produce trisomics.

(b) Types and Meiotic behaviour: Trisomics are classified into different types depending on the nature of the extra chromosome.

Primary trisomics: In primary trisomics, the extra chromosome is an unmodified chromosome. These have been extensively studied in jimson weed *Datura stramonium*. *Datura* has a haploid number of 12 chromosomes with the consequence that 12 different trisomics are possible. All have been identified and each produces a certain characteristic phenotype (Fig. 9.4). Each of the 12 haploid chromosomes has been designated by labeling their ends. Thus the chromosomes become 1.2, 3.4, 5.6 . . . . . 23.24. The trisomic rolled for example has the 1-2 chromosome in triplicate. The three homologues generally pair to form a trivalent or a bivalent with univalent may be formed. A closed ring of three chromosomes is never formed. This requires two chiasmata in each arm. Lakshmi *et al.*, (1990) reported two primary trisomics in chilli with 2n=25 chromosomes. The origin of these is from interchange heterozygotes and revertant diploids. The priamry trisomics of chillies are characterised by reduced leaf size and high sterility (Fig. 9.5).

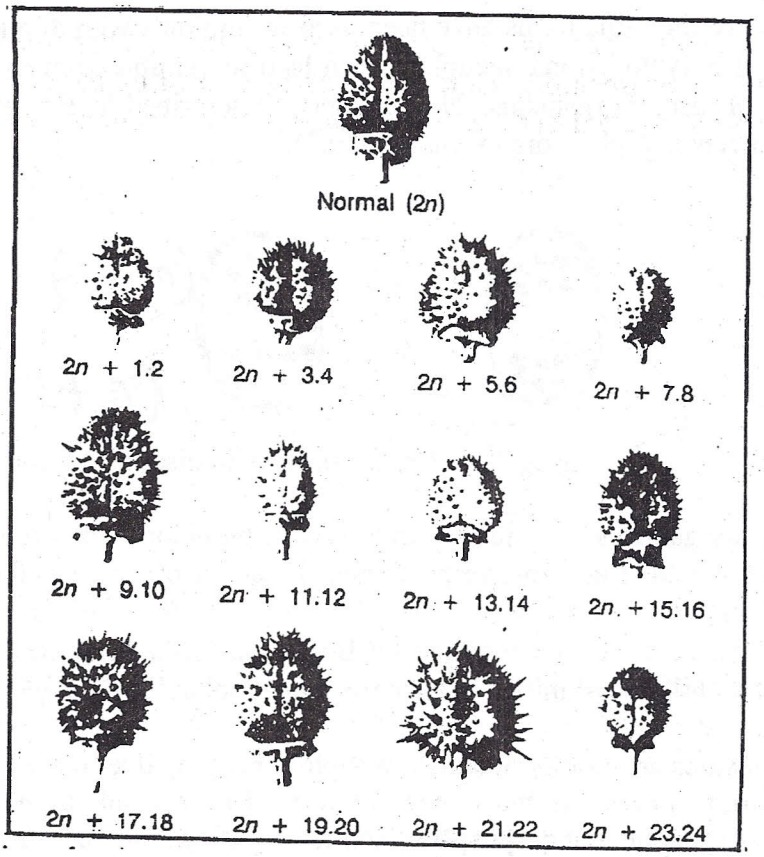
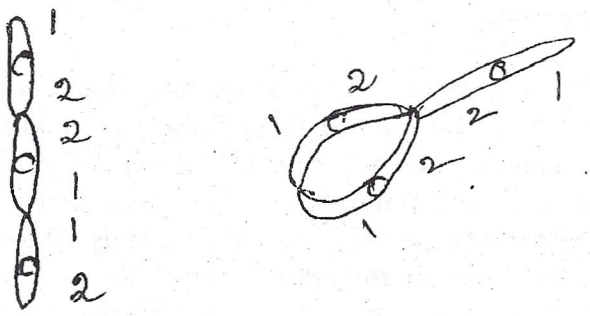


Fig. 9.4. Morphology of seed capsules of normal and primary trisomic types of the jimson weed. *Dautra stramonium*. In 'rolled' the extra chromosome is 1.2, in 'glossy' the extra chromosome is 3.4 and so on, upto 'ilex' with extra chromosome 23.24 (Modified from Avery *et al*., 1959).



Chain type Frying-pan type

Fig. 9.5 Meiotic configurations (trivalents) possible for a primary trisomic

Secondary trisomics: These are also identified in *Datura pennisetum* (Lakshmi and Vishnuvardhan, 1981) and *Capsicum* (Nalini Kumari and Lakshmi, 1988). These form closed rings

of three chromosomes since the extra chromosome in this has identical ends. The extra chromosome may be an isochromosome or it may be formed by the occurrence of a single cross over in a pericentric inverted chromosome. Each primary trisomic can produce two secondary trisomics (Fig. 9.6).

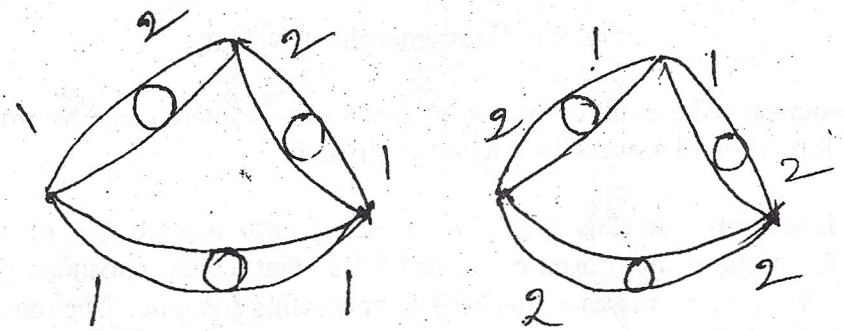


Fig. 9.6 Closed ring of three chromosomes in secondary trisomics

The origin of secondaries and telocentrics is often by mis-division of the centromere in meiosis of primary trisomic or normal parents that have one or more univalents. In *Dautra*, all the 24 secondary trisomics are described and identified.

Tertiary trisomics: Here, the extra chromosome is formed as a result of translocation possessing the ends of two non-homologous chromosomes. These can be identified by the characteristic chains that are produced by the conjugation of five chromosomes. Double frying-pans are also formed. Lakshmi and Nalini (1989) reported a tertiary trisomic with dimorphic leaves in chilli (Fig. 9.7).

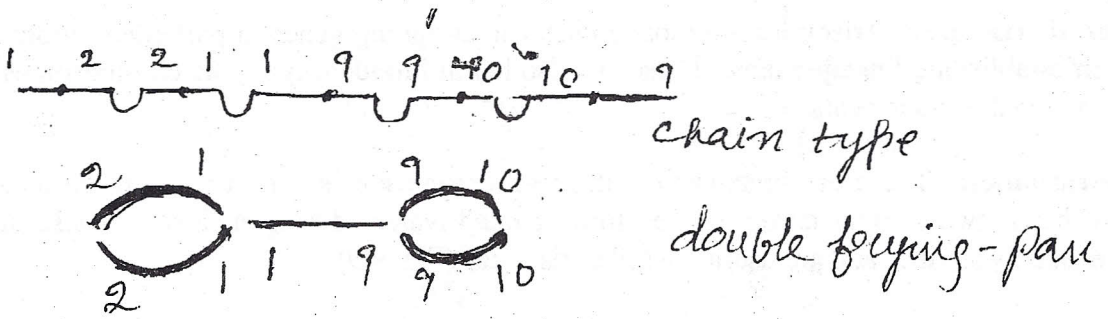


Fig. 9.7 Configurations of a tertiary trisomic

Telotrisomics or Monotelotrisomics: The extra chromosome is a telocentric chromosome. The trivalent formed is a heteromorphic one. Lakshi and Yacob (1980) reported a telotrisomic for chromosome 7 in pearl millet from an open pollinated progeny of translocation heterozygote produced by γ-irradiation (Fig. 9.8).

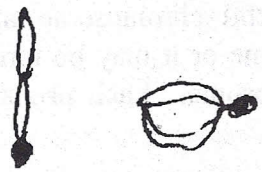


Fig. 9.8 Heteromorphic trivalents

nterchange trisomics: The extra chromosome occurs in an interchange heterozygote. It can be dentified by the formation of a quadrivalent and a univalent.

Compensating trisomics: In this type, one missing chromosome 1.2 of this trisomic was compensated by two tertiary chromosomes 1.9 and 2.5 so that the chromosomal formula of Nubbin being 2n – 1.2 + 1.9 + 2.5. So it was a ditertiary compensating trisomic. The maximum associatior in such a type being a chain of seven chromosomes.

(c) Phenotypic effects: A number of trisomics have been identified in human beings. Increased age of the mother is sometimes responsible for the appearance of more number of trisomis at birth. Each trisomic is associated with a specific phenotypic effect. Downs syndrome trisomy of 21 chromosome is characterized by severe mental retardation, slanting eyes, saddle nose etc. In *Datura* rolled trisomic which is a primary trisomic of chromosome 1 is characterised by the presence of narrow inrolled leaves and small capsules with short spines. Sugar loaf, a secondary trisomic (2.2) for chromosome one is characaterised by shorter plants, larger leaves and large conical capsules with short spines. The primary trisomic of chilli is characterised by smaller leaves while the tertiary trisomic exhibited dimorphic leaves. All the trisomics are associated with sterility. Trisomics are mostly unstable and the progeny consists of mostly diploids.

(d) Uses of trisomics: Trisomics are mostly useful in assigning genes to particular chromosomes i.e. in establishing linkage maps. These are also helpful in identifying the chromosomes which are involved in translocations.

(iii) Tetrasomics: These are organisms with 2n+2 chromosomes. The two extra chromosomes along with the two normal chromosomes form a quadrivalent during meiosis. These are also characterised by sterility and are useful just like trisomics (Fig. 9.9).

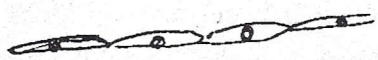


Fig. 9.9. Chain quadrivalent

A tetrasomic chilli with 2n=26 chromosomes was reported by Lakshmi and Nalini, 1987.

(iv) Monosomis and Nullisomis: Both monosomics and nullisomics are aneuploids. The organism is monosomic if it is lacking a single chromosome (2n-1) and nullisomic if a pair is absent (2n-2). In diploids these are viable only in exceptional cases. However, in allopolyploid they may be viable and fertile and offer special opportunities for chromosome manipulation and genetic engineering. In allotetraploid tobacco monosomics could be isolated and studied. All 21 monosomics and nullisomics have been isolated and studied by Sears in the Chinese spring variety of wheat. Nullisomy is tolerated by the hexaploid wheat species *Triticum aestivum*.

(a) Origin: The origin is similar to that of trisomics. If an 'n' gamete unites with (n-1) gamete monosomics are formed. If 'n' unites with (n-2) gamete nullisomics are formed.

n x (n-1) = 2n-1 (monosomic)
n x (n-2) = 2n-2 (nullisomic)

Nullisomics can be obtained by self fertilization of monosomics also.

(n-1) x (n-1) = 2n-2 (nullisomic)

(b) Cytology: During meiosis in monosmics, the monosome remained as a univalent. In rare cases, a trivalent may be formed when the monosome pairs with two homeologous chromosomes, it may pass to one of the poles undivided where it may or may not be included in telophase I nucleus or it may divide equationally with sister chromatids going to the same pole or to the opposite poles or it may misdivide. The proportion of n and (n-1) spores varies. Nullisomics yield (n-1) spores only.

(c) Uses: Just like trisomics, monosomics and nullisomics can also be used in gene location and identification of individual chromosomes. They can also be used in practical breeding programmes for transferring a whole chromosome or segment of a chromosome from one variety into another or from one species to another. They have also been used in wheat to develop substitution lines. In oats several nullisomics are male sterile. Hence, they can be used for the production of F_1 hybrid seed for commercial use.

5. SUMMARY

Variation in chromosome number produces two kinds of individuals viz., euploids and aneuploids. In euploid individuals, the somatic complements are the exact multiples of the basic chromosome number while aneuploids are individuals with irregular chromosome numbers. Euploids consist of haploids diploids and polyploids while aneuploids are made up of polysomics such as trisomics, tetrasomics, double trisomics, multiple trisomics and monosomics and nullisomics. In a true haploid only single set of chromosomes (one genome) is present. In diploid two similar genomes will be present. If more than two genomes are present they are designated as polyploids, the individuals may be triploid (3 genomes), tetraploid (4 genomes), pentaploid (5

genomes) etc. If all the genomes are similar they are called autopolyploids, if dissimilar, allopolyploids.

Individuals with gametic chromosome number are haploids and if they have basic number they are monoploids. Haploids cannot always be monoploids, but monoploids are always haploids. Haploidy may be a normal or abnormal condition. Haploids are characterised by small size and high sterility. During meiosis only univalents are formed. The presence of bivalents in haploid meiosis indicates presence of duplication and/or polyploidy. Haploids may originate by parthenogenesis, from intergeneric crosses from twin embryos by semigamy, by alien cytoplasm by anther culture and by chromosome elimination. Haploids are useful in crop improvement for the development isogenic pure lines, for the development of inbreds and disease resistant lines. Indirectly they are useful for the production of monosomics, nullisomics and mutants. High cost of production and difficulty in the production of haploids are the limitations of haploid breeding.

Aneuploids such as trisomics and monosomics can originate in disomics by the phenomenon of non-disjunction. Trisomics are organisms with 2n+1 chromosomes and they are of different types such as primary, secondary, tertiary, interchange, telo and compensating depending on the nature of the extra chromosome present. For example in primary trisomics, the extra chromosome is an unmodified chromosome while in secondary it is a chromosome with identical end regions. Each trisomic is associated with a specific phenotype. The meiotic behaviour of these trisomics vary depending upon the type. Tetrasomics are organisms with 2n+2 constitution. During meiosis they form a quadrivalent. Double trisomics 2n + 1 + 1 and multiple trisomics 2n + 1 + 1 + . . . n are other types of polysomics. Monosomics are organisms with 2n-1 chromosomes and nullisomics are 2n-2. These are not viable in diploids but they are viable in polyploids. During meiosis of monosomics, the monosome remained as a univalent. Two types of spores are formed n and n-1, while in nullisomics, the spores are always n-1. All these aneuploids are useful in gene location and identification of individual chromosomes but however, monosomics and nullisomics are useful in practical breeding programmes for the transfer of characters such as disease resistance from one variety to another and also in the development of substitution lines.

QUESTIONS

1. What are haploids? Describe various types of haploids and their role in crop improvement..
2. What is the difference between monoploids and polyhaploids? How would you induce them artificially? What is their importance in plant breeding?
3. Distinguish between monoploid and haploid. Explain various methods of developent of haploids in crop plants.
4. Discuss briefly the role of haploids in crop improvement.
5. Define aneuploidy. Give a brief account of aneuploids in crop plants with suitable examples.
6. Define monosomics, nullisomics and trisomics. Describe their origin, cytogenetics and uses.
7. Give an account of different types of trisomics, their origin and their role in crop improvement.
8. Differentiate between:
   (a) Monoplois and haploids

(b) *Monosomics and nullisomics*
(c) Primary and secondary trisomics
(d) Trisomics and tetrasomics

9. Write a short notes on:
   (a) Haploids
   (b) Monosomics
   (c) Nullisomics
   (d) Double trisomics
   (e) Tertiary trisomic
   (f) Ditertiary compensating trisomic

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CYTOLOGY

LESSON-X

AUTO AND ALLOPOLYPLOIDY AND EVOLUTION OF MAJOR CROPS

OBJECTIVES

1. Introduction
2. Autopolyploids
3. Effects of Autopolyploidy
4. Allopolyploidy
   i). Segmental allopolyploids
   ii). Amphidiploids
5. Evolution of major crop plants
   i). Natural Allopolyploid
   ii). Artificial Allopolyploids
6. Induction of polyploidy
7. Effects of polyploidy
8. Applications in crop improvement
9. Limitations of polyploidy
10. Summary

1. Introduction

An organism or individual having more than two basic or monoploid sets of chromosomes is called polyploid and such condition is called polyploidy. It is estimated that about $^1/_3$ species of flowering plants are polyploids. In wild species of grass family polyploidy has been reported upto 70%. In animals polyploidy is rare because of its lethal effects. It is found only in those species of animals which develop partheno-genetically like aphids. Polyploidy is of two types viz., (i) autopolyploidy, and (ii) allopolyploidy (Fig. 10.1).

2. Autopolyploids

These are polyploids with similar genomes. If the diploid species has its two similar genomes designated as AA, then an autotriploid becomes AAA and the autotetraploid AAAA. Autopolyploids include triploids (3x) tetraploids (4x), pentaploids (5x), hexaploids (6x), septaploids (7x), octoploids (8x) and so on. Autotriploids have three sets of chromosomes of the same species. They could arise as an offspring of a tetraploid and diploid parent or from diploid parents by the union of an unreduced and reduced gametes.

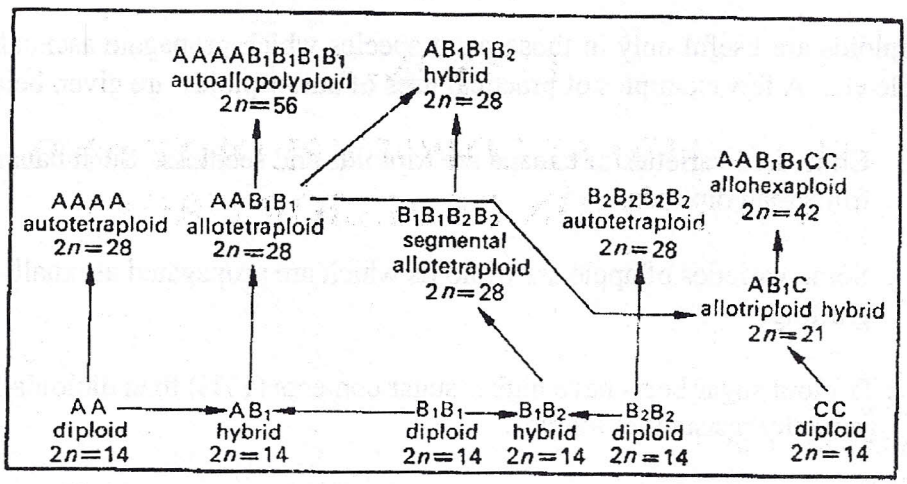
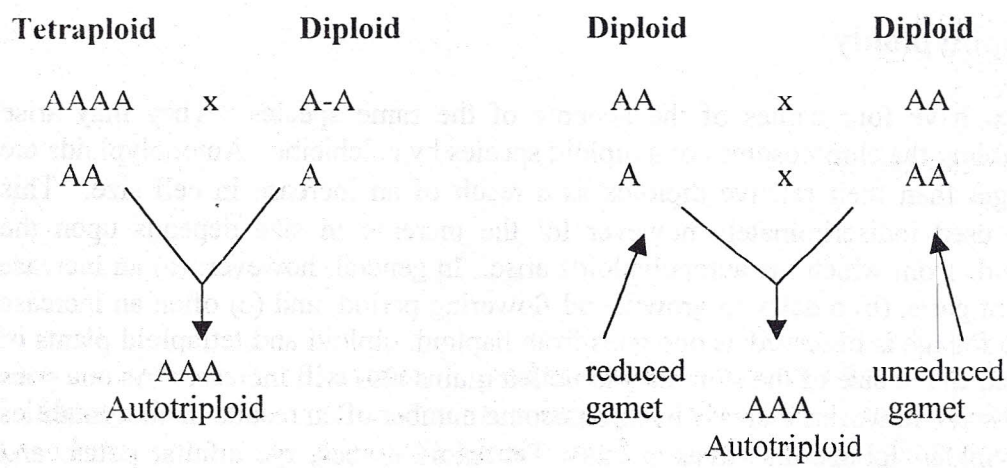
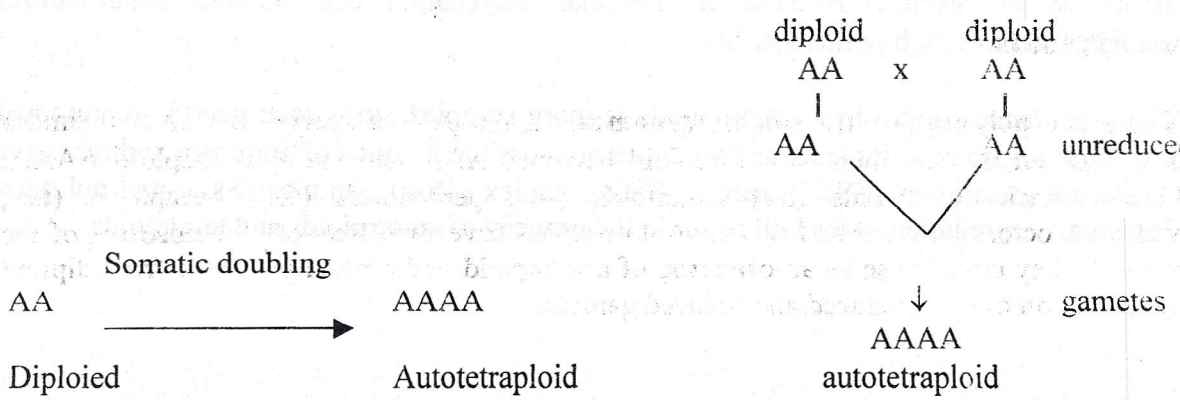


Fig. 10.1. Different kinds of polyploids and their derivation from one or more basic diploid species



The autotetraploid AAAA can originate directly from the diploid by doubling of its chromosome number by somatic doubling or by union of two unreduced diploid gametes.



Autotriploids are useful only in those plant species which propagate asexually like banana, sugarcane, apple etc. A few examples of practical uses of autotriploidy are given below:

a. Banana : Cultivated varieties of banana are triploids and seedless. Such bananas have large fruits than diploids.

b. Apples : Some varieties of apple are triploids which are propagated asexually by budding or grafting.

c. Sugarbeet : Triploid sugar beets have higher sugar contents (13%) than diploids (8%) and are generally resistant to moulds.

d. Watermelon: Triploid watermelons are seedless or have rudimentary seeds like cucumber. These are produced by crossing tetraploid female with diploid male. However, the reciprocal cross is not successful.

3. Effects of Autopolyploidy

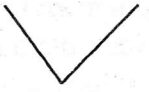
Autotetraploids have four copies of the genome of the same species. They may arise spontaneously by doubling the chromosomes of a diploid species by colchicine. Autopolyploids are in many respects larger than their relative diploids as a result of an increase in cell size. This criterion can not be used indiscriminately however for the increase in size depends upon the genotype of the diploids from which the autopolyploids arise. In general, however: (a) an increase in size of various plant parts, (b) a delay in growth and flowering period, and (c) often an increase in the darkness of the foliage is observed as one goes from haploid, diploid and tetraploid plants of the same genetic stock, d) the size of the stomata and pollen grains also will increase. As one goes beyond the tetraploid level, however, increase in chromosome number often results in abnormalities such as dwarfing, wrinkled foliage and weak plants. Tetraploid grapes, rye alfalfa, potato and coffee are well known examples of importance. Cytologically, the autopolyploids are characterised by the presence of multivalents formed at diakinesis and metaphase I stages of meiosis. In autotriploids the three homologues pair with each other to give trivalents and in autotetraploids, quadrivalents would result. Because of irregular segregation and meiotic abnormalities autotetraploids will have high pollen sterility.

The inheritance pattern in autotetraploids is more complex since each gene is represented four times. With regard to a single locus five stages are possible in terms of dominant and recessive genes. These are quadruplex (SSSS), triplex (SSSs), duplex (SSss), Simplex (Ssss) and nulliplex (ssss). Polysomic segregation ratios will result in the progeny of autotrploids and tetraploids.

4. Allopolyploidy

A polyploid organism which originates by combining complete chromosome sets from two or more species is known as allopolyploid or alloploid and such condition is referred to as allopolyploidy. This is a ploidy in which there is hybridization of two or more number of species followed by doubling of the chromosome number. An allopolyploid which arises by combining genomes of two diploid species is termed as allotetraploid.

diploid species x **diploid species**
AA x BB



AB sterile F_1
↓ doubling of ch. no.
AABB Allotetraploid

Allopolyploidy can be developed by interspecific crosses and fertility is restored by chromosome doubling with colchicine treatment.

Allopolyploids of more complex nature can arise in nature. A possible origin of allohexaploid is fusion of an AB gamete from allotetraploid with a 'C' gamete from species 'C' will give an F_1 triploid ABC which has three different genomes from three different species. If there is no or very little synapsis among the chromosomes high sterility is expected. Doubling of the chromosome number will give rise to the allohexaploid (AA BB CC) characterised by high fertility.

AABB x CC
AB C

ABC
↓ doubling
AA BB CC

The doubling of an allotetraploid will give an octoploid individual would lead to a complete polyploid type having the characteristics of both auto and allopolyploidy. Stebbins has referred to such an individual as auto-allo octoploid with the genomic formula AAAA BBBB. Such a genotype could arise through hybridization of two autotetraploids followed by doubling of the chromosome number. Most allopolyploids however appear to be allohexaploids having a general formula AA AA BB. **eg**:- *Phleum pratense* and *Solanum nigrum*.

i) Segmental allopolyploidy: In all the above allopolyploids, the chromosomes of 'A' genome have few or no homologies with the chromosomes of 'B' or 'C' and only autosyndesis (pairing between chromosomes of similar genomes) characterises the meiotic behaviour. However, sometimes there may be partial or complete synapsis between A & B genomes leading to quadrivalent formation during meiosis. Such a type of pairing between chromosomes of A & B genomes is called allosyndesis and Stebbins has referred to such individuals as segmental allopolyploids.

Most of the allopolyploids present in nature such as wheat, cotton and tobacco are the segmental allopolyploids only. Clearly therefore auto and allo polyploids are extreme spectrum of meiotic homologies ... autopolyploids are those with complete homology between all the sets of the chromosomes while allopolyploids represents the other end where there is complete lack of homology. The segmental allopolyploids fall in between the auto and allopolyploids where there are certain homologies between A & B genomes. Such a pairing between chromosomes of dissimilar genomes is called homoeologous pairing instead of homologous pairing. *Primula kewensis, Tradescantia canaliculata, Delphinium gypsophylum* are some examples of segmental allopolyploids.

ii) Amphidiploids: A plant which possesses two genomes from each of the two species is known as amphidiploid, since it is diploid for both parental genomes. (It is also allotetraploid but a plant combining three genomes from one parent with one of the other is likewise allotetraploid but not an amphidiploid). Amphidiploid hybrids are of special importance because they are usually fertile occur rather widely among angiosperms.

Amphidiploidy commonly arises through doubling of the chromosome number in the somatic cells of diploid hybrid. Such doubling may occur spontaneously and in numerous cases it has been induced with colchicine.

Advantages of amphidiploidy

The most valuable feature of amphidiploidy is the increased fertility over the diploids from which they arose. In addition, the plants may show certain characters associated with tetraploidy itself, such as sturdier habit or greater flower size. They also exhibit pronounced hybrid vigour.

Example: (1) *Primula kewensis*

One of the earliest known amphidiploid hybrids appearing in culture was the fertile *Primula kewensis* with 36 somatic chromosomes. A cross between *P. floribunda* (2n=18) and *P. verticellata* (2n=18) had yielded the sterile diploid hybrid *P. kewensis* (2n=18) with one genome from each parent species. From a lateral bud on this plant there arose spontaneously a tetraploid shoot with two genomes from each parent and this proved to be fertile. The numerical changes may be represented as follows:

P. floribunda x *P. verticellata*
FF 2n = 18 VV 2n = 18

9 9

2n =18 Primula kewensis
F V sterile
↓ Chromosome doubling
Fertile *Primula kewensis*
2n=36 FF VV

(2) *Raphano brassica*: 2n = 36 chromosomes

Raphanus sativus x *Brassica oleracea*
RR BB

n = 9 n = 9

doubling
Sterile RB ⟶ RRBB
n = 9 n = 18

(3) *Nicotiana tabacum* : 2n = 48

N. sylvesris x *N. otophora*

12 + 12 x 2 = 48

Plants spoken of as amphidiploids have arisen from crosses of species differing in chromosome number. Example *N. digluta* (2n = 72) arose from a cross of *N. glutinosa* (2n = 24) and *N. tabacum* (2n = 48) (12 + 24) x 2 = 72. *N. tabacum* is a tetraploid with two genomes from each of the species and *N. digluta* in terms of the basic number for the genus 12, would be allo-hexaploid with four genomes from another species. The genomic formula in such cases could be (n+2n) x 2 = 6n. Whether such plants which carry the combined somatic complements of the parents are to be called amphidiploids or allohexaploids because of their number of basic genomes is still under debate.

Origin of amphidiploids: In all the above examples amphidiploids originated as a result of hybridization between two different diploid species followed by somatic doubling. Amphidiploids sometimes arise in ways other than somatic doubling. Unreduced diploid gametes some times may

unite and form amphidiploids. Eg. *Madia,* a genus of western composites has arisen by the union of unreduced gametes of *Madia nutans* n = 9 and *Madia rammi* n = 8.

The fertile amphidiploid is with 2n = 34 chromosomes 18 from one parent and 16 from another.

A third important method of obtaining amphidiploids is by crossing two diploid autotetraploid strains. Eg: *Antirrhinum.*

5. Evolution of major crop plants

(i) Natural Allo-polyploids: Allo-polyploidy is found in about 50% of crop plants. Some important natural allopolyploid crops are wheat, cotton, tobacco, mustard, oats etc.

(a) Wheat: The bread wheat (*Triticum aestivum*) is an allopolyploid. It is believed that a genome of wheat has come from *T. monococium*, 2n=14, D genome from *T. tauschi* (2n=14) and B genome from unknown source probably from an extinct species 2n=14. Thus hexaploid wheat has two copies of the genomes from three species. First allotetraploid *T. turgidum* developed from a cross between *T. monococcum* and unknown species of B genome. Then cross between *T. turgidum* and *T. tauschi* resulted in the development of hexaploid wheat *T. aestirum.*

(b) Tobacco: There are two cultivated species of tobacco viz., *N. tabacum* x *N. rustica. N. tabacum* is an amphidiploid between *N. synvestris* (2n=24) and *N. tomentosa* (2n=24). *N. rustica* is believed to be amphidiploid between *N. paniculata* and *N. undulata.* Each of these species has 2n=24.

(c) Cotton: The tetraploid American cotton (*Gossypium hirsutum*) is believed to be an amphidiploid between *G. africanum* and *G. raimondii.* Both these species are diploid with 2n=26. The chromosomes of *G. africanum* are larger than *G. raimondii.*

(d) Oat: The cultivated Oat (*Arena sativa* n=21) is an allohexaploid which is considered to have originated form a cross between *A. barbata* (tetraploid n=14) and *A. strigosa* (a diploid n=7).

(e) Brassica: In *Brassica* there are three basic species viz., *B. nigra* (BB n=8), *B. oleracea* (CC, n=9) and *B. campestris* (AA, n=10). The cross between *B. nigra* and *B. oleracea* gave rise to *B. carinata.* Cross between *B. campestris* and *B. oleracea* led to the development of *B. napus* and cross between *B. campestris* and *B. nigra* resulted in the development of *B. juncea.* All the resulting species are amphidiploids.

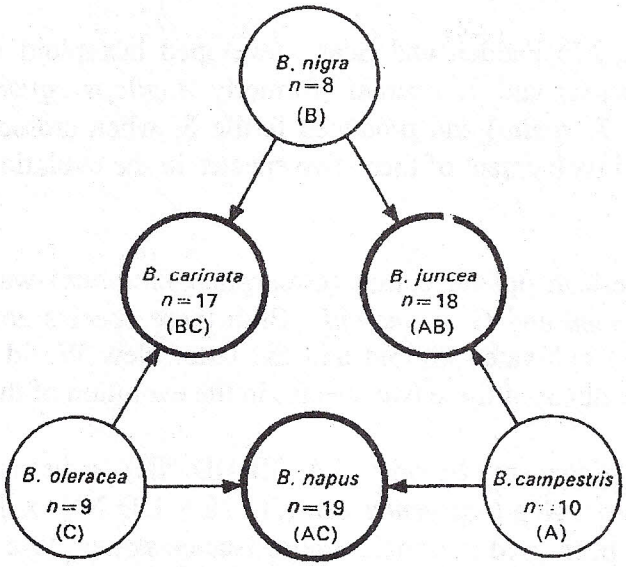


Fig. 10.2. Triangle of U explaining the origin of three tetraploid cultivated *Brassica* species from three diploid species

(ii) Artificial Allopolyploids: Artificial allopolyploids have been synthesized in some important crop plants to understand the process of origin of natural allopolyploids and also to explore the possibilities of creation of new species. A few examples of synthesized allopolyploids are given here.

(a) Raphanobrassica: This is a classical example of artificially synthesized allopolyploid. This was developed between radish (*Raphanus sativus* n=9) and cabbage (*Brassica oleracea* n=9) by Russian geneticist karpechenko (1926). He wanted to develop a fertile hybrid species between these two species with roots of radish and leaves of cabbage. But he got a fertile amphidiploid (4n=36) by spontaneous chromosome doubling which unfortunately had roots of cabbage and leaves of radish. Thus it was of no economic use.

(b) Tobacco: Clausen and Good speed synthesized a new hexaploid species of tobacco (*Nicotiana*) from a cross between *N. tabacum* (2n=48) and *N. glutinosa* (2n=24). The F_1 was sterile with 2n=36 chromosomes. This was made fertile by doubling of chromosome number through colchicine treatment. The new species is known as *N. digluta*.

(c) Triticale: Triticale is a new crop species which has been synthesized from a cross between wheat *T. aestivum* and rye (*Secale cereale*, n=7). Some triticales are developed from cross between tetraploid wheat (*Triticum turgidum*) and rye and some from cross between hexaploid wheat (T. aestivum) and rye. The F_1 was sterile which was made fertile by colchicine treatment. Triticales produced from tetraploid and hexaploid wheat are hexaploid and octoploid respectively. Triticale is now commonly grown in Canada, Mexico, Hungary and some other countries.

(d) Wheat: In wheat, Mc Fadden and Sears developed hexaploid between *Triticum turgidum* (formerly *T. dicoccum*) and *T. tauschi* (formerly *Aegilops squarrosa*). This resembled *T. aestivum* (formerly *T. spelta*) and produced fertile F_1 when crossed with natural *T. aestivum*. This suggested the involvement of these two species in the evolution of *T. aestivum* in the long past.

(e) Cotton: The American upland cotton (*Gossypium hirsutum*) was synthesized from a cross between *G. herbaceum* and *G. raimondii*. Both these species are diploid with 2n=26. The former is old world cultivated diploid and the latter New World wild diploid. This clearly indicated the involvement of these two species in the evolution of the upland cotton.

(f) *Aegilotricum*: *Aegilotricum* (2n=56 = AA BB DD NN) is the name given to an amphiploid derived from the cross *Aegilops ventricosa* (2n=28 = DD NN) x *Triticum turgidum* (2n=28 = AA BB). This has been used to transfer the resistance against 'eye spot' disease from *Aegilops ventricosa* to wheat. By repeated backcrossing of the amphiploid with wheat a new resistant cultivar of wheat viz., 'Roazon' was produced.

(g) *Agrotrichum:* *Agrotrichum* was obtained as an amphiploid from a cross of *Agropyron intermedium* 2n = 42 E_1 E_1 E_2 E_2 NN x Triticum aestivum (2n=42 = AA BB DD). The vigour and fertility of this hybrid were however low. Through repeated backcrossing of the F_1 with 6x wheat a partial amphiploid TAF_{46} was obtained which had 56 chromosome and genomic formula: AA BB DD XX where X is a new reconstructed genome. This partial amphiploid was later used to obtain alien addition lines for *A. intermedium* chromosomes to wheat which were successfully utilized for transfer of resistance against rusts to wheat.

6. Induction of polyploidy

Polyploidy was mainly induced by treatment with an alkaloid chemical colchicine. This is obtained from the seeds of a plant, *Colchicum autumnale* of the family Liliaceae. Colchicine does not affect *Colchicum* from which it is extracted because this plant has an anticolchicine substance.

Colchicine is applied in very low concentrations since high concentrations are highly toxic to the cells. For effective induction of polyploidy, usually the concentrations of 0.01% to 0.5% are used in different plant species. The colchicine induced polyploidy is known as colchiploidy. In plants colchicine is applied to growing tips, meristematic cells, seeds and axillary buds in aqueous solution or mixed with lanolin. The duration of treatment varies from 24 h – 96 h depending upon the species.

Colchicine induces polyploidy by inhibiting formation of spindle fibers. The chromosomes do not line up on the equatorial plate and divide without moving to the poles due to lack of spindle fibers. The nuclear membrane is formed around them and the cell enters interphase. Thus the nucleus has double the chromosome number.

7. Effects of Polyploidy

Polyploidy has marked effects on the morphology of the plants. The main features of autopolyploidy are increase in general vigour and size of various plant parts such as stems, leaves, flowers etc. Such a feature is generally referred as giagantism. Autopolyploids generally have the following important features:

i) Stems are thicker and stouter.
ii) Leaves fleshy, thicker, larger and deeper green in colour.
iii) Roots are longer and stronger
iv) Flowers, pollen, seeds stomata and guard cells are larger than in diploids.
v) Maturity duration is longer and growth rate is slower than in diploids.
vi) Water content are higher than diploids etc.

8. Applications in crop Improvement

Polyploidy plays an important role in crop improvement. Both autopolyploidy and allopolyploidy are useful in several ways. However, allopolyploidy has wider applications than autopolyploidy. Applications of autopolyploidy and allopolyploidy in crop improvement are briefly represented below.

i). Autopolyploidy: Both triploids and tetraploid have been used in crop improvement. However, their applications have been limited to few species only. Autotriploids have been developed in sugarbeets and weater melon. Triploid sugarbeets have larger roots and higher sugar content than diploids. Triploid water melons are seedless or have rudimentary and soft seeds like cucumber. The triploid seed is produced by using tetrapoid as female and diploid as male. The reciprocal cross is not successful. Moreover, triploid water melons have irregular shape and fresh seeds have to be made every year.

Autotetraploids have been developed in forage crops like berseem, alfalfa and rye, vegetables like radish, turnip, cabbage, *Capsicum* and fruits like grapes. Tetraploid varieties of rye have been grown in Sweden and Germany. They have larger seeds and higher proteins than diploids. Tetraploid grapes have been developed in California, USA, which have larger fruits and fewer seeds per fruit than diploids. Tetraploid varieties of alfalfa are better than diploid in yield and recovery after grazing. However, tetraploid cabbage and turnips have higher water contents than diploids.

Allopolyploidy: Allopolyploidy is useful in four principal ways viz., (i) in tracing the origin of natural allopolyploids, (ii) in creating new species, (iii) in interspecific gene transfer, and (iv) as a bridging species.

(i).Tracing the origin of crop species: Allopolyploidy plays an important role in determining the origin of natural allopolyploids. Study of chromosome pairing in a cross between allopolyploid and

a diploid species helps in tracing the origin of polyploid species. The affinity in pairing indicates the involvement of diploid species in the evolution of such polyploid. Lack of pairing between chromosomes of the species rules out the involvement of diploid species in the origin of polyploid under study.

(ii) Creation of New Species: Allopolyploidy sometimes leads to the creation of new species. Triticale is the best example which is an allopolyploid between wheat and rye. It combines the desirable qualities of both the species i.e. grain quality of wheat and hardiness of rye. Triticales are of two types viz., primary triticales and secondary triticales. Primary triticales are derivatives of cross involving either tetraploid wheat or hexaploid wheat with rye. Secondary triticales are derivatives of the cross either between two primary triticales or between primary triticale and wheat. These are superior to primary trtiticales in several aspects. Presently cultivated strawberry originated from a cross between North and South American species in the middle of 17^{th} century in Europe. Longanberry was developed from a cross between rasp berry and blackberry in 1880 in California, USA.

(iii) Interspecific gene transfer: When the desirable character is not found within a species it is transferred from the related species. Interspecific gene transfer is made in two ways viz., by alien addition and alien substitution. In the case of alien addition, one chromosome of wild species is added to the normal complement of a cultivated species. In the case of alien substitution one pair of chromosome is substituted in cultivated species with those of wild donar species. Such type of gene transfer is generally made for disease resistance. This type of gene transfer has been achieved in crops like wheat, tobacco, cotton and oats. In cotton lint strength has been transferred from *G. thurburi* to *G. hirsutam*. However, in such gene transfer several undesirable genes are transferred along with desirable ones. In tobacco, mosaic resistance has been transferred from *N. glutinosa* to *N. tabacum* through alien addition. Similarly leaf rust resistance has been transferred from *Aegilops* to wheat through alien addition and substitution.

(iv) Bridging cross: Sometimes direct cross between two species is not possible due to sterility in F_1. In such a case, first an amphidiploid is made between such species and then the amphidiploid is crossed with the recipient species. Such bridging crosses have been made for transfer of genes from wild species especially in crops like tobacco and cotton.

9. Limitations of Polyploidy

Polyploidy has several limitations. They are

(i). Limited use: The single species polyploidy has limited applications. It is generally useful in those crop species which propagate asexually like banana, potato, sugarcane, grapes etc.

(ii) Difficulty in maintenance: The maintenance of polyploidy is not possible in the case of sexually propagating species because of the formation of aneuploid gametes and sterility associated.

(iii) Undesirable characters: The characters associated with polyploidy are sometimes undesirable as in the case of *Raphano brassica*.

(iv) Some other defects: Induced polyploids have several defects such as low fertility, genetic instability, low growth rate, late maturity etc.

(v) Chances of developing new species through allopolyploidy are extremely low.

10. Summary

Organisms with more than two genomes are called polyploids. Polyploidy is of two types autopolyploidy and allopolyploidy. Polyploids with similar genomes are called autopolyploids. They may be autotriploids, tetraploids, hexaploids, octoploids etc. Autopolyploids are larger than their relative diploids with delayed flowering and dark green foliage. They are characterised by the presence of multivalents. The inheritance pattern in autopolyploids is complex since each gene is represented many times. Tetraploid grapes, triploid watermelons, sugar beets are polyploids of commercial importance.

Allopolyploidy is a type of polyploidy imposed on hybridisation. Here the genomes are dissimilar since they are developed by interspecific crosses and fertility is restored by chromosome doubling with colchicine treatment. Complex allopolyploids, segmental allopolyploids and amphidiploids are allopolyploids of considerable importance. Natural allopolyploidy is found in about 50% of crop plants. Some important crop plants like wheat cotton, tobacco, mustard, oats etc., are natural polyploids. *Rahhano brassica*, tobacco Triticale, wheat, cotton, *Aegilotricum, Agrotrichum* are allopolyploids which are synthesized artificially. Colchicine the polyploidising agent is obtained from the seeds *Colchiceme autumnale* which belongs to the family Lilaceae. Allopolyploidy has wider applications than autopolyploidy in crop improvement. Allopolyploidy is useful in tracing the origin of natural polyploids, in creating new species, in interspecific gene transfer and as a bridging species. In auto and allopolyploids autosyndesis is a rule while in segmental allopolyploids allosyndesis takes place.

Questions

1. Compare and contrast between autopolyploids and allopolyploids giving suitable examples.
2. Discuss the origin, occurrence, meiosis and genetics of autopolyploids.
3. Discuss the role of allopolyploidy in the evolution of crop plants.
4. Write critical notes on:
   (a) Amphidiploids
   (b) Segmental allopolyploids
   (c) Autosyndesis
   (d) Allosyndesis

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LESSON-XI

PLANT INTRODUCTION

OBJECTIVES

1. Introduction
2. Means & Introduction
3. Sources of Plant Introduction
4. Types of Plant Introduction
5. Uses of Plant Introduction
6. Procedure of Introduction
7. Plant Introduction Organizations
8. Acclimatization
9. Domestication
10. Achievements of Plant Introduction
11. Merits and Demerits of Plant Introduction
12. Summary

1. Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into new environment where they were not grown before. In other words, it is the process of introducing crop plants into new areas. Plant Introduction is an ancient method of crop improvement. Introduction may be from outside the country or within the country between two climatic regions. Maize, beans, potato, tomato, tobacco, American cotton, rubber, pepper etc., are introduced into Old world from New world. Crops like wheat, barley, oats, rice, sugarcane, bananas, coffee and many fruits, vegetables etc., were brought to the new world. Some examples of within the country introduction are the popularisation of grape cultivation in Haryana, of wheat in West Bengal and of rice in Punjab. It can be applied to all the three groups of crops namely self pollinated, cross pollinated and asexually propagated species.

2. Means of Introduction

Earlier introduction of plants from one place to another used to be done by travellers, traders, invaders and merchants. Now specific organisations like IPGRI (International Plant Genetic Resource Institute) and NBPGR (National Bureau of Plant Genetic Resources) etc., have been established for this work.

The plant material is obtained through expeditions, personal visit, correspondence, exchange of material, purchase or as a gift. The introductions may be in the form of seeds for seed propagated crops or in the form of cuttings or propagules in vegetatively propagated crops.

3. Sources of Introduction

Collection of plants for introduction is from five important sources. They being:

(i) From centres of diversity,
(ii) From gene or germplasm banks,
(iii) From gene sanctuaries,
(iv) From seed companies, and
(v) from farmer's fields

4. Types of Plant Introduction

On the basis of adaptation, plant introduction is classified into two types viz., primary and secondary. Based on utilization it is classified into direct and indirect types.

i) Primary Introduction

If the introduced variety is well suited to the new environment, it is released for commercial cultivation without any alteration in the original genotype. This constitutes primary introduction. There are several examples of crops in which direct use of introduced material is successful. In wheat, Sonara 64, Lerma Rojo are examples of direct release of introduced material in India. Similarly, in semi-dwarf rice varieties Taichung Native 1, IR 8, IR 20 and IR 36 are examples of primary Introduction. Many more examples of this type can be cited from other field crops and fruit crops.

Introductions that are immediately adopted to the changed environment are known as direct introduction. Hence primary introduction can also be called direct introduction. Any foreign variety directly recommended for commercial cultivation in a new country is called exotic variety.

ii) Secondary Introduction

The introduced variety, instead of direct utilization if subjected to selection to isolate a superior variety, or if it is hybridized with local varieties to transfer one or few characters from this variety to the local ones, it is known as secondary introduction. Examples of secondary introduction are wheat varieties Kalyana, Sona and Sonalika were released after selection from mexican wheat varieties semi dwarf wheat and rice varieties developed through hybridization with introduced varieties. Several other examples can be cited from other crops. Secondary introduction is much more common than primary introduction. Secondary introduction is also called indirect introduction as it take some years for adaptation to the new environment.

5. Uses of Plant Introduction

Introduction is mainly for carrying out 5 purposes. They being:

(i) For economic use
(ii) For the study of origin and evolution of crop plants
(iii) For conservation of diversity
(iv) For genetic improvement of crop plants
(v) For satisfaction of aesthetic interest.

(i) **Economic use:-** New crop species introduced may serve as new sources of food, vegetables, oil, fiber, wood or timber and fruits. In other words, new crop species are added to enrich the existing field and horticultural crops, forest species and medicinal plants of economic use.

(ii) **For the study of origin and evolution:-** Plant introduction serves to help in the study of origin and evolution of crop plants and their wild relatives in different geographical regions. The study also brings to light about their place of origin based on distribution of crop plants in different regions of the world Vavilov described centres of origin of cultivated species and other investigators identified ancestral stocks of different crop species.

(iii) **Conservation of diversity:-** The genetic diversity of crop plants is gradually being eroded due to clean cultivation, deforesting, development of townships and various other factors. Introduction is helpful in conservation of plant genetic diversity and saving certain species from extinction.

(iv) **For genetic improvement of crop plants:-** New plants are introduced for genetic improvement of crop plants. The introduced material is used for the transfer of desirable characters to the cultivated species.

Sometimes, the crop is introduced into a new area to protect it from diseases and pests. Coffee was introduced in South America from Africa to prevent losses from leaf rust. Hevea rubber on the other hand was brought to Malaya from South America to protect it from a leaf disease.

(v) **Aesthetic interest:-** Ornamentals, shurbs and lawn grasses are introduced for beautification of gardens, parks, offices, houses, bungalows and roads in big cities to satisfy the finer sensibilities of man. These plants are used for decoration and are of great value in social life.

6. Procedure of Introduction

In India, plant introduction for different crop plants is carried out by NBPGR (National Bureau of Plant Genetic Resources), New Delhi. The indenter has to inform the details of plant material to be introduced and the place where the material is present to the NBPGR. Then the NBPGR with the help of IPGRI arranges introduction of such material. The sender has to issue phyto-sanitary certificate along with the material. This certificate indicates that the material is free from insects, diseases and weed seeds. The imported material is examined by quarantine department of the imported country for absence of pests, diseases and absence of weed seeds before supplying to the indenter. The material of seed propagated species is imported in the form of seeds and those of asexually propagated species in the form of cuttings or propagules. Thus export and import of plant material is routed through NBPGR.

7. Plant Introduction Organizations

There are two types of plant introduction organizations, international and national. International Plant Genetic Resource Institute (IPGRI) located at Rome, Italy deals with global plant introductions. Various other international crop Research Institutes also undertake plant introduction work but only in collaboration with IPGRI. In India, plant introduction work was undertaken by NBPGR, New Delhi and deals mainly with the introduction of Agricultural and Horticultural crops. NBPGR has four substations for the testing of introduced plant material which are located in different agroclimatic zones of India. They are located at:

1. Simla, situated in Himachal Pradesh and represents temperate zone which is approximately 2,300 m above sea level.
2. Jodhpur, Rajasthan. It represents arid zone.
3. Kanyakumari, Tamil Nadu – represents the tropical zone.
4. Akola, Maharashtra. It represents the mixed climatic zone. It was recently shifted from Amaravathi.

In addition, a new substation at Shillong has been recently established for collection and testing of germplasm from North-East India.

In addition to these two, more organizations viz., (1) Forest Research Institute (FRI), Dehradun and BSI (Botanical Survey of India), Calcutta are also concerned with Plant Introduction work. These institutes are concerned with introduction of forest, botanical and medicinal plants.

8. Acclimatization

Acclimatization refers to adaptation or adjustment of an introduced variety to the new environment. The existence of variability in the introduced material helps in faster

acclimatization. Thus races and varieties of cross pollinated species have better acclimatization capacity than pure lines and inbreds. Natural selection operates in the process of acclimatization. Mode of pollination also affects acclimatization. The better acclimatization capacity of cross pollinated species has been traced to the existence of more genetic variability in these species.

9. Domestication

Domestication is the process of bringing wild and weedy species under human management. The process of domestication started when man started selecting superior plants for his use. Domestication of wild species is still being done and is likely to continue in future also. This is because human needs are likely to change with time. Consequently, the wild species of little importance today may assume great significance tomorrow. This is true for micro-organisms producing antibiotics involved in nitrogen fixation. Man has domesticated various plants such as food crops, vegetable crops, fibre crops, medicinal plants, timber trees, useful microbes etc. Through domestication, man has improved plants for various attributes such as yield, quality, earliness, low toxin contents, resistance to biotic and abiotic stresses and several other characters. A notable case of recent domestication is that of several members of Euphorbiaceae producing latex. The latex of these plants may be commercially used for the extraction of petroleum products including petrol and diesel. The Department of Science & Technology, Government of India has initiated a project for cultivation of jojoba (*Simmondsia* sp.) in arid zones since the seeds contain oil which is highly suitable as an industrial lubricant. The improvements under domestication vary from species to species.

10. Achievements of Plant Introduction

New crop species introduced into India include important crops such as potato, maize, groundnut, chillies, coffee, rubber, guava, grape, pineapple, papaya etc. Several ornaments like Gulmohar, Phlox, Salvia, Aster etc., are introductions, Soyabean, Sugar beet, oil palm and jojoba are some other recent introductions.

Crop improvement has been achieved through introduction:

(i) direct as a variety
(ii) as a variety after selection
(iii) as a parent in hybridizatien

(i) **Direct as variety:-** Semi dwarf wheat varieties Sonara 64 and Lerma Rojo were released directly for cultivation. TNI rice was introduced from Taiwan and directly released as a variety. Other introduced rice varieties are IR 81, IR 21 and IR 36 from IRRI Phillippines. Brass and Lee varieties of Soyabean, Ridley wheat and Kent Oats from **Australia**, introductions of vegetable crops such as onion, cow pee, cauli flower, lettuce, sioux tomato from USA, are directly released as varieties.

a) Varieties selected from Introductions

Many varieties have been selected after Introduction. Egyptian cotton variety Sujata was released after selection from Egyptian variety Karnak. Variety PRS 72 in cotton was selected from Russian material. Kalyana Sona and Sonalika are the result of selection from mexican wheats. Other selections include Jam Nagar Giant and Improved Ghana bajra, Pusa Lal and Pusa Suchari sweet potato, Pusa Basmati vegetable cow pea and Japanese white and 40 days radish etc.

b) Varieties developed through hybridization

Introductions have contributed to crop varieties through hybridization in almost all important agricultural and horticultural crops. All semi dwarf varieties of wheat and paddy are developed through the use of introduced material. Many hybrids of maize, sorghum and pearl millet have been developed using introduced material as one of the parents. The pioneer cotton hybrid H_4 has been developed from a cross between Gujarat 67 x American Nectariless.

11. Merits and Demerits of Plant Introduction

Merits:

(1) It provides entirely new crop plants.

(2) It provides superior varieties either directly or after selection or hybridization.

(3) This is a good method of collection and conservation of germplasm of different crops to protect the same from genetic erosion.

(4) Species having the danger of extinction can be saved from shifting them to other suitable areas.

(5) It is a beautiful method of crop improvement applicable to all the three types of plants viz., cross pollinated, self pollinated and vegetatively propagated species.

(6) Plants may be introduced in new disease free areas to protect them from damage as in the case of coffee and rubber.

Demerits:

The disadvantages of Plant Introduction are associated with the entry of weeds, diseases and pests along with the introduced material. For example, weeds like *Argimone mexicana, Eichornia crassipes* and *Phylaris minor*, diseases like late blight of potato, coffee rust, bunchy top of banana, pests like potato tuber moth, wooly aphis of apple and fluted scale of citrus were all entered into India along with introductions.

In addition, some introduced species may disturb the ecological balance in their new home and may cause serious damage to the ecosystem. Example being rapid depletion of sub soil water reserves by the introduction of *Eucalyptus* species from Australia.

However, the entry of weeds, diseases and pests occurred during a period when quarantine was almost non-existent. Now chances of entry of new insects, diseases and weeds are remote due to rigid quarantine laws and check-up. But this underlines the necessity of vigilance by the officials responsible for enforcement of quarantine laws. An error, lapse or carelessness on their part may allow entry to a serious disease, pest or weed species which may cause considerable economic losses to the country.

12. Summary

Plant Introduction is an ancient method of crop improvement which is applicable to all three types of plants viz., self pollinated cross pollinated and asexually propagated plants. Plant introduction consists of taking a plant species or variety into a new environment where it has not grown before. Plant introduction is of two different types, primary and secondary. In primary, the introduced material without any alteration of the original genotype is released directly as a variety whereas in secondary, the introduced variety is subjected to either selection or hybridization with local varieties to be released as a variety. Introduction is mainly carried out for economic use, for conservation of diversity, to know the origin of a crop, for genetic improvement and finally for the satisfaction of aesthetic interest. At present, Plant Introduction is the responsibility of NBPGR situated at IARI, New Delhi. The bureau through it's substations carries out introduction, evaluation, multiplication and maintenance of germplasms. Quarantine of the introduced germplasms is also done by the bureau. The bureau supplies germplasm to scientists on request.

Questions

1. Describe in brief, the purpose of plant introduction and outline the various steps involved in plant introduction.
2. Describe some important achievements of plant introduction. Discuss the merits and demerits of plant introduction as a breeding method.
3. Write short notes on:
   (a) Plant introducing agencies
   (b) Quarantine
   (c) Uses of Plant introduction
   (d) Achievements of Plant introduction
   (e) Acclimatisation and domestication

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LESSON-XII

PURE-LINE SELECTION AND MASS SELECTION

OBJECTIVES

I. MASS SELECTION

1. Introduction
2. Types of mass selection
3. Procedure of mass selection
4. Main features of varieties developed by mass selection
5. Merits and Demerits
6. Achievements

II. PURE-LINE SELECTION

1. Introduction
2. Characteristics of pure-lines
3. Applications of pure-line selection
4. General procedure for pure-line selection
5. Advantages of pure-line selection
6. Disadvantages of pure-line selection
7. Differences between mass selection and pure-line selection
8. Summary

I. MASS SELECTION

1. Introduction

Mass selection is the oldest method of crop improvement commonly used in cross-pollinated crops than in self pollinated species. In this large number of plants of similar phenotype are selected and their seeds are mixed together to constitute a new variety.

2. Types of mass selection

There are two types of mass selection viz., positive and negative.

i) Positive:- When desirable plants are selected from a mixed population and their seeds are mixed together to grow further generation it is referred to as positive mass selection. This is the common type practised in land races for several years.

ii) Negative mass selection:- When only undesirable off type plants are removed from the field and the rest are allowed to grow further, it is known as negative mass selection. this is generally used for varietal purification in seed production and certification programmes. This helps in maintaining high level of genetic purity in the varieties especially in self-pollinated species.

3. Procedure of Mass selection

The procedure for mass selection is indicated schematically (Fig. 12.1).

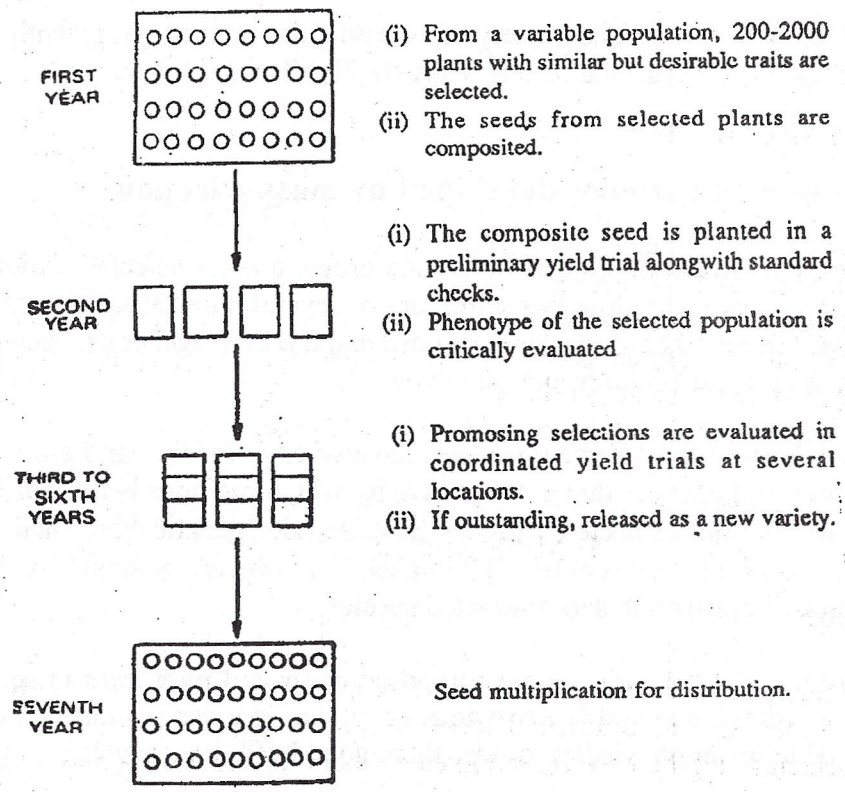

Fig. 12.1 Mass selection in self-pollinated crops as used to develop new varieties. For Maintaining the purity of pureline varieties, operations of the first year may be repeated every year or after every few years.

First year:- A large number of phenotypically similar plants are selected for their vigour, plant type, disease resistance and other desirable characteristics. The number of plants selected may vary from few hundred to few thousand. If too many plants are selected the improvement is likely to be small. But if too few plants are selected, the adaptation of the variety may become poor. Seeds from the selected plants are composited to raise the next generation.

Second year:- The crop is grown from the bulk seed of selected plants in a separate field using standard variety as a check for comparison of performance. In other words, the material is evaluated in preliminary yield trial. If mass selection is used for purification of old mixed variety the same old variety can be used as check for comparison.

Third to sixth year:- The performance of bulk is evaluated for yield and adaptation in main yield trials for 3-4 years using standard check for comparison.

Seventh and eighth year:- The variety is released and named in seventh year and seed is multiplied. In the eighth year, the seed is ready for distribution.

4. Main features of varieties developed by mass selection

(i) **Genetic constitution:-** In self pollinated crops, a mass selected variety is homozygous but heterogeneous because it is a mixture of several pure-lines. In cross pollinated crops such varieties are homo and heterozygous and are heterogeneous, because they consist of several homo and heterozygous genotypes.

(ii) **Adaptation:-** Mass selected varieties have wide adaptation and are more stable against environmental changes due to heterogeneity which provides better buffering capacity. In other words, mass selected varieties have broader genetic base than pure lines. They exhibit more or less stable performance. However, adaptability is more in cross pollinated crops than in self pollinated species.

(iii) **Variation:-** These varieties are composed of several pure lines in self-pollinated crops and of several homo and heterozygous genotypes in cross pollinated crops. Hence, there is heritable variation which provides them good buffering capacity.

(iv) **Selection:-** Selection is effective in case of mass selected varieties of self pollinated crops due to heritable variation. However, further selection in the mass selected varieties of cross pollinated crops may lead to inbreeding depression.

(v) **Quality:-** A variety developed by mass selection is less uniform in the quality of seed than pure lines due to presence of heritable variation.

(vi) **Resistance:-** Mass selected varieties are less prone to the attack of new diseases due to genetic diversity. In other words, they are more resistant or tolerant to new diseases. Periodic removal of off-type plants is essential to maintain the yield of mass selected varieties.

(vii) **Genetic basis:-** Mass selection is used both in self and cross pollinated species. In self pollinated crops a mass selection variety is a mixture of several pure lines. Thus it constitutes a homozygous but heterogenous population. In cross pollinated crops, a mass selected variety is a mixture of several homozygotes and heterozygotes. Thus such a variety is a heterozygous and heterogenous population.

5. Merits and Demerits

Merits:

(i) This is a good method for improvement of old varieties and land races. This is also used for purification of improved varieties.

(ii) Mass selected varieties are more stable in their performance than pure lines. In other words, they have more buffering capacity than purelines due to heterogeneity.

(iii) Mass selected varieties provide good protection against diseases.

(iv) Mass selection is a simple and quick method of crop improvement. It takes about 8 years for the release of new variety.

(v) This method is applicable to both self and cross pollinated species.

Demerits:

(i) The selection is based on phenotypic performance. The superior phenotype is not always an indication of superior genotype. Progeny test is not carried out in mass selection hence the real breeding value can not be ascertained.

(ii) Because of cross pollination, the selected plants are pollinated by both superior and inferior pollen parents. This results in rapid deterioration of variety developed by mass selection.

(iii) Large number of plants are to be selected for bulking because small sample will lead to inbreeding depression.

(iv) The produce of varieties developed by mass selection is less uniform than pure lines. This is because mass selected varieties are mixture of several pure-lines in self pollinated crops and consists of several genotypes in cross-pollinated species.

(v) In self-pollinated species, pure-line selection is more effective than mass selection, because pure line selection leads to isolation of the best line from a mixed or heterogenous population.

In order to overcome the defects of mass selection, three modifications have been suggested. These are:

(a) rejection of inferior pollen parents,
(b) use of composite pollen,
(c) stratification of field.

6. Achievements

Mass selection has played more significant role in developing new varieties in cross pollinated species than in self pollinated species. In India, mass selection has been useful in the development of improved varieties in cross pollinated crops like maize, pearl millet, mustard, cotton and sorghum. In self pollinated crops, it has been rarely used since pure line selection is more effective in such crops than mass selection.

II. Pure-line selection

1. Introduction

A pure line is the progeny of a single homozygous self-pollinated plant. As a result, all the individuals within a pure line have identical genotype and any variation present in a pure line is solely due to environment. In pure line selection a large number of plants are selected from a self-pollinated crop and are harvested individually. Individual plant progenies are evaluated and the best progeny is released as a pure line variety.

2. Characteristics of purelines

(i) All the plants within a pureline have the same genotype because, the present plant was homozygous and self-fertilized.

(ii) The variation within a pureline is environmental and non-heritable.

(iii) Purelines become genetically variable with time. The genetic variation is produced by mechanical mixtures, natural hybridization and mutation.

Pure lines are useful as a variety, as parents in hybridization programme, in studies of mutation and in biological investigations such as medicine, immunology, physiology, biochemistry, nutrition etc.

3. Applications of pure-line selection

(i) **Improvement of local varieties:-** A large number of varieties have been developed in local varieties. Some examples being NP4, NP52 wheat, NP11 and NP12 linseed, T1 Cowpea, Pusa Sawani Bhindi etc.

(ii) **Pure line selection in introduced varieties:-** Kalyana Sona wheat variety, shining mung-1, PS-16 etc., are examples of introduction from pure-line selection.

(iii) **Improvement of old purelines:-** Purelines will become variable with time. Selection is used to isolate new varieties from such genetically variable pure lines. Chafa gram, Jalgoan 781, Khargone, 1, Pusa Baisakhi, CO_2 and CO hung, B 76 urid were some examples. A dwarf off type from tall rice variety kali moonch 64 is selected and released as variety Shyama.

(iv) **Selection for a new characteristic in a pure-line:-** An example of selection of a new characteristic being isolation of jowar variety resistant to root rot caused by *Periconia cercinati*.

4. General procedure for pure-line selection

Pureline selection has three steps (Fig. 12.2).

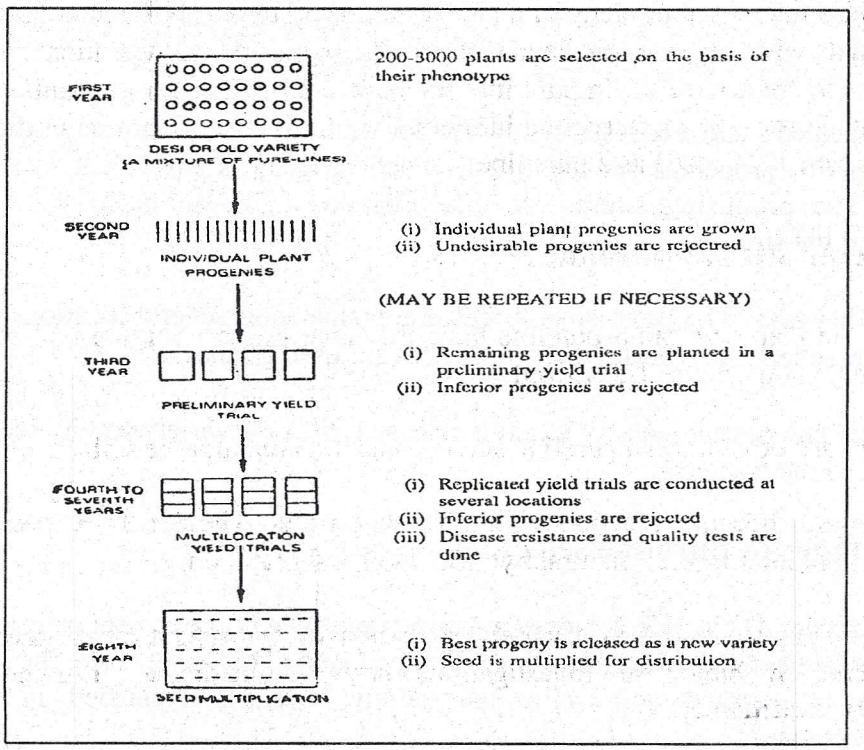

Fig. 12.2 Schematic representation of pureline selection in self-pollinated crops

Step One : First Year

The first step consists of selection of individual plants from a local variety. A large number of plants (200-3000) are selected from a desi or local variety or some other mixed population and their seeds are harvested separately. Superior genotypes are to be selected from the mixed population. It is advisable to select for easily observable characters such as flowering and maturity times, disease resistance, presence of awns, plant height etc. If desirable types are not included in the initial selection they cannot be expected to be present in the progeny.

Step Two : Second year

Progenies from individual plants are grown separately with proper spacing. The progenies are evaluated visibly. Poor, weak and defective progenies are discarded. Selection for the desired characters has been made. The superior progenies are harvested separately. The number of progenies should be reduced to facilitate replicated yield trials.

Step Three : Third year

This step consists of a replicated yield trial for a critical evaluation of the selected progenies. The best variety is used as a check and should be planted at regular intervals. **e.g.:** after every 20-25 progenies for ease in comparison. If enough seed is available, a preliminary yield-trial may be conducted.

Fourth year

Replicated yield trials are conducted for desirable characters using the best variety as a check. Superior progenies are identified. Each progeny is equivalent to a strain and it is a pureline. The promising strains are then included in co-ordinated yield trials for further evaluation of the strains.

Fifth-Eighth year: The promising strains are evaluated at several locations along with strains from other breeders. The best released varieties are used as checks.

Ninth year: The best progeny or strain is released as a new variety and its seed is multiplied for distribution to the farmers.

5. Advantages of pureline selection

(i) This type of selection achieves maximum possible improvement over the original variety.
(ii) They are extremely uniform since all the plants in the variety have the same genotype.
(iii) Due to extreme uniformity, the variety is easily identified in seed certification programmes.

6. Disadvantages of pureline selection

(i) These varieties do not have wide adaptation and stability in production possessed by the local varieties from which they are developed.

(ii) The procedure requires more time, space and more expensive yield trials than mass selection.

(iii) The upper limit on improvement is set by the genetic variation present in the original population.

7. Differences between mass selection and pureline selection

S.No.	Particulars	Mass selection	Pureline selection
1	Application	Used in both cross and self pollinated species	Used mainly in self pollinated species
2	Genetic constitution	Homozygous but heterogenous in self pollinated species. Heterozygous and heterogenous in cross pollinated species	Homozygous and homogeneous
3	Component lines	Mixture of several purelines in self pollinated species. In cross pollinated species, mixture of several open-pollinated genotypes	It is a progeny of single homozygote
4	Genetic variation	Present	Absent
5	Adaptation	has wide adaptation	narrow adaptation
6	Genetic base	has broad genetic base	narrow genetic base
7	Produce of a variety	less uniform	highly uniform
8	Progeny testing	not required	essential
9	Basis of selection	based on phenotype of plants	based on the progeny performance of the plants
10	Time required	6-7 years for the release of a variety	9-10 years
11	Vulnerability to new race of a pathogen	Low	High

8. Summary

Mass selection consists of selection of few hundred to few thousand phenotypically superior plants whose seeds are composited without progeny testing. Mass selection is simple, easy and less demanding. The new variety is stable and widely adapted.

Pure line selection consists of a large number of plants whose individual progenies are tested and the best progeny is released as a variety. These are extremely uniform genotypically and phenotypically. This type of selection achieves maximum improvement over the original variety. In India, a large number of varieties have been developed through this method.

Questions

1. Compare and contrast mass selection and pure line selection.
2. Define mass selection. Give its types important features, merits, demerits and achievements with examples.
3. Define pure line selection. Discuss briefly various steps involved in the development of a pureline variety.
4. Discuss briefly merits, demerits and practical achievements of pureline selection.
5. Write short notes on:
   (a) Pureline
   (b) Main features of mass selection
   (c) Types of mass selection
   (d) Merits and demerits of mass and pureline selection.

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LESSON-XIII

PEDIGREE METHOD, BULK METHOD, BACKCROSS METHOD, RECURRENT AND CLONAL SELECTION

OBJECTIVES

I. CLONAL SELECTION

1. Introduction
2. Breeding procedure for clonal selection
3. Merits and Demerits
4. Achievements
5. Summary

II. PEDIGREE METHOD

1. Introduction
2. Main features
3. Breeding procedure
4. Mass Pedigree Method
5. Merits and Demerits
6. Achievements
7. Summary

III. BULK BREEDING METHOD

1. Introduction
2. Main features
3. Breeding procedure
4. Merits and Demerits
5. Achievements
6. Single seed descent method
7. Summary

IV. BACKCROSS METHOD

1. Introduction
2. Main features
3. Breeding procedure
4. Achievements
5. Summary

V. RECURRENT SELECTION

1. Introduction
2. Main features
3. Types of Recurrent selection
4. Merits and Demerits
5. Achievements
6. Summary

I. CLONAL SELECTION

1. Introduction

Progeny of a single plant obtained by asexual reproduction is known as clone. The procedure of selecting superior clones from the mixed population of asexually propagating crops is referred to as clonal selection. Crops which are propagated asexually or by vegetative means are known as asexually propagated or vegetatively propagated or clonal crops. There are some agricultural (sugarcane, potato, sweet potato etc.,) and horticultural (banana, mango, citrus, apple etc.) crops that propagate by asexual means. The main reasons of asexual reproduction are: (a) reduced flowering and seed set, (b) non-flowering in many cases, (c) to avoid inbreeding depression in certain crops, and (d) apomixis in some species.

(a) **Non-flowering species:** This group includes garlic, ginger, betel and several yams.

(b) **Low seed setting species:** Sugarcane, potato, sweet potato etc.

(c) **Normal flowering and seed setting species**: Citrus, mango, pear, peach, apple. These are highly heterozygous and vegetative propagation is essential to maintain the heterozygous balance.

(d) **Apomictic species:** Seeds develop asexually in such species. Many fruit crops exhibit apomixis.

Main Features of clones: Clones have many important features such as: (1) homogenous constitution, 2) heterozygosity, (3) vigorous growth, (4) wider adaptation etc.

2. Breeding procedure of clonal selection

Improvement of asexually propagated crops by selecting superior clones is known as clonal section. Superior clones can be obtained from three types of material viz., (1) local variety, (2) introduced variety, and (3) intercrossed population. In other words, clonal selection can be practised in the above three types of material. The general procedure of clonal selection is as follows:

(a) In the first year, superior plants are selected from a mixed population of vegetatively propagated crop. Superior plants are selected on the basis of yield, maturity, disease resistance etc.

(b) In the second year, the progeny of each selected plant is asexually propagated and grown separately for seed increase and evaluation. Clones superior to the check variety are selected and evaluated in replicated preliminary trials in the third year.

(c) Multilocational trials are conducted in 3 & 4 years using standard variety as check. Superior clones are identified for release.

(d) The best clone is released as a variety and its seed is multiplied for distribution. Thus release of new variety by this method takes 9-10 years.

Besides clonal selection interspecific hybridization and mutation breeding are also used for improvement of clonal crops. In sugarcane modern cultivars have been derived from crosses of *Saccharum officinarum* with *S. spontaneum* or *S. barberi*. These crosses are useful in combing high sugar content of the first species with disease resistance, cold tolerance and vigour of last two species. Backcross method is used for transfer of desirable genes from wild species to the cultivated species in sugarcane and potato.

3. Merits & Demerits

a. Merits:

1. This method conserves heterosis for several generations. The variety retains the characteristics of parental clone for several years.

2. They are highly uniform like purelines. They are highly stable as there is no risk of deterioration due to segregation and recombination.

3. It is useful for isolation of best genotypes from a mixed population of asexually propagated crops.

b. Demerits:

(i) The varieties developed by clonal selection are highly prone to diseases.
(ii) It can not create variability and therefore genetic make up cannot be improved without hybridization.

4. Achievements

In India, new varieties have been developed in potato, sugarcane, banana, citrus and grapes. Varieties kufri red and kufri safed in potato, KO11, KO22 and Neelam in mango; and Bombay green, pride monthan and high gate in banana have been developed by clonal selection. Several varieties of sugarcane (CO 541, CO 5510, CO 1148, CO 1558 etc.) and potato (Kufri Sindhuri), Kufri Kuber, Kufri Kundan, Kufri Chamatkar etc.,) have been developed by interspecific hybridization followed by clonal selection.

5. Summary

Progeny of a single plant obtained by asexual reproduction is called a clone. The procedure of selecting superior clones from a mixed population of asexually propagating plants is called clonal selection. Clones have homogeneous constitution, heterozygosity, vigorous growth and wider adaptation. The variety released by clonal selection is highly uniform. Clonal selection can be practised in local varieties, introduced varieties as well as in intercrossed populations. The method is useful for retention of heterosis for several generations. They are highly stable but they are highly prone to new races of a disease. In India, clonal selection has been successfully used for developing new varieties in potato, sugarcane, banana, citrus and grapes.

II. PEDIGREE METHOD

1. Introduction

There are three methods for handling of segregating populations after hybridization. These are pedigree breeding, bulk breeding and backcross breeding. A brief description of pedigree method is given below:

2. Main features

Pedigree refers to record of the ancestry of an individual selected plant. Pedigree breeding is a method of genetic improvement of self pollinated species in which superior genotypes are selected from segregating generations and proper record of the ancestry of selected plants are maintained in each generation. In other words, it is a selection procedure in segregating population of self pollinated species that keeps proper record of plants and or progeny selected in each generation. Main features of this breeding method are given below:

(i) **Application:-** This method is widely used for the improvement of self-pollinated crops. It is used when both the parents involved in hybridization are well adapted and have good agronomic characters. It is more commonly used for the improvement of polygenic characters than oligogenic ones. In cross pollinated species, it is used for the development of inbred lines.

(ii) **Maintenance of pedigree record:-** Proper record of ancestry of each selected plant or plant progeny is maintained for all generations of selection. Important characters of each selected plant and progeny are recorded.

(iii) **Selection:-** In this method only human selection or artificial selection is used. Natural selection is allowed to operate only in modified form of pedigree breeding called mass pedigree method.

(iv) **Time taken:-** Generally taken 14-15 years.

(v) **Genetic constitution:-** The variety developed is homozygous and homogeneous because it is the progeny of single homozygote.

3. Breeding procedure

After selection of parents, the cross is made between selected parents. The F_1 material is grown using wide spacings. The dominance behaviour for various characters is recorded. In F_2 also the material is grown using wide spacings. Individual plant selection is practised in F_2. The progeny of each selected plant is grown separately which constitutes F_3 generation. In F_3 and F_4 generations, selection is practised within and between families. From F_3-F_8 between progeny selection is practised and superior progeny are identified and isolated in F_8. These progeny constitute strains. These strains are evaluated in replication multilocation trails for a period of 3-5 years. Based on superior performance, the strain is released as a variety. Thus release of new variety by this method takes 14-15 years. The number of plants to be grown and selected in each generation is not fixed but may slightly vary from crop to crop.

4. Mass pedigree Method

It is a modification of pedigree method developed by Harrington in 1937. Mass pedigree method refers to growing of segregating material by bulk method when conditions are unfavourable for selection and use of progeny testing when conditions are favourable for selection. For example, in wheat dry season is not suitable for selection f straw length, plant height, earliness and resistance to some diseases and shattering. The breeder has to wait for normal season for effective selection for above characters in wheat.

5. Merits and Demerits

Merits:

(i) This method provides information about the mode of inheritance of various qualitative characters which is not possible by other breeding methods.
(ii) There are chances of recovering transgressive segregants by pedigree method.

Demerits:

(i) The selected material becomes so large that handling becomes very difficult.
(ii) Maintenance of records is a time consuming tedious process.
(iii) Large number of progeny are rejected in this method so there are chances of elimination of some valuable material.

6. Achievements

Pedigree method has been extensively used for developing improved varieties in several self-pollinated crops like wheat, rice, pulses, barley, cotton and various vegetable crops. Some examples are given below:

Rice:- Ratna, Krishna, Sabarmati, Bala etc.

Wheat: HO2281, NO2402, Janak, Pratap etc.,

Cotton: LH 900, LH 1556, F 1378, H 1098, Anjali, Surabhi, Savin etc.

Pegion pea: T_{21}, Prabhat

Greengram: T_2, T_{44}, T_{51}, Sheela etc.

Chick pea: T_1, T_2, T_3, T_5 Radhey etc.

Pea:- Pant Matar 2, Jawahar Matarl etc.

7. Summary

Pedigree breeding is a method of improvement of self pollinated species in which superior genotypes are selected from segregating generations and proper record of the ancestry of selected plants are maintained in each generation. Several improved varieties have been developed in several self pollinated crops like wheat, rice, pulses, cotton and various vegetable crops.

III. BULK BREEDING METHOD

1. Introduction

This method is a selection procedure in which the segregating population of self pollinated species is grown in bulk (F_1-F_5) with or without selection, a part of the bulk seed is used to grow the next generation and individual plant section is practised in F_6 or later generations. This method is also termed as mass or population method.

2. Main Features

The concept of bulk breeding method was developed by Nilsson Ehle in 1908. The main features are as follows:

(i) **Application:** Applicable to the genetic improvement of self-pollinated crop plants. It is used when both parents have good agronomic characters and are adapted and when these characters are governed by polygenes.

(ii) **Handling of material:** The material is handled by bulk method from F_2-F_5 and by individual plant selection as in pedigree method from F_6 onwards. In crop breeding a 5-6 year period of bulking is usually adapted.

(iii) **Selection:** In this method, both natural and as well as artificial selection operate. Natural selection operates during bulk period and human selection operates in the later generations when individual plant selection is practised.

(iv) **Adaptation:-** It is an evolutionary method of crop improvement. Natural selection favours genotypes with better surviving capacity. But these genotypes may not be good agronomically. Hence, artificial selection should be practised during bulk period. The varieties developed by this method are more stable and more adapted.

(v) **Genetic constitution:** The end product of this method is a homozygous and homogenous population because it is the progeny of a single homozygote.

3. Breeding procedure

In this there are 4 important steps, viz., (i) Bulking period, (ii) Progeny selection and isolation of superior progeny, (iii) multi-location trials of superior progeny, and (iv) release of the best progeny as a variety.

(i) **Bulk period:** The F_1 plants are grown and their F_2 seeds are harvested in bulk. This process is repeated until the desired level of homozygosity is achieved. In general, bulk period is allowed upto F_5 generation. Undesirable genotypes are eliminated during this period.

(ii) **Progeny selection:** In F_6, the material is space planted and individual plant selection is practised. The progeny of each selected plant is grown separately in F_7 and superior progeny are selected and isolated in F_7 and F_8.

(iii) **Multi-location testing:** The selected progeny constitute strains. In eighth year, preliminary yield trial is conducted. From 10^{th}-14^{th} year multilocation testing is carried out and the best performing strain is identified on the basis of 3-4 years performance in the multilocation trials. The best strain is released and multiplied for seed distribution in the 15^{th} year. Thus bulk method takes 15-16 years for release of a new variety.

The bulk method should be tested under such environment which is expected to favour desirable genotypes. For example, screening for disease resistance should be done in the disease prone area under natural field conditions.

4. Merits and Demerits

Merits:

(a) It is simple convenient and less expensive method of crop improvement.

(b) In this method, natural selection operates which results in elimination of undesirable genotypes from the bulk population and increases the frequency of desirable plants.

(c) The chances of obtaining transgressive variants is more because of large number of plants grown.

(d) This method is useful to study the competitive ability of various genotypes in the population.

(e) This method leads to improvement in adaptation of genotypes due to bulk period

(f) There is no need to maintain pedigree records in this method.

Demerits:

(a) This method does not provide information about the mode of inheritance of oligogenic characters.

(b) This method is not preferred by plant breeders since it takes 20-30 generations for long term bulking and 6-10 generations for short term bulking.

(c) Natural selection sometimes may favour undesirable genotypes than favourable ones.

(d) It is difficult to assess the variability because they change in each generation of bulking.

5. Achievements

This method has been used in the improvement of barely in USA. More than 50 varieties have been developed from composite crosses by this method. In India, only one variety 'Narendra Raj' has been developed in 'Brown Mustard' by this method. The bulk method has limited application in practical plant breeding.

6. Single seed descent method

Single seed descent method is a modification of the bulk method in which only one seed is selected randomly from each plant in F_2 and subsequent generations. The selected seed is bulked and is used to grow the next generation. This is repeated upto F_5 to achieve the desired level of homozygosity. In F_6 large number of single plants are selected (400-500) and their

progeny are grown separately. In F_7 & F_8 superior progeny are isolated and grown in multilocational trials and the best progeny is released as a variety.

7. Summary

Bulk breeding is a selection procedure which is used in segregating population of self pollinated species in which material is grown in bulk plot from F_2-F_5 with or without selection, next generation is grown from bulk seed and individual plant selection is practised in F_6 or later generations. This method has limited application in practical plant breeding.

IV. BACKCROSS METHOD

1. Introduction

In backcross method, F_1 is crossed with either of its parents. When the F_1 is crossed with homozygous recessive parent it is called *test cross*. A system of breeding in which repeated backcrosses are made to transfer a specific character to a well adapted variety for which the variety is deficient is referred to as backcross breeding.

2. Main features

i) This method is used for improvement of specific characters such as disease resistance. It is commonly used for transfer of monogenic or oligogenic characters than polygenic characters. It is applicable to all three groups of crop plants viz, self pollinated, cross pollinated and asexually propagated.

ii) Parental material:- Backcross method involves two types of parents viz., recipient parent and donar parent. The parent which receives a desirable character is known as recipient parent. The recipient parent is repeatedly used in the backcross method, hence it is called recurrent parent. The recipient parent is generally a well adapted high yielding variety of an area which is deficient in one or few characters. The parent which donates the desirable characters is called donar parent. It is also called non-recurrent parent as it is used only once in crossing. This donar parent is generally poor in agronomic characters. Thus backcross method is used when one of the parents is an unadapted type.

iii) Genetic constitution:- It retains the genotype of the original variety except for the character which is improved by backcrossing. In other words, the new variety resembles the parent variety in all the characters except for the character under transfer.

iv) Number of backcrosses:- Generally 5-6 backcrosses are sufficient to retain the genotype of original variety with new character.

v) Basic requirements:- The basic requirements to start a backcross programme are: (1) recurrent parent, (2) donar parent, and (3) high heritability of the character under transfer.

3. Breeding Procedure

If the character is controlled by a dominant gene, the F_1 is backcrossed to the recurrent parent 6-8 times to obtain identical plants with the added gene. All the true breeding plants are bulked and new variety is released. For recessive gene transfer it is necessary to self the population after each backcross to obtain homozygous recessive plants while with dominant gene continuous backcrosses are made.

4. Achievements

Backcross method has been widely used for the development of disease resistant varieties in both self and cross pollinated species. It has also been used for interspecific gene transfer and development of multiline varieties in self pollinated species. Several disease resistance varieties have been developed by this method in wheat, cotton and several other crops. In cotton V 797, Digvijay, Vijalpa and Kalyan varieties which belong to *Gossipium herbaceum* have been developed by backcross method.

5. Summary

Crossing of F_1 with either of its parents is called backcrossing. When the F_1 is crossed with homozygous recessive parent it is known as test cross. The transfer of a specific character such as disease resistance from the donar parent to the recipient parent is called backcross breeding. This method is applicable to all the three types self cross pollinated and asexually propagated plants. It is the only method which is sued for interspecific gene transfer.

V. RECURRENT SELECTION

1. Introduction

It is a cyclic selection that is used to improve the frequency of desirable alleles for a character in a breeding population. This method was developed initially by Hayer and Garber (1919) and East and Jones (1920).

2. Main features

It is a modified form of progeny selection. It differs from progeny selection in two main aspects. Firstly, the selected plants are self pollinated in recurrent selection whereas they are open pollinated in progeny selection. Secondly, the progeny of selected plants are intercrossed in all possible combinations in this method whereas they are open pollinated in progeny selection.

(i) **Application:-** It is a method developed for improvement of cross pollinated crops but it is also used in self pollinated species.

(ii) ***Base population:-*** *A heterozygous base population is required to start recurrent* selection. The base population may be (a) an open pollinated variety, (b) a synthetic variety, (c) progeny of intercrosses among selected in breds, (d) a double cross, and (e) single cross.

(iii) **Important steps:-** A simple recurrent selection consists of 5 steps: (a) selection of superior plants from base population, (b) selfing of selected plants, (c) growing of progeny of selfed seed, (d) intermating among progeny, (e) bulking of crossed seed in equal quantity. This completes original cycle of recurrent selection. The bulk seed is used for next cycle of selection which also involves five steps.

(iv) **Use of end product:-** The population developed by recurrent selection can be used in 3 main ways: (a) In producing homozygous inbreds by selfing, (b) In the production of hybrid varieties, and (c) In the production of synthetic varieties.

(v) Basic assumption, Recurrent selection is based on 3 assumptions namely absence of epistatis, absence of multiple alleles and absence of linkage equilibrium.

(vi) **Impact:** Recurrent selection is used to improve the frequency of desirable alleles for a character in a population. The heterozygosity that is last due to selfing is recovered by intermating of selected progeny.

3. Types of recurrent selection

There are four types of recurrent selection, namely: (i) simple recurrent selection, (ii) recurrent selection for general combining ability (GCA), (iii) recurrent selection for specific combining ability (SCA), and (iv) reciprocal recurrent selection.

i. Simple Recurrent selection

In the first year, the superior plants for the character under improvement are selected from the heterozygous base population. These plants are grown from the selfed seed and intermating is done among the progeny. The crossed seed is bulked in equal quantity. This completes original cycle of selection. In the third year, bulked seed is grown and superior plants are selected and selfed like first year. In the fourth year, progeny of selected plants are grown from selfed seed and intermating is done like first year. The crossed seed is composited in equal quantity for use in the next cycle of selection. This completes first cycle of simple recurrent selection. Thus selection cycles may be repeated till the desired improvement is achieved. (Fig. 13.1)

The main features of this selection are:

(a) Tester is not used.
(b) It does not measure the combining ability.
(c) The selection is based on phenotype.
(d) The method is useful for only those characters which have high heritability.
(e) It requires only two seasons for completion of one cycle.

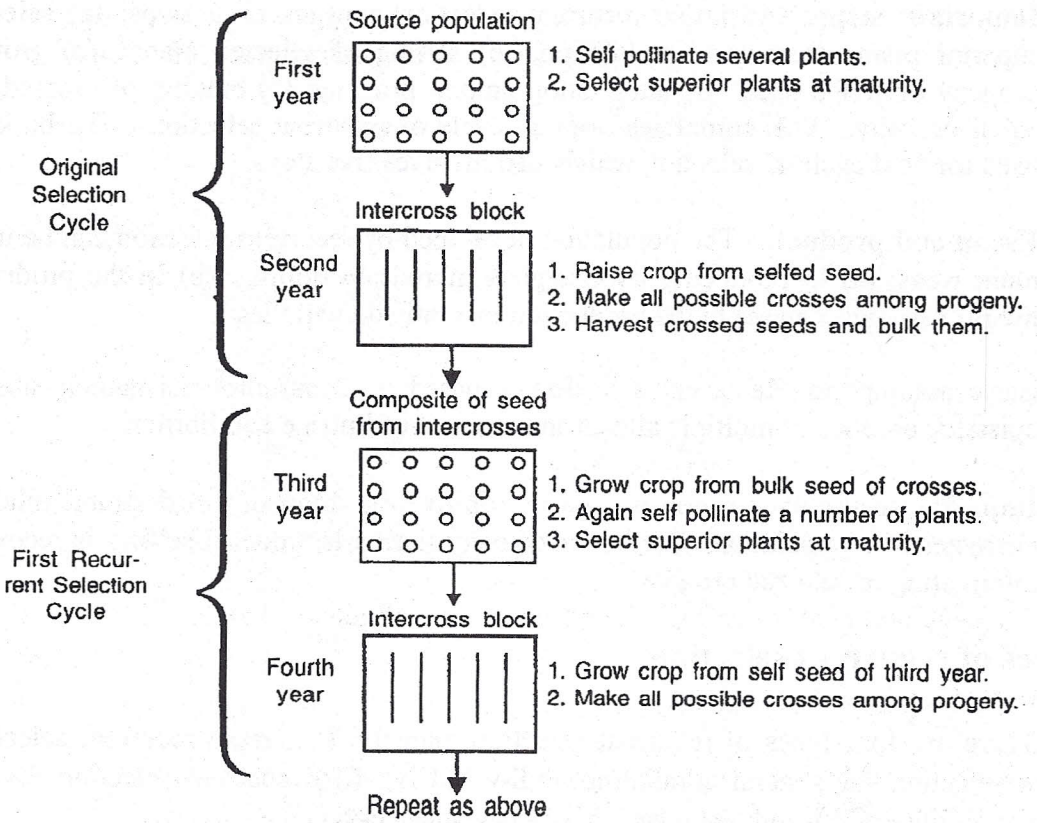

Fig. 13.1 Procedure of simple recurrent selection (from Allard, 1960)

The form of recurrent selection that is used to improve the general combining ability of a population for a character and includes heterozygous tester is called recurrent selection for general combining ability. If selection has been made for specific combining ability using homozygous tester it is called recurrent selection for specific combining ability. The form of recurrent selection that is used to improve both GCA & SCA using two heterozygous tester stocks is known as reciprocal recurrent selection.

4. Merits & Demerits

Merits:

(i) It is an efficient breeding method for increasing the frequency of superior genes in the population for various economic characters. Thus it is an important method of population improvement.

(ii) Repeated intermating of heterozygous progeny provides greater opportunities for recombination to occur. Thus this method helps in breaking repulsion phase linkages.

(iii) This method also helps in maintaining high genetic variability in a population due to repeated intermating of heterogenous plants.

(iv) The selection has been made on the basis of test cross performance (except in simple recurrent selection) and only selected plants are allowed for intermating.

Demerits:

(i) This method is not used directly for the development of new varieties. This is only a method of population improvement.

(ii) This method involves lot of selection, crossing and selfing work.

(iii) This method permits selfing which leads to loss of genetic variability.

5.Achievements

Recurrent selection has been successfully used for the improvement of oil content in maize, fiber strength in cotton and sugar content in sugar beet and sugar cane.

6. Summary

It is a modified form of progeny selection. It is a cyclic selection used to improve the frequency of desirable alleles for a character in a breeding population. There are 4 types recurrent selection: (1) simple, (2) recurrent selection for GCA, (3) recurrent selection for SCA, and (4) reciprocal selection. In simple recurrent selection tester is not used. Recurrent selection has been successfully used in maize, cotton, sugar beet and sugarcane for improvement of specific characters.

Questions

1. Explain briefly important features and procedures of pedigree breeding.
2. Give a brief comparison of pedigree and bulk method of breeding.
3. Define pedigree breeding and describe briefly its advantages and disadvantages.
4. Enumerate important features, procedure, merits, demerits and achievements of bulk breeding method.
5. Describe briefly various applications, merits, demerits and achievements of backcross method.
6. Define recurrent selection. Give important features of recurrent selection and its uses in plant breeding.
7. Define clone? What is clonal selection? Describe main features of clonal selection.
8. Write short notes on:
   (a) Clone
   (b) Clonal selection
   (c) Pedigree breeding
   (d) Bulk method
   (e) Backcross
   (f) Test cross
   (g) Simple recurrent selection.

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LESSON-XIV

HYBRIDIZATION

OBJECTIVES

1. Introduction
2. History of Hybridization
3. Objectives of Hybridization
4. Types of Hybridization
5. Hybridization programme
6. Procedure of Hybridization
7. Raising of F_1 generation
8. Difficulties in Hybridization
9. Consequences of Hybridization
10. Summary

1. Introduction

Hybridization is a technique to create new genetic variability. The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. In plants crossing is done by placing pollen grains from one genotype, the male parent on to the stigma of flowers of the other genotype, the female parent. The seeds as well as the progeny resulting from hybridization are known as hybrid or F_1. The progeny of F_1 are called segregating generations.

2. History of Hybridization

Compared to selection, hybridization is of recent origin. There is evidence that Babylonians and Assyrians hand-pollinated date palm as early as 700 B.C. for metaxenic effects of pollen. Sex in plants was discovered by Camararious in 1694. In 1717, Thomas fair child produced the first artificial hybrid by crossing sweet Willium (*Dianthus barbatus*) with carnation *Dianthus caryophyllus*. Subsequently, a large number of scientists used hybridization for scientific studies as well as for crop improvement. The first intergeneric hybrid *Raphano brassica* was produced in 1926 by Karpechenco.

3. Objectives of Hybridization

The chief objective of hybridization is to create genetic variation. The degree of variation produced in the segregating generations would depend on the number of heterozygous genes present in the F_1. This in turn depends on the fact that whether the parents are closely related or not. The aim of hybridization is to transfer one or few qualitative characters or improvement in

one or more quantitative characters or use of F_1 as hybrid variety. These objectives may be achieved through combination breeding, transgressive breeding and development of hybrid varieties.

a. Combination breeding

The main aim of this breeding is the transfer of one or more characters into a single variety from other varieties. These characters may be governed by oligogenes or polygenes. A familiar example of combination breeding is that for disease resistance. In this type of breeding, the genetic divergence between parents is not the major consideration. What is important is that one of the parents must have in a sufficient intensity the characater(s) under transfer, while the other parent is generally a popular variety.

b. Transgressive breeding

Transgressive breeding aims at improving yield or its contributing characters through transgressive segregation. Transgressive segregation is the production of plants in F_2 generation that are superior to both the parents for one or more characters. Such plants are produced by the accumulation of plus or favourable genes from both the parents as a result of recombination. The parents involved in this cross must combine well with each other and should preferably be genetically diverse.

c. Hybrid varieties

In most self pollinated species, F_1 is more vigorous and high yielding than the parents. Wherever it is commercially feasible, F_1 may be used directly as a variety. In such cases, it is important that the two parents should produce an outstanding F_1.

4. Types of Hybridization

Based on taxonomic relationship of the two parents hybridization may be classified into two broad groups: (a) intervarietal, and (b) distant hybridization.

a. Intervarietal hybridization

It is known as intraspecific hybridization. The parents belong to the same species, they being two strains, races or varieties of the same species. In crop improvement programmes this is the most commonly used method. These intervarietal crosses may be simple or complex.

Simple cross

In this, two parents are crossed to produce the F_1. The F_1 is selfed to produce F_2 or is used in backcross programme.

A x B → F_1 (A x B)

Complex cross

More than two parents are crossed to produce the hybrid. This is also called a convergent cross because genes are converged i.e. brought together from different parents (Fig. 14.1).

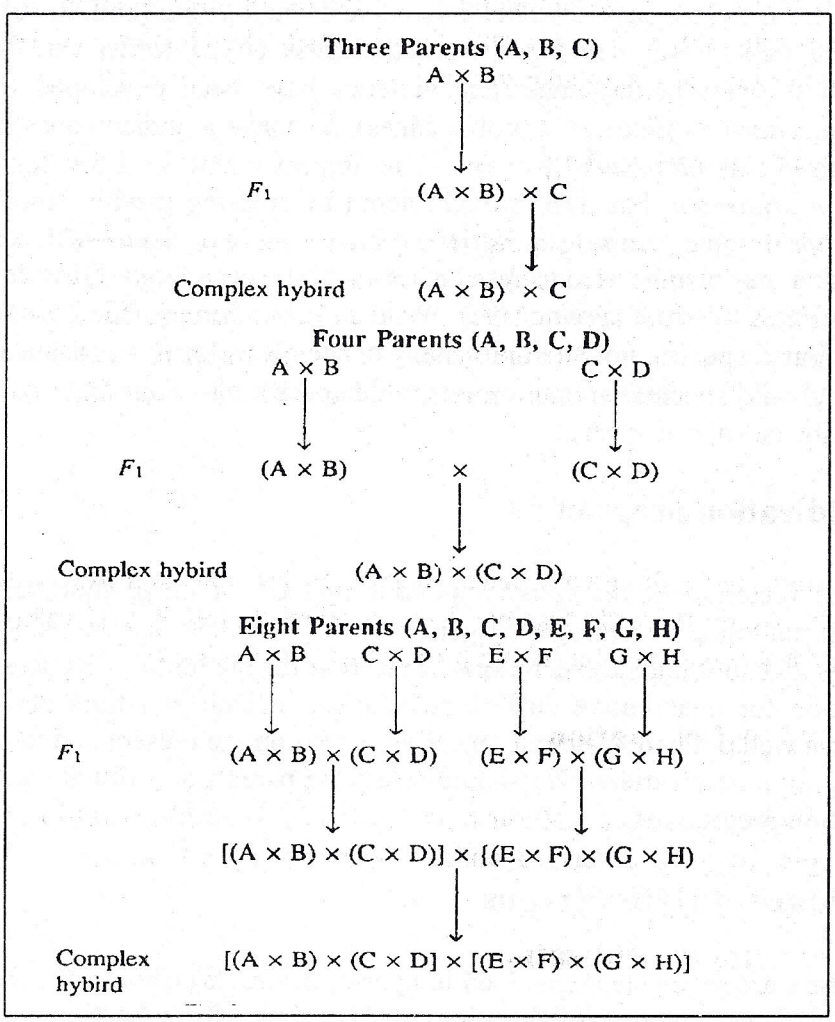

Fig.14.1 Complex crosses involving 3, 4 and 8 parents

Examples:- Breeding of highly improved self-pollinated crops like wheat and rice. Complex crosses are a common practise today. Complex crosses would become a routine in near future for the improvement of self-pollinated species.

b. Distant Hybridization

Distant hybridization includes crosses between different species of the same genus or of different genera, it is termed as intergeneric hybridization. Generally, the objective of this type of hybridization is to transfer one or few simply inherited characters like disease resistance to a crop species. This may be used to develop a new variety. **Eg:-** Clinton Oat variety was developed from a cross between *Avena sativa* x *A. byzantitina* (both hexaploid Oat species) and CO 31 rice variety was developed from the cross *Oryza sativa* var. indica X *O. perennis*. Almost all the present day sugarcane varieties have been developed from complex crosses between *Saccharum officinarum* (noble canes), *S. barberi* (Indian canes) and other *Saccharum* species eg. *S. spontanesum* (Kans). The improvement in fiber length in Indian cotton (*Gossypium arboreum*) has been brought about by crossing it with American cultivated cotton (*G. hirsutam*); many improved varieties have resulted from such crosses. Intergeneric hybridization may also be used to develop a new crop species **eg:-** Triticale from a cross between *Triticum* species x *Secale cereale* (rye). Wild species often provide genes which are not present in the cultivated species. For example, many of the genes for rust resistance in wheat are derived from related wild species. In many cases, wild species may contribute valuable 'yield genes' as well as to the cultivated species.

5. Hybridization programme

Hybridization is the most important method of crop improvement. The difficulty involves in handling of segregating generations. Selection for desirable plant type has to be made in F_2 and subsequent generations. Selection for qualitative characters is quick and simple but selection for quantitative characters is often difficult and time consuming. The breeder should have well defined, clear cut objectives based on the present and the expected future needs in developing a new variety. He should select the parents accordingly and use them in suitable hybridization programme.

6. Procedure of Hybridization

There are seven steps involved in hybridization. (a) Choice of parents, (b) Evaluation of parents, (c) Emasculation, (d) Bagging, (e) Tagging, (f) Pollination, and (g) Harvesting and storage of F_1 seed.

a. Choice of Parents

The choice of parents mainly depends upon the objectives of the breeding programme. In addition to other objectives increased yields are always an objective of the breeder. Therefore, atleast one of the parents involved in a cross should be a well adapted and proven variety in the area. The other variety should be having the characters that are absent in this variety. In combination breeding genetic diversity of parents is not important but in transgressive breeding it is of great importance. Further, the breeder has to see that the parents differ for many genes affecting yield or some other character of importance. Further, parents with good combining ability are to be selected for hybridization programme.

It is essential that all the characters sought to be improved are present in the parents involved in the hybridization programme. If necessary three or more parents may be included and a complex crossing programme is to be launched. One should remember that a character absent in the original programme generally will not appear in the segregating generations except in the case of transgressive segregation and complementary gene interaction. Thus the choice of parents is the basic step in a hybridization programme which determines success or failure of the programme.

b. Evaluation of parents

Evaluation of parents is not necessary if the performance of parents is known in a particular area. But if the performance of parents is not known in a particular area evaluation of parents for disease resistance and for heterozygosity and such other characters is necessary. If the crop species shows about 5% cross pollination it may be necessary to self-pollinate a parent for one or more generations, if it is suspected to be heterozygous.

c. Emasculation

The removal of stamens or anthers or the killing of pollen grains of a flower without affecting in any way the female reproductive organs is known as emasculation. The purpose of emasculation is to prevent self-fertilization in the flowers of the female parent. In dioecious plants, male plants are removed while in monoecious species, the male flowers **eg.** in castor or the male inflorescence, eg. in maize are removed to prevent self-pollination. But emasculation is essential in bisexual flowers. It may be done in any one of the several ways. The method suitable for a species is largely determined by the size of the flowers, the amount of seed needed, the number of seeds set per fruit and the purpose for which the hybrid seeds are required.

In species with relatively large flowers hand emasculation may be adequate. On the other hand in species with small flowers generally hand emasculation is generally difficult, tiring and

time consuming. Similarly, where large quantities of seed are needed, hand emasculation is virtually impractical. For accurate genetic studies hand emasculation is desirable because with other methods there may be some self-pollination. In addition to hand emasculation, the other methods used for emasculation are suction method, hot water emasculation, alcohol treatment, cold treatment and genetic emasculation by male sterility. Whatever may be the method of emasculation damage to the female reproductive organs is to be avoided.

d. Bagging

Immediately after emasculation, the flowers or the inflorescences are enclosed in suitable bags of appropriate size to prevent random cross pollination. The bags may be made of paper, butter paper, glassine or fine cloth. Cloth bags are generally not preferred since they permit some degree of cross pollination. 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 designed for the purpose. The moisture and temperature are generally higher inside the bags as compared to the outside. Therefore, bagging may promote fungus development on the fruits or the spikes. This may be prevented by removing the bags usually 2-3 days after pollination i.e. after the danger of cross pollination is over.

e. Tagging

The emasculated flowers are tagged after bagging with the following information: (i) Date of emasculation, (ii) Date of Pollination, (iii) Names of the female and male parents. Female parent is always denoted first.

f. Pollination

In case of pollination fertile and viable pollen should be placed on a receptive stigma to bring about fertilization. The duration of pollen viability after anther dehiscence varies with the species. eg:- a few minutes in wheat and a few hours in maize. Therefore always it is advisable to use fresh pollen from mature anthers for pollination. The time of anther dehiscence always coincides with stigma receptivity with the opening of flowers. Anthers usually dehisce during morning the exact time varies with the species.

The pollination procedure consists of collecting pollen from freshly dehisced anthers of the male parent and dusting this pollen onto the stigmas of the emasculated flowers. Pollen grains may be collected in a bag and are used for dusting the emasculated stigmas in maize and bajra or anthers or collected and the liberated pollen may be applied to stigma with the help of a brush or forceps. Sometimes in species like maize the male inflorescence may be detached and enclosed in the bag containing the female inflorescence.

g. Harvesting and Storing the seed

The crossed heads or pods should be harvested. The seeds should be dried properly and stored. Proper care is to be taken to avoid from contamination of hybrid seeds with other seeds. The seeds from each cross are to be kept separately.

7. Raising of the F_1 generation

The identification of selfed seed in the F_1 generation can be made with the help of dominant characters which express themselves in the F_1 itself. The size of F_1 generation may be small or large. The F_1 is usually allowed to self-pollinate but in a backcross programme, it is crossed to one of the parents.

8. Difficulties in Hybridization

Ordinarily intervarietal hybridization presents little problem but distance hybridization is often beset by many difficulties such as hybrid inviability, hybrid sterility and hybrid necrosis. The difficulties may be overcomed by adapting certain methods of surpassing interspecific sterility barriers.

9. Consequences of Hybridization

Several new phenotypes and genotypes are produced by hybridization. The number of phenotypes with and without dominance increases rapidly with an increase in the number of segregating genes. In F_2 vast majority of plants would be heterozygous for one or more genes. Selection on individual plant basis can be made from F_5 or F_6 generations as the population becomes more homozygous. In the case of quantitative characters recombination may produce genotypes superior to the two parents. For example, if a genotype AA bb CC dd is crossed with another variety with the genotype aa BB cc DD, the F1 would be Aa Bb Cc Dd, and in F_2 plants with genotypes AA, BB, CC, DD and aa bb cc dd would be produced. These plants would be superior and inferior respectively to both the parents. Such recombinations are known as transgressive segregants. Breeding for yield generally aims at the recovery of transgressive segregants, but the frequency of such segregants will be very low which is 2 out of 4^n plants, where 'n' is the number of genes segregating.

10. Summary

Hybridization consists of crossing of two plants or lines with different genotypes. The purpose of hybridization is to bring together in F_1 genes from two parents for the purpose of creating variability and breeding for desirable genes. The F_1 may be used as a variety where it is commercially feasible. The two parents involved in the cross may belong to the same variety,

different varieties of the same species (intervarietal or intraspecific hybridization) or to two different species of the same genus or to different genera (distant hybridization). The intervarietal hybridization may involve two (simple crosses) or more parents (complex cross). The function of distant hybridization generally is to transfer specific characters from wide species to cultivated species.

The breeder should have clear cut objectives in making a cross. The parents are to be selected accordingly and are to be evaluated for various characteristics before being crossed. Flowers of the parent to be used as female are emasculated bagged and tagged. These are pollinated by hand next morning. It is desirable to have a large F_1 population if resources permit to provide the maximum chance for recombination. Segregation and recombination produce large number of genotypes in F_2. The number of different genotypes increases geometrically with an increase in the number of segregating genes. Homozygosity increases with continued selfing. Transgressive segregation may occur but usually the recovery of such recombinants will be more difficult because of low frequency of occurrence.

Questions

1. What is hybridization? Briefly describe the different types of hybridization. Discuss in detail the objectives of hybridization.
2. Describe in brief the various steps involved in hybridization.
3. Write short notes on:
   (a) Emasculation
   (b) Trangressive breeding
   (c) Distant hybridization
   (d) Complex crosses

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LESSON: 15

PRINCIPLES OF MENDELIAN INHERITANCE AND INTERACTION OF GENES

OBJECTIVE:

This lesson explains the laws of Inheritance and interaction of genes.

15.1. Introduction
15.2. Mendel – Biographical sketch
15.3. Mendel and his experiments
15.4. Mendel's laws of Inheritance
  a. Law of segregation
  b. Law of Independent assortment
15.5. Interaction of genes
  a. Incomplete dominance (or) Intermediate inheritance
  b. Co-dominance
  c. Epistasis
  d. Complimentary genes
  e. Duplicate genes
  f. Polymeric genes
15.6. Summary
15.7. Key terms
15.8. Questions
15.9. Reference books

15.1 Introduction

Genetics is a branch of science that deals with heredity and variation. Man's quest to understand the heredity, is as old as human by itself. The offspring resemble parents has long been known. Yet the rules, which govern heredity, were not understood. Early hypotheses sought to explain heredity as a mixing up or dilution of parental traits in the progeny. One of the earliest views on heredity are those of Hippocrates (400 B.C.), who believed that the reproductive material is handed over from all parts of the body of an individual, so that the characters are directly handed over to the progeny. Aristotle (350 B.C.) believed in direct inheritance of characters. Most speculative and philosophical ideas on heredity were proposed prior to the advent of light microscope and discovery of sexuality. The discovery of sexuality led to the belief that the hereditary traits must be transmitted either through egg or through sperm or both.

J.Swammerdam (1679) proposed 'Preformation theory' that the development of an organism is a simple enlargement of a minute but preformed individual (homunculus) and it could be present in the sperm or in the ovum.

K.F.Wolff, (1738-1794) proposed the theory of 'epigenesis' stating that the gametes contained undifferentiated living substance capable of forming the organized body after fertilization.

V.B.Lamarck (1744-1829) proposed the theory of inheritance of acquired characters with the belief that the characters which acquired during the life time of an individual are inherited.

Charles Darwin (1809-1882), through his 'theory of pangenesis' tried to suggest the physical basis of heredity. He suggested that every part of the body produces very small invisible bodies (gemmules or pangenes) which are transported and assembled there into the gametes.

Wiseman (1834-1914) proposed 'germplasm theory' to account for heredity. Of the two types, the somatoplasm and germplasm, the latter is meant for the reproductive purpose only, so that any change occurring in the germplasm will influence the progeny. Aforesaid theories neither explain satisfactorily the physical basis of heredity nor they could be verified.

Gregor Johann Mendel in 1865 discovered the fundamental laws of inheritance based on the precise quantification of experimental crosses and exceptional abstract thinking.

15.2 G.J.Mendel (1822-1884) – Biographical sketch

Gregor Johann Mendel is known as the *father of Genetics* for his pioneering experiments with garden pea (Pisum sativum) to formulate the *'laws of heredity'*. These laws have been tested and stood the test of time.

Mendel born in 1822 in the Czech village of Heinzendorf, then part of Austria (now in Czechoslovakia). His father was a (peasant) farmer, who had great love for plants and expertise in fruit gardening. This undoubtedly influenced Mendel to develop a passion in experimenting with plants. After having his school education, Mendel later had a two-year course in philosophy. He then entered a Augustinian monastery as monk in 1843 and was ordained as parish priest in 1847. He was sent to University of Vienna in 1851 for training in Physics, Mathematics and Natural sciences. It was at Vienna that Mendel was influenced by two scientists, Franz Unger a plant physiologist and Christian Doppler a physicist. After completion of his studies at Vienna he returned to Brunn in 1854 where he worked as a teacher and as priest in the monastry for 14 years. He became an abbot of the monastry in 1868. His interest in plant hybridization made him to carry out hybridization experiments with the garden peas, in the monastry garden. He took eight years (1856-1864) to formulate his laws of inheritance. Mendel presented the results of his experiments in 1865 at the Natural History Society of Brunn, which were published in the society proceedings in 1866. The English translation of his German original paper *'Versuche uber pflanzen hybriden'* (Experiments in plant hybridization) was published by William Bateson in 1901 which was read by the whole scientific world. Although Mendel presented his results in 1865, it was not until 35 years later the significance of them was realized when three biologists, Devries (Holland), Carl Correns (Germany) and Tschermak (Austria) independently rediscovered Mendel's laws in the year 1900.

15.3 Mendel's Experiments

In 1856 Mendel began his experiments on plant hybridization with garden peas to study the inheritance of characters. He was not the first to perform hybridization experiments in plants. Earlier, Kolreuter (1763) in Nicotiana, Knight (1799) and Goss (1824) in peas performed hybridization experiments.

These investigators could not understand the mechanism of inheritance, as they have considered observations on all inherited characters at a time, instead of collecting and analyzing data in a system-

atic and mathematical way. Mendel's foresight and experimental astuteness enabled him to prove and show that unit characters of organisms singly or collectively heritable realities that could be dealt with in a precise, quantitative and predictable manner.

It is the genius of Mendel that a few but quantitatively predictable results enabled him to postulate that an abstract entity the 'element' or factor as he called it, the gene as we designate it was responsible for the phenotypic character he was following in his breeding experiments.

Mendel's vividness in thinking, precision in selection of material and experimentation and shrewdness in collection of data and analysis mathematically can be seen in his endeavours to understand and formulate laws of inheritance.

Mendel's hypotheses

All living organisms contain definite number of unit characters. Units characters are expressed in two or more clear cut phenotypic traits.

A Pair of elements or factors are responsible for every unit character of the individual.

The factor or element was singly represented in gametes and doubly so in zygotes and somatic tissues. In true breeding individuals pair of factors of an unit character are similar (Homozygous).

Factors when together in pairs, exhibit dominance or recessive ness in expression.

Choice of the Material

Mendel wanted to perform hybridization between the two clear cut alternate traits or forms of a unit character and to observe the expression of pair of factors in the F_1 hybrid (F_1 = First Filial generation), F_2 and F_3 progeny. He chose garden pea for his hybridization experiments, because of the following ideal features such as;

a) It is an annual plant
b) It has well defined characteristics
c) Predominantly self-fertilized and
d) Easy to perform artificial hybridization.

Mendel selected the following seven characters each with two contrasting traits.

Character	Contrasting Traits	
A. Seed shape	Round	Wrinkled
B. Color of cotyledons	Yellow	Green
C. Seed coat color	Grey	White
D. Form of the pod	Inflated	Constricted
E. Color of the pod	Green	Yellow
F. Position of flower	Axial	Terminal
G. Plant height	Tall	Dwarf

In garden pea each character breeds true, as the plants are naturally self fertilized. Hence, Mendel worked with 14 pure breeding varieties belonging to seven characters with contrasting traits.

MONOHYBRID CROSS:

Monohybrid cross is one that produces hybrid offspring by crossing parents that differ in only one character.

Mendel concentrated his attention on a single character and performed monohybrid crosses. He crossed a true breeding *tall* plant (female parent) with a true breeding *dwarf* (male parent) plant. The population obtained as a result of crossing is called first filial generation or F1 (Filial means progeny). The F1 hybrid progeny plants were tall resembling one parent. Thus the trait that appeared in the F1 hybrid, Mendel named it as dominant and the other trait which was hidden (not expressed) as 'recessive' trait. Self-fertilization of the F1 hybrids produced the F2 progeny consisting of a total of 1064 plants of which 787 were *tall* and 277 were *dwarf*. That is tall and dwarf plants appeared in F2 in the proportion of 2.81 : 1 which is roughly equal to 3:1. When he performed the reciprocal cross using dwarf plants as female parent and tall plants as the male parent in the cross, the same results were obtained showing thereby that it did not matter which plant was used as male or as female parent.

Similarly, Mendel crossed pea plants differing in other characters such as seed shape (Round versus wrinkled), color of cotyledons (Yellow versus green), seed coat color (Grey versus White) and position of flower (Axial versus Terminal). In each case, Mendel found one parental trait (Dominant) in the F1 hybrid, and after self fertilization in F2 generation both parental traits (dominant and recessive) appeared in the proportion of three-fourths to one-fourth.

The dominance and recessive relationship of the two traits of each character is shown in the table 15.1:

Table 15.1 : Actual data obtained by Mendel in *monohybrid* crosses

S.No	Character Studied	Cross	F1 hybrid	F2 generation Dominant Vs Recessive	Ratio
1	Seed shape	Round x Wrinkled	Round	Round (5474) Wrinkled(1850)	2.96:1 (3:1)
2	Color of Cotyledons	Yellow x Green	Yellow	Yellow (6022) Green (2001)	3.01:1 (3:1)
3	Seed Coat Color	Grey x White	Grey	Grey (705) White (224)	3.15:1 (3:1)
4	Form of pod	Inflated : Constricted	Inflated	Inflated (882) Const.(299)	2.95:1 (3:1)
5	Pod color	Green x Yellow	Green	Green (428) Yellow (152)	2.82:1 (3:1)
6	Flower position	Axial x Terminal	Axial	Axial (651) Term.(207)	3.14:1 (3:1)
7	Plant Height	Tall x Dwarf	Tall	Tall (787) Dwarf (277)	2.84:1 (3:1)

Numbers in parentheses are number of plants of each kind.

Mendel used English alphabets to represent the 'factors' responsible for the character. A capital letter to signify dominant member and lower case letter signifying the recessive member. Mendel's monohybrid cross results of tall and dwarf plants can be summarized as in figure 15.1

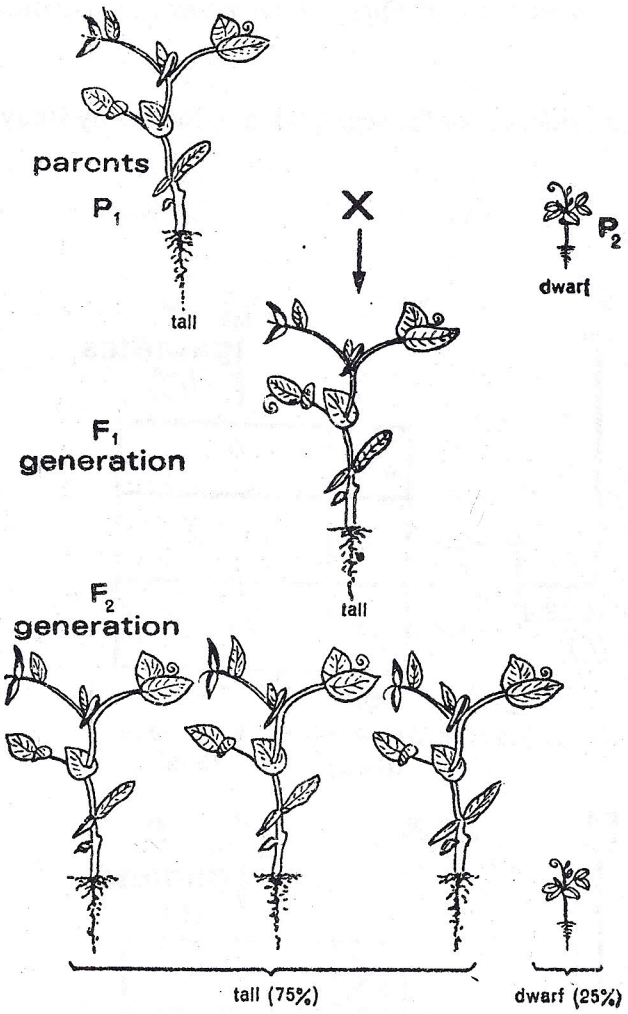


Fig. 15.1 Inheritance of a Mendelian character in F_1 and F_2 generations.

15.4 Mendel's Laws of inheritance

a) Law of segregation (or) Law of purity gametes.

Having studied the above said crosses; Mendel concluded that each character is controlled by a pair of factors. Pure breeding individual contains a pair of identical factors (for eg. pure breeding tall DD and dwarf dd). Each parent contributes one factor for a character to the F1 hybrid. In this way the

F1 hybrid has two factors (dominant factor and recessive factor) one each from the parents. There is no blending or mixing-up of these Mendelian factors in F1, but they stay together and only one is expressed. When the F1 hybrid forms gametes, the two factors separate or segregate from each other. The gametes that are formed are always pure for a particular character. A *gamete* may carry either the dominant factor or the recessive factor, but not both as in F1 individuals. This is why it is called '*Principle of Segregation*' or as '*Law of Purity of Gametes*'. This concept is also often called as Mendel's first principle.

Mendel further added evidence for the segregation of factors by studying the F2 progenies. (Fig 15.2).

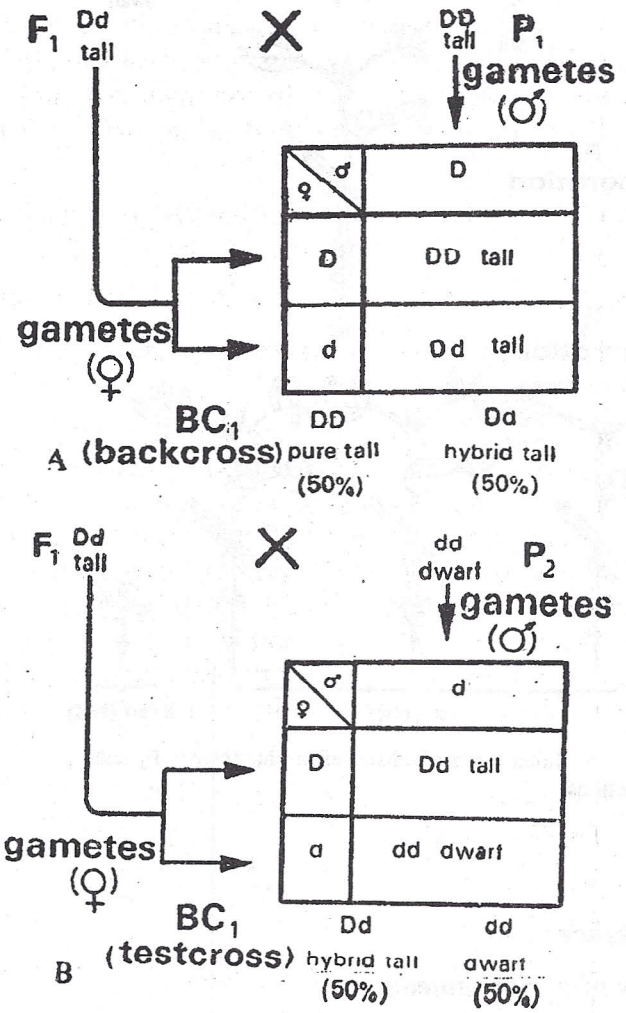


Fig. 15.2 Inheritance of plant height in a backcross (A) and a testcross (B).

The appearance of parental characters in the F2 in the ratio of 3:1 dominant and recessive traits supports the law of segregation of factors. The appearance of hidden factor (recessive type) in F2 is a proof for its segregation.

Factors which control the contrasting expression (form or trait) of a character are said to be 'alleles'. For example, the factor responsible for tall form 'D' and dwarf form 'd' are alleles of a character 'plant height'. The terms *'Homozygous' and 'heterozygous'* are coined by Bateson and Saunders (1902). When both factors are identical such as DD and dd, the individual is said to be homozygous for that character. When the factors are different (for example Dd) the term heterozygous is used.

Mendel's factors were later replaced by the term 'gene' by a Danish botanist Johannsen (1909). The word *'genotype'* refers to the genetic constitution of an individual (for example genotype of pure tall plant is DD) where as the 'phenotype' refers to external appearance or manifestation of a character (For example tall or dwarf).

Mendel also added evidence for segregation of factors by his observations on F_3 progenies. Selfed progenies of recessive F2 plants bred true (i.e. produced only recessive plants), while only one third of dominant plants bred true (i.e. produced again dominant plant only). The rest (two thirds) of dominant plants were F1 hybrid like again segregated in the ratio of 3:1 dominant and recessive phenotypes.

Therefore the F2 ratio 3:1 would be more correctly stated as 1 true breeding dominant (DD) : 2 hybrid like or segregating dominant (Dd): 1 true breeding recessive (dd).

The test-cross as evidence: Not satisfied with his work. Mendel subjected his results to a test:

The cross of an F1 hybrid with a homozygous recessive individual is a test-cross. This helps to test and verify the F_1 individual genotype.

In the Mendel's monohybrid cross of tall plants with dwarf plants, the F1 hybrids were all phenotypically tall but their genotypes were not DD but Dd. When this F1 hybrid forms gametes, the law of segregation states that the factors 'D' and 'd' segregate into the gametes in a 1:1 ratio. This means that 50% gametes carry the factor 'D' and 50% the factor 'd'. Mendel crossed such a hybrid plant (Dd) with a plant of the true breeding, dwarf (dd). All the gametes of the true breeding dwarf plant carried the recessive factor 'd'. Every gamete of the recessive parent has 50% chance of combining with a gamete carrying D and 50% chance to combine with a 'd' gamete from F1 heterozygous parent. This should result in 50% of progeny showing the tall phenotype and genetic constitution Dd, where as 50% of the progeny should be phenotypically dwarf with genotype dd as illustrated below and Fig.15.4.

P :	Tall (DD)	×	Dwarf (dd)	
Gametes :	*D(50%),d(50%)*		*All d*	
	Tall :	Dwarf :	: 1 : 1	
Test Cross Progeny :	*(Dd)*	*(dd)*		
	50%	50%		
	1 :	1		

Fig. 15.3 Monohybrid test cross

Indeed, Mendel's results of this cross agreed with the theoretical expectations thus providing additional experimental proof of the correctness of his interpretations, on the principle of segregation.

b. Law of Independent Assortment

As earlier pointed out, one of Mendel's success was that he considered only one character at one time and unlike earlier workers did not study the individual as a whole. Such an approach soon enabled him to discover the principle of segregation. From this simplest situation, Mendel tried to analyse cases where two characters with alternative traits were simultaneously studied.

Mendel tried to find out whether the factors of one character behave independently of the factors of other character. For this he made a dihybrid cross, in which inheritance of two characters is considered. He used true breeding plants for two characters, as parents. The plants with yellow and round seeds (pure) were crossed with those having green and wrinkled seeds (pure). The F1 seeds were yellow and round. This was in conformity with earlier results of monohybrid cross, where round seed shape was found to be dominant over wrinkled shape and yellow color was dominant over green color. The F2 progeny obtained from the selfed F1 hybrids showed all the four possible combinations table 15.2).

Table 15.2 F2 Progeny phenotypes of a dihybrid

Plant character		
Color of Seed	Seed Shape	Resulting combination
Yellow (Dominant)	Round (Dominant)	Yellow Round
	Wrinkled (Recessive)	Yellow Wrinkled
Green (Recessive)	Round (Dominant)	Green Round
	Wrinkled (Recessive)	Green Wrinkled

The four kinds of seeds i.e. (i) yellow round seeds, (ii) yellow wrinkled, (iii) green ground and (iv) green wrinkled were obtained in 9:3:3:1 ratio (figure 15.4).

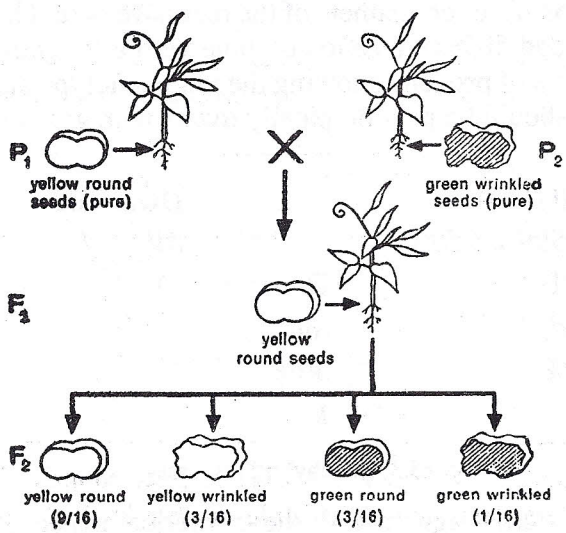


Fig 15.4 Independent assortment of seed colour and seed shape.

Mendel explained above results based on the 'Independent assortment of factors'. The pair of factors for roundness must be behaving independently of the pair of factors for yellow color of seeds. In other words one factor for a *character* must be passing independently of a factor for *another character*. If the individual character segregates 3:1 ratio of *dominant* and *recessive* types, the law of probability states that the chance of two or more independent events occurs together will be the *product* of the chances of their separate occurrences, i.e., (3:1) (3:1)=9:3:3:1

(Round:wrinkled)	(Yellow:green)=	Round : Yellow	Round: green	Wrnkled : yellow	Wrinkled : green
3 : 1	3 : 1	9	3	3	1

The probabilities of the occurrence of above four combinations (phenotypes) can then be calculated on the basis that for each character $3/4^{th}$ of the progeny is dominant and $1/4^{th}$ recessive (table 15.3).

Table 15.3 **Relative proportions of four combinations in a dihybrid cross as derived from monohybrid crosses.**

Cross	Phenotype	Probability in F_2
1. Monohybrid cross (seed colour)	yellow seeds	3/4
	green seeds	1/4
2. Monohybrid cross (seed shape)	round seeds	3/4
	wrinkled seeds	1/4
3. Dihybrid cross (seed colour & seed shape	yellow and round seeds	3/4×3/4=9/16
	yellow and wrinkled seeds	3/4×1/4=3/16
	green and round seeds	1/4×3/4=3/16
	green and wrinkled seeds	1/4×1/4=1/16

Table 15.4 **Segregation for seed colour among different classes of seed shape.**

Population analysed	Phenotype	Proportion	Ratio
1. Whole F_2	yellow	12/16	3 : 1
	green	4/16	
2. Among round seeds	yellow	9/12	3 : 1
	green	3/12	
3. Among wrinkled seeds	yellow	3/4	3 : 1
	green	1/4	

Table 15.5 **Segregation for seed shape among different classes of seed colour.**

Population analysed	Phenotype	Proportion	Ratio
1. Whole F_2	round	12/16	3 : 1
	wrinkled	4/16	
2. Among yellow seeds	round	9/12	3 : 1
	wrinkled	3/12	
3. Among green seeds	round	3/4	3 : 1
	wrinkled	1/4	

In the ratio 9 (yellow round) : 3 (yellow wrinkled) : 3 (green round) : 1 (green wrinkled) one can analyse them for single characters separately as in Table – (table 15.4,5). In this analysis it is obvious

that when one character is fixed the other character exhibits a clear 3:1 ratio. Therefore it can be concluded that the two characters under consideration are assorting (giving rise to different combinations) in an independent manner.This principle is known as Mendel's law of Independent assortment. A dihybrid cross of round yellow seeds with those having wrinkled green. F2 genotypes and phenotypes are shown in fig. 15.5.

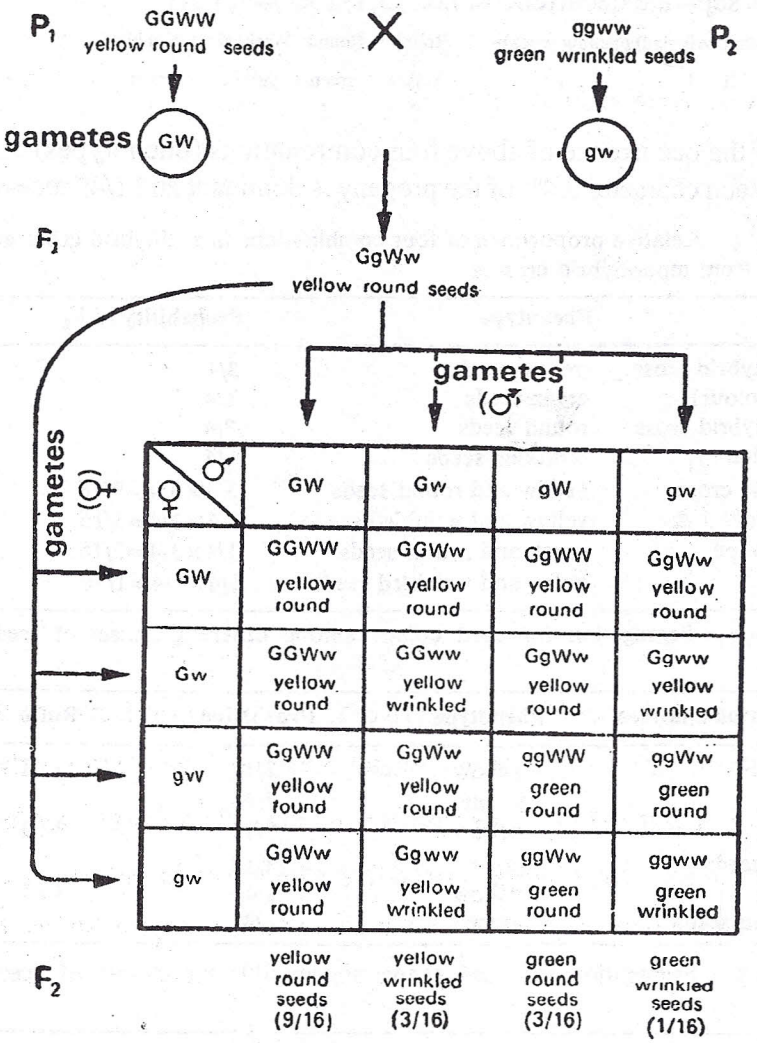


Fig 15.5 A checkerboard showing 16 combinations obtained in a dihybrid cross involving characters for seed colour and seed shape.

As in the case of monohybrid cross, Mendel verified his results of independent assortment by performing test cross. He crossed the F1 dihybrid, heterozygous (Yy Rr) for both characters with a double recessive parent (rryy) which should produce only one type of gamete (ry). The uniformity in the gametes of the recessive parent determines the difference in the type of gametes produced by the heterozygous parent. If the dihybrid Rr Yy produced gametes carrying RY, Ry, rY, ry with equal

frequency, and if all these four types of gametes unite with ry gamete of the recessive parent, the resulting progeny should show all the four combinations of characters also in equal proportions. Indeed, Mendel observed the test-cross progeny to consist of Round Yellow, Round Green, Wrinkled Yellow and Wrinkled Green plants in the ratio 1:1:1:1. (fig. 15.6)

F1 hybrid — Double recessive parent

Round Yellow(Rr Yy) X Wrinkled Green(rr yy)

↓

Gametes RY, Ry, rY, ry — ry

F_2 Progeny

RY/ ry	Round Yellow	1
Ry/ ry	Round Green	1
rY/ ry	Wrinkled yellow	1
ry/ ry	Wrinkled Green	1

Fig. 15.6

From the results of his dihybrid crosses Mendel discovered the following facts. At the time of gamete formation the segregation of alleles R and r into gametes occurs independently of the segregation of alleles Y and y. Because of this the resulting gametes contain all possible combinations of these alleles, i.e., RY, Ry, rY, ry. In this way Mendel proved that when two characters are considered in a cross, there is independent assortment of genes for each character and this is knows as *'Law of Independent Assortment.*

Trihybrid and Multihybrid crosses:

Mendel extended his observations to trihybrid crosses, involving three characters each with two alternative clear cut dominant and recessive forms.When a cross is made between a true breeding tall yellow round seed plant (TT YY RR) and a true breeding dwarf green, wrinkled seed plant (tt yy rr), the F1 hybrid will be tall yellow round (Tt Yy Rr) which is heterozygous for the three genes. The F2 segregations of a trihybrid and phenotypic ratio (27:9:9:9:3:3:3:1) are here represented by forked line method (Fig. 15.7).

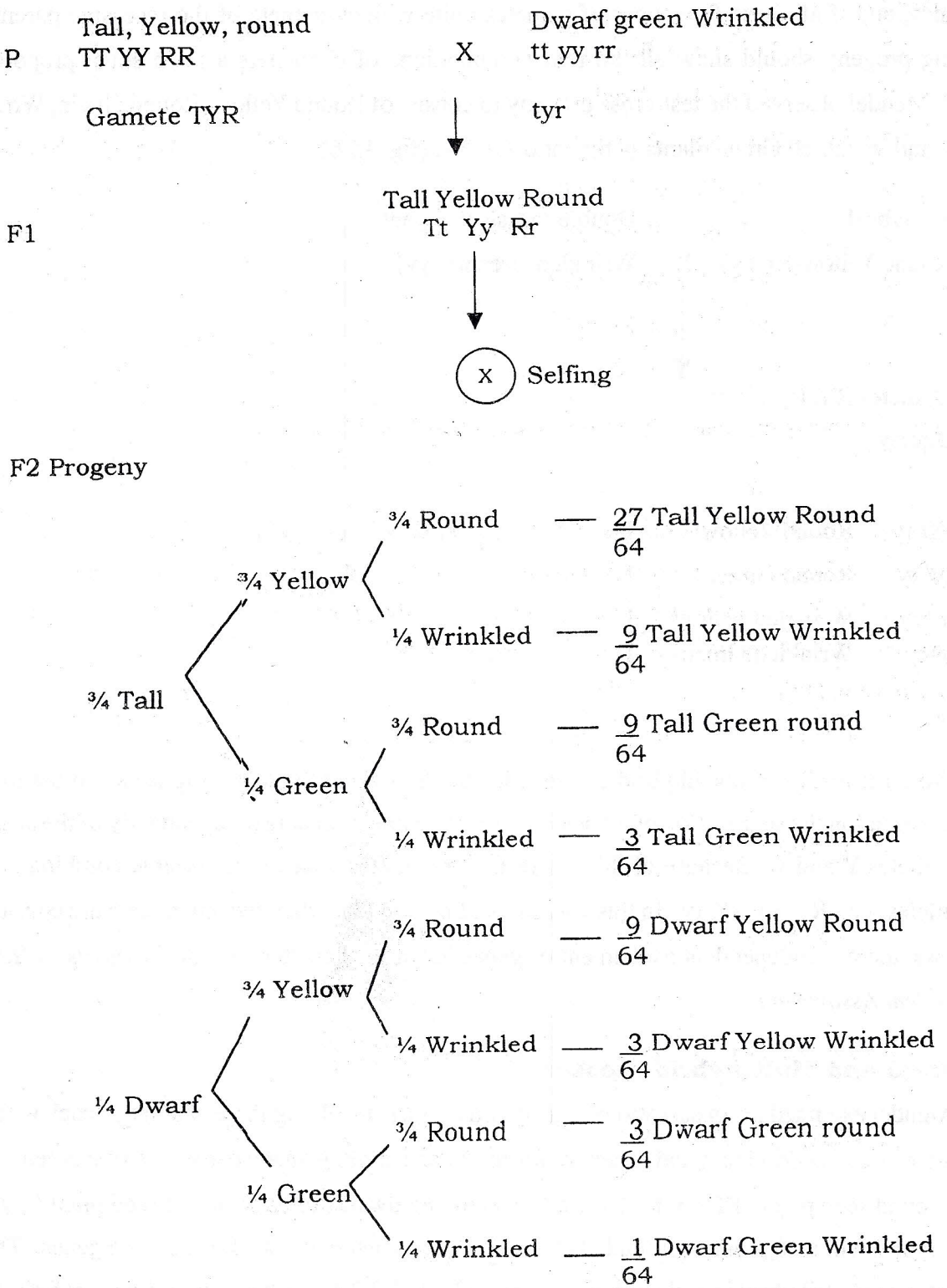


Fig. 15.7 Phenotypic classes and their proportions of a trihybrid F2 progeny.

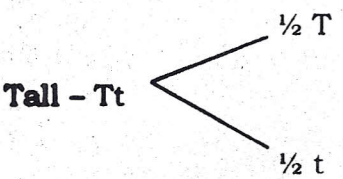
Phenotypic ratio:

27	-	Tall Yellow Round
9	-	Tall Yellow Wrinkled
9	-	Tall Green Round
9	-	Dwarf Yellow Round
3	-	Tall Green Wrinkled
3	-	Dwarf Yellow Wrinkled
3	-	Dwarf Green Round
1	-	Dwarf Green Wrinkled

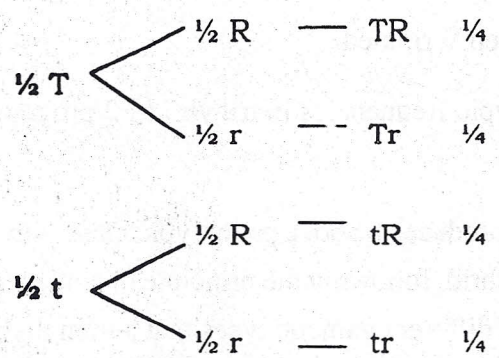
Figure 15.7 shows the genotypic frequencies in trihybrid F2 progeny, presented by forked-line method.

The testcross of F1 tribybrid produces 8 above phenotypic classes in equal proportions, as eight different gametes obtain from trihybrid, following the principle of 'Independent assortment of factors of three characters. The number of different gametic types that a monohybrid, dihybrid and trihybrid produce are shown here by forked-line method Fig 15.8) and phenotypic and genotypic types produced are shown in table 15.6.

Monohybrid 2 gametes



Dihybrid 4 gametes
Tall Round Tt Rr



Trihybrid 8 gametes
Tall Yellow Round
Tt Yy Rr

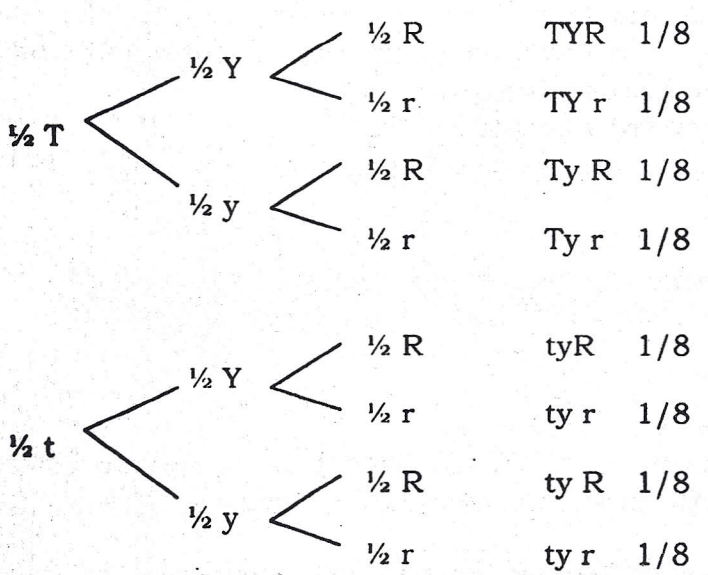


Fig. 15.8 Showing the different gametes produced by a mono hybrid, dihybrid and trihybrid.

Table (15.6) showing number of gametic phenotypic, genotypic classes produced in monohybrid, dihybrid, trihybrid F2 progeny.

Type of hybrid	No. of Characters Involved	No. of Possible Phenotypic Classes	No. of Gametic Types	No. of Possible genotypes
Monohybrid	1	2	2	3
Dihybrid	2	4	4	9
Trihybrid	3	8	8	27
Tetrahybrid	4	16	16	81
Multihybrid	n	2^n	2^n	3^n

15.5 INTERACTION OF GENES

Mendel demonstrated that one gene (factor) controls one character and of the two alleles of a gene, one is completely dominant over the other allele, due to this the dominant homozygote (for eg. TT) has a phenotype identical to that of a heterozygote (for e.g. Tt). Soon after the Mendel's laws were rediscovered, instances came to light where some deviations observed in relation to dominant and recessive relationship of alleles of a gene and hybrid phenotypic ratios that can be suitably explained on the basis of interaction of genes. These observations led to the development of a hypothesis that genes interact with each other to produce novel phenotypes. Many genes interact in some complex fashion to produce the trait. There are two main principles that constitute interaction viz., I) more than one gene may be responsible for the full expression of a particular character, ii)and a single factor/gene effects more than one visible character.

15.5 Types of Gene interactions:

a) Incomplete dominance (or) Intermediate inheritance
b) Co-dominance
c) Epistasis
d) Complementary genes
e) Duplicate genes
f) Polymeric genes

a. Incomplete dominance:

In monohybrid crosses, if the F_1 hybrid shows incomplete or partial dominance with no phenotypic resemblance to either parent, it comes under *incomplete dominance*.

A monohybrid cross between red-flowered (RR) snapdragon (*Antirrhinum majus*) and a white flowered (rr) variety does not produce red or white flowered F_1 plants as expected from Mendelism. This is because neither red color (allele R) nor white (allele r) is dominant, but each allele has its influence in color development and the hybrid appears *pink*.

Fig. 15.9 Incomplete Dominance in Snapdragon for Flower Colour in the F_1 and F_2 Generations

$P_1 \rightarrow$ Red Flower *RR* × White Flower *rr*

↓

$F_1 \rightarrow$ Pink Flower *Rr*

↓ ⊗ Selfing

$F_2 \rightarrow$

♀ \ ♂	*R*	*r*
R	*RR* Red	*Rr* Pink
r	*Rr* Pink	*rr* White

F_1 — Pink Flower (Incompletely Dominant)
F_2 — 1 Red : 2 Pink : 1 White

when the F_1 hybrid was selfed, there was segregation of 1 Red : 2 Pink : 1 White in F_2 generation (fig. 15.9). It may be recalled that this is the same genotypic ratio that Mendel obtained in garden pea. The deference is that in the present case the heterozygotes are distinct in appearance from homozygotes. The heterozygous nature of F_1 and progeny plants can be verified by test crossing with recessive parent. The pink flowered plants when crossed with white flowered ones produced pink and white in 1:1 ratio, proving that it is a heterozygote (Rr) producing two types of gametes. The heterozygous individual (Rr) is pink, the homyzygote (RR) carrying 'R' allele is red and r allele is white. This comes under inter-allelic interaction.

b. Co-dominance:

In co-dominance the heterozygote expresses both the parental types equally. For example, in human beings blood group system is controlled by multiple alleles of a dominant gene I, each allele producing a different antigen. The genotype of A blood group is $I^A I^A$ or $I^A i$ that produces antigen A. The genotype of B blood group is $I^B I^B$ or $I^B i$ that produces antigen B. The recessive allele i with genotype ii produce O blood group that produces no antigen. A cross between A and B blood groups produce F_1 hybrid that is neither A type nor B type. The blood group of F_1 is AB type where it produces antigen A and B, so that the expression of both the genes present (Fig. 15.10).

$I^A I^A$ X $I^B I^B$

Blood Group A ↓ Blood group B

$I^A I^B$

F_1 AB Blood group

Fig. 15.10. Explaining co-dominance of I^A and I^B alleles

Another example where the heterozygote can be recognized distantly is offered by the 'roan' (reddish grey) coat color of short-horn cattle. When homozygous red-haired cattle are crossed with

homozygous white haired type, the F_1 has reddish grey hair and it is designated as 'roan'. It must be noted that some hair are all red and others all white, so that the final effect is a reddish grey coat color. In this genes responsible for red and white hair are expressed in heterozygous individual (Fig. 15.11).

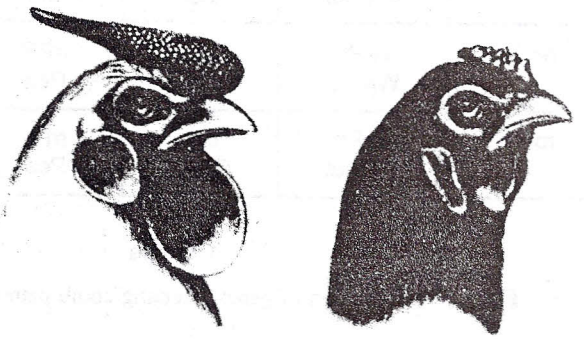
Parents: Red $r_1 r_1$ X White $r_2 r_2$

F_1 hybrid: $r_1 r_2$
Roan (Reddish grey)

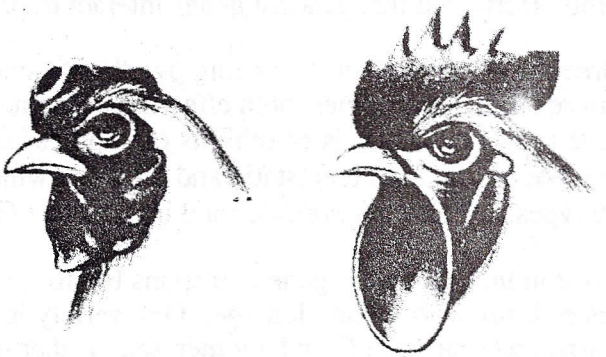
F_2 progeny: 1 Red($r_1 r_1$) : 2 Roan ($r_1 r_2$):1 white ($r_2 r_2$)

Fig. 15.11. Codominance of r_1 and r_2 alleles

A third example where two genes influence a single character was discovered by Bateson and Punnet (1905) in fowls. There are four types poultry or fowls viz., Walnut, Rose, Pea and single (Figure 15.12).



1. Rose comb 2. Pea comb



3. Walnut comb 4. Single comb

Comb types characteristic of different breeds of chickens: (1) Rose, Wyandottes; (2) Pea, Brahmas; (3) Walnut, hybrid from cross between chickens with Rose and Pea combs; and (4) Single, Leghorns.

Fig. 15.12 Types of combs in fowls.

Two genes are responsible for this character. One gene gives rise to rose comb and the other gene gives rise to pea comb. These two comb types are dominant over single comb. However a curious result was obtained when rose comb was crossed to pea comb, the F_1 hybrid was neither rose

nor pea but 'walnut' a new phenotype was obtained. When the F_1 was selfed, the F_2 produced 9 walnut 3 Rose, 3 Pea and 1 Single comb. This resembles typical dihybrid ratio where two comb types rose and pea were controlled by two independent genes and shows co-dominance when present in the same individual (Figure 15.13).

$P_1 \rightarrow$ Rose comb RRpp × Pea comb rrPP

↓

$F_1 \rightarrow$ RrPp Walnut

↓ ⊗ Selfing

F_2

♀ \ ♂	RP	Rp	rP	rp
RP	RRPP Walnut	RRPp Walnut	RrPP Walnut	RrPp Walnut
Rp	RRPp Walnut	RRpp Rose	RrPp Walnut	Rrpp Rose
rP	RrPp Walnut	RrPp Walnut	rrPP Pea	rrPp Pea
rp	RrPp Walnut	Rrpp Rose	rrPp Pea	rrpp Single

9 Walnut
3 Rose

3 Pea
1 Single

Fig. 15.13 Interaction of genes affecting comb pattern in fowls.

The independent dominant genes P and R are jointly responsible for the walnut comb. When present alone they produce pea and rose comb. The recessive alleles of rose and pea combs produce the fourth type single comb. Here two independent genes interact to produce a novel phenotype.

c. Epistasis: This is a Greek word that means "standing over" or "Conceals". The phenomenon of epistasis refers to two different nonallelic genes, both effecting the same character in such a way that the expression of one gene masks or conceals or inhibits or suppresses the expression of the other gene. The gene that suppresses is said to be epistatic and the gene which remains obscure is hypostatic. Epistasis is of two types: i)*Dominant epistasis* and ii)*recessive Epistasis*.

i) Dominant Epistasis: A dominant epistatic gene is responsible for this epistasis. In poultry there are two different varieties of birds with white plumage. One variety is called white leghorn, represented by IICC, which carries a color gene C and another gene I, that inhibits the expression of the color gene C. The other variety is white wyandotte whose genotype is represented by genes recessive to the two genes(C,I) i.e. iicc and so it is white or colorless. When a cross is made between the two white varieties, leghorn and Wyandotte the F_1 hybrid was also white (IiCc) and when the F_1 hybrid was selfed and the F_2 generation raised there was a segregation of 13 white and 3 colored. In the F_2 progeny, only the iiCC, iiCc, iiCc genotypes produce colored birds. The white birds obviously contain a gene I which in the dominant state inhibits or suppress the expression of the dominant color gene C, resulting white plumage.

ii) Recessive Epistasis: Epistasis is here due to a recessive gene. Modified dihybrid F_2 ratio 9:3:4 is observed when homozygosity for a recessive 'allele' with respect to one gene masks the expression of a different gene. Foreg in mice albinism (white fur) is produced by a recessive gene aa. There is a different dominant (B) gene that produces greyish coat color called 'agouti' characterized by the presence of a horizontal band of yellow pigment just beneath the tip of each hair. Its recessive gene bb leads to black coat color. The recessive gene for albinism (aa) is found to be epistatic to the gene for agouti (BB or Bb) and also to its recessive homozygous allele (bb) for black Hence aa genotype has the same phenotype regardless of whether the genotype is BB, Bb or bb, and the 9:3:4 ratio results.. The dominant allele (AA) of the epistatic gene allows the expression of gene B so that agouti (BB & Bb) and black (bb) coat colors can be produced.The details of parents, F1 and F2 progeny are shown in Figure 15.14.

P: Agouti × Albino

AABB *aabb*

Gametes: *AB* *ab*

F_1: Agouti × Agouti

AaBb *AaBb*

Gametes: *AB, Ab, aB, ab*

A B	AB	Ab	aB	ab
AB	AABB Agouti	AABb Agouti	AaBB Agouti	AaBb Agouti
Ab	AABb Agouti	AAbb Black	AaBb Agouti	Aabb Black
aB	AaBB Agouti	AaBb Agouti	aaBB Albino	aaBb Albino
ab	AaBb Agouti	Aabb Black	aaBb Albino	aabb Albino

F_2: 9 Agouti : 3 Black : 4 Albino

Fig. 15.14. Recessive Epistatic gene interaction resulting a modified 9 : 3 : 4 ratio

d. Complementary genes

Complementation between two genes means that both genes are necessary for the production of a particular phenotype. Bateson and Punnet observed that when two white flowered varieties of sweet pea (*Lathyrus odoratus*) were crossed, F_1 produced had colored flowers. On self-polthination the F_1 plants gave and F_2 progeny of 9 plants with colored flowers and 7 with white flowers. This result can be explained by assuming two genes (non-alletic) for red color, which must be present together acting in a complementary way to each other. Thus each gene independently contributes some thing different but essential for synthesis of red pigment. If one of the two genes for red color is absent, the result is a white flower (fig 15.15).

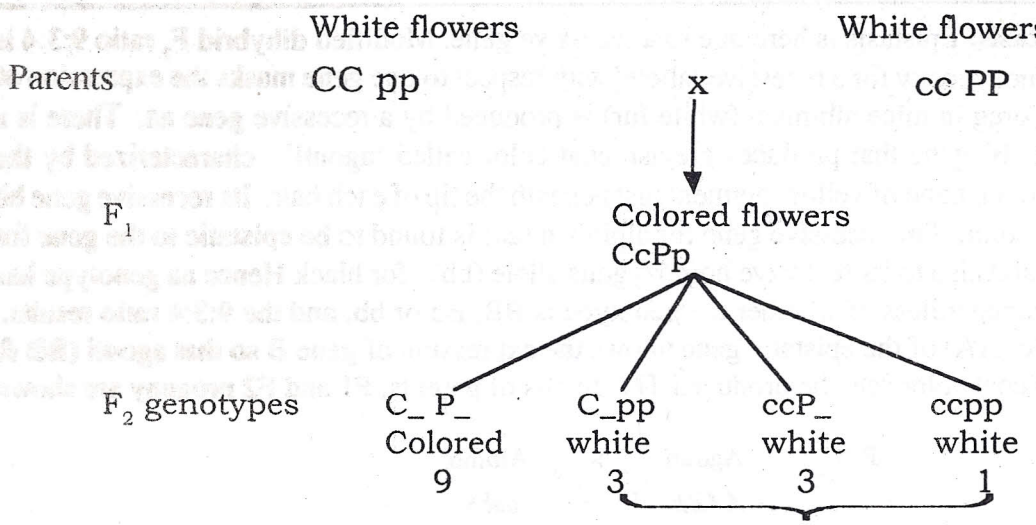


Fig. 15.15 Complementary gene action resulting 9:7 ratio.

e. Duplicate Genes: When two or more genes have the same effect on a given trait, such genes are reflected as 'duplicate' genes. Then the dihybrid ratio modifies into 15:1 dominant and recessive phenotypes. G.H.Shull observed two kinds of capsules (triangular & top shaped) in a plant known as shepherd's purse belonging to the genus *Capsella.* When races with these two phenotypes were crossed, in F_1 generation only triangular capsules were observed. The selfed progeny of F_1 produced plants with triangular capsules and top shaped capsules in 15:1 ratio. If A and B are two dominant genes, each individually capable of producing triangular capsules, presence of both dominants has the same effect. The double recessive genotype only produces top shaped capsules (fig 15.16).

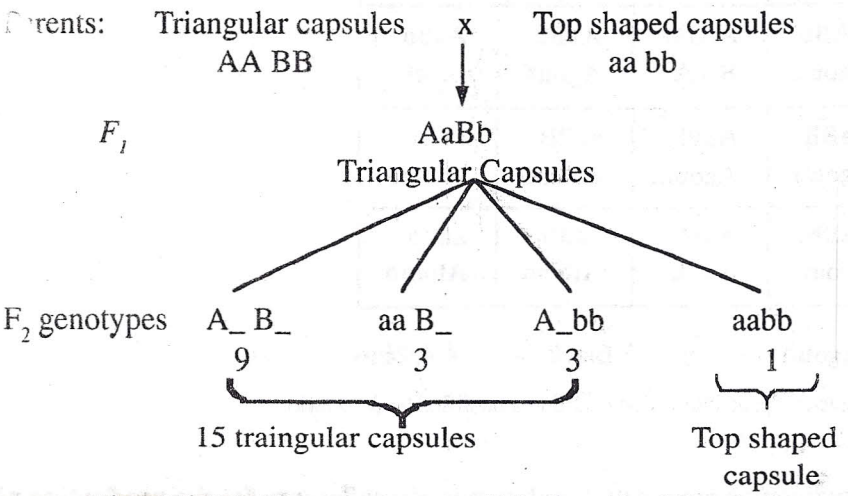


Fig. 15.16 Showing a modified ratio 15 : 1 due to duplicate gene action

f. Polymeric genes:

There are two different varieties in summer squash *(Cucurbita pepo)* that produce spherical fruits. When two such spherical fruit varieties are crossed, the F_1 progeny showed a novel fruit shape *'discoid'.* Selfed F_2 progeny from F_1 plant contained discoid spherical and cylindrical shapes in the ratio of 9:6:1. This result is explained on the basis of presence two independent genes each with an additive (polymeric) effect (Fig. 15.17).

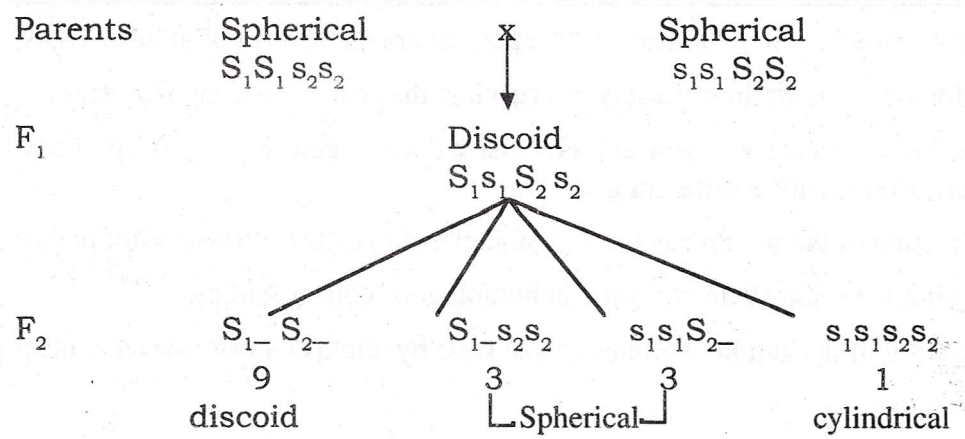


Fig. 15.17 Explains polymeric Gene interaction

15.6 Summary

Genetics is a branch of science that deals with and heredity variation. Gregor Johann Mendel (1865) known as the 'Father of Genetics' first demonstrated the way in which characters are transmitted from one generation to another. Through his experiments on plant hybridization with garden pea discovered the 'laws of inheritance' : i) *the law of segregation* and ii) *the law of Independent assortment*. He proposed and provided experimental proof that every unit character of an individual is determined by a pair of factors contributed by one each from the male and female parent. The factor responsible for one character is now coined as a *gene*, which exist in allelic forms i.e. dominant allele and recessive allele. Pair of factors of a character segregate equally into gametes, making the gamete pure for that character. The separation of pair of factors one each into the gametes is known as *the law of segregation*. The factors of one character behave independently from the factors of the another character and result in all possible combinations of gametes and phenotypes, which is known as *'law of independent assortment'*. Mendel's laws of inheritance enable us to understand the inheritance of characters and predict with precision the phenotypic and genotypic proportion in the progenies. Subsequent to rediscovery of mendels laws several deviations to 3:1 and 9:3:3:1 ration were observed. These were explained through gene interaction.

Many genes interact in complex fashion, to express the phenotype. More than one gene may be responsible for the full expression of a particular character or a single gene effect more than one visible character. Different types of gene interactions namely *incomplete dominance, co-dominance, epistasis, complementary, duplicatory and polymeric* gene actions are described.

15.7 Key terms

Gene: The hereditary unit responsible for the expression of a unit character in organisms.

Allele: A gene can mutate to various forms and such alternative forms of a gene called alleles of that gene.

Monohybrid: The offspring of a cross between genotypes that are hymozygous for two different alleles of a gene.

Reciprocal cross: A cross in which the sexes of the parents are the reverse of another cross.

True-breeding: Homozygous strain or variety of organism that yields progeny like itself.

Test-cross: A cross between a heterozygote and a recessive homozygote, resulting in progeny in which each phenotypic class represents a different genotype.

Segregation: Separation of the members of a pair of alleles into different gametes in meiosis.

Homozygous: Having the same allele of a gene in homologous chromosomes.

Epistasis: The masking of the action of alleles of one gene by allele combination of another gene.

15.8 Questions

1. Discuss Mendel's laws of inheritance?
2. Explain the law of Independent assortment, with examples and add your coment on its importance?
3. Explain the deviations of dihybrid phenotypic ratios in the light of different gene interactions?
4. Write a detailed account on interaction of genes?

15.9 Reference Books

1. KB Ahluwalia (1985) *Genetics:* Willey Eastern Ltd., New Delhi.
2. AVSS Sambamurthy (1999) *Genetics:* Narosa Publ. House, New Delhi.
3. D.L.Hartl & E.W. Jones 1998 Principles and Analysis *Genetics:* , Jones & Bartlett Publishers, London (1998).

LESSON: 16

LINKAGE, RECOMBINATION AND GENE MAPPING

OBJECTIVE:

To define and describe linkage of genes, crossing over and chromosome mapping.

16.1. Introduction
16.2. Coupling and Repulsion Hypothesis
16.3. Linked genes and Linkage
  i) Linkage in Maize
  ii) Linkage in *Drosophila melonogaster*
  iii) Absolute linkage – Absence of crossing over
  iv) Crossing over and Recombination
  v) Frequency of crossing over
16.4. Linear order of the genes and Genetic mapping
  i) Map distance in two-point test cross
  ii) Map distance in three point test cross
  iii) Interference
16.5. Other methods determining recombination in dihybrid F_2 progeny
  i) Square-root Method
  ii) Product-ratio Method
16.6. Mapping by Tetrad Analysis
  i) Analysis of un-ordered tetrads
  ii) Analysis of ordered tetrads
16.7. Somatic or Mitotic crossing over
16.8. Chromosomal evidence for crossing over
16.9. Significance of crossing over & factors influencing
16.10. Summary
16.11. Key terms
16.12. Questions
16.13. Reference Books

16.1 Introduction

Mendel's law of independent assortment suggests that when two or more than two genes are considered the segregation of the members of any pair of alleles is independent of the segregation of the other pair/pairs in the formation of reproductive cells. The separation of *paired homologues* and independent *transmission* of *chromosomes* in meiosis were shown as parallel physical proof for the segregation and independent assortment of Mendelian genes.

The number of pairs of chromosomes of an organism or species is constant. In *Drosophila melanogaster* for example the number is four, in human beings 23, in the mouse 20. The discovery of an increasing number of mutant genes greater than the number of pairs of chromosomes of that indi-

vidual species contains, led to realize that some of these genes must occupy positions on the same chromosome. Each of these when tested individually for transmission and segregation confirmed the Mendelian expectations. But, when studied in groups of two or more than two, they yielded ratios that frequently departed from the distributions expected on the basis of random assortment. Bateson and Punnet (1906) described a dihybrid cross in sweet pea, where a deviation from independent assortment was exhibited. Thus, Mendelian law of random assortment although not invalid, was not as universally applicable as was once believed. Where the number of known genes exeeds the haploid number of chromosomes, some of these genes must inevitably occupy positions on the same chromosome. The genes that reside in the same chromosome are linked genes and their tendency to inherit together is linkage. The phenomenon of linkage, recombination and gene mapping will be discussed in this chapter.

16.2 Coupling and Repulsion Hypothesis

Bateson & Punnet (1906) crossed the sweet pea plants of a variety having blue flowers (B) and long pollen (L) with those of another variety having red flowers (b) and round pollen (l). The F_1 individuals (BbLl) had blue flowers and long pollen. These were test crossed with plants having red flowers and round pollen (double recessive). Normally, if independent assortment takes place one should expect 1:1:1:1 ratio in a test cross. Instead 7:1:1:7 ratio was actually obtained in which the maximum represented groups were parental types where two dominant alleles remain together in one group. Similar was the case with recessive alleles of other parent. They have coined the term 'coupling' to explain the presence of two dominant alleles together present in one parent and also recessive alleles in the other parent. In another cross when parents were chosen such that they contain one dominant trait of one character and one recessive trait of another chracter (i.e. Blue flowers, round prollen in one parent and red flowers long pollens in another parent), the test cross progeny of F_1 hybrid again produced 1:7:7:1 ratio in which again parental combinations namely, blue flowers round pollen (7/16) and redflowers long pollen (7/16) appeared in highest frequency. They coined the term 'repulsion' to explain a case that two dominant genes or recessive genes come from two different parents. The lack of independent assortment of genes in the above experiment has been attributed to 'coupling' and 'repulsion' of genes by Bateson and Punnet.

16.3 Linked genes and Linkage

A team of geneticists led by T.H.Morgan (1910-1915) performed experiments with *Drosophila* and found that coupling and repulsion were not complete. Morgan proposed that the two genes are found in coupling phase or repulsion phase, because these were present in the same chromosome (coupling) or on two different homologous chromosomes (repulsion). He called those genes present in one chromosome as *linked genes* and the phenomenon of such genes to inherit together in a group in their original combination is coined as *linkage*. Both coupling and repulsion pertains to the arrangement of linked genes. Results obtained, when linked genes are in coupling or in repulsion are shown in fig. 16.1 and 16.2

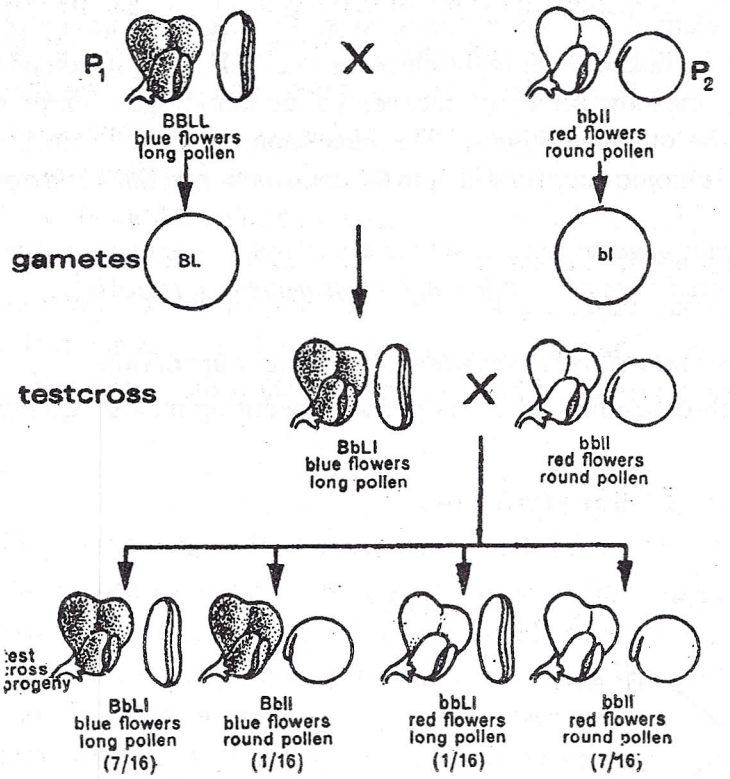


Fig. 16.1 Results obtained by Bateson in sweet pea when two characters, blue flowers and long pollens, were present in coupling phase.

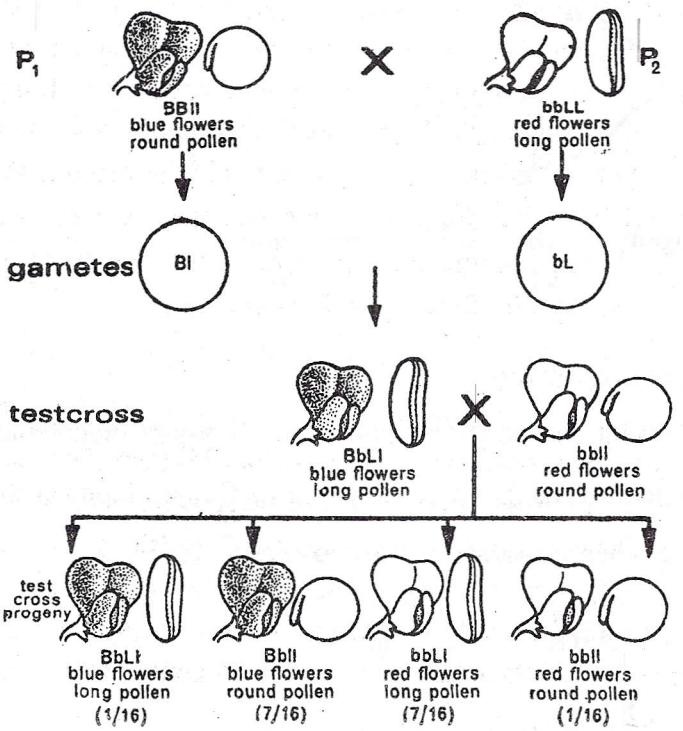


Fig. 16.2 Results obtained by Bateson in sweet pea when two characters, blue flowers and long pollen, were present in repulsion phase.

Complete linkage is relatively rare among organisms. The degree of strength of linkage depends upon the distance between the linked genes in the chromosome. T.H.Morgan coined the term "crossing over" i.e. exchange of chromosome segments between paired homologous chromosomes to explain the origin of recombination of linked genes. The idea soon developed into the theory of linear arrangement of genes in the chromosomes and later to the construction of linkage maps of chromosomes.

"*Linkage is the tendency of genes located in the same chromosome to be associated in inheritance more frequently than expected from their independent assortment in meiosis*".

Explanation for the genes that exhibit independent assortment or linkage

Genes on different chromosomes assort independently during meiosis giving a 1:1:1:1 test cross ratio

Parents: Aa Bb x aa bb

Gametes: A → B: AB, b: Ab ; a → B: aB, b: ab ; ab

Test Cros Progeny : AaBb : Aabb : aaBb : aabb

1/4 1/4 1/4 1/4

1 : 1 : 1 : 1

Linked genes (i.e. present on one chromosome) do not assort independently. But tend to stay together in the same combination as they were in parents. (Genes on one chromosome are separated by slash from those on the other homologous chromosome).

Parents: AB/ab x ab/ab

Gametes: (AB)(ab) (ab)

F_1 Progeny:	AB/ab	:	ab/ab
	1/2		1/2
	1	:	1

Large deviations from 1:1:1:1 ratio in the test cross progeny of a dihybrid could be used as evidence for *linkage.*

16.3.1 Linkage in maize

Hutchinson crossed a variety of maize having that were colored, normally filled-out(full) seed to one with colorless and shrunken seeds. Color gene [C] is dominant over colorless[c], and full grain (S) is dominant over shrunken seed (s). Accordingly the parents genotypes were CC SS and cc ss. The F_1 obtained was colored, full with genotype Cc Ss. When genes are linked they must be represented together. Hence the F_1 genotype is to be written as CS/cs. If C and S assort independently in accordance with Mendel's second principle, these F_1 plants should produce four types of gametes CS,Cs,cS, cs in equal numbers. When a test cross is made (F_1 hybrid x double recessive parent), the test cross progeny should segregate in a 1:1:1:1 ratio of all the four phenotypes. But in actual cross the following progeny was obtained.

Cross	CS/cs	x	cs/cs
Test cross progeny:			
Colored full	CS/cs- 4,032		Parental combination
Colored shrunken	Cs/cs - 149		Recombinants
Colorless full	cS/cs - 152		
Colorless shrunken	cs/cs 4,035		Parental combination

The parental combinations are predominantly more (4032+4035=8067→96.4%) than the recombinations (149+152=301→3.6%).Hence, the classical 1:1:1:1 test cross ratio was not obtained. It is obvious that the two genes and their alleles have not assorted independently and they remained combined or linked (in coupling phase) i.e. (C and S on one chromosome and c and s on corresponding homologous chromosome) in 96.4%. They are recombined (C with s and c with S) only in 3.6% of the gametes. (fig. 16.3)

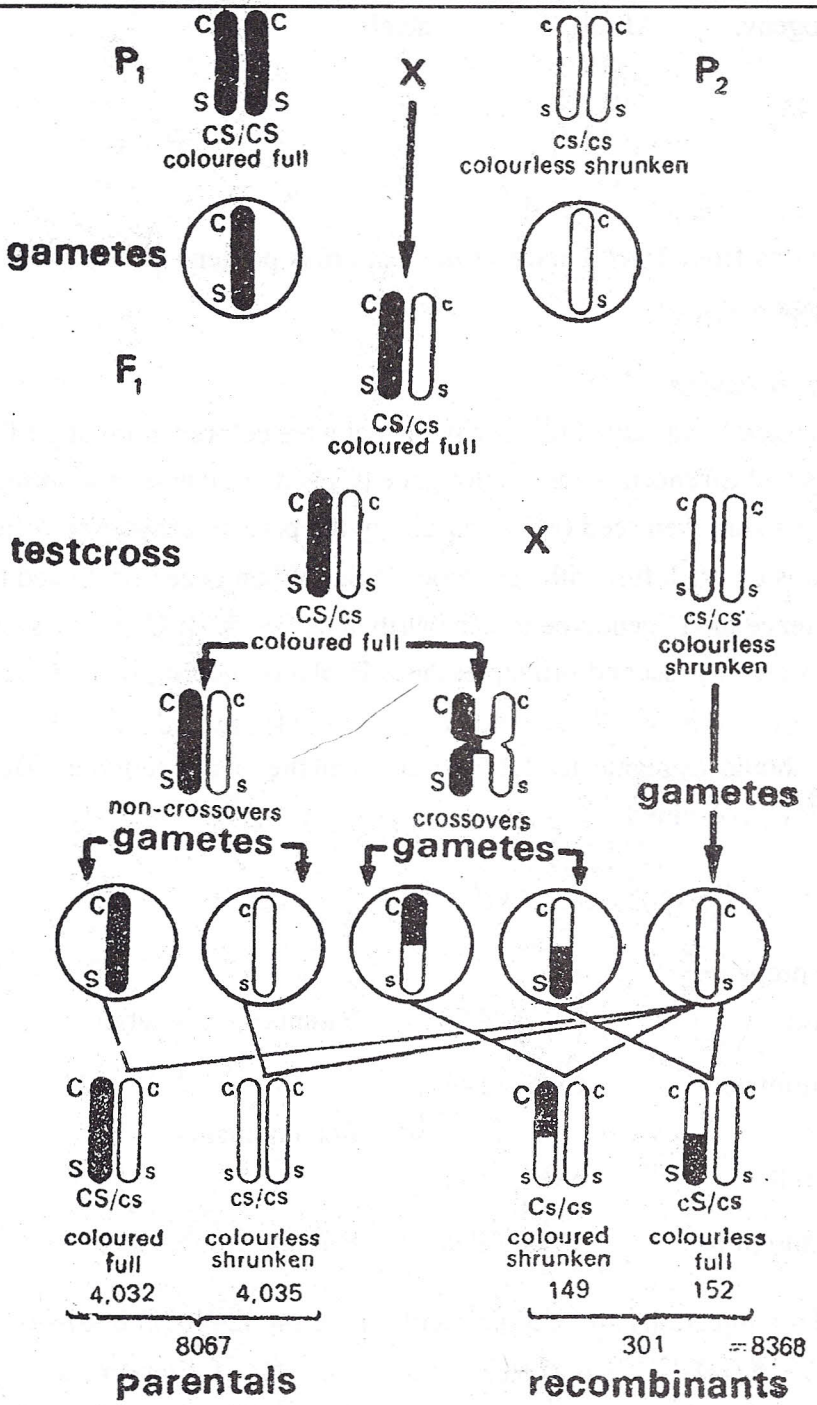
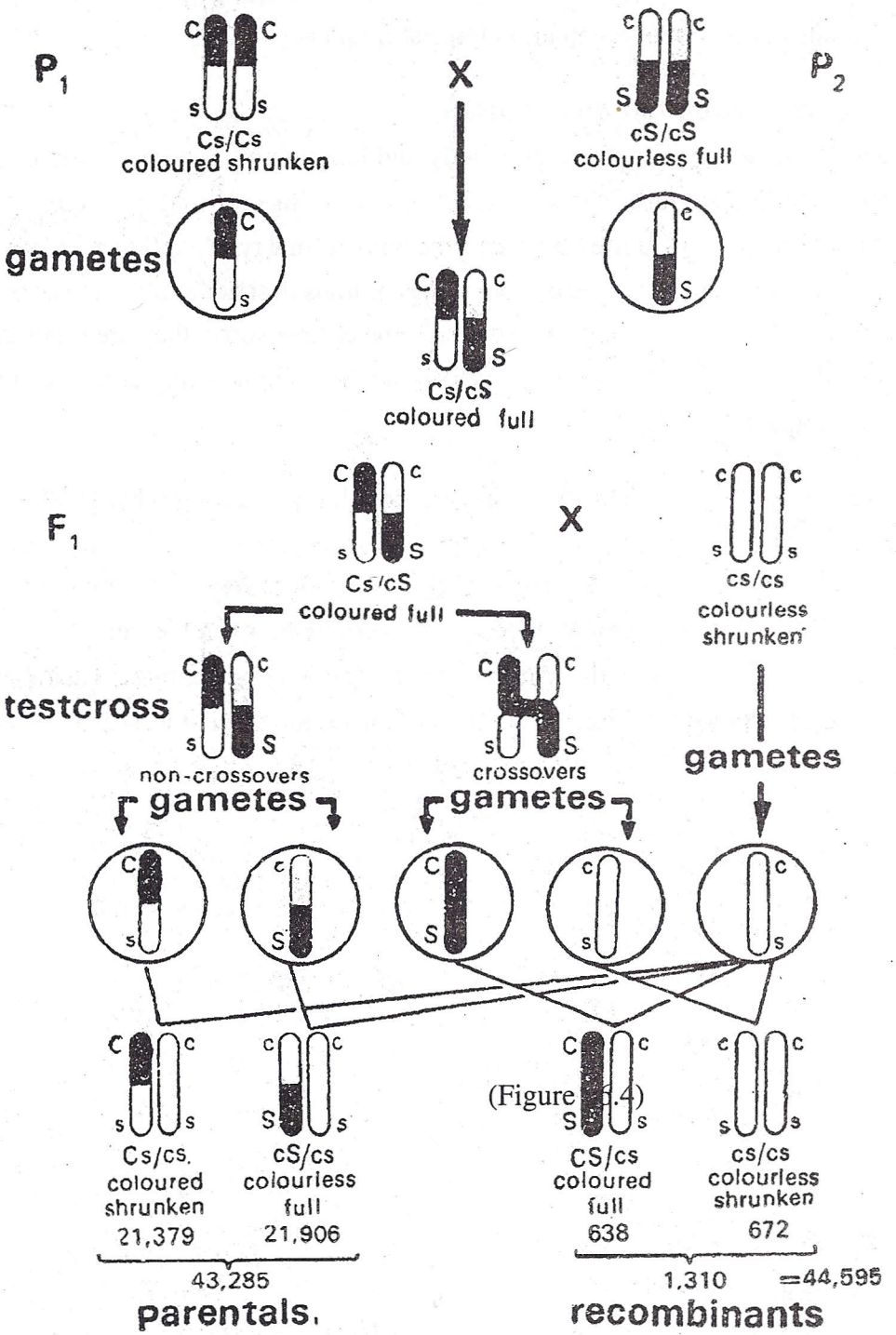


Fig. 16.3 Results obtained in maize by Hutchinson, when characters coloured seeds and full seeds were taken in coupling phase.

Final proof for linkage of genes obtained from another experiment in which parents with colorless full seeds were crossed with those with colored shrunken seeds. The F_1 was with colored full seeds. In the test cross progeny again parental combinations (97.06%). were predominants. (fig. 16.4)



(Figure 16.4)

Fig.16.4 Results obtained by Hutchinson in maize, when characters coloured seeds and full seeds were taken in repulsion phase.

It is clear that whatever be the parental combinations of linked genes on homologous chromosomes, linkage tends to keep them together, in highest frequency.

16.3.2 Linkage in *Drosophila melangaster*

In *Drosophila* the normal flies have grey body and long wings. There are some recessive mutants due to which the body color of the fly is black and the wings are under-developed or vestigeal. C.B.Bridges proposed that in linkage experiments the wild normal type of allele be represented by the sign +, and mutant recessive to wild type by abbreviated initials in small letters such as b for black and v for vestigial. Since linked genes are present on the same chromosome, they are represented by their symbols above and below a horizontal line, genes on one homologue being above the line and those on the other homologue below the line.

Bridges crossed a wild type female fly having grey body and long wings with a male having black body and vestigeal wings. The F_1 was wild type with grey body and long wings. These F_1 females were test crossed with double recessive male i.e. black vestigeal. Now, if the genes are assorting independently this cross would yield all four combinations in the ratio of 1:1:1:1. But the actual results showed deviation from the expected that the parental type grey long and black vestigeal were more and the recombinations (grey vestigial and black long) were less than expected (Figure 16.5). The observed data of test cross progeny is given table 16.1.

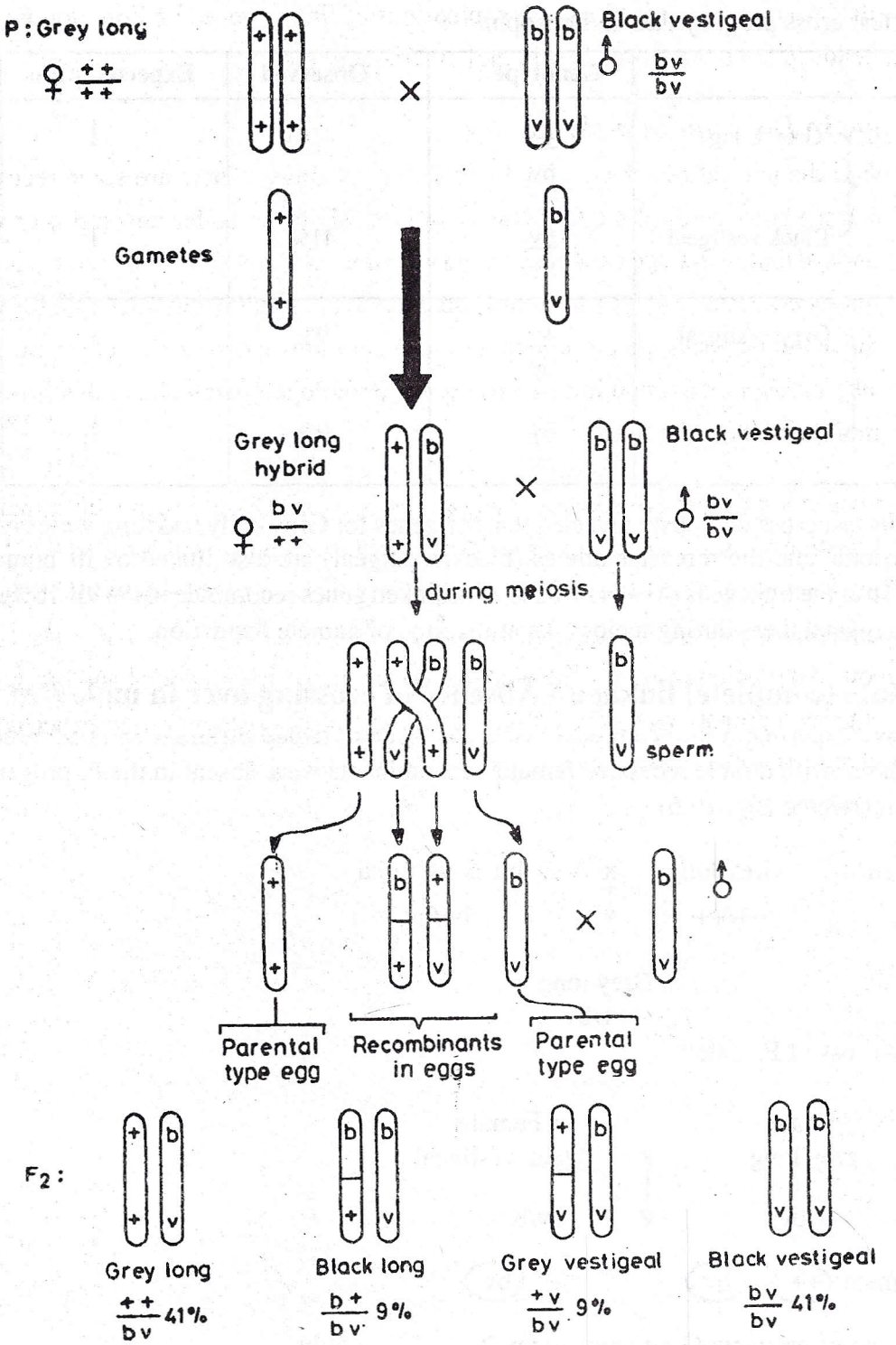


Fig. 16.5 Cross made by Bridges demonstrating linkage in *Drosophila*.

Table 16.1 the test cross progeny data in Drosophila

Phenotype		Genotype	Observed	Expected ratio
Parental Types	Grey Long	++ / bv	41%	1
	Black vestigeal	bv / bv	41%	1
Recombinant Types	Grey vestigeal	+v / bv	9%	1
	Black long	b+ / bv	9%	1

The result of this test cross obviously revealed that the genes for Grey body and long wings are linked on one chromosome and the recessive alleles (black, vestigeal) are also linked on its homologous, chromosome. Thus the linkage is (41+41) = 82%. The linked genes recombine in (9+9)=18%, through crossing over between them during meiosis i.e at the time of gamete formation.

16.3.3 Absolute (complete) linkage – Absence of crossing over in male *Drosophila*

In the above experiment the reciprocal test cross results yielded different results. When an F_1 male is test crossed with double recessive female, recombinants were absent in the F_2 progeny. Only praental types recovered (fig. 16.6)

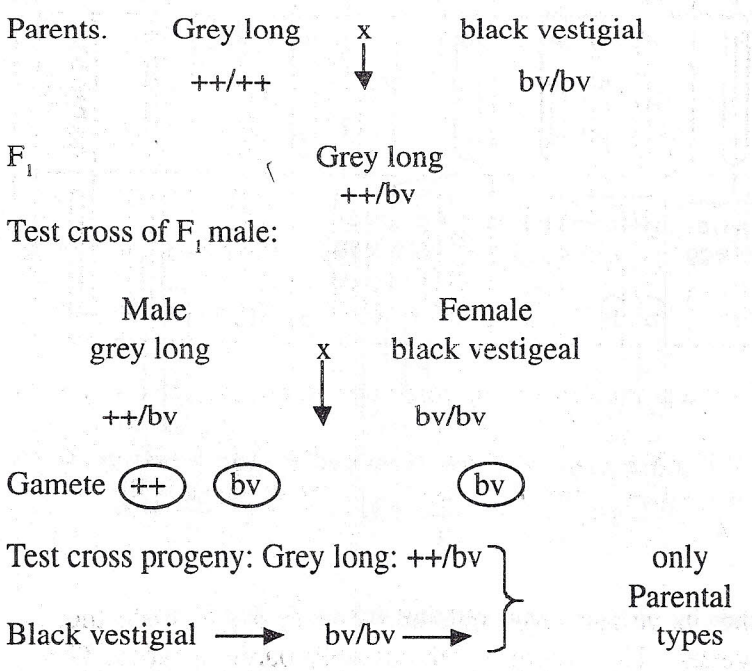


Fig. 16.6. Absolute (complet linkage in male Drosophila)

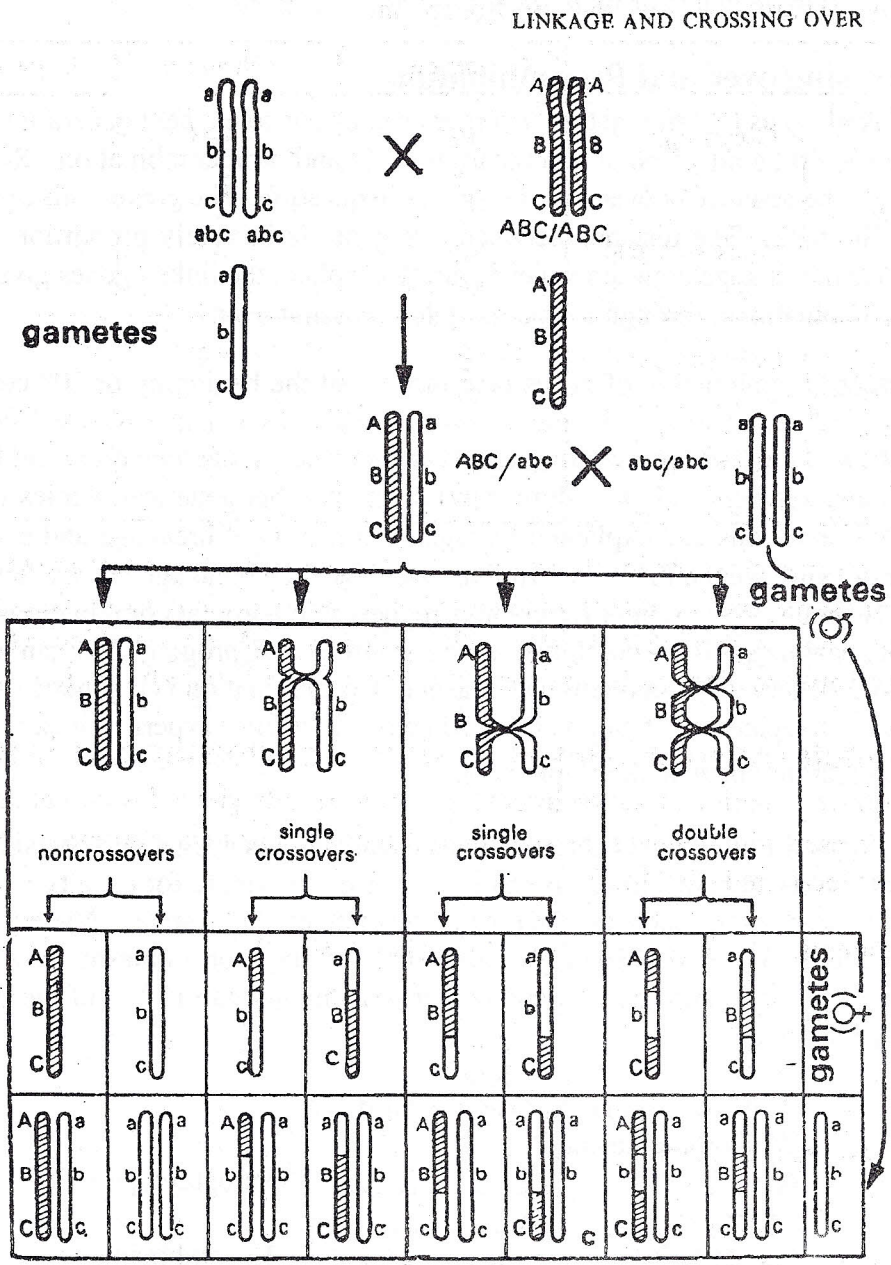


Fig. 16.7. A three-point testcross involving three hypothetical genes A,B and C.

criteria : if Z=X+Y, order of genes is A—B—C; if Z=X—Y, order of genes is A—C—B, and if Z=Y—X, order of genes is B—A—C.

Absence of recombinants in the above test cross indicates that in the F_1 male there is absence of crossing over between linked genes. The progeny contains only parental types. This is also an ex-

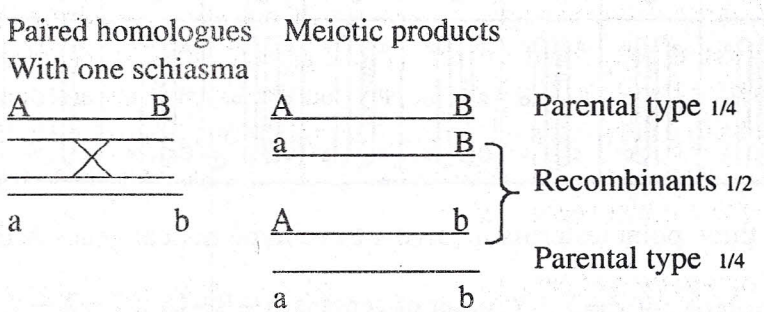
ample for absolute (complete) linkage in male *Drosophila* flies. The female silk moth (*Bombyx*) is another such example where crossing over does not take place.

16.3.4 Crossing over and Recombination

The linked genes inherit together in larger proportions to the next generation. If linkage is complete, there should be all parental combinations only and no recombination. Strength of linkage is determined by the distance between the two genes in question. The greater this distance, lower will be the linkage strength. The distance between any genes is inversely proportional to the strength of linkage. Since the linkage between genes is rarely absolute, the linked genes give rise to new combinations (recombinations) through a process called crossing over.

How does recombination of genes take place? At the beginning of 20th century, Janssens had made cytological observations on meiotic chromosomes in salamanders. He found that paired chromosomes showed cross-shaped configurations and suggested that they represent break and exchange of chromosome segments. Later Morgan supplemented his genetical studies on *Drosophila* with cytological observations and explained linkage on the basis of breakage and exchange in synapsed chromosomes. He coined the term *'crossing over'* for such breakage and exchange of chromosome segments. He could thus account for greater frequency of parental combinations and also why linkage was not absolute so that recombinant types occurred in F_2 progeny. Morgan termed cross-shaped configurations observed by Janssens as Chiasma. Recombination is a genetic outcome of breakage and exchange of segments. It can be inferred genetically from experiments and estimated from the frequency of recombinants in the F_2 progeny.

Every pair of homologous chromosomes usually forms atleast one chiasma somewhere along its length. There is a characteristic average number of chiasmata for each type of chromosome. In general, the longer the chromosome the greater the number of chiasmata. Moreover, farther apart two genes are located on a chromosome, the greater the chance for a chiasma to occur between them. When chiasma forms in one cell between two gene loci, only half (50%) of meiotic products will be cross over recombinant type.



Therefore chiasma frequency is twice the frequency of cross over products. The percentage of cross over (recombinant) gametes formed by a given genotype indicates the frequency of chiasma formation between the genes in question.

Chiasma % = 2 x cross over %

Cross over % = 1/2 chiasma %

for example if a chiasma forms between the loci of genes A and B in 30% of the tetrads (paired homologous chromosomes) of an individual of genotype AB/ab, then 15% of the gametes will be recombinant (Ab or aB) and 85% will be parental (AB or ab). Further the map distance between A and B would be 15 units apart. Each chiasma produces 50% cross over products. Fifty percent cross over is equal to 50 map units. If the average number of chiasmata is known for a chromosome pair, the total length of the genetic map for that linkage group may be predicted.
Total length = mean number of chiasmata x *50.*

16.3.5 Frequency of crossing over

In experiments on linkage, the proportion of parental types and new combinations can be counted. From the percentage of new combinations or recombinants, the amount of crossing over can be calculated. In the cross between colored full (CC SS) and colorless shrunken (cc ss) maize the F_1 hybrid is colored full (CS/cs). In the test cross progeny of F_1 hybrid new combinations obtained were 149+152=301. Out of total progeny 8368, new combinations observed are 301. To get the percentage of recombination 301 is divided by total i.e. 8368 and multiplied with 100.

$$\% \text{ Recombination} = \frac{301}{8368} \times 100 = 3.6$$

Therefore, percentage of recombination or frequency of crossing over between two linked genes C and S is 3.6. Recombination between two linked genes can not exceed 50% even when multiple cross overs occur between them.

16.4 Linear order of the genes and Genetic mapping

It is possible to locate genes on chromosomes, on the basis of their cross over frequency or recombination percentage. The distance between genes is measured in map units. According to Sturtevant one map unit is equal to 1% crossing over. In other words if one gamete out of 100 gametes i.e. 1% carries a cross over chromosome for two linked genes, we say that the two genes are one map unit apart. There are two major aspects to genetic mapping: 1)the determination of the relative distances between the genetic units (*gene distance*) and 2)the determination of the linear with which genetic units are arranged with respect to one another (*gene order*).

16.4.1 Map distance in two-point test cross:

The easiest way to detect cross-over gametes in a dihybrid is through test cross progeny. Suppose we test-cross dihybrid in coupling phase (AC/ac) and find in the progeny the following phenotypes

Dominant at both loci AC/ac = 37%
Recessive at both loci ac/ac = 37%

Dominant at the first locus and
Recessive at the second locus Ac/ac = 13%

Recessive at the first locus and
Dominant at the second locus aC/ac = 13%

Obviously the last two groups (genotypically Ac/ac and aC/ac) were produced by crossover gametes from the dihybrid parent. Thus 13+13=26% of all gametes were cross over types and the distance between the loci A and C is estimated to 26 map units. Map units are often referred to as *centimorgans* (cM) in honour of the work of Thomas Hunt Morgan, a famous *Drosophila* geneticist.

16.4.2 Map distance in three point test cross

A.H.Sturtevant devised a test where by the position and linear order of a third gene could be determined with reference to two other genes on the same chromosome. Because three genes are used simultaneously, the test is known as a *three-point cross.* The underlying basic principle involved in the preparation of linkage map or genetic map can be explained by using a hypothetical example.

Let us presume that there are three genes A, B and C present on the same chromosome (linked genes). There could be *'three'* possible linear orders in which these genes may be present on a chromosome.

A B C or A C B or B A C

As is obvious, that in one case B is present in the middle, while in second C is in the center and in third case A is at the middle position. Therefore in finding out linear order, one has to really find out the gene which is in the center. Presuming A B C as the order of the genes, the results expected in the trihybrid test cross can be represented as in Fig 16.7. The frequencies of eight types of genotypes can be listed as in table 16.2

Table 16.2 Frequencies of different genotypes and phenotypes obtained in a trihybrid test cross of the genes is A-B-C;

State of Crossing over		Genotype	Phenotype	Supposed frequency
Non cross overs	A B C / a b c	ABC/abc	ABC	A
		abc/abc	abc	b
Crossing-over between A-B	A B C / a b c	Abc/abc	Abc	c
		aBC/abc	aBC	d
Crossing-over between B-C	A B C / a b c	ABc/abc	ABc	e
		abC/abc	abC	f
Crossing-over between (A-B) & (B-C)	A B C / a b c	aBc/abc	aBc	g
		Abc/abc	AbC	h

Total = T

The following procedure will be followed for preparation of linkage map.

If crossing over or recombination percent between A and B is called X, B and C is called Y and that between AC is called Z, then

a) Crossing over percentage between A-B (when A and B separated) $X = \frac{c+d+g+h}{T} \times 100$

b) Crossing over percentage between B-C (where B and C separated) $Y = \frac{e+f+g+h}{T} \times 100$

c) Crossing over percentage between A-C (when A and C separated) $Z = \frac{c+d+e+f}{T} \times 100$

From the above values of X, Y and Z order of the genes can be worked out and linkage map can be prepared by using the following criteria if Z = X + Y, order of the genes is A-B-C;

if Z=X-Y, order of the genes A-C-B and if Z=Y-X, order of the genes is B-A-C.
The preparation of a 'linkage map' can be further illustrated by using an example from maize involving three-point cross.

The genes brown midrib (bm), red aleurone (pr) and virescent seedling (v) are located in chromosome 5 and the data obtained from linkage studies is as follows:

Parents: $\frac{+\ +\ +}{+\ +\ +} \times \frac{bm\ pr\ v}{bm\ pr\ v}$

F_1 $\frac{+\ +\ +}{bm\ pr\ v}$

Test Cross progeny:

+ + +	232
bm pr v	235

Non cross-overs —— Parents = $\frac{467}{1109} \times 100 = 42.1\%$

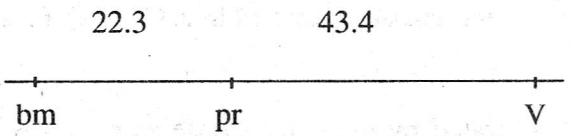
+ pr v 84
bm + + 72 } Single cross-overs between bm and pr = 161 x 100 = 14.5%
1109

+ + v 201
bm pr + 194 } Single cross overs between pr and v = 395 x 100 = 35.6%
1109

+ pr + 40
bm + v 46 } Double cross overs between bm and pr and between pr and b = 86 x 100 = 7.8%
1109

From the above it is clear that the non-cross over types are those that preserve the original parental gene grouping (linked genes), the least frequent groups represent double cross overs, representing those having lowest probability of occurrence. This group provides the information on the linear order of the genes. With respect to bm and b, the gene pr has shifted position with its allele and is therefore between bm and v. Then the order of the genes is bm - pr-v. The two remaining classes constitute the single cross over types, one class representing the cross over taking place between bm and pr and the other class representing cross over between pr and v.

In calculating genetic distance between two genes, all cross overs (single and double) that have occurred in a particular region must be taken into consideration, because we are attempting to construct an accurate representation of the genetic map from cross over data. The map distance between bm and pr, then is not 14.5 but (14.5+7.8) = 22.3. Similarly the total crossover frequency for the pr-v region is 35.6 + 7.8 = 43.4. The total distance from bm to v would be 22.3 + 43.4 = 65.7, because units of map can be successively added to indicate genetic distance. Thus the genetic map of the three genes on 5th chromosome of maize is shown here



Through accumulation of genetic data on the frequencies of crossing over, it has been possible to construct genetic or chromosome maps in which the serial order of the genes and their genetic spacing have been accurately determined. Figure 16.8 shows linkage map of the four chromosomes of Drosophila melanogaster

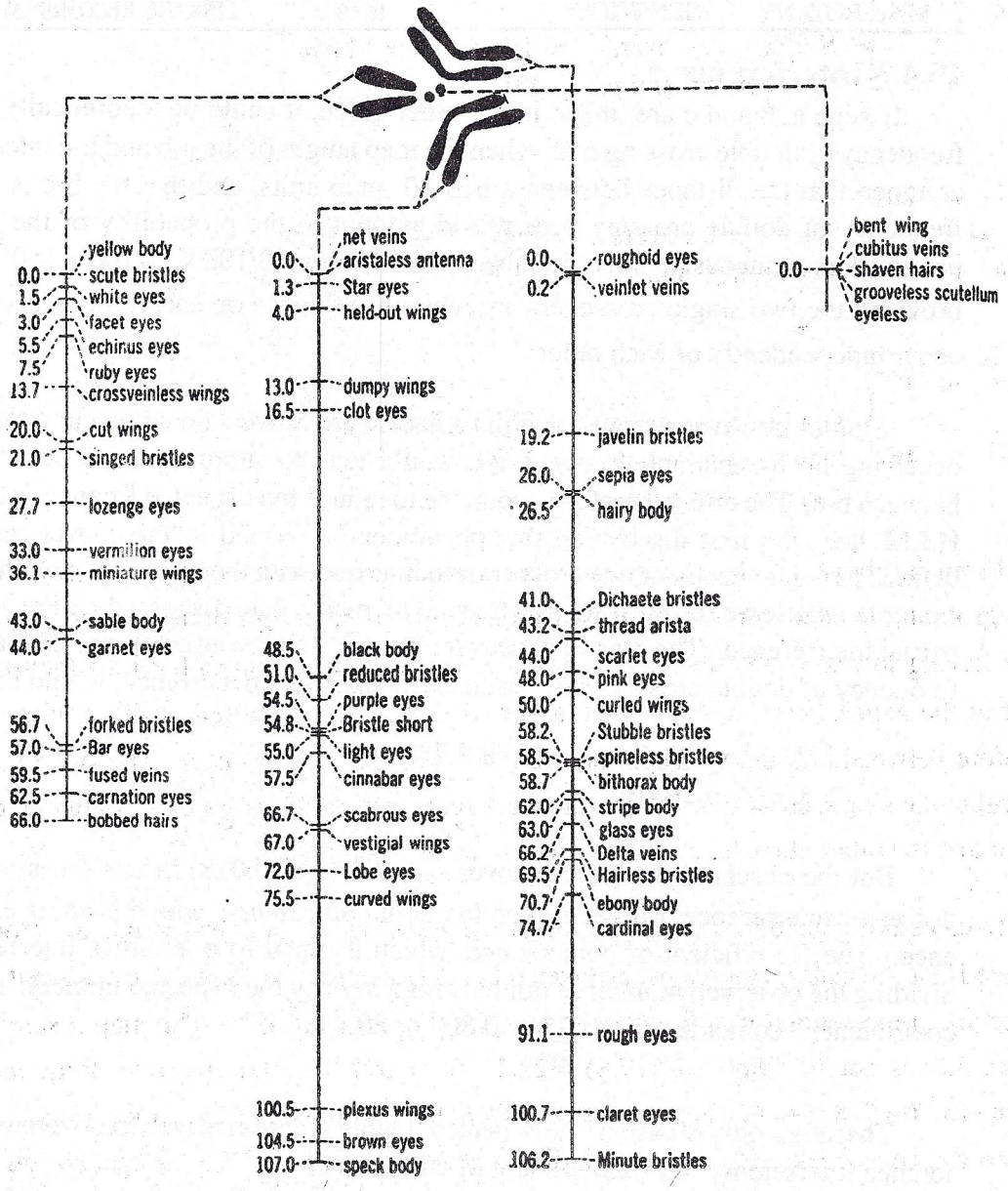


Fig. 16.8 A genetic or linkage map of the four chromosomes of *Drosophila melanogaster,* showing the relative positions of some of the more important genes. Figures refer to distances from the upper end of the chromosome as determined from the percentages of recombination observed in linkage experiments.

Genetic maps and physical maps:

The actual physical distance between linked genes separated by nucleotide base pairs (bp) is the *physical map.* The genetic map is one that shows the gene order and distances between adjacent genes, where distance is proportional to the rate of recombination or crossing-over between genes. It is drawn in centimorgan (cM) units. There is no direct relationship to the map distances calculated in the above two ways.

16.4.3 Interference

If gene a, b and c are linked in the order given, it could be theoretically possible to predict the frequency of double crossing over when the map length of the a-b and b-c intervals are known. If it is assumed that the distance between a-b is 10 map units, and that the b-c is 15 map units, then the frequency of double crossing over would amount to the probability of the two single cross overs occrung simultaneously. This could be 10% of 15% or 10/100 x 15/100 = 150/10000 = 0.015 or 1.5%, provided the two single crossovers are without influence on each other's occurrence, that is, if they occur independently of each other.

Studies however revealed that the adjacent cross overs do not occur independently and that one occurring in, for example between a-b would tend to suppress those occurring simultaneously in between b-c. The effect therefore, would be to reduce the expected frequency of double crossing over. H.J.Muller who first discovered this phenomenon, termed it 'cross-over interference'. It may be defined as the tendency of one cross over to interfere with the occurrence of another in its vicinity. An example will serve to illustrate its effect. The maize data discussed earlier in this chapter illustrate partial interference. The map distances for bm-pr and pr-v intervals are 22.3 and 43.4. The expected frequency of double cross- overs, based on independent occurrence, would be

$$\left[\frac{22.3}{100}\right] \times \left[\frac{43.4}{100}\right] = 0.097 \text{ or } 9.7\%$$

But the observed double crossovers are 7.8% (or 0.078) in test-cross progeny. To express the degree of interference, Muller coined the term *coincidence,* which is an inverse measure of interference. The Co-efficient of coincidence, which is equal to one minus interference, is calculated by dividing the observed number of double crossovers by the expected number. In the above experiment coefficient of coincidence $\frac{0.078}{0.097} = 0.804$ or 80.4% .

Therefore only 80.4% of the expected double crossovers were recovered; indicating partial interference Interference = 1 - Coefficient of coincidence

= 1 - 0.804 = 0.196 or 19.6%

16.5 Other methods of determining linkage and recombination in dihybrid F_2 progeny:

The estimation of gene distance and linkage has been already dealt by utilizing dihybrid test-cross progeny data. An alternative to the test cross method, there are two other methods, 1)Square-Root Method and 2)Product Ratio Method, to estimate map distance or recombination frequency from dihybrid selfed progeny.

16.5.1 Square-root Method: If the genes in F1 are in *coupling* phase, the frequency of double recessive phenotypes are used as an estimator of frequency of *non-cross over gametes.* When the genes in F_1 are in repulsion phase, double recessive phenotypes are used as an estimator for the frequency of *crossover gametes.*

If F_1 in coupling phase:

% Non-cross over gametes = $2\sqrt{\text{frequency of double recessives}}$

If F_1 in repulsion phase:

% cross-over gametes = $2\sqrt{\text{frequency of double recessives}}$

For example from the F_2 data we know that double recessives percentage is 16% . Suppose the F_1 dihybrid is in coupling phase. Then the percentage of recombination can be calculated in two steps.

Step 1. non-crossovers = $2\sqrt{\text{freq. Double recessives.}}$

$$= 2\sqrt{\frac{16}{100}} = 2\sqrt{0.16} =$$

$$= 2(0.4) = 0.8 \text{ or } 80\%.$$

Step 2. If the percentage of non-crossovers is 80, then crossover (recombination) percentage = 100-80=20%. Thus the two genes are 20 maps units apart.

Similarly if the double recessive are 1% and the F_1 is in repulsion phase then:

0% cross over gametes = $2\sqrt{\text{Freq. Double recessives}}$

$$\% \text{ cross over gametes} = 2\sqrt{\frac{1}{100}}$$

$$= 2 \times 0.1 = 0.2 \text{ or } 20\%$$

There fore, recombination between two genes is 20%

16.5.2 Product-Ratio Method

In the dihybrid F_2 progeny the four phenotypes of eg. R-S-, R-ss, rr S-, rrss are appearing in the frequency of a, b, c, d respectively. The ratio of crossovers to parental types is called the product-ratio, is a function of recombination.

For coupling data $\chi = bc/ad$

For Repulsion data $\chi = ad/bc$

The recombination fraction represented by the value of χ may be read directly from a **product – ratio table 16.3.** calucation recombination by using product ratio method is shown with an example.

Table : 16.3 **Recombination Fraction Estimated by the Product-Ratio Method**

Recombination Fraction	Ratio of Products		Recombination Fraction	Ratio of Products	
	ad/bc (Repulsion)	*bc/ad* (Coupling)		*ad/bc* (Repulsion)	*bc/ad* (Coupling)
0.00	0.000000	0.000000	0.26	0.1608	0.1467
0.01	0.000200	0.000136	0.27	0.1758	0.1616
0.02	0.000801	0.000552	0.28	0.1919	0.1777
0.03	0.001804	0.001262	0.29	0.2089	0.1948
0.04	0.003213	0.002283	0.30	0.2271	0.2132
0.05	0.005031	0.003629			
0.06	0.007265	0.005318	0.31	0.2465	0.2328
0.07	0.009921	0.007366	0.32	0.2672	0.2538
0.08	0.01301	0.009793	0.33	0.2892	0.2763
0.09	0.01653	0.01262	0.34	0.3127	0.3003
0.10	0.02051	0.01586	0.35	0.3377	0.3259
0.11	0.02495	0.01954	0.36	0.3643	0.3532
0.12	0.02986	0.02369	0.37	0.3927	0.3823
0.13	0.03527	0.02832	0.38	0.4230	0.4135
0.14	0.04118	0.03347	0.39	0.4553	0.4467
0.15	0.04763	0.03915	0.40	0.4898	0.4821
0.16	0.05462	0.04540	0.41	0.5266	0.5199
0.17	0.06218	0.05225	0.42	0.5660	0.5603
0.18	0.07033	0.05973	0.43	0.6081	0.6034
0.19	0.07911	0.06787	0.44	0.6531	0.6494
0.20	0.08854	0.07671	0.45	0.7013	0.6985
0.21	0.09865	0.08628	0.46	0.7529	0.7510
0.22	0.1095	0.09663	0.47	0.8082	0.8071
0.23	0.1211	0.1078	0.48	0.8676	0.8671
0.24	0.1334	0.1198	0.49	0.9314	0.9313
0.25	0.1467	0.1328	0.50	1.0000	1.0000

Source: F. R. Immer and M. T. Henderson, "Linkage studies in barley," *Genetics*, 28: 419–440, 1943.

Example

Parents RS/RS x rs/rs

↓

F1 RS/rs (in coupling phase)

F_2	Phenotype	Numbers
a	R_ S_	1221
b	R-ss	219
c	rr S-	246
d	rrss	243

χ (for coupling data) = $\frac{bc}{ad} = \frac{219 \times 246}{1221 \times 243} = 0.1816$

χ lies between the values of product-ratio table 16.3 which corresponds to recombination fractions of 0.28 and 0.29 respectively. Therefore the recombination is approximately 28%.

16.6 Mapping by Tetrad Analysis

The term *tetrad* refers to the four chromatids that make up a pair of homologous chromosomes in meiotic division and the four haploid products of a single meiosis.

Tetrad analysis is a method for analysis of linkage and recombination using the four haploid products of single meiotic division.

In some fungi (*Yeast* and *Neurospora)* a sae-like structure 'ascus' contains all the four haploid products of meiosis included in reproductive cells called 'ascospores'. The advantages of these fungi to study recombination and genetic analysis are: 1) the potential for analyzing all of the products from each meiotic division 2)the haploid nature organisms, so dominance is not a complicating factor because the genotype is expressed directly in the phenotype and 3) large number of progeny is produced enabling to detect rare events and to estimate their frequency.

One of the simplest ascomycetes is the unicellular baker's yeast *Saccharomyces cerevisiae* (Fig. 16.9).

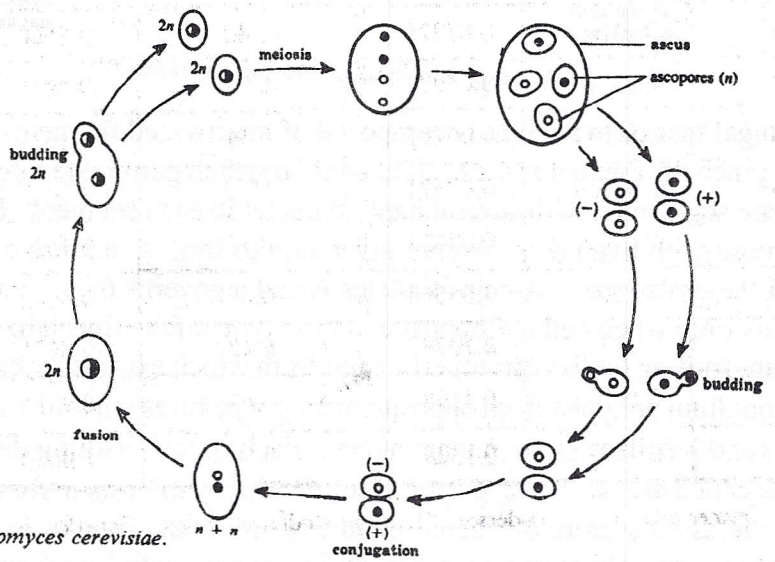


Fig. 16.9 Life cycle of *Saccharomyces cerevisiae.*

Asexual reproduction is by budding, a mitotic process usually with unequal cytokinesis. The sexual cycle involves the union of entire cells of opposite mating type forming a diploid zygote. The diploid cell may produce diploid progeny asexually by budding or haploid progeny by Meiosis. The *four haploid* nuclei form ascospores enclosed by the ascus. Rupture of the ascus releases the haploid spores, which then germinate into new yeast cells.

Another ascomycete of interest to geneticists is the bread mold *Neurospora crassa* (Fig.16.10).

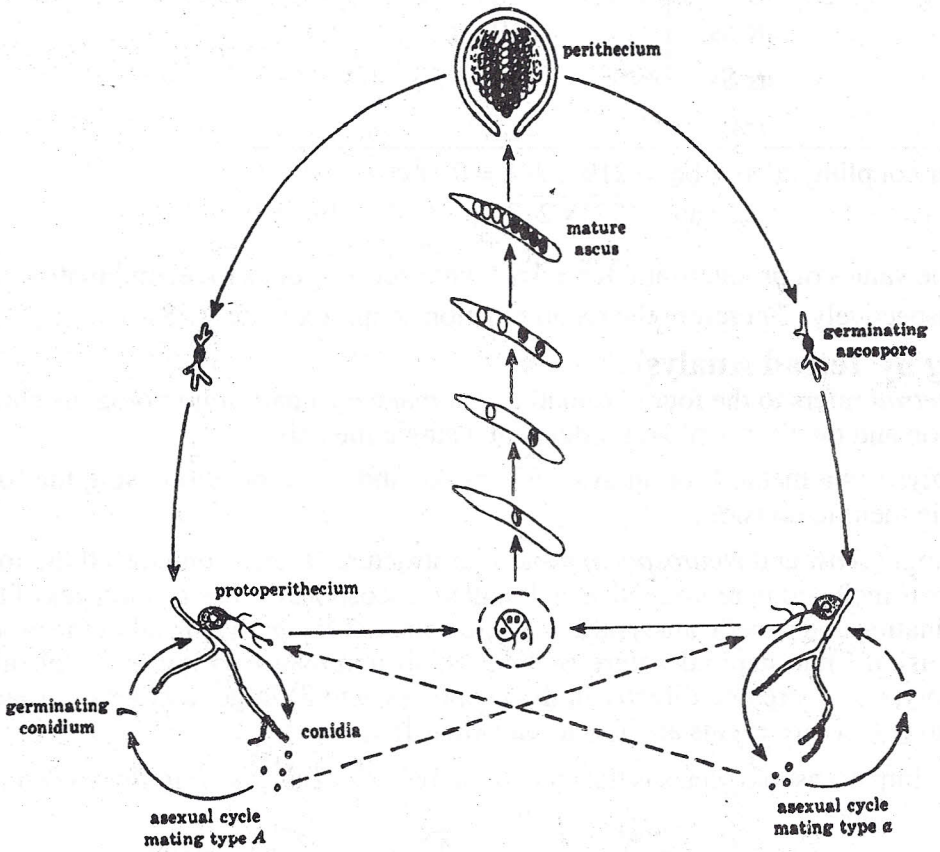


Fig. 16.10 Life cycle of *Neurospora crassa.*

The fungal mat or mycelium is composed of intertwined filaments called hyphae. The tips of hyphae may pinch off asexual spores called *conidia*, which germinate into more hyphae. The vegetative hyphae are segmented with several haploid nuclei in each segment. Hyphae from one mycelium may anastomose with hyphae of another mycelium to form a mixture of nuclei in a common cytoplasm called '*heterokaryon*". A pair of alleles A and a governs the two mating types. Sexual reproduction occurs only when cells of opposite mating type unite. Specialized region of mycelium produce immature fruiting bodies (protoperithecia) from which extrude receptive filaments called '*trichogynes*'. A conidium or hypha from opposite mating type fuses with the trichogyne, undergoes several karyokineses and fertilizes many female nuclei. Each of the resulting diploid zygotes lies within an elongated sae called ascus. The zygote nucleus divides by meiosis to form four nuclei, followed by a mitosis that yields four pairs of nuclei, maturing into eight ascospores. A mature fruiting body (Perithecium) may contain over 100 asci, each containing eight ascospores.

In case of yeast the ascospores representing the four chromatids of meiosis are in no special order (*un-ordered),* but in *Neurospora* the ascospores are linearly *ordered* in the ascus in the same sequence as the chromatids were on the meiotic metaphase plate. It is because of the confines of the ascus force the polar organization of division to orient lenghth-wise in the ascus and prevent the meiotic and mitotic products from slipping past each other. Each of the four chromatids of first meiotic prophase are now represented by a pair of ascospores in tandem order within the ascus.

16.6.1 The analysis of unordered Tetrads:

In the tetrad, when two pairs of alleles are segregating, three *patterns* of segregations are possible. For example, in the cross AB x ab the three types of tetrads are 1)*Parental ditype*(PD) 2)Non *parental ditype* (NPD) and 3)*Tetra type* (TT).

Parental ditype contains only two genotypes and their alleles have the same combinations found in the parents. Non-parental ditype contains two genotypes only but their alleles have non-parental combinations. Tetra type contains all four of the possible genotype. The figure 16.11

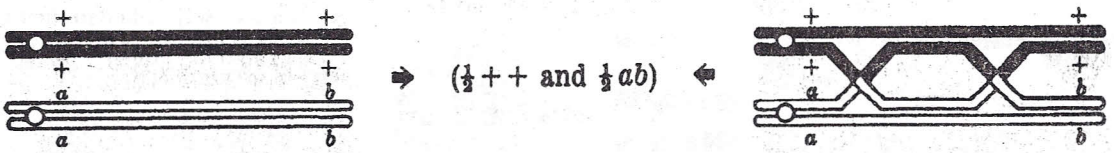


Fig. 6-7. Parental ditypes.

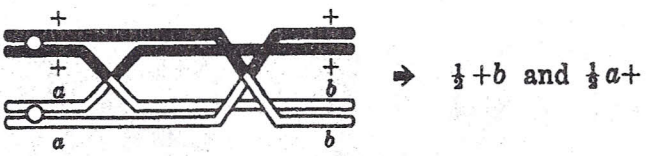


Fig. 6-8. Non-parental ditypes.

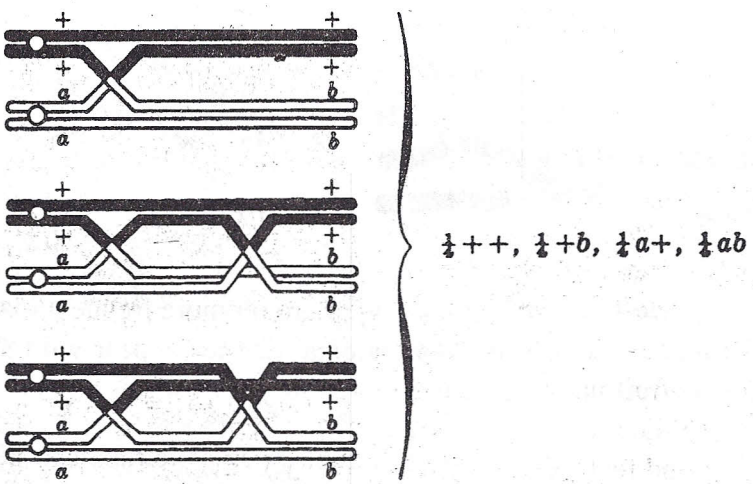


Fig. 16.11 Showing two strand, three strand and four strand single and double cross overs resulting PD, NPD and TT.

shows the types of number of two strand, three strand, four strand single and double cross-overs occurring between the four chromatids resulting three different patterns of segregations.

Tetrad analysis is the effective way to determine whether the two genes are linked or not.

"When the genes are unlinked, the parental ditype (PD) tetrads and non-parental ditype (NPD) tetrads are expected in equal frequencies (PD=NPD)" (Figure 16.12).

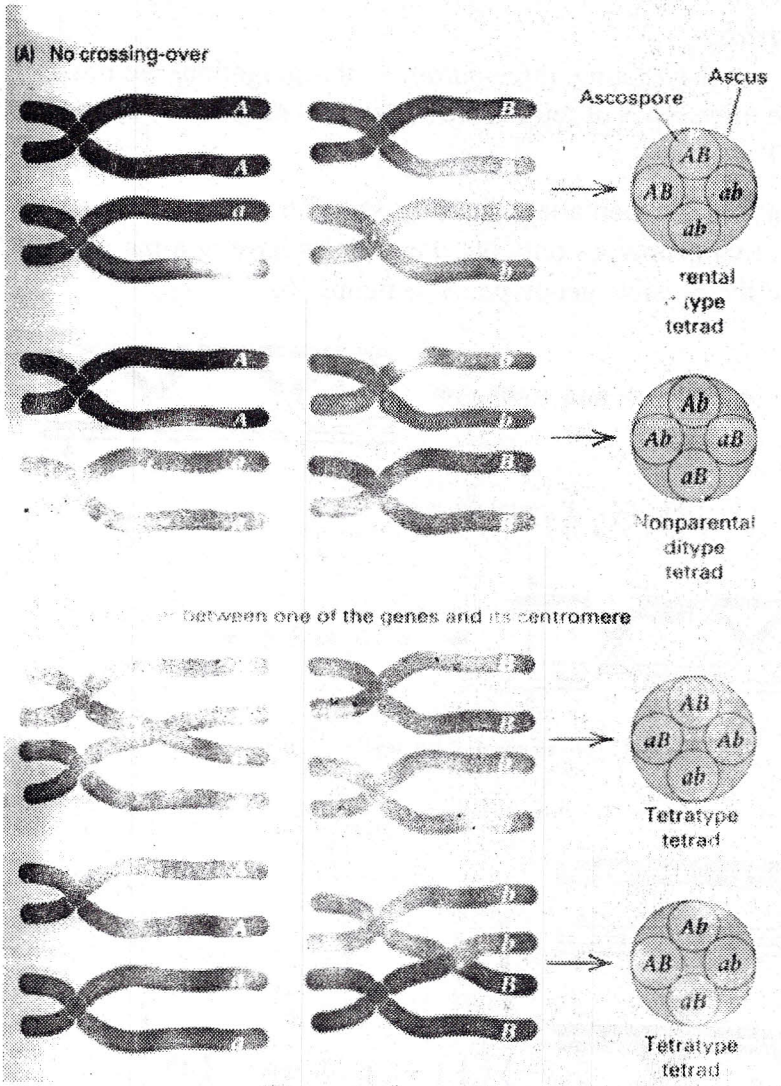


Figure 16.12
Types of unordered asci produced with two genes in different chromosomes. (A) In the absence of crossing-over, random arrangement of chromosome pairs at metaphase I yields two different combinations of chromatids, one yielding PD tetrads and the other NPD tetrads. (B) When crossing-over takes place between one gene and its centromere, the two chromosome arrangements yield TT tetrads. If both genes are closely linked to their centromeres (so that crossing-over is rare), then few TT tetrads are produced.

When genes are linked, parental ditypes are far more frequent than nonparental ditypes. Linkage manifestation is also one that non-parental ditypes appear with a much lower frequency than parental ditype tetrads (NPD <<PD).

The relative frequencies of the different types of tetrads can be used to determine the map distance between two linked genes, assuming that the genes are sufficiently close enough that triple cross overs and higher levels of crossing overs can be neglected.

The number of double cross-overs expected are four times equal to that of **NPD tetrads (Fig. 16.13)**

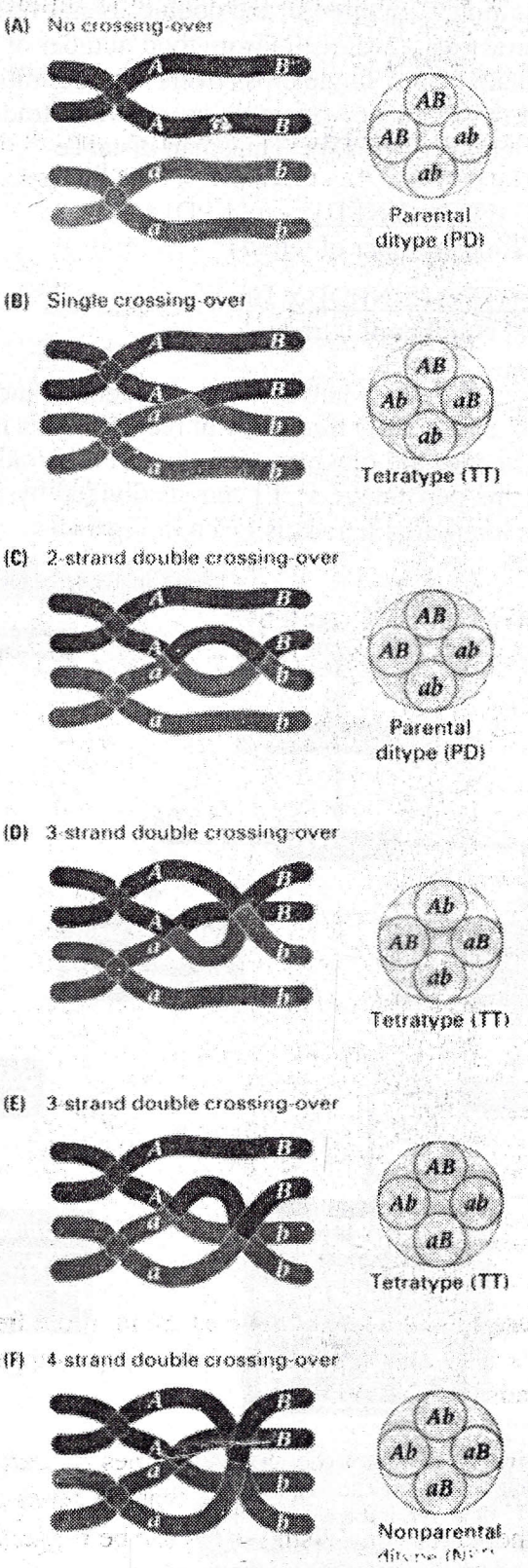


Figure 16.13
Types of tetrads produced with two linked genes. In the absence of crossing-over (A), a PD tetrad is produced. With a single crossover between the genes (B), a TT tetrad is produced. Among the four possible types of double crossovers between the genes (C, D, E, and F), only the four-strand double crossover in part F yields an NPD tetrad.

and TT tetrads resulting from three strand double cross-overs should be equal to twice the number of NPD tetrads. Subtracting 2 x (NPD) from total number of TT tetrads yield the number of tetratype tetrads that origninate from single cross overs. By definition the map distance between two genes equals one-half of the frequency of single cross-over tetrads (TT) – 2 (NPD) plus the frequency of double cross over tetrads (4[NPD]). Thus map distance

$$= \frac{(1/2)\,([TT] - 2[NPD]) + 4\,[NPD]}{\text{Total Number of tetrads}} \times 100$$

$$= \frac{(1/2)\,([TT] + 3[NPD])}{\text{Total Number of tetrads}} \times 100$$

In the above equation only half the tetra type tetrads and 3[NPD] tetrads are recombinants out of the total number of tetrads. The frequency of recombinants is calculated in percentage. In the special case where linked genes are close enough that NPD tetrads = o (no double cross overs), the map distance equals to the percentage of TT tetrads divided by 2. A tree diagram for analyzing linkage relationships from unordered tetrads is given in Fig 16.14.

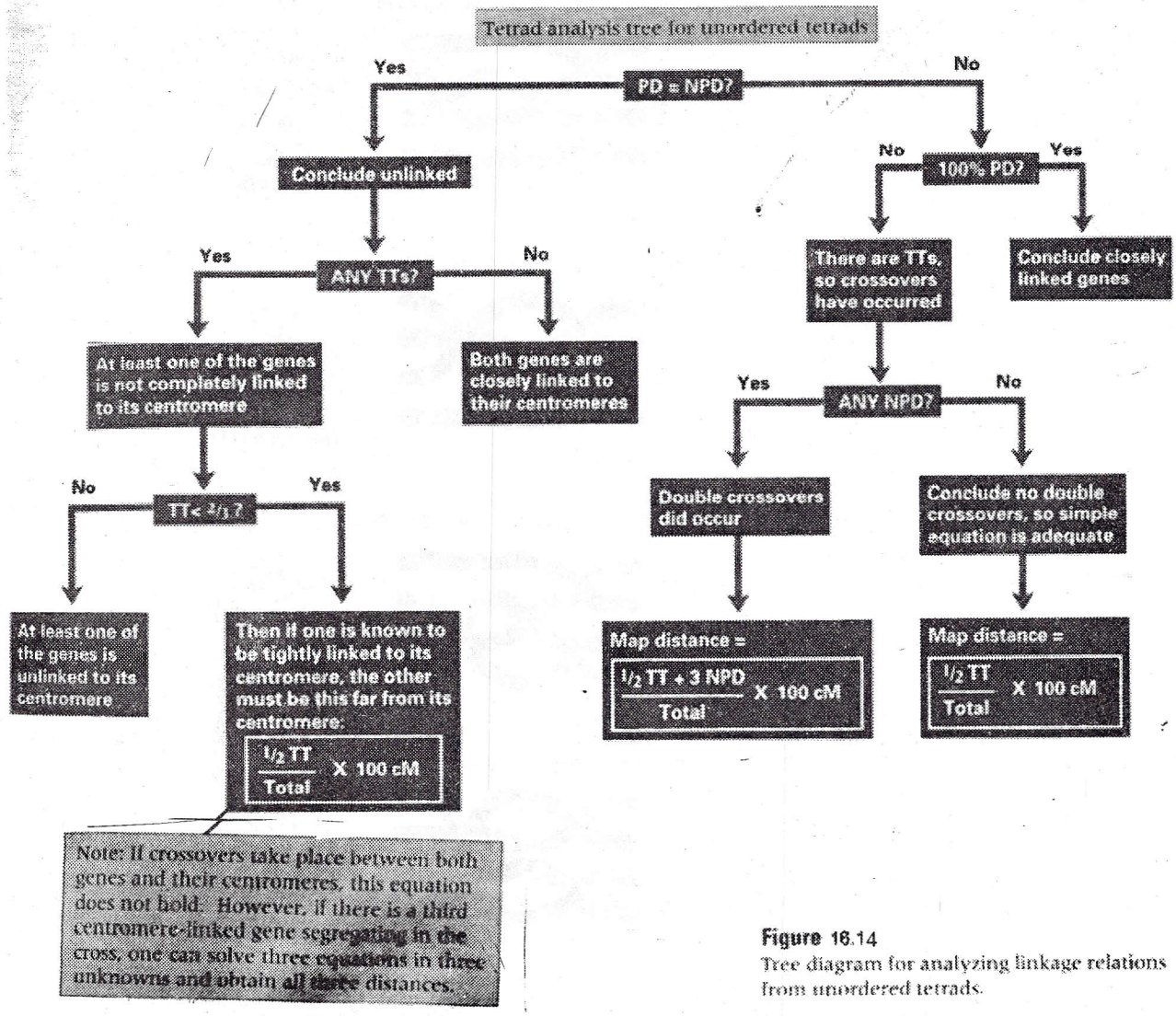


Figure 16.14
Tree diagram for analyzing linkage relations from unordered tetrads.

16.6.2 Analysis of ordered tetrads

Neurospora cressa is extensively used in genetic investigations. In this species the products of meiosis are contained in an ordered array of ascospores. The four nuclei produced by meiosis from zygote nucleus are in a linear order, ordered sequence in the ascus, and each of them undergoes a mitotic division to form two genetically identical and adjacent, ascospores. Each mature ascus contains eight ascospores arranged in four pairs, each pair derived from one of the products of meiosis. The ascospores can be removed one by one from an ascus and each germinated in a culture tube to determine its genotype.

Ordered asci also can be classified as PD, NPD, or TT with respect to two pairs of alleles. Hence tree diagram in figure 16.14 can be used to analyse linkage data.

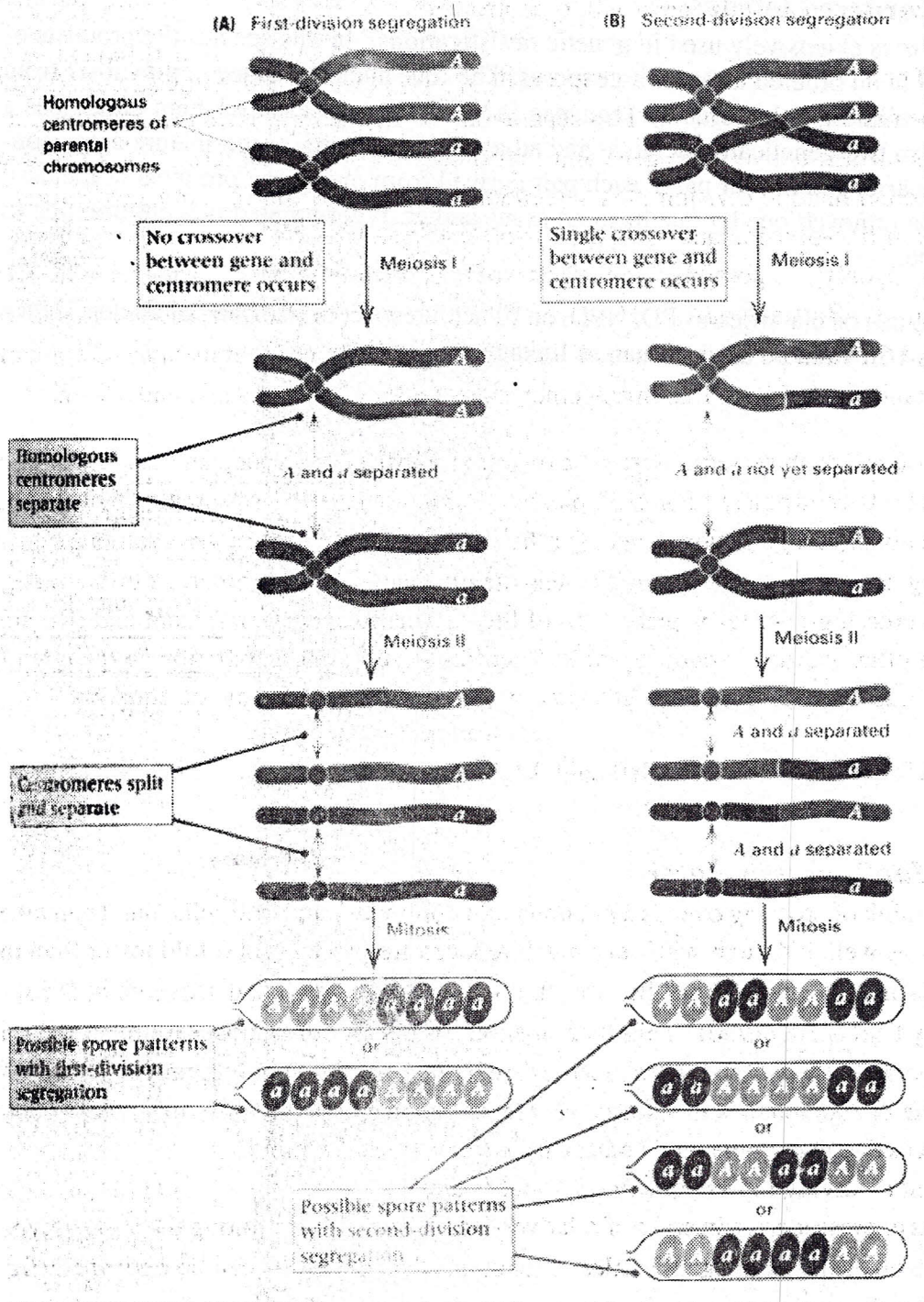


Figure 16.15
First- and second-division segregation in *Neurospora*. (A) First-division segregation patterns are found in the asci when crossing-over between the gene and centromere does not take place. The alleles separate (segregate) in meiosis I. Two spore patterns are possible, depending on the orientation of the pair of chromosomes on the first-division spindle. (B) Second-division segregation patterns are found in the asci when crossing-over between the gene and the centromere delays separation of *A* from *a* until meiosis II. Four patterns of spores are possible, depending on the orientation of the pair of chromosomes on the first-division spindle and that of the chromatids of each chromosome on the second-division spindle.

In addition, the ordered arrangement of meiotic products makes it possible to determine the recombination frequency between any particular gene and its centromere.

In the absence of crossing over between a gene and its centromere, the alleles of the gene (A and a) must separate in the first meiotic division. This separation is called *"first-division segregation"*. If instead, a crossover is formed between the gene and centromere, the A and a alleles do not become separated until the second meiotic division, this separation is called *'second division segregation'*. The distinction between first-division and second-division segregation is shown in the figure 16.15. As shown in figure16.15, only two possible arrangements of the products of meiosis can yield through first-division segregation: AAaa and aaAA. However, four patterns of second-division segregation are possible because of the random arrangement of homologous chromosomes at metaphase I and of the chromatids at metaphase II. These four arrangements are Aa Aa, aA aA, Aa aA, and aA Aa.

The percentage of asci with second-division segregation pattern for a gene can be used to map the gene with respect to its centromere. For example, let us assume that 30% of sample of asci from a cross have a second division segregation pattern for the A and a alleles. This means that 30 percent of the cells undergoing meiosis had a crossover between the A gene and its centromere. Further more, in each cell in which crossing-over takes place, two of the chromatids are recombinant and two are non-recombinant. In other words a frequency of 30% corresponds to recombination frequency of 15%. Therefore, the map distance between a gene and its centromere is given by the equation

$$\frac{(1/2)\text{ Asci with second division segregation}}{\text{Total number of asci}} \times 100$$

16.7 Somatic or Mitotic crossing over

We customarily think of crossing over as a phenomenon confined to meiotic cells, but it can take place in somatic cells as well, although with very much reduced frequency (1000 fold lower than in meiosis). Mitotic crossing over leads to mitotic recombination. This was first discovered in *Drosophila melanogaster* by Curt Stern (1936). This phenomenon has been studied most carefully in fungi such as yeast and *Aspergillus,* in which the frequencies are higher than in other organisms. In *Drosophila melanogaster* the phenomenon was detected by the occurrence of single or twin spots of body tissue displaying a recessive phenotype on an otherwise wild type back ground.

In a typical experiment involving the use of a y + / + sn female
fly, the mosaicism was expressed as twin spots of relatively equal size, one exhibiting the y phenotype (yellow body) and the other the sn (singed bristles) phenotype. These results can be explained (fig. 16.16) by assuming that the twin spots arose by a cross over after somatic pairing in four strand stage.

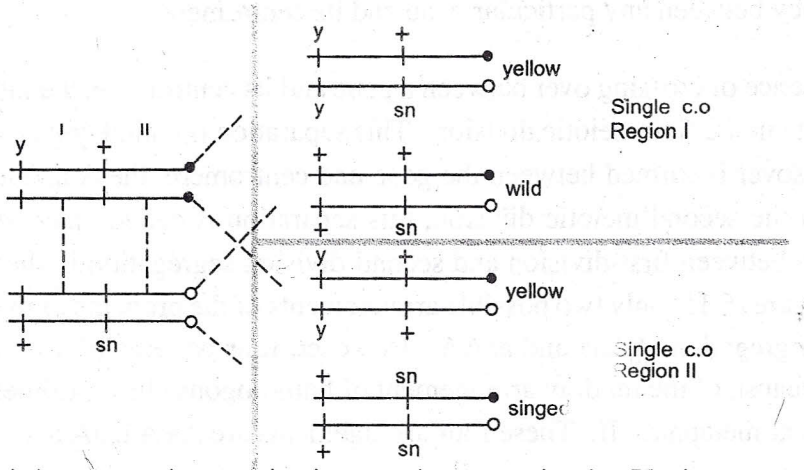


Figure 16.16 explaining somatic or mitotic crossing over in the X chromosome of *Drosophil melanogaster.*

A single cross over between y and sn (region I) will produce a yellow spot if chromatids segregated as indicated in Fig. 16.16. A single crossover between sn and centromere (region II) will produce adjacent twin spots (yellow, singed).

16.8 Chromosomal evidence for crossing over

Curt stern (1931) working with *Drosophila melanogaster* and Creighton and McClintock (1931) working with maize, provided experimental evidence for cytological crossing over. Stern's experiment in *Drosophila* is explained here.

Drosophila flies carrying translocations were utilized to produce a female *drosophila* having a part of the Y chromosome attached to one of the two X Chromosomes. The other X chromosome of this female fly was also marked (identifiable due to distinct morphology) and consisted of two approximately equal fragments each carrying its centromere. These two X chromosomes in the female fly could be distinguished not only from each other but also from the normal X chromosome, under the microscope. In such female fly, one of the two fragments of an X chromosome carried mutant allele for carnation eye ('car' is recessive showing light eye color) and barred eye ('B' is dominant showing narrower eyes). The other X chromosome, having a part of Y attached, carried normal alleles of these two genes, so that the female heterogygote for these two genes (car B/++) had barred eyes. (but normal eye color since car is recessive to +). Such females were crossed with male flies having recessive alleles for both genes (car, +). In such a situation, this makes a simple test-cross. If no crossing over takes place between the two genes in question two types of gametes i.e. car, B and ++ will be produced from the female flies. Crossing over will give two additional types of gametes i.e. car + and + B (Fig. 16.17). Due to fertilization of two types of non-crossovers and of the other two types cross-over gametes by the male gamete carrying X chromosome (car +), four kinds of female flies will be produced. Similarly four kinds of male flies will also be produced due to fertilization by gametes carrying Y chron osome.

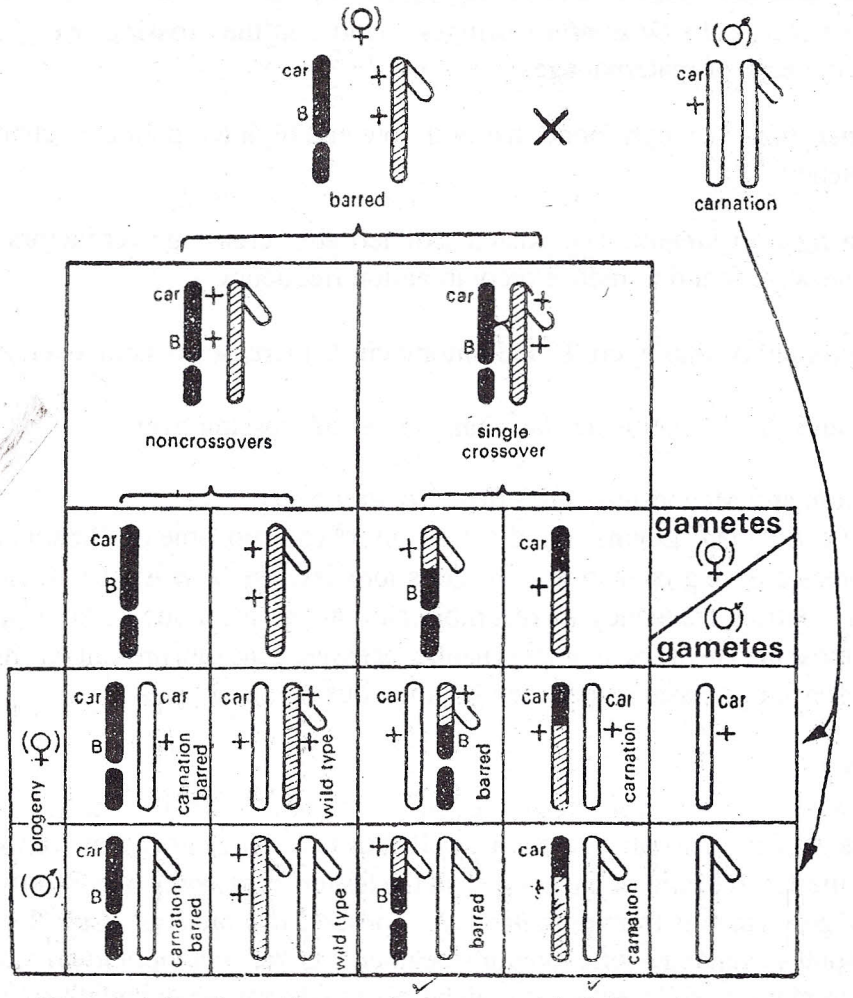


Fig. 16.17 Stern's experiment to demonstrate cytological crossing over.

The flies, which are, classified as cross overs on the basis of phenotype i.e. carnation with normal eye shape and barred with normal eye color, were studied cytologically. It was found that carnation flies did not have any fragmented X-chromosome but rather had normal X-Chromosome. On the other had barred flies had a frangmented X-chromosome with a segment of Y chromosome attached to one of the two fragments of X-chromosome. Such a cytological obervation provided evidence that genetic crossing over was accompanied with an actual exchange of chromosome segments.

16.9 Significance of crossing over & factors influencing

Crossing over occurs in all organisms ranging from viruses to man. Its widespread occurrence in organisms ensures exchange of genes and production of new types, which increase diversity. Genetic diversity accompanied by phenotypic variation at the species level confers adaptive advantage to wider range of habitats. This finally enhances the species potential for evolutionary success.

The following external factors can influence the frequency of crossing over:

a) ***Maternal age***: In *Drosophila* Bridges found that the crossing over frequency decreased with increase of maternal age.

b) ***Temperature:*** Plough found that both low and high temperatures changed the cross over frequency.

c) ***Crossing-over factors:*** The existence of heritable, crossing over factors in the cytoplasm of female were found to reduce recombination frequency.

d) ***Antibiotics:*** Actinomycin-D and mitomycin-C increase crossing over.

e) Heat and shock treatments also change rates of crossing over.

f) Calcium and Magnesium ions effect crossing over.

g) ***Gene controlled***: Levine found a gene on 3rd chromosome of *Drosophila* that completely suppress crossing over in homozygous females. In *Neurospora cressa* a recessive gene rec-1 controls frequency of recombination at the his-1 locus, both present on the same chromosome. In maize mutant synaptic recessive gene (as) present on chromosome 1 results in absence of chiasmata between homologous chromosomes.

16.10 Summary

Genes located in the same chromosome tend to remain together in meiosis rather than undergo independent assortment. This phenomenon is called *linkage.* The indication of linkage is a significant deviation from the 1:1:1:1 ratio of phenotypes in the test cross progeny of a F_1 dihybrid. The *recombination* of linked genes results from *crossing-over,* a process in which nonsister chromatids of homologous chromosomes exchange corresponding segments in the first *meiotic prophase.* The frequency of recombination between different genes can be used to determine the relative *order and location* of the genes in chromosomes. This type of analysis is called *genetic mapping.* Distance between adjacent genes in such a map (genetic or linkage map) is difined to be proportional to the frequency of recombination between them. The unit of map distance (the map unit or Centimorgan) is defined as one percent recombination. When many genes are mapped in a particular species, they form linkage groups. The number of linkage groups is equal to the haploid chromosome number of the species. In Ascomycetes fungi the ascospores are present orderly or un-orderly in the asci. The ascospores represent the four products of each meiotic division. The analysis of haploid products (Ascospores) of meiosis for linkage and recombination estimation is known as *tetrad analysis.* Curt Stern (1931) working with *Drosophila melanogaster* and Creighton and McClintock (1931) working with maize provided cytological proof for crossing over.

16.11 Key terms

Coefficient of Coincidence: An experimental value obtained by dividing the percentage of observed double crossovers by the percentage expected double crossovers.

Coupling: Arrangement of alleles in which mutant alleles are on the same chromosome and wild type alleles on the homologue.

First division Segregation: Separation of a pair of alleles into different nuclei in the first meiotic division, happens when there is no crossing over between the gene and the centromere in a particular cell.

Interference: The tendency of crossing over to inhibit formation of another crossing over nearby.

Linkage: The tendency of genes located in the same chromosome to be associated in inheritance more frequently than expected from their independent assortment in meiosis.

Mitotic crossing over: Occurrence of crossing over with very low frequency in mitotic division of somatic cells.

Physical map: A diagram showing the relative positions of physical landmarks in a DNA molecule, commonly restriction sites and particular DNA sequences.

Second division segregation: Segregation of a pair of alleles into different nuclei in the second meiotic division as the result of crossing over between the gene and the centromere of the pair of homologous chromosomes.

Tetrad analysis: A method for analysis of linkage and recombination using the four haploid products of single meiotic division.

Tetratype(TT): An ascus containing spores of four different genotypes ___ one each of the four genotypes possible with two alleles of each of two genes.

16.12 Questions

1. What is linkage? Explain linkage with suitable examples?
2. Enumerate various methods of calculation of recombination from a dihybrid test-cross and selfed progienies?
3. Describe in detail the determination of gene order and map distance employing a three-point testcross.
4. Explain the calculation of recombination between linked genes through analysis of ordered and unordered tetrads.
5. ***Write short notes***:
   a) Negative interference
   b) First-division and Second-division segregation
   c) Mitotic crossing over
   d) Coupling and repulsion phases.

16.13 Reference Books

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LESSON: 17

MULTIPLE ALLELES

Objective:

To know about the nature of multiple alleles and mode of their inheritance - Some illustrative examples.

17.1. Introduction
17.2. Multiple alleles – Mode of inheritance: Some examples.
  a) Eye color in Drosophila
  b) Coat color in rabbits
  c) Purple coloration of plant parts in maize
  d) Blood groups in man
  e) Self-Incompatibility in plants.
17.3. Complementation test for Allelism
17.4. Pseudo alleles
17.5. Recombination within genes: Gene concept
17.6. Intragenic recombination: Experimental proof
17.7. Summary
17.8. Key terms
17.9. Questions
17.10. Reference Books

17.1 Introduction

G.J Mendel first advocated the existence of a pair of factors for each character that determine two alternative forms (dominant and recessive) of the character. These factors were described as *dominant factor* and *recessive factor* respectively. The same was demonstrated later in many organisms and the pair of factors are designated as "*alleles*" of a gene. Therefore the different forms of a particular gene are called alleles. Each member of the pair of alleles is said to occupy an identical position or locus on each of the two homologous chromosomes in an organism. In Mendel's experiments the gene controlling height of the pea plants has both its alleles as TT or Tt or tt. Here T is dominant allele and t is recessive allele. But due to mutations that have taken place in the past, several alternative states, more than two have been detected for the same gene. *When there are more than two allelic forms of a given gene in the individuals of a population, such alleles are described as "multiple alleles".*

17.2 Multiple alleles, mode of inheritance : Some examples

A large number of multiple alleles is theoretically possible in a population of individuals and whenever more than two alleles are identified at a gene locus in a population, they are grouped as '*multiple allele series*'. In a given individual only two of these alleles occur one derived from each parent. A capital letter is commonly used to designate the allele that is dominant to all others in the series. The corresponding lower case letter designates the allele that is recessive to all others in the series. Other alleles intermediate in their degree of dominance between these two extremes are usually assigned the lower case letter with some suitable superscript.

17.2.a Eye color in Drosophila

T.H.Morgan (1910) reported the first case of multiple alleles of a gene controlling eye color in *Drosophila* during his studies on mutation. In a vial of flies with normal red eyes he found a fly with white eyes, that had arisen due to a mutation in the gene, which produces red color eyes in the normal flies. By performing genetic experiments, the position of this gene was determined to be on the X chromosome. Later on some other flies were discovered with eye color resembling the biological stain eosin. The gene for eosin color was also found to be located in the same locus on the X-chromosome. When crosses were made between eosin flies and red, and between eosin and white it turned out that the gene for eosin was an allele to both red and white genes. This proves that the genes producing red, white and eosin eyes in different flies are alleles, of each other. In other words, gene controlling eye color in *Drosophila* has multiple alleles. Later on a series of alleles producing eye pigment in different shades and intensities between red and white were found. Each shade has a different name. The different multiple alleles of white-eye in *Drosophila* are shown in table 17.1)

Table 17.1 showing the list of different multiple alleles and their symbols in Drosophila

Allele	Symbol	Allele	Symbol
White	w	apricot	w^a
Ivory	w^i	cherry	w^{ch}
Pearl	w^b	eosin	w^e
Tinged	w^t	blood	w^{bl}
Buff	w^{bf}	coral	w^{co}
Honey	w^h	Red (wild)	W

The wild type (red) is dominant to all other alleles. Each allele in the system except white (w) can be considered to produce pigment but successively less, is produced by alleles as in the order shown here.

$W > w^{co} > w^{bl} > w^e > w^{ch} > w^a > w^h > w^{bf} > w^t > w^p > w^i > w$.

Some times phenotypes produced by different alleles are not markedly different, Such alleles with similar effects are described as *"isoalleles"*.

17.2.b Coat color in rabbits

The Himalayan rabbit is a classical example for multiple alleles. This was first studied by Sturtevant in 1913. The wild type rabbit has grey fur and is called *agouti.* Rabbits with white fur, pink eyes are called *albino.* The *Himalayan* has white fur on the body, but its feet, tail, ears, and tip of the nose are balck or dark brown. There is another variety known as *chinchilla,* which is less intense in coat color than the wild type (Agouti). The inheritance of these types of coat color is as follows.

When crosses are made between agouti and albino, and between agouti and Himalayan both albino and Himalayan were found to behave as recessive to agouti. In F_2, a ratio of 3:1 dominant and recessive were produced. When Himalayan crossed with albino F_1 were all Himalayan and in F_2 3 Himalayan: 1 albino were produced. When chinchilla is crossed with albino the F_1 are light grey (intermediate) and in the F_2 there is a segregation of 1 chinchilla: 2 light grey: 1 albino. This shows that the heterozygous genotype for chinchilla and albino produces light grey phenotype. The gene controlling coat color in rabbits thus have alternative forms responsible for various coat colours constituting multiple allele series. The genotypes and phenotypes of multiple allele series of coat color in rabbits is summarized in table 17.2.

Table 17.2 The different genotypes and phenotypes of multiple alleles for coat color in rabbits

Genotype	Phenotype
CC	Wild type (Agouti)
Cc^{ch}, Cc^{h}, Cc^{a}	Wild type (Agouti)
c^{h} c^{h}	Himalayan
c^{ch} c^{ch}	Chinchilla
c^{ch} c^{h}, c^{ch}, c^{a}	Light greny
c^{h} c^{a}	Himalayan
c^{a} c^{a}	Albino

17.2.3 Purple coloration of plant parts in Maize

L.J.Stadler described a series of multiple alleles in maize that determine the purple coloration of different parts of plant. Four of these alleles have the following effects in maize.

R^{r} → colored seed, colored plant

R^{g} → colored seed, colorless plant

r^{r} → colorless seed, colored plant

17.2.d Blood groups in man

The blood groups in man form a multiple allelic series. If blood from two persons is allowed to mix outside the body, some times it will mix well and at other times there is clumping (agglutination) of red blood cells. Landsteiner (1900) studied the reaction for agglutination. Blood can be separated into red cells and the colorless serum or plasma. Landsteiner found that the red cells of one sample would mix smoothly with the serum of the same person. But when red cells of one person are mixed with plasma of another person, some times the two would mix well, at other times there would be clumping of red cells. On the basis of these reactions Landsteiner identified four blood group types in man namely A,B, AB and O.

Later, on Landsteiner found that human erythrocytes carry on their surface two distinct types of muco-polysaccharides called as antigen A and B, which can stimulate the production of specific serum antibodies. The type of polysaccharide present on the surface of the red blood cell determines the type of blood. The alleles of (I^{A}, I^{B}, I^{O}) a gene I encode transferase enzyme that modifies the precursor substance into a specific polysaccharide (*antigen*). For example people of genotype I^{A} I^{A} produce red

blood cells having only the A polysaccharide and are said to have blood type A. Those of $I^B I^B$ genotype, have red blood cells with only B polysaccharide and have blood type B. Heterozygous $I^A I^B$ people have red cells with both the A and B polysaccharides on the red blood cells. $I^A I^B$ genotype illustrates co-dominance i.e. presence of both A and B polysaccharides on the red blood cells. The third allele I^O, does not show codominance. It encodes a defective enzyme that leaves the precursor substance unchanged; neither A nor B type of polysaccharide is produced. Homozygous $I^O I^O$ persons therefore lack both the A and B polysaccharides; they are said to have blood type O. In $I^A I^O$ heterozygotes presence of the I^A allele results the production of the A polysaccharide; and $I^B I^O$ heterozygotes, presence of I^B allele results in production of the B polysaccharide. The result is that $I^A I^O$ persons have blood type A and $I^B I^O$ persons have blood type B, so I^O is recessive to both I^A and I^B. (Table 17.1) Thegenotype and phenotypes of different blood group types are as follows:

Table 17.3 Various blood groups and their genotypes

Blood groups	Genotypes
A	$I^A I^A$ or $I^A I^O$
B	I^B or $I^B I^B$ or $I^B I^O$
AB	$I^A I^B$
O	$I^O I^O$

Human blood contains antibodies to either the A or the B polysaccharide. An *antibody* is a protein that is made by the immune system in response to a stimulating molecule called an *antigen* and is capable of binding to the antigen. An antibody is usually specific in that it recognizes only one antigen. People of blood type O make both anti-A and anti-B antibodies; those of blood type A make anti-B antibodies: those of blood type B make anti-A antibodies; and those of blood type AB make neither type of antibody. Some antibodies combine with antigen and form large molecular aggregates that may precipitate to cause agglutination. When blood cells agglutinate many blood vessels are blocked and the individual may die. The occurrence of antigens (also called agglutinogens) and antibodies (agglutinins) in different blood group types and genotypes is shown in table17.4. Antibodies causing agglutination is shown in Figure17.1

Table 17.4 The presence of different antigens and antibodies in genotypes of ABO blood groups

Genotype	Antigens present on red blood cells	ABO blood goup phenotype	Antibodies present in blood fluid	Blood types that can be tolerated in transfusion	Blood types that can accept blood for transfusion
I^AI^A	A	Type A	Anti-B	A & O	A & AB
I^AI^O	A	Type A	Anti-B	A & O	A & AB
I^BI^B	B	Type B	Anti-A	B & O	B & AB
I^BI^O	B	Type B	Anti-A	B & O	B & AB
I^AI^B	A & B	Type AB	Neither anti-A nor anti-B	A, B, AB & O	AB only
I^OI^O	Neither A nor B	Type O	Anti-A & anti-B	O only	A, B, AB & O

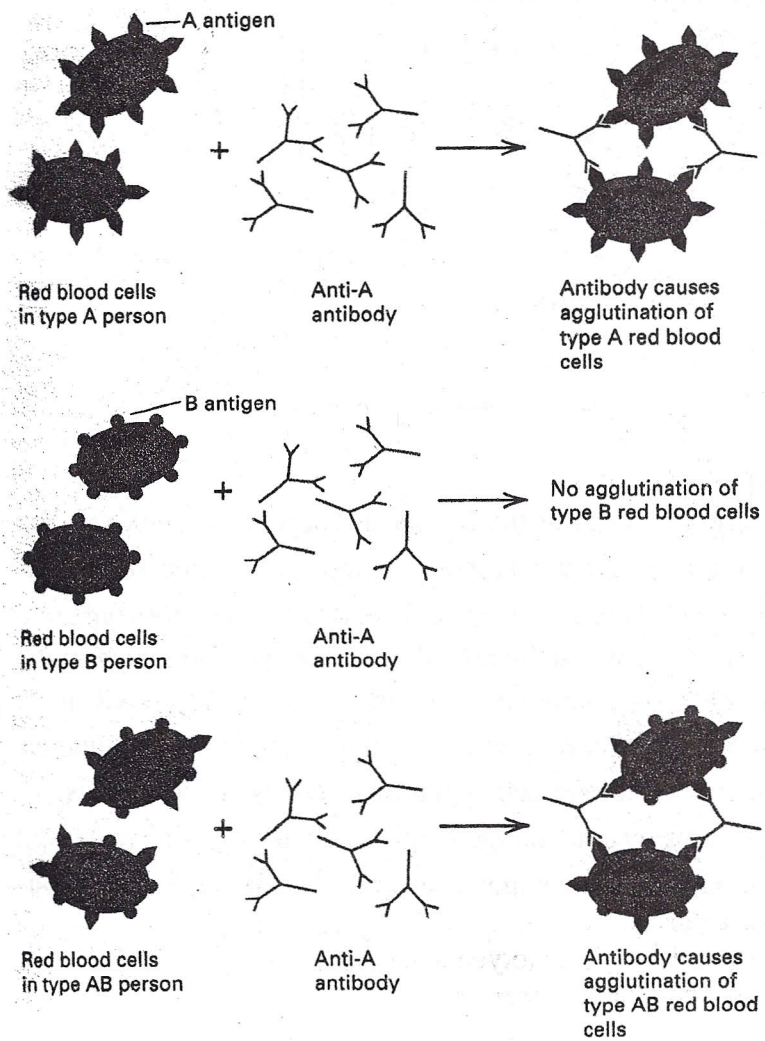


Figure : 17.1
Antibody against type-A antigen will agglutinate red blood cells that carry the type-A antigen, whether or not they also carry the type-B antigen. Blood fluid containing anti-A antibody will agglutinate red blood cells of type A and type AB, but not red blood cells of type B or type O.

People of O blood type have been described as *'universal donors'* as they can donate blood to persons of A, B, and AB types. The AB blood type people are known as *'universal receipients'*, as they can accept blood from A, B and O blood group persons.

The blood group genes are inherited in the Mendelian fashion in the case of A, B and O groups. The AB blood group however results from codominance of A and B alleles: The blood groups of offspring can be determined from their parent's genotypes (Table 17.5)

Table 17.5. Showing the genotypes of parents and offspring and their blood groups

Genotypes of parents	Genotypes of offspring	Blood group of offspring
I^AI^A x $I^A I^O$	I^AI^A, $I^A I^O$	A
I^AI^O x I^AI^O	I^AI^A, I^OI^O	A,O
I^AI^A x I^BI^B	I^AI^B	AB
I^AI^A x I^AI^B	I^AI^A, I^AI^B	A, AB
I^AI^O x I^AI^B	I^AI^A, I^AI^O, I^AI^B, I^BI^O	A,B, AB
I^AI^O x I^BI^B	I^AI^B, I^BI^O	AB, B
I^AI^O x I^BI^O	I^AI^B, I^AI^O, I^BI^O, I^OI^O	AB, A,B,O
I^AI^O x I^OI^O	I^AI^O, I^OI^O	A,O
I^OI^O x I^OI^O	I^OI^O	O
I^OI^O x I^BI^B	I^BI^O	B
I^OI^O x I^AI^A	I^AI^O	A

17.2.e Self-Incompatibility in plants

In some monoecious plants, pollen from a plant will not be able to bring about fertilization in the ovules of the same plant. The pollen grains fail to germinate on the stigma or even if pollen tubes are formed their growth may be inhibited. Such condition is known as *self-incompatibility* or *self-sterility*. This is controlled by multiple alleles of a single gene, and may be of two types – *gametophytic* and *sporophytic*. The gametophytic self-incompatibility (GSI) is controlled by the genotype of the pollen grains. This type is quite common in families like Liliaceae, Gramineae, and Solanaceae. Sporophytic self incompatibility (SSI) is controlled by the somatic or sporophytic cells of the plant which produce pollen grains, as observed in families Cruciferae and Compositae.

East and Mangelsdorf (1925) designated the gene controlling incompatibility by 'S' and its multiple alleles by a series S_1, S_2, S_3, S_4 ..... so on. The plant with genotype $S_1 S_2$ produces pollen grains with either S_1 or S_2 allele. In the GSI system these pollen grains will not be able to pollinate the pistil with genotype $S_1 S_2$, but pollinate the pistil with S_3S_4 genotype and fertilization takes place. In the plants belonging to SSI system, even one of the alleles present in the sporophytic tissue of the male

parent is present in the 'pistil of the female parent, then all the pollen grains produced by the male parent fail to effect fertilization of the pistil. Thus if male parent has the genotype $S_1 S_2$, it would be in compatible with the plants having $S_1 S_2$, $S_1 S_3$, $S_2 S_3$, $S_2 S_4$, $S_2 S_5$ genotypes. It is cmpatible with $S_3 S_4$, $S_3 S_5$, $S_4 S_5$ plants (Fig. 17.2). A large number of alleles of the incompatibility genes are known to be present in plants. *Oenothera* has 37 and red clover has more than 200 different self-sterility alleles.

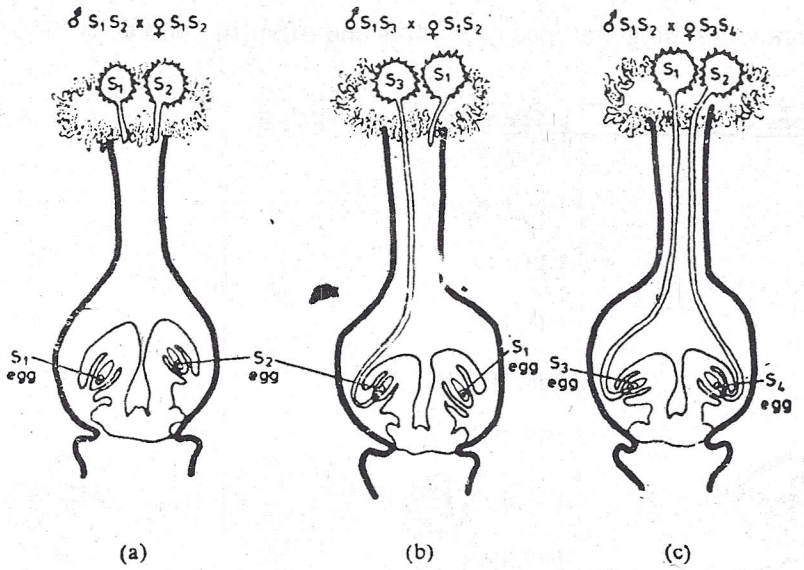


Fig. 17.2 Diagrammatic representation of incompatibility envisioned for plants of the GSI and SSI systems; a and b Gamtophyfic self in compatibility & C : Sporophytic self incompatibility

17.3 Complementation test for allelism

One test of allelism to find out whether two mutant alleles in question are allelic to each other or not, is that the progeny of a cross between two mutants should not give rise to the wild type in the F_1 generation. For example if a and b are two alleles, allelic test may be shown in the following way.

aa x bb

↓

F_1 a/b

i) If wild type appears, a and b are non-allelic.

ii) If no wild type appears a and b are allelic.

The isolation of a set of mutants all of which show the same type of defect in phenotype is called a *mutant screen.* For instance, a genetic analysis of flower color in peas might yield one or more new mutations that changed the wild type P allele into a defective p allele that prevents the formation of the purple pigment. Each of the P⟶p mutation might differ in DNA sequence, but all of the newly isolated p alleles whould be defective forms of p that prevent formation of the purple pigment. The issue of the possible allelism of the mutants is resolved by observing the phenotype of the progeny produced from a cross between the varieties. As indicated in the figure17.3 there are two possible out comes to the cross.

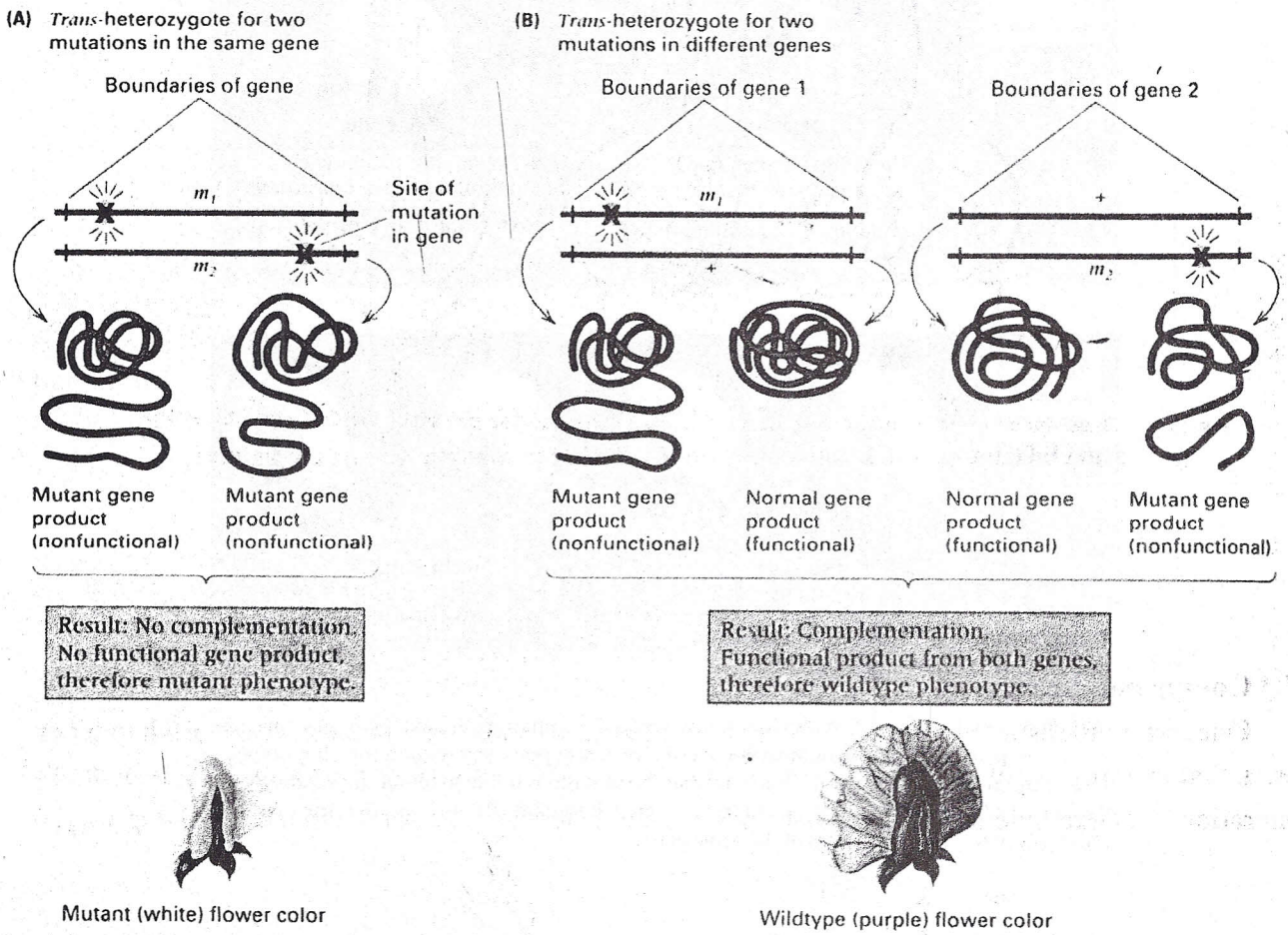
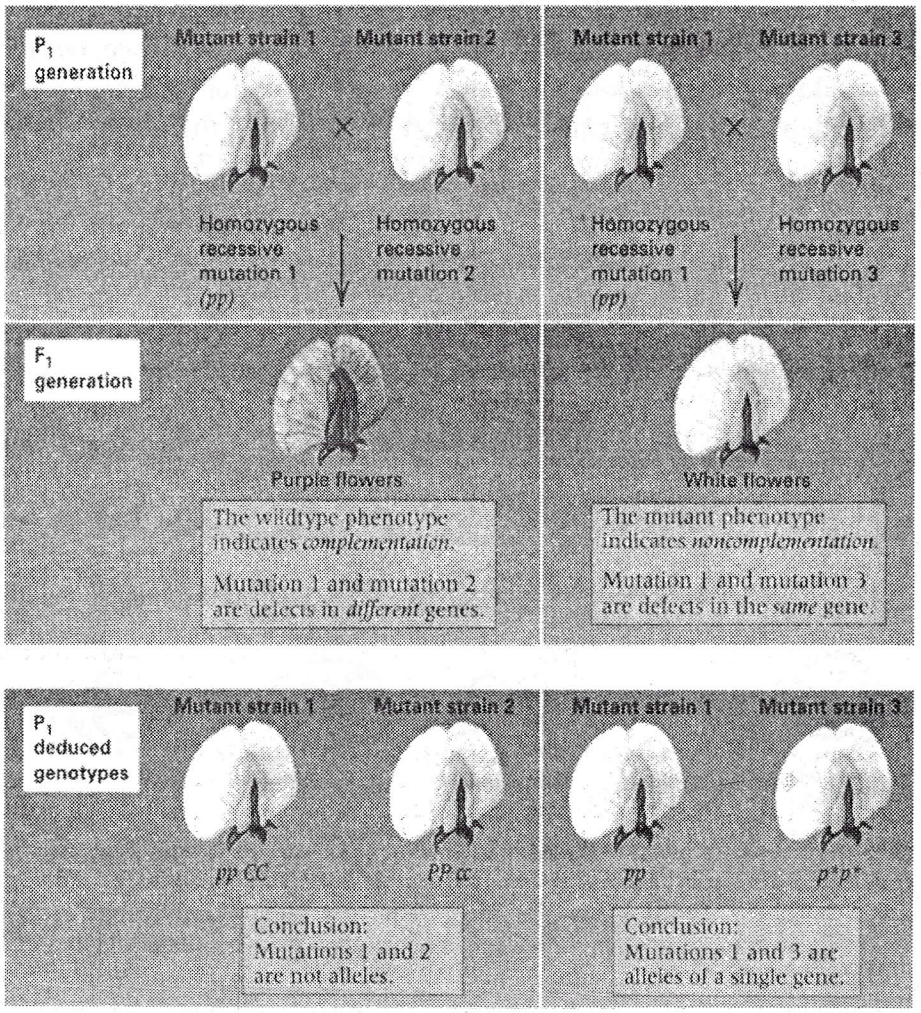


Fig. 17.3



Figure

The complementation test reveals whether two recessive mutations are alleles of the same gene In the complementation test, homozygous recessive genotypes are crossed. If the phenotype of the F_1 progeny is nonmutant (A), it means that the mutations in the parental strains are alleles of different genes. If the phenotype of the F_1 progeny is mutant (B), it means that the mutation in the parental strains are alleles of the same gene.

The F_1 progeny have either the wild type phenotype or mutant phenotype. If the progeny have wild type phenotype it means that the mutations in parental individuals are in different genes; this result is called *complementation*. It implicates two different genes are needed for wild type phenotype. If the progeny have mutant phenotype, it implies that the mutations in each of the two different strains are alleles of the same gene. This result is called *non-complementation*. The lack of complementation reveals that both parental strains have a mutation in the same gene, because neither mutant strain can provide the genetic function missing in the other.

17.4 Pseudo alleles

In 1940 and later, C.P.Oliver and his student Green conducted experiments using different mutants in the lozenge (lz) gene locus. Among progeny of the cross between *two alleleic* mutants at lozenge locus *contained* a very low frequency of *wild type* phenotype appeared that could not be explained easily because of the following reasons.

1) The gene was believed to be the smallest unit of genetic material capable of changing by mutation and the smallest unit of inheritance.
2) The established view through genetic analyses was that genes are in linear array joined in some way resembling a string of beads constituting a chromosome.
3) The recombination was observed to be between genes.
4) Unaware of intragenic recombination.

Later in 1951 E.B. Lewis reported results of one of his experiments where from a cross of *apricot* and *white eye* (both recessive mutants), he obtained F_1 having intermediate eye color. In F_2 he expected segregation for apricot and white. To his surprise he recovered wild type showing that the two alleles are non-allelic. Lewis explained above result by a phenomenon called *pseudoallelism.* The allelic genes, if they behaved as they were non-allelic, called *pseudoalleles,* and the phenomenon '*pseudoallelism.* Pontecarvo later preferred to call it 'Lewis effect'. The problem posed by recombination between alleles led to coin the term "Pseudoallele". However, we know that there is nothing 'Pseudo' about pseudo alleles. They are perfectly legitimate alleles that are far apart along the DNA to allow detection of recombination.

17.5 Recombination within genes: Gene concept:

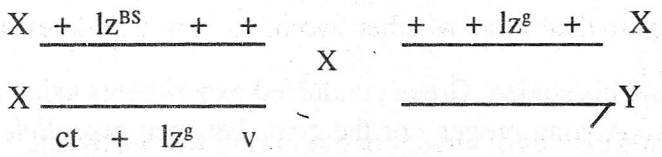
The appearance of wild type in allelic mutant crosses F_2 generation could be explained on the basis of *intragenic recombination.* Usually in the allelic forms there will be lack of complementation as a result of which the F_1 individuals will not be wild type. Since intragenic (inter-allelic) crossing over is possible, in the F_2 generation wild type individuals appear. In the F_1 individual the arrangement of mutant alleles a+/+b within the same gene does not allow the wild type to be expressed due to position effect (trans position). As a result of crossing over ++ and ab gametes are produced from the F_1 individuals and in the F_2 generation ++/++ or ++/ab will express (cis position) the wild type. Hence, within the gene cis-trans arrangements of alleles exhibit different phenotypes. cis trans arrangement of a and b alleles

$$\frac{+\ +}{a\ b} \qquad \frac{a\ +}{+\ b}$$

(wild) cis trans (mutant)

17.6 Intragenic recombination: Experimental Proof

C.P. Oliver observed a gene *Lozenge* (lz) in the X-chromosome of *D.melanogaster.* Mutant alleles of *lozenge* are recessive and their phenotypic effects include a disturbed arrangement of the facets of the compound eye and a reduction in the eye pigments. Numerous lz alleles with distinguishable phenotypes are known.He crossed two mutants for lz allele and examined a large number of progeny in which appeared exceptional flies with normal (wild type) eyes. The cross-carried out was



in which lz^{BS} and lz^{g} are mutant alleles of Lozenge, and ct (cut wings) and v (vermilion eye color) are genetic markers that map 7.7 units to the left and 5.3 units to the right respectively of the lozenge locus. From among more than 16,000 progeny of this cross 134 males and females with wild type eyes were found. These exceptional progeny could not be explained on the basis of reverse mutation of lz allele to lz^{+}. Moreover the genetic constitution of the maternally derived x chromosomes in the *normal eyed* offspring was in agreement with the explanation that there was reciprocal intragenic recombination. That is all of the rare non-lozenge progeny had an X-chromosome with constitution ct + + + and male offspring had *cut wings*. In other five male progeny that had lozenge phenotype distinctly different from the phenotype resulting form the presence of either lz^{BS} or lz^{g} allele alone, the X chromosome was detected with v (vermilion) allele (+ lz^{BS} lz^{g} v), thus the exceptional males also had vermilion eyes, as expected from reciprocal recombination.

The above observation of intragenic recombination indicated that genes have a fine structure. The multiplicity of allelic forms of some genes might be due to mutations at different sites in the gene. These sites are the nucleotide constituents that make up DNA. In a gene *cistron* is a functional unit, whose alleles exhibit cis-trans phenomenon and the smallest unit capable of undergoing recombination is called *recon* which is sub divisible into smaller units of mutation. Since mutation takes place due to a single base replacement, a single nucleotide pair will be the ultimate limit of a *muton.* Therefore, cistron, recon and muton are the three units with in the gene in descending order of size.

17.7 *Summary*

The Existence of more than two alternative forms of a gene in the different individuals of a population is known as Multiple allelism. Mutations bring about change in the wild type allele to form a defective recessive allele that is mutant allele. Many such recessive mutations that might differ in DNA sequence give rise to multiple alleles. The coat color in rabbits, eye color in *Drosophila*, Blood group types in humans are determined by multiple alleles. Complementation test is used to determine whether recessive mutations in each of the two strains are alleles of the same gene. Genetic analysis of exceptional wild types appeared in F_2 progenies of mutant crosses led to the finding of rare recombination within the gene. This has changed the classical concept of the gene and to recognize sub units within the gene *cistron, muton* and *recon.*

17.8 Key terms:

Allele: Any of alternative forms of a given gene.

Antigen: A substance able to stimulate the production of antibodies.

Antibody: A blood protein produced in response to a specific antigen and capable of binding with the antigen.

Complementation test: A genetic test to determine whether two mutations are alleles (are present in the same functional gene).

Agglutination: The clumping or aggregation as of viruses or blood cells caused by antigen – antibody interaction.

Multiple alleles: The presence of more than two alleles of a gene in a population.

Mutant screen: A type of genetic experiment in which the geneticist seeks to isolocate multiple new mutations that effect a particular trait.

Universal Donor: A person of blood group type O, who can donate blood to a person of any ABO types.

Universal Receipient: A person of blood group type AB, who can receive blood from a person of any other ABO types.

17.9 Self-Assessment Questions:

1. What are multiple alleles? Explain multiple allelism with suitable examples?.
2. Describe the complementation test and its importance?
3. What is intragenic recombination? How will it be analysed?
4. "Recombination within genes changed the classical concept of the gene". Justify the statement.

17.10 Reference Books

1. Elrod, S.L. and Stansfield, W.D.2002. Theory and Problems of Genetics, Tata McGraw-Hill Publishing Company Ltd. New Delhi.
2. Hartl, D.L. and Jones, E.W. 1998. *Genetics: Principles and Analysis, Jones* and Bartlett Publishers, London.

LESSON: 18

PROBABILITY LAWS AND THEIR APPLICATION TO GENE SEGREGATION

OBJECTIVE:

To learn probability rules and their application in genetic analyses.

18.1. Introduction
18.2. Probability Definition
18.3. Mutually Exclusive Events: The Addition Rule
18.4. Independent events: The Multiplication Rule
18.5. The Binomial Expansion
18.6. The Chi-square test: Goodness of fit
18.7. Yates correction
18.8. Summary
18.9. Key terms
18.10. Model Questions
18.11. Reference books

18.1 Introduction

In the field of Genetics and plant breeding data recording on qualitative as well as quantitative traits is an essential component of experimental work. Analysis and interpretation of data requires methods that enable us to draw logical conclusion from experiments. For meeting this need, we often make use of statistics, a science, pure and applied of creating, developing and applying techniques such that uncertainty of inductive inferences may be evaluated. A working knowledge of the rules of probability for predicting the outcome of chance events is basic to understand the transmission of hereditary characteristics. The concept of probability is knowing situation and predicting possible future outcomes.

18.2 Probability

The Probability of an event may be considered as equivalent to the proportion of times that the event is expected to be realized in numerous repeated trails.

If there are n trails and the event occurs on the average m times during n trails, then the probability of that event is m/n. It is hardly possible to predict the appearance of certain event. But what we can say is that there is a certain probability of occurrence of a given event.

If a coin is tossed, there are two possible out comes (Head or Tail) out of which the probability for realization of an outcome of interest i.e. head or tail is 1/2. In the language of probability an out come of interest is typically called an 'event'.

For example, in the F_2 generation of hybrid between round seeds and wrinkled seeds, Mendel observed 5474 round seeds and 1850 wrinkled seeds. In this case the proportion of wrinkled seeds was

$\frac{1850}{1850+5474} = 1/3.96$ o. ery nearly 1/4. We may therefore regard 1/4 as the approximate proportion of wrinkled seeds to be expected among a large number of progeny from this cross. *Equivalently, we can regard 1/4 as the probability that any particular seed chosen at random will be wrinkled.* Similarly progeny produced by self-pollination of an Aa plant, four equally likely progeny genotypes (out comes) are possible AA, Aa, aA, aa. Two of the four possible out comes are heterozygous, so the probability of a heterogygote is 2/4 or 1/2.

Hence the proportion of times that the event is expected to be realized in numerous repeated trails *is equivalent to the probability that it is realized in a single trail.*

18.3 Mutually exclusive events: The Addition Rule

Some times, there may be two or more possibilities for an out come of interest (event). For example if we toss a dice, six possible outcomes (1,2,3,4,5,6) are there each with a probability of p=1/6. Similarly a seed with phenotype of 'round' may have either of two genotypes RR and Rr. A seed that is round cannot have both genotypes at the same time. Only one event can be realized in any one organism and the realization of one event in an organism *precludes* the realization of others in the same organism. When one event occurs, the other can not occur. The realization of the genotype RR in a plant precludes realization of the genotypes Rr in the same plant. Events that exclude each other in this manner are said to be '*mutually exclusive*'. When events are mutually exclusive, their probabilities are combined according to the addition rule.

Addition Rule: The probability of the realization of one or the other of two mutually exclusive events A or B, is the sum of their separate probabilities.

Prob (A or B) = prob (A) + Prob (B)

Eg. The proportion of round seed expected from cross Rr x Rr is prob {RR or Rr} = prob {RR} + prob {Rr} = 1/4 + 1/2 = 3/4 . This is the probability of an individual seed being round.

18.4 Independent events: The multiplication Rule

Events that are not mutually exclusive may be independent, which means that the realization of one event has no influence on the possible realization of any others. For example two coins are tossed simultaneously so that four possible out comes (HH, TT, HT, TH) are there. The out come of first coin tossing is independent of the out come of the second coin tossing. Similarly, in Mendel's crosses for seed shape and color, the two traits are independent. The proportions of round yellow seed or round green seed can be obtained by considering the traits separately, because they are independent. Considering only seed shape, we can expect the F_2 generation to consist of 3/4 round and 1/4 wrinkled seeds. Considering only seed color, the F_2 generation to consist of 3/4 yellow and 1/4 green. Because the traits are inherited independently, among 3/4 of the seeds that are round, there should be 3/4 that are yellow, so the over all proportion of round yellow seeds is expected to be 3/4 x 3/4 = 9/16. The principle is that when events are independent the probability that they are realized together is obtained by multiplication.

Multiplication rule: The probability of two independent events A and B, being realized simultaneously is given by the product of their separate probabilities.

Prob{A and B} = Prob (A) . Prob(B).

Example 1. What is the probability that both offspring from the mating Aa x Aa have the dominant phenotype? The probability that any particular off spring has dominant phenotype equals 3/4. Thus the multiplication rule says that the probability that both of the two offspring have dominant phenotype is (3/4) (3/4) = 9/16.

Example 2. In self-fertilized progeny of genotype AaBbCcDd the probability of quadruple heterozygote is $(1/2)(1/2)(1/2)(1/2)=(1/2)^4$ or 1/16 as the individual probability of Aa, Bb, Cc and Dd alleles is 1/2.

The probabilities of the individual outcome of segregation or fertilization are multiplied to obtain the overall probability.

18.5 THE BINOMIAL EXPANSION

Many problems in genetics concern not only the probability that certain event will occur, but also the probability that a certain *combination* of events will occur. It means the addition and multiplication rules are used together. For example in two tosses of a coin with p=heads and q=tails, there are four following possibilities.

First toss	Second toss		Probability
Heads(p)	(and] Heads(p)	=	$p \times p=P^2$
Heads(p)	(and) Tails(q)	=	$p \times q=pq$
Tails(q)	(and) Heads(p)	=	$q \times p=pq$
Tails(q)	(and) Tails(q)	=	$q \times q=q^2$

i.e. $1p^2$ + $2pq$ + $1q^2$
(two heads) (1 head, I tail) (two tails)

This result is similar to $(p+q)^2 = P^2+2pq+q^2$

Similarly when a coin is tossed *three* times, the probabilities for any combination of heads and/or tails can be found from $(p+q)^3$.

i.e $(P+q)^3 = p^3 + 3p^2q + 3pq^2 + q^3$

If probability of heads is 1/2 and tails is 1/2 then the probabilities for any combination of head and tails will be the same expression to that of $(p+q)^3$ and it is summarized here under.

Combination of events:

No.of Heads	No.of Tails	Term	Probability
3	0	P^3	$(1/2)(1/2)(1/2)=1/8$
2	1	$3p^2q$	$3(1/2)(1/2)(1/2)=3/8$
1	2	$3pq^2$	$3(1/2)(1/2)(1/2)=3/8$
0	3	q^3	$(1/2)(1/2)(1/2)=1/8$

Thus $(p+q)^n$ represents the binomial, where p and q represent the probabilities of alternative independent events and the power *n* to which the binomial is raised represents the number of trails. A short method for expanding (p+q) to any power (n) may be performed by following these rules ———

1. The coefficient of the first term is 1. The power of the first factor (p) is n, and that of (q) is 0. (Note: Any factor to the zero power is 1).

2. There after in each term, multiply the coefficient by the power of p and divide by the number of that term in the expansion. The result is the coefficient of the next term.

3. Also, thereafter, the power of p will decrease by 1 and the power of q will increase by 1 in each term of the expansion.

4. The fully expanded binomial will have (n+1) terms the coefficients are symmetrical about the middle term(s) of the expansion.

For example the expansion of the binomial $(p+q)^5$ is given hereunder.

1st term: 1 p^5q^0 :Coefficient of 2nd term = (5)(1)/1=5

2nd term: 5 p^4p^1 :Coefficient of 3rd term = (4)(5)/2=10

3rd term: 10 p^3q^2 :Coefficient of 4th term = (3)(10)/3=10

4th term: 10 p^2q^3 :Coefficient of 5th term = (2)(10)/4=5

5th term: 5 p^1q^4 :Coefficient of 6th term = (1)(5)/5=1

6th term: 1 p^0q^5

Summary: $(p+q)^5 = p^5+5p^4q+10\ p^3q^2+10p^2q^3+5pq^4+q^5$

The coefficients of the binomial expansion represent the number of ways in which the conditions of each term may be satisfied. The number of combination [C] of *n* different things taken *k* at a time is expressed by

$$ {}_nC_k = \frac{n!}{(n-k)!\,k!} $$

Where n! (called "factorial n") = n(n-1)(n-2) ..... 1(0!=1 by definition)

For example if n=4, then n! = 4(4-1)(4-2)(4-3) = 4x3x2x1=24.

For example, the number of ways to obtain two heads in three tosses of a coin is

$$ {}_3C_2 = \frac{3!}{(3-2)!2!} = \frac{3x2x1}{(1)(2x1)} = 3 $$

These three combinations are HHT, HTH and THH. The probability to obtain two heads in three tosses of a coin is $3p^2q = 3x(1/2)^2.(1/2) = 3/8$

Then the probability that in n trails a success will occur 's' times and a failure will occur f times can be given by the following formula

$$\left(\frac{n!}{s!\ f!}\right)p^s q^f$$

The following problem can be solved by using the above formula.

A total of 160 families with 4 children each were surveyed with the following results.

Girls	4	3	2	1	0
Boys	0	1	2	3	4
Families	7	50	55	32	16

Is the family distribution consistent with the hypothesis of equal number of boys and girls?

Let p probability of a girl = 1/2 and q probability of a boy = 1/2

Probability of 4 girls and 0 boy

$$= \frac{4!}{4!0!}.(1/2)^4 (1/2)^0 = \frac{4x3x2x1}{(4x3x2x1)(-1)}.(1/2)^4 (1/2)^0 = \frac{1}{6}$$

Probability of 3 girls and 1 boy

$$= \frac{4!}{3!1!}.(1/2)^3 (1/2)^1$$

$$= \frac{4x3x2x1}{(3x2x1).(1)}.\frac{1}{2}.\frac{1}{2}.\frac{1}{2}.\frac{1}{2} = \frac{4}{16} or \frac{1}{4}$$

Probability of 2 girls and 2 boys

$$= \frac{4!}{2!\ 2!}(1/2)^2 (1/2)^2$$

$$= \frac{4x3x2x1}{(2x1)2x1)}.\frac{1}{2}.\frac{1}{2}.\frac{1}{2}.\frac{1}{2} = \frac{6}{16}$$

Probability of 0 girl and 3 boys

$$= \frac{4!}{1!\ 3!}(1/2)^1 (1/2)^3$$

$$= \frac{4x3x2x1}{(1)(3x2x1)} 1/2.1/2.1/2.1/2 = \frac{4}{16} or \frac{1}{4}$$

Probability of 0 girls and 4 boys

$$= \frac{4!}{0!\,4!}(1/2)^0\,(1/2)^4$$

$$= \frac{4x3x2x1}{(0!)(4x3x2x1)}\left(\frac{1}{2}\right)^0\left(\frac{1}{2}\right)\left(\frac{1}{2}\right)\left(\frac{1}{2}\right)\left(\frac{1}{2}\right) = \frac{1}{16}$$

Then, Expected numbers in 160 families with different combinations can be obtained by multiplying the probability of respective class with total number of formities.

4 girls and 0 boy = $\frac{1}{16}\ x160 \quad = 10$

3 girls and 1 boy = $\frac{1}{4}\ x160 \quad = 40$

2 girls and 2 boys

= $\frac{6}{16}x160 \quad = 60$

1 girl and 3 boys = $\frac{1}{4}\ x160 \quad = 40$

0 girl and 4 boys = $\frac{1}{16}\ x160 \quad = 10$

$$Chi-square(\chi^2) = \Sigma\frac{observed-expected)^2}{expected}$$

$$= \frac{(7-10)^2}{10} + \frac{(50-40)^2}{40} + \frac{(55-60)^2}{60} + \frac{(32-40)^2}{40} + \frac{(16-10)^2}{10}$$

$$= \frac{9}{10} + \frac{100}{40} + \frac{25}{60} + \frac{64}{40} + \frac{36}{10} = 9.02$$

This value is close to, but less than critical value 9.49 from table against 4 degrees of freedom

Table 18.1 Chi-Square Distribution

Degrees of Freedom	Probability										
	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59
	Nonsignificant								Significant		

Source: R. A. Fisher and F. Yates, *Statistical Tables for Biological, Agricultural and Medical Research*, 6th ed., Table IV, Oliver & Boyd, Ltd., Edinburgh, 1963, by permission of the authors and publishers.

We may therefore accept the hypothesis that the family distribution is consistent with the boys and girls are equal in numbers. The details of chi-square (χ^2) test are given below.

18.6 The Chi-Square test: Evaluating the fit of Observed Results to Theoretical Expectations.

Geneticist often need to decide whether an observed ratio is in satisfactory agreement with a theoretical prediction. Suppose for example in the progeny of a cross purple flowers X white flowers, 12 plants are with purple flowers and 8 are with white flowers. Is this result close enough to be accepted as 1:1 ratio? Is this result consistent with a 1:1 ratio? To know whether observed results deviate too far from a theoretical expectation, a test for 'goodness of fit' is used. The word fit means how closely the observed results 'fit' or 'agree' with the expected results. A conventional measure of goodness of fit is a value called 'chi-square' (symbol-χ^2) which is calculated from the number of progeny observed in each of various classes, compared with the number expected in each of the classes on the basis of some genetic hypothesis.

The procedure for testing goodness of fit of a genetic hypothesis by Chi-Square (χ^2) method is as follows:

1. *State the genetic hypothesis in detail, specifying the genotypes and phenotypes of the parents and the possible progeny.* For example in a cross between plants with purple flowers and those with white flowers, we may be interested in testing the hypothesis that purple flower parent is heterozygous for a pair of alleles and white flower parent is homozygous recessive. The genotypes in the cross pruple x white could be symbolized as Pp x pp. The possible progeny genotypes are Pp and pp.

2. *Use the rules of probability to make explicit that should be observed if the genetic hypothesis is true. Convert proportions to numbers of progeny (Percentages are not allowed in* (χ^2) *test).* For example, if the hypothesis of above said flower-color cross is true, then we should expect the progeny genotypes Pp and pp to occur in a ratio 1:1. Among 20 progeny the expected numbers are 10 purple and 10 white.

3. *For each class of the progeny in turn, subtract the expected number from the observed number, square this difference and divide the result by the expected number.*
In the present example the calculation for the purple flower progeny is $(12-10)^2/10 = 0.4$, and that for white flower progeny is $(8-10)^2/10 = 0.4$.

4. *From the result of the numbers calculated in step-3 for all classes of progeny, the summation is the value of chi-square value for these data.*
In the present example the sum for the purple and white classes of progeny is 0.4+0.4=0.8. This is chi-square value for the experiment, calculated on the assumption that our genetic hypothesis is correct.

Thus chi-square can be represented by the expression. $\chi^2 = \Sigma \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$

(Σ means summation over all the classes of progeny)

Note that χ^2 value is calculated using the observed and expected numbers, not the proportions, ratios or percentages. The χ^2 value is smaller as the observed and expected numbers are very close and implies that the observed number fit the expected numbers perfectly.

Once the chi-square value is calculated, the next step is to interpret whether this value represents a good fit or a bad fit to the expected numbers. Each chi-square value has a certain probability occurrence, depending on the number of degrees of freedom (*df*) in the experiment. The degrees of freedom have to be decided as n-1, where 'n' is number of classes of data of the experiment. For example in the present example there are two classes of progeny types with genotype Pp and pp. Hence the degrees of freedom are 2-1 =1 for this example. Once the degrees of freedom in an experiment have been determined, the calculated chi-square value is interpreted with the probabilities of χ^2 – table 18.1. The table contains χ^2 values and probabilities for each degree of freedom, that chance alone would produce a fit as bad as or worse than that actually observed. The critical values of p are conventionally chosen as 5% and 1% level. When the calculated chi-square value of a particular degree of freedom falls in the critical area, the correctness of the genetic hypothesis considered is very doubtful. If the chi-square value falls in high probability greater than 5% level, the goodness of fit to the hypothesis is judged to be satisfactory. The chi-square value 0.8 of the present example has the probability value between 0.2 – 0.3. This means that chance alone would produce a chi-square value as great as or greater than 0.80 in about 30% of experiments. Hence, because the p-value is not in the critical area (5% to 1% level, significant or highly significant) the goodness of fit to the hypothesis of 1:1 ratio of Pp:pp genotypes is judged to be satisfactory.

18.7 Yates correction

The Yates correction should be routinely applied in χ^2 value calculation whenever only 1 degree of freedom exists or in small samples where each expected frequency is between 5 and 10.

Deduction of 0.5 from the absolute value of the (observed – expected) value, whether it positive or negative is called Yates correction. χ^2 value in the present example after Yates correction is as followed:

Purple = (Observed – expected) = 12-10=2

Yates correction = 2 - 0.5 = 1.5

$$\frac{(\text{Observed} - \text{expected})^2}{\text{expected}} = \frac{(1.5)^2}{10} = \frac{2.25}{10} = 0.225$$

White =(Observed – Expected) = (8 – 10) = -2

Yates correction = -2 – 0.5 = -1.5

$$\frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}} = \frac{(-1.5)^2}{10} = \frac{2.25}{10} = 0.225$$

Chi-Square value = 0.225+0.225=0.450

If the corrected and uncorrected methods of χ^2 value calculation each leads to the same conclusion, there is no difficulty. However, if these methods do not lead to the same conclusion, then either more data need to be collected or a more sophisticated statistical test should be employed.

18.8 Summary

The probability of an event may be considered as equivalent to the proportion of times that event is expected to be realized in numerous repeated trails.

***Addition rule*:** The probability of the realization of one or the other two mutually exclusive events A or B is the sum of their separate probabilities i.e Prob (Aor B) = Prob (A) + Prob (B).

***Multiplication rule*:** The probability of two independent events A and B, being realized simultaneously is given by the product of their separate probabilities i.e. Prob (A and B) = Prob (A) . Prob (B).

Some times in solving genetic problems both rule of addition and rule of multiplication may be used together. The probability of certain combination of events can be obtained by the expansion of '*binomial*' $(p+q)^n$, where p and q are the probabilities of alternate independent events and n represents the number of trails. Thus the general rule for repeated trails of events with constant probabilities is as follows.

$$\frac{n!}{s!\,f!}\,p^s\,q^f$$

in which out of '*n*' trails 's' times realized one event and 'f' times the other event.

Goodness of fit and χ^2 *-method*:

Genetics often need to decide whether an observed ratio is in satisfactory agreement with a theoretical prediction. This can be done by a test called *'test for goodness of fit,'* through a measure of value called 'Chi-square' (χ^2).

$$\chi^2 = \Sigma \frac{(observed - expected)^2}{expected}$$

18.9 Key Terms

1. *Addition rule:* The principle that the probability that any one of a set of mutually exclusive events is realized equals the sum of the probabilities of the separate events.

2. *Binomial theorem:* The theorem that gives the terms of the expansion of a binomial raised to a particular power.

3. *Goodness of fit:* The extent to which observed numbers agree with the numbers expected on the basis of some specified genetic hypothesis.

4. *Independent events:* An event that has no influence on the possible realization of any other event.

5. *Multiplication rule:* The principle that the probability that all of a set of independent events are realized simultaneously equals the product of the probabilities of the separate events.

6. *Mutually exclusive event:* An event that excludes the realization of all other events.

7. *Probability:* The proportion of times an event may be expected to occur if infinitely large number of trails are made

8. Chi-*Square*(χ^2): A statistical quantity calculated to assess the goodness of fit between observed numbers and theoretically expected numbers.

18.10 Model questions

1. Define probability and explain the multiplication rule and addition rule with suitable examples?

2. Distinguish the mutually exclusive events from independent events and describe the calculation of their probabilities?

3. What is a binomial? Describe its application in the expectation of probabilities of alternate independent events?

18.11 Reference books

1. Hartl, D.L and Jones E.W. 1998. *Gnetics: Principles and Analysis,*) Jones and Bartlett publishers, London.

2. Susan Elrod and William Stansfield 2002. *Theory and problems of Genetics.* McGraw-Hill Publ.Corp., New Delhi.

LESSON: 19

SEX DETERMINATION

Objective:

To explain the various mechanisms of sex determination in plants, animals and man. Also to describe the inheritance of sex-linked characters.

19.1. Introduction
19.2. Sex Determining Mechanisms
- i) Sex chromosome mechanisms
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19.1 Introduction:

Sex is universal occurrence in all organisms whether plants or animals. The two sexes may be reside in deferent individuals or within the same individual. An animal possessing both male and female reproductive organs is referred to as a *hermaphrodite.* In plants where *staminate* (male) and *Pistillate* (female) flowers occur on the same plant, the term of preference is *monoecious.* Moreover, most flowering plants have both male and female parts within the same flower (perfect flower). *Uni-sexual* or *dioecious* organisms are those having male and female elements in different individuals. The different kinds of genetic sex determinating mechanisms present in *dioecious* organisms is dealt here in detail.

It is pertinent to know about some of the important findings that have led to the understanding of mechanism of sex determination. What determines, whether an offspring will be male or a female, a problem that puzzled many biologists for longtime. With the advent of the microscope the cytological basis of sex began to be understood. Oscar Hertwig in 1876 observed fusion of two nuclei during fertilization, one derived from the egg and the other from the spermatozoan in the sea urchin *Toxopneusteus lividus*. These observations were confirmed by Van Beneden (1883) in *Parascaris equorum,* a parasite on the horse and by Boveri (1890) in the sea urchin *Echinus microtuberculatus*. Henking (1891) observed an intensely stained chromatin body that lagged behind other chromosomes during anaphase of dividing spermatocytes of the plant bug *Pyrrhocorus apterus*. As its function was unknown, Henking called it the 'X' chromosome. Later Mc Clung (1901) was first to suggest that this chromosome was concerned with determination of sex.

After Sutton's hypothesis in 1903, that Mendelian factors are carried on chromosomes, many scientists got engaged in the study of chromosome cytology in various plants and animals. The chromosomes present in pairs during meiosis and each pair consists of two homologues. When such pair of homologues did not have identical chromosomes (Heteromorphic), it was easy to observe segregation of this pair. Wilson (1905) found that in some male insects the heteromorphic chromosome pair present while in others the females had this pair. Montgomery (1904) called the chromosomes of heteromorphic pair as *heterochromosomes* and the other chromosomes were called *autosomes*. Wilson used the terms X and Y-chromosomes for the hetero chromosomes. Steven (1905) called male dipterans flies that had heteromorphic pair as *heterogametic* and females with two X chromosomes as *homogametic*.

The establishment of the chromosomal basis of sex determination brought to light some other types of chromosomal arrangements underlying sex determination. Later on it was found that sex determination mechanisms are not always chromosomal, they may be sometimes genotypic, hormonal, cytoplasmic and environmental.

19.2 Sex Determining mechanisms

19.2.i Sex-chromosome Mechanisms

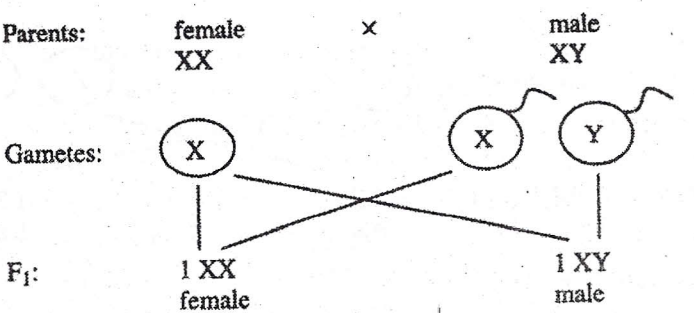
19.2.i a Heterogametic Males:

i) XY Method of sex determination: In humans and most other mammals, there are two different or *'heteromorphic'* sex chromosomes the X and the Y chromosomes. The presence of the Y chromosome determines maleness. For example normal human males have 22 pairs of autosomes and an X and a Y sex chromosome; females also have 22 autosomal pairs but have two X chromosomes. Since the male produces two kinds of gametes as far as the sex chromosomes are concerned, he is said to be the *'heterogametic'* sex. The female producing only one kind of gamete, is the *homogateic sex.* Thus assuming unbiased segregation and equal success of each type of gamete during fertilization, an equal number of males and females should be produced in each generation. The proportion of males to females is referred to as the 'sex ratio'. This method of sex-determination is commonly referred to as the XY method. (Fig. 19.1). In some plants such as *Rumex, Coccinia, Melandrium* and *Sphaerocarpus* this XY method of sex-determination was observed.

In the plant species as *Malandrium album* and *Coccinia indica* males are heterogametic sex with XY chromosomes and females homogametic (XX). Here Y chromosome determines maleness just as it does in humans. The research studies revealed that a plant is male when one or more Y chromosomes are present and is female when Y chromosome is absent.

In *Melandrium,* the Y chromosome is longer than X chromosome. The X and Y chromosomes have a common segment that helps in pairing and regular disjunction. The reminder of Y chromosome has three segments responsible for suppressing femaleness, initiating anther development and controlling late' stages of another development. The X chromosome also has a differential segment that should promote femaleness in the absence of female suppressing segment on Y chromosome.

Figure : 19.1 XY Method of sex determination.

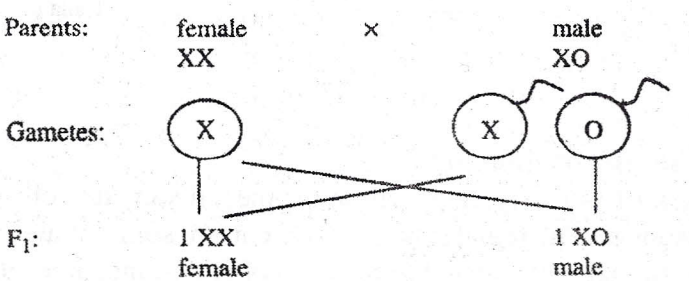


A gene called SRY (Sex-determining region Y) has been identified on the short arm of the Y chromosome in mammals. It encodes a gene product often referred to as the TDF (testis-determining factor). SRY seems to be highly conserved in mammals. This gene in combination with several other genes such as the autosomal DAXI, WTI, SFI and SOX9 genes, encodes a DNA-binding protein that appears to control the expression of one or more other genes that are involved in testicular development and fertility (sperm production).

ii) XO method of sex determination:

In some insects, especially those of the orders *Hemiptera* (true insects) and *Orthoptera* (grasshoppers and roaches), males are also heterogametic, but produce either X-bearing sperm or gametes without a sex chromosome. In males of these species one X chromosome is present and Y chromosome is absent. Thus males exhibit an odd number in their chromosome compliment. The one X condition determines maleness and two X condition femaleness respectively. This method of sex determination is commonly referred to as XO method (Fig. 19.2) where O symbolizes the lack of a chromosome analogous to the Y of the XY system. This mechanism was first discovered in the bug *Protenor,* where males have 13 chromosomes and females have 14.

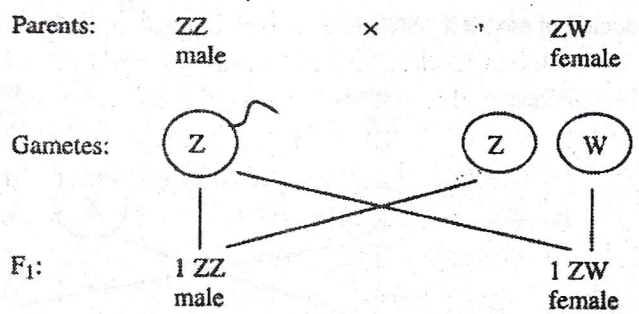
Fig. 19.2 XO Method of sex determination.



19.2.i.b Heterogametic females:

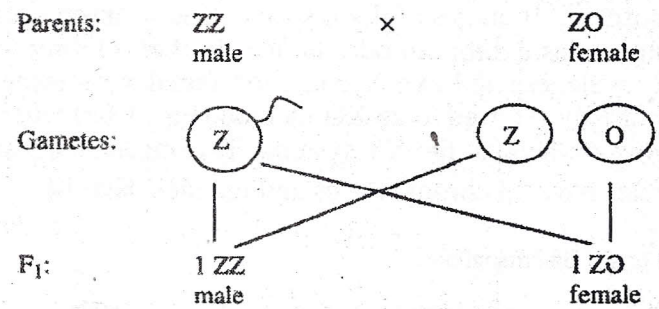
i) ***ZW method of sex determination:*** The females of domestic chickens have a chromosome similar to that of the Y in humans. In this case the chromosomes are sometimes labbelled Z and W instead of X and Y respectively, in order to call attention to the fact that the female (ZW) is heterogametic sex and the male (ZZ) is the homogametic sex (Fig. 19.3). In only one plant species *(Fragaria elatior)* hetegametic females with XY chromosomes were observed.

Fig. 19.3 ZW Method of sex determination.



ii) ***ZO method of sex determination:*** *The 1Z and 2Z condition* determines femaleness and maleness. (Fig. 19.4) This type of sex determination is found in comparatively large group of insects including butterflies, moths, caddis flies, silkworms and in some birds and fishes. In this system of sex-determination, males exhibit even number and females exhibit odd number in their chromosome complement.

Fig. 19.4 ZO Method of sex determination.



19.2.ii Balance theory of sex determination:

Drosophila melanogaster has four pairs of chromosomes in somatic cells of which the fourth pair represents sex chromosomes. The females have two X chromosomes males have XY. However, the genetic basis of sex determination is related to the ratio of X chromosomes to autosomes.

In 1910 T.H.Morgan discovered a white-eyed mutant in *Drosophila* which was recessive to the normal red-eyed condition of the wild type. After making several crosses between red and white-eyed flies, Morgan concluded that the gene for white eyes was located on the X chromosome while the Y chromosome carried no genes (empty).

Calvin B.Bridges (1916) discovered certain males of *Drosophila* lacking a Y chromosome (XO). Such males are sterile but normal in appearance and behavior. Females that carry an extra Y chromosome (XXY) are normal and fertile. This situation proves that the females have 2X's and males one X for the sex to be determined. The presence of the Y chromosome, although it is essential for male fertility, apparently has nothing to do with the determination of sex. In fact, it is ratio of X chromosomes to haploid sets of autosomes that determines sex in *Drosophila.* Using the letter A to represent a haploid set of chromosomes a normal female (2X : 2A), has an X:A ratio of 2:2 or 1.0, and therefore the balance is in favour of femaleness. When only one X chromosome is present in a normal male (XY:2A), the ratio is 1:2 or 0.5. Several abnormal combinations obtained from a cross of triploid (3A + 3X) X diploid (2A + XY) have confirmed this hypothesis. Bridges compared the phenotypes of the embryos of above cross with the corresponding chromosomal constitution and found correlation between the ratio of X chromosomes to complete sets of autosomes that determines phenotypic sex. The four types of gametes (2A XX, 2AX, AXX, AX) from triploid female unite with two types of gametes (AX, AY) from normal male to produce the following zygotes.

Chromosomal Constitution	Ratio of X chromosomes to autosomal sets (X/A)	Phenotype
3AXXX	3/3 = 1.00	Triploid female
2AXY	1/2 = 0.5	Diploid male
3AXX	2/3 = 0.67	Triploid intersex
3AXY	1/3 = 0.33	Meta male
2AXX	2/2 = 1.00	Diploid female
3AXXY	2/3 = 0.67	Triploid intersex
2AXXX	3/2 = 1.5	Metafemale
2AXXY	2/2 = 1.0	Diploid female

A ratio of 2:3 or 0.67 in between the ratios for normal maleness and femaleness, produces *intersex* that is sterile and sexual characteristics intermediate between the male and female. Ratios above 1.0 produce sterile *metafemales* (super females) and ratios below 0.5 produce sterile *metamales* (supermales).

The ratio determines sex by activating sex-specific gene expression of several genes, such as *sexual – lethal* (sxl), *transformer* (tra) and *double sex (dsx).* Simply stated 'in females, the *sxl* gene is active and leads to the production of an active *tra* gene product. This results in further production of a female-specific *dsx* gene product and development of female flies. The X/A chromosome ratio in normal males does not result in production of *sxl* gene product; thus the *tra* protein is not produced and the default, or male development pathway is followed. A recessive *tra* gene, when homozygous, can transform a diploid female into sterile male, since the absence of tra^+ *gene.* This mutation has no effect in normal males.

19.2.iii Sex-determination by Haplo diploidy:

This mechanism of sex-determination is characteristic of the in ect order *Hymenoptera* including ants, bees, wasps etc. Male bees are known to develop *Parthenogenetically* (without union of gametes) from unfertilized eggs (*arrhenotoky)* and are therefore haploid. Females (both workers and queens) originate from fertilized eggs and they are diploid. The quantity and quality of food available to the diploid larvae determines whether that female will become a sterile worker or a fertile queen. Thus the environment determines sterility or fertility but does not alter genetically determined sex.

19.2.iv Single gene controlled sex determination:

Some members of the insect order 'Hymenoptera' are known to produce males by homozygosity at a single gene locus. This has been confirmed in the tiny parasitic wasp *Bracon hebetor* (often called *Habrobracon juglandis)* as well as in bees. Atleast nine sex-alleles are known at this locus in *Bracon* and represented by S^a, S^b, S^c.....S^i. A wasp that gets a combination of any two different alleles such as S^aS^b, S^aS^c, S^dS^f etc. becomes female. Thus all females must be heterozygotes. If an individual is homozygous for any of these alleles such as S^aS^a, S^bS^b, S^cS^c etc., it develops into a diploid male (usually sterile). Haploid males, of course would carry only one of the alleles at this locus e.g., S^a, S^b, S^c, etc. Because of the number of different alleles is large, most matings result heterozygotes (females). The homozygous condition is rarely obtained (Fig. 19.5).

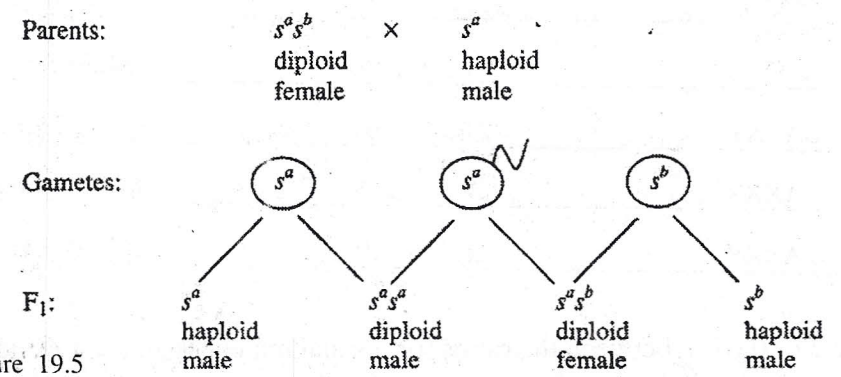


Figure 19.5
Among the diploid progeny we expect 1 s^as^a male : 1 s^as^b female.
Among the haploid progeny we expect 1 s^a male : 1 s^b male.

19.2.v Gene induced sex reversal: A gene is known to induce sex reversal in human beings. An X-linked dominant gene, which has no effect on females but in males, it acts and makes the target tissues inactive to the androgen hormone. This condition is known as *testicular feminization.* The individual has XY chromosomes but is phenotypically female, internally they do not have uterus, fallopian tubes or ovaries, instead they have undescended, degenerate testes and vasdeferens. They have normal levels of the male hormone.

Maize is monoecious with male inflorescence (tassel) and the female inflorescence (silk) located on the same plant. But a recessive gene called tassel-seed(ts) when homozygous, the individuals of genotype ts/ts are functionally reduced to a single sex that of the female. Another recessive gene called "*silkless*" (sk) when homozygous (sk/sk) produce individual plants that are reduced to perform only male functions (production of pollen in tassel).The ts gene epistatic to sk locus recessive homozygous genes. It is possible to induce dioecism by using these in corn plants. In dioecious forms the female plants will have the genotype ts/ts, sk/sk and male plants ts^+/ts^+, sk sk. They will segregate again in 1:1 ratio.

19.2.vi Environmental control of sex:

The Sea Worm Bonellia is a sexually dimorphic organism. The female is large about 10 cm long and the male is only 3 cm long. The male lives parasitically inside the cloaca of the female. The eggs on fertilization produces a free-swimming larvae. If there are not females around, the larvae settle down on the bottom of the sea and develop into females. But if mature females are available, the larva attaches itself to its proboscis and grows in to males by drawing nourishment from the female. It then migrates down the proboscis to reside in the cloaca of the female.

In the marine annelid *Dinophilus* also the female worm is much larger than the male. The eggs are of two different sizes. The larger eggs on fertilization develop into females and the small ones into males. Here, it appears that the quantity of egg cytoplasm determines sex. Similarly temperature plays a vital role in influencing sex. Thus high temperatures during incubation promotes females production in the turtle *chrysema picta* and males in the lizard *Agama agama.*

19.2.vii Hormones and sex determination:

a) **The *Crew's hen:*** Crew (1923) reported a case of sex reversal. Hormones are known to play a role in such sex reversal. It is often observed that a good egg-laying hen starts showing male characteristics in old age. Not only does the camb develop on the head, but it crows like a rooster and even starts mating with hens. In poultry, females have a single functional ovary. In the center of this ovary is a small mass of tissue similar to the testes. The female hormones produced by the ovary ensure the normal development and functioningof female characters, at the same time suppressing the growth of the small testes-like tissue. If however either due to damage, infection or due to age factor, the ovarian tissues cease to function, the small testicular tissue gets released from inhibition. It grows and produce male hormones due to which the hen starts showing a shift towards maleness. It is possible to change the sex by administering the male hormone testosterone to newborn chicks. If the normal functioning of ovaries is suppressed the bird becomes a male.

b) **The Free-Martin**: In dizygotic twins consisting of one male and one female, it is found that in most cases the female twin shows male characteristics and is sterile. Such a genetically female but expressing a male phenotype is called a 'free martin' after the name the worker. The condition originates when there is fusion of the two fetal membranes resulting in joining of the blood vessels of the male and female twins. The male calf starts producing sex hormones earlier than the female calf. The male hormones cross the placenta and reach the female to induce the development of male characteristics.

19.3 Sex-linked inheritance:

Mendel's crosses revealed that the reciprocal crosses give the same results. It did not matter, whether a particular trait present in the male parent or in the female parent. One of the earliest exception to this rule was found by Thomas Hunt Morgan (1910) in an early study of a mutant in the fruit fly *Drosophila melanogaster* that had white eyes. The wild type eye color is a brick-red combination of red and brown pigments. In a mating of white-eyed male with red-eyed female all the F1 progeny of both sexes had red eyes, which showed that the allele for white eye is recessive. In the F2 progeny from the mating of F1 males and females, Morgan observed 2459 red-eyed females, 1011 red-eyed males and 782 white eyed males. The white-eyed phenotype was some how connected with sex, because all of the white-eyed flies were males. A key observation came from the results of reciprocal cross of white eyed females with red-eyed (wild) males. All the female progeny had red-eyes, but all the male progeny had white eyes. Morgan realized that receiprocal crosses yield different results if the allele for white eyes were present in the X chromosome. The reason is that the X chromosome is transmitted in a different pattern by males and females. Male transmits his X chromosome to his daughters, where as a female transmits one of her X chromosome to the offspring of both sexes. *Hence reciprocal crosses yield different results for the X chromosome determined traits. A gene on the X chromosome is said to be X – linked or sex-linked.* Figure 19.6 shows the transmission of sex chromosome (X) from two parents to the daughters and sons. In addition to the gene for eye color, at least 140 other genes in *Drosophilae* exhibit the same mode of transmission and are carried on X chromosome.

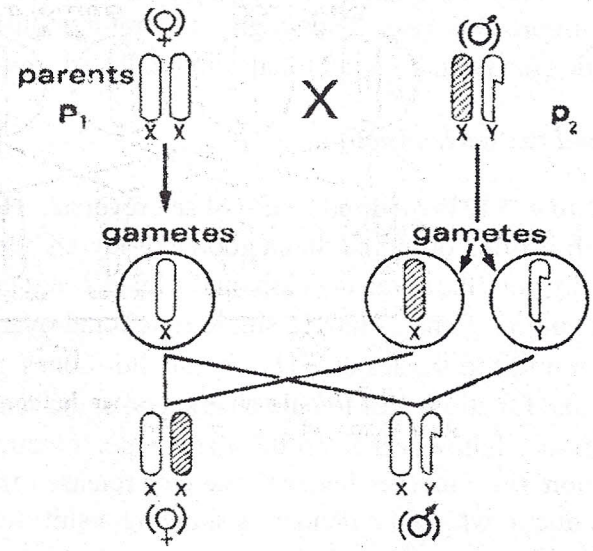


Fig. 19.6 Transmission of sex chromosome X from two parents to the daughters and sons.

The sex linked character from the father goes to the daughter (F1) and then from the daughter to the grandson in the next generation (F2). This *criss-cross* mode of inheritance is characterstic of six-linked genes (Fig. 19.6).

The inheritance of X-lined eye color in receprocal crosses is shown in figures 19.7& 19.8 The reciprocal cross where the sex-linked recessive gene appears in the male results in the disappearance of the trait in the F1 and its appearance only in the males of the F2. This type of *skip generation* inheritance also characterizes sex-linked genes.

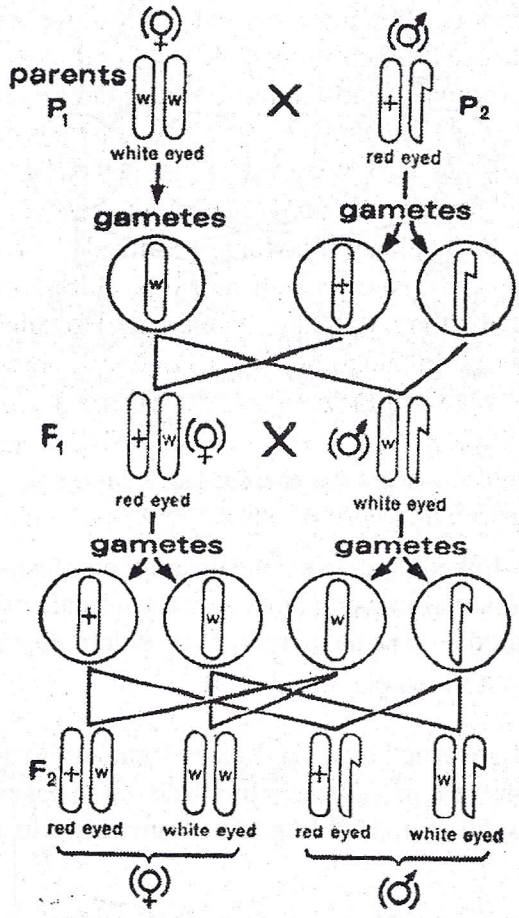


Fig. 19.7 Inheritance of eye colour in *Drosophila* from white eyed ♀ × red eyed ♂.

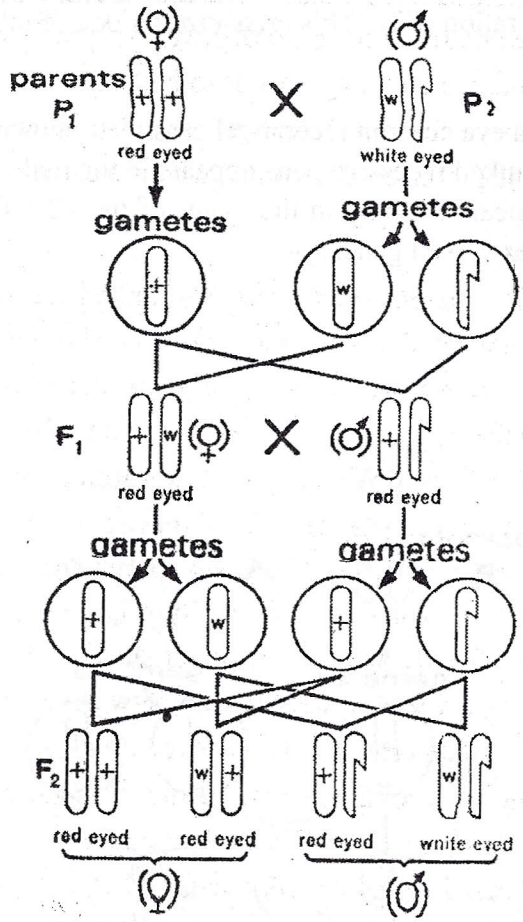


Fig. 19.8 Inheritance of eye colour in *Drosophila* from a cross red eyed ♀ × white eyed ♂.

A trait governed by a sex-linked recessive gene usually manifests itself in the following manner: 1) it is usually found more frequently in the male than in the female of the species 2) it fails to appear in females unless it also appeared in paternal parent, 3) it seldom appears in both father and son, then only if the maternal parent is heterozygous.

On the other hand, a trait governed by a sex-linked dominant gene usually manifests itself by 1) being found more frequently in the female than in the male of the species 2) being found in all female offspring of a male that shows the trait, 3) failing to be transmitted to any son from a mother that did not exhibit the trait itself.

Some other examples of Sex-linked traits:

19.3.i Hemophilia: A recessive sex-linked gene h prolongs the blood-clotting time resulting in what is commonly called '*bleeder's disease*' or *hemophilia* in human beings. In normal persons the blood clots immediately in wounds and prevents from excessive hemorrhage leading to death. In hemophilia effected persons, even a small injury can lead to death due to loss of blood. These persons lack a blood-clotting protein called *factor VIII* needed for normal clotting.

Hemophilic men, if they survive and reach the reproductive age, produce daughters all of whom are normal but carriers of hemophilia, which they transmit to half their sons (grandsons of male hemophilic. One half of the daughters of the female carriers are also heterozygous carriers. A female hemophilic, could theoretically be produced if a woman who is a carrier marries a man who is hemophilic.

***19.3.ii Colorblindness*:** In human beings there is an X – linked recessive gene that causes color blindness. The usual form of color blindness is *'red-green color blindness'*. Human color vision is mediated by three light sensitive *Protein pigments* present in the cone cells of the retina. Each of the pigments is related to *rhodopsin* the prigment that is found in the rod cells and mediates vision in dim light. The light sensitivities of the cone pigments are toward blue, red and green. These are our primary colors. We perceive all other colors as mixtures of these primaries. The gene for blue pigment resides in 7q22-qter (7q22 to the terminus) and the genes for red and green pigments are on the X – chromosome at Xq28 separated by less than $5c^M$ (or 5 Mb of DNA). These red and green pigment genes arose from duplication of a single ancestral pigment gene. Hence they are 96% identical in amino acid sequence. The process of unequal crossing over between the two genes is the genetic basis of *red-green color blindness*. Unequal crossing over between red-pigment gene and green-pigment gene yields a *chimeric gene*, which is a composit gene. If the chimeric gene resulted contains mostly red-pigment gene the chrosome causes *green blindness* (deuteranopia = an inability to perceive green). If in the chimeric gene most of the red pigment gene is replaced with the green pigment gene it causes *'red-blindness'* (*Protanopia* = an inability to perceive red).

The preponderance of affected males with red-green colorblindness immediately suggests X-linked inheritance. Pedigree studies revealed that affected males have normal sons and carrier daughters. The carrier daughters have 50% affected sons and 50% carrier daughters.

19.3.iii Barred plumage: The inheritance of barred plumage in poultry is a classical example of sex-linkage. The gene for barred plumage is dominant over black or red plumage and is X-linked. This gene follows same *criss-cross inheritance*. The only difference from *Drosophila* is that, in fowls that the sex-linked gene (B) goes from mother (XY) to her sons only where as the father (XX) transmits the gene to both sons and daughters.

19.4 Sex-Influenced traits:

A trait whose expression depends on the sex of the individual, is known as *sex-influenced trait.* The genes governing sex-influenced traits may reside on any of the autosomes or on the homologous portions of the sex chromosomes. The gene for *pattern baldness* and the gene for *short index finger* are the two examples of sex-influenced genes. These two genes are *dominant* in men and *recessive* in women as shown here in the table.

Genotype	Phenotype of	
	Males	Females
B^1B^1	Bald	Bald
B^1B^2	Bald	Normal
B^2B^2	Normal	Normal
F^1F^1	Short index fingered	Short-index finger
F^1F^2	Short-index finger	Long-index finger
F^2F^2	Long-index finger	Long-index finger

The gene governing the presence of horns in sheep is sex-influenced gene exhibiting dominant in males but acts recessively in females.

19.5 Sex-limited traits:

A trait expressed in one sex and not in the other sex is a sex-limited trait. Some autosomal genes may only come to expression in one of the sexes either because of differences in the internal hormonal environment or because of anatomical dissimilarities. For example bulls have many genes for milk production that they may transmit to their daughters, but *they* or their *sons* are unable to express this trait. Cock feathering in chicken is a sex-limited autosomal recessive gene. It expresses only in males. The genotype hh produces cock-fea thering in males and hen feathering in females.

19.6 Sex chromatin or Barr body: Dosage compensation by X-chromosome inactivation:

Sex-linked traits are not expressed more intensely in females (XX) with two doses of X-linked genes than in males (XY) with only one X chromosome. At a particular stage early in the development of females, one of the two X chromosomes in a cell becomes inactivated as a *dosage compensation mechanism.* According to Dr.Mary Lyon the inactivation of any X chromosomes in excess of one per cell results from *condensation* or *heterochromatinization.* This inactivated X chromosome can be identified in many interphase cells of females as a dark-staining mass that is attached to the nuclear membrane and described as *sex-chromatin* or *Barr body* (after Dr.Murray L.Barr). Thus extra X chromosomes have milder effects than extra autosomes in mammals because of all X chromosomes except one are genetically inactivated. Similarly the effects of extra Y chromosomes are also relatively mild because the Y chromosome in mammals is largely heterochromatic and contains very few genes.

The number of *barr bodies* in a cell is always one less to the actual number of X chromosomes present in that cell. Although all mammals use X chromosome inactivation for dosage compensation in females, the choice of which chromosome to inactivate is not always random. In marsupial mammals which include the Kangaroo, the Koala, and the Wombat, the X chromosome that is inactivated

is always the one contributed by the father. As a result the female marsupial expresses the X-linked genes that she inherited from her mother. In non-marsupial mammals, there is random inactivation of maternal and paternal X chromosome. This results in genetic mosaicism of maternal and paternal X-linked genes in females. One example is the "*calico*" pattern of coat coloration in female cats. In cats one allele present in X-chromosome results in an orange coat color, the other allele in a black coat color. The male cat has only one X chromosome and it has either the orange or the black allele. A female can be heterozyous for orange and black and in this case the coat color is 'calico' - a mosaic of orange and black patches mixed with patches of white. The orange and black patches result from X chromosome inactivation. In cell lineages in which the X chromosome bearing the orange allele is inactivated, the X chromosome with the black allele is active and so the fur is black. In cell lineages in which the X-chromosome with black allele is inactivated, the orange allele in the active X chromosome results in orange fur.

19.7 Summary

Sex determination mechanisms in dioecious organisms are different kinds. In human beings and in most other mammals *heteromorphic* sex chromosomes (X and Y) are present. Based on the presence of XX and YY chromosomes in different sexes they are classified as *homogametic* sex and *heterogametic* sex respectively.

Heterogametic Males: In humans and other mammals the males are heterogametic with XY chromosomes and females are homogametic with XX chromosomes. This type of XY sex determination mechanism is also present in different plant taxa viz., *Coccinia, Rumex and Melandrium.*

In some insects especially those of the orders Hemiptera and *Orthoptera* (grass hoppers and roaches) males are heterogametic with only one X chromosome and without Y chromosome. Where as females are homogametic with two XX chromosomes. This method of sex determination is described as XO method.

Heterogametic females: In some other organisms females are heterogametic sex. In domestic chicken female is heterogametic (ZW) and male is homogametic (ZZ). In butterflies, moths, caddis flies, silk worms, birds and fishes females are with one sex chromosome (ZO) and males are with two sex chromosomes (ZZ).

Balance theory of sex determination: The ratio of X chromosomes to haploid sets of autosomes determines the sex in *Drosophila.* In females the ratio is 1 and in males it is 0.5.

Haplo-diplody: In the insects of the order Hymenoptera (wasps, ants, bees) haploidy and diploidy determines the male and female sex respectively.

Gene controlled sex-determination: In some organisms a single gene determines the sex. For eg. insects namely *Bracon hebetor* homozygosiy at a single gene locus produces males. Heterozygosity of the allele produces female sex.

An X-linked dominant gene in human males is known to induce sex-reversal into phenotypically female, but it has no effect on females.

Environmental and Hormonal sex determination: Some times environmental factors control sex determination (e.g. sea worm Bonnelia, turtles and lizards). Hormones also determine the sex of an individual (e.g. the crew's hen and Free-martin twin).

Sex-linkage: If the genes of the traits are present on X-chromosome, they are called X linked genes or sex-linked genes. Their inheritance pattern is different from that of autosomals genes. Hemophilia and colorblindness are the best examples for sex-linked traits.

Sex-influenced and sex-limited traits: Some autosomal genes are expressed differently in males and females and are called sex-influenced genes. The genes for baldness, in human beings and genes for horn production in sheep are the examples of sex-influenced genes. Some genes are expressed in one sex only (sex-limited)

Dosage compensation – X chromosome inactivation: In mammals where males are with one X chromosome and females with two X chromosomes, the gene dose of X chromosome is balanced through genetic inactivation of extra X chromosome in females. The inactivated X-chromosome appears as sex-chromatin or Barr body in the interphase cells. In marsupial mammals the paternal X chromosome becomes inactivated always. But in others the paternal and maternal X-chromosomes inactivate randomly resulting in genetic mosaicism of X-linked genes.

19.8 Key terms:

Dioecious: Male and female sexes present in different individuals.

Heterogametic: The production of dissimilar gametes with respect to the sex chromosomes.

Sex chromosome: A chromosome such as the human X or Y, that has a role in the sex determination.

Sex-linked trait: A trait determined by a gene on a sex-chromosome usually the X.

Sex-limited trait: A trait expressed in one sex and not in the other

Sex-influenced trait: A trait whose expression varies in male and female individuals.

Criss-cross pattern of inheritance: Transmission of X-linked genes from mother to son and from son to his daughters is criss-cross type of inheritance.

Barr body: A darkly staining body found in the interphase nucleus of certain cells of female mammals, consists of condensed and inactivated X chromosome.

Dosage compensation: A mechanism regulating X-linked genes such *that their activities are equal in males and females.*

Colorblindness: In human beings X-linked recessive gene caused *red or green – vision* defect is known as colorblindness.

Protanopia: An inability to perceive red.

Deuteranopia: An inability to perceive green.

Holandric inheritance: Inheritance of Y-linked genes.

19.9 Questions

1. Explain the sex-determination mechanisms by sex-chromosomes in plants and animals?
2. What is the balance theory of sex determination in D*rosophila* Explain?
3. Describe gene controlled and hormone controlled sex determination mechanisms?
4. What is sex-linkage? Explain sex linked inheritance with examples?
5. Write about sex-influenced and sex-limited characters?

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LESSON – 20

CYTOPLASMIC AND MULTIPLE FACTOR INHERITANCE

Objective: To know about the mode of cytoplasmic inheritance and multifactor inheritance with some illustrative examples.

20.1 Cytoplasmic Inheritance

20.2 Examples of cytoplasmic Inheritance

A. Plastid color

B. Male sterility in plants

C. Cytoplasmic inheritance in Chlamydomonas

D. Petites in fungi

E. Killer particles in Paramecium

20.3 Chemical basis of cytoplasmic Inheritance

20.4 Multiple factor OR Polygenic inheritance

20.5 Polygenic inheritance : Some examples

A. Seed color in Wheat

B. Corolla length in Nicotiana longiflora

20.6 Summary

20.7 Key terms

20.8 Questions

20.9 Reference Books

20.1 Cytoplasmic inheritance:

The inheritance of organism's characters takes place by genes located at fixed loci in the chromosomes which inturn situated in the nucleus. Contrary to this, there is a mechanism in which cytoplasmic particles or inclusions play a part in the inheritance of characters from one generation to next is known as *'cytoplasmic inheritance* or *Extra chromosomal* or *Extra nuclear inheritance.* It is controlled by genes which are contained in DNA threads of cytoplasmic organelles like chloroplasts and mitochondria. These organelles are semi-autonomous, self-replicating and possess their own protein synthesizing machinery.

In the case of nuclear genes, reciprocal crosses give identical results because the nuclear contributions of male and female parents are equal. But in the case of cytoplasmic inheritance reciprocal crosses give different results, because the cytoplasmic contributions of parents are not equal. Most of the cytoplasm of the zygote is contributed by the egg rather than by pollen or sperm. Thus cytoplasmically inherited characters show maternal inheritance or the progeny resemble female parent.

20.2 Examples of cytoplasmic inheritance

A) Plastid color

Plastids are cytoplasmic organanelles. The plastid number per cell varies depending on the species. Plastids arise from proplastids. The plastids are self-replicating organelles and never arise *deno vo.* Their duplication is independent of other organelles. They are distributed more or less equal in the daughter cells. A plastid with green pigment is a chloroplast. Some plastids fail to develop into green plastids in the presence of light. Such plastids are known are *leucoplasts.* Correns (1908) observed non-Mendelian inheritance of plastid color in *Mirabilis jalapa.* This plant has green, white or variegated leaves. Correns found that seeds borne on green leaved branches produced only green plants, where as those borne on white branches produced only white leaved plants seeds and borne on variegated branches produced branches only green or only white or variegated leaves on branches. In all these cases it is immaterial whether the pollen has been donated by a flower borne on the green, white or variegated leaved branch. The progeny always resemble the branch on which the seed is borne (Fig. 20.1). Inheritance of plastid color in *Mirabilis Jalapa* is strictly maternal. Pollen of this plant has very little cytoplasm with out plastids or proplastids. On the other hand eggs contain plastids in their cytoplasm. Therefore the seeds borne on green branches contain primordia for green plastids only, those borne on white branches, only white and those borne on variegated branches may contain either green or colorless or both the types of plastids. A random distribution of plastids from

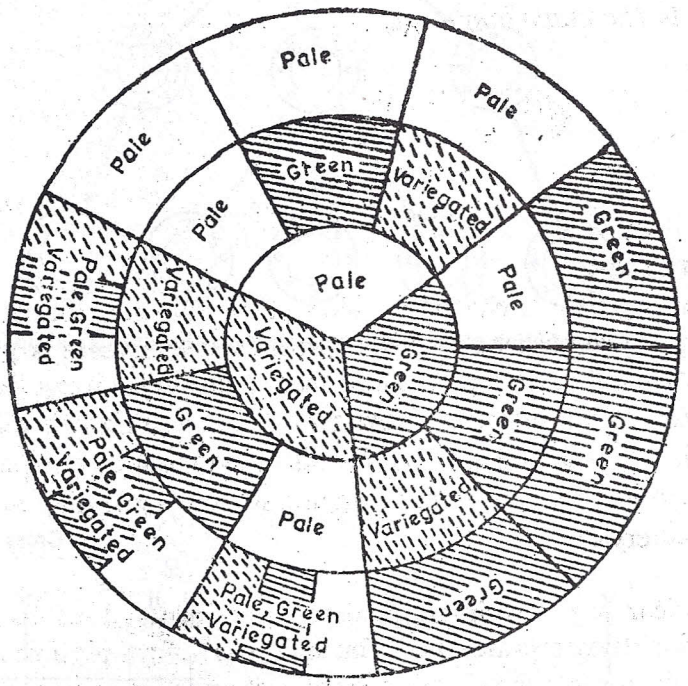
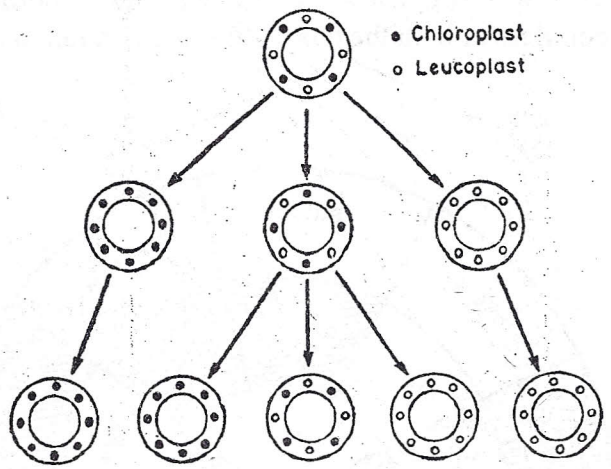


FIG. 20.1 Maternal inheritance of plastid colour in *Mirabilis jalapa.* The central circle represents the type of branch that produces flowers which are pollinated. The intermediate circle shows the branch from which pollen is used and the outer circle represents the type of progeny.

a cell containing both green and colorless plastids may give rise to an egg with only green or only colorless or a mixture of green and colorless plastids. Maternal inheritance of plastids has also been identified in rice beans, *Epilobium,* mosses etc. Because of their location outside the nucleus i.e. in cytoplasm, such factors are variously described as extra-chromosomal factors, plasmagenes, plasmons, plasmids, etc.

In maize, plastid color is determined by cytoplasmic as well as nuclear genes (Rhoades 1943). Some maize plants have mosaic leaves with green and white stripes. A recessive nuclear gene iojap (ij) governs the striped leaves. Once formed, the striped character is transmitted as if it is controlled by a cytoplasmic factor. Reciprocal crosses between striped and normal plants give different results (fig. 20.2). When normal green plant is used as female parent, in the cross, the progeny produced is only green. But when striped plant is used as female parent, the progeny contained green, white and striped plants. It has been postulated that the eggs of striped plants (ij ij) contain proplastids for green plastids but as development proceeds, some of them mutate and thus give rise to colorless plastids. In the absence of dominant gene (Ij), the mutation frequency of the plasma gene increases thus giving rise to striped leaves.



Distribution of green and colourless plastids during cell division.

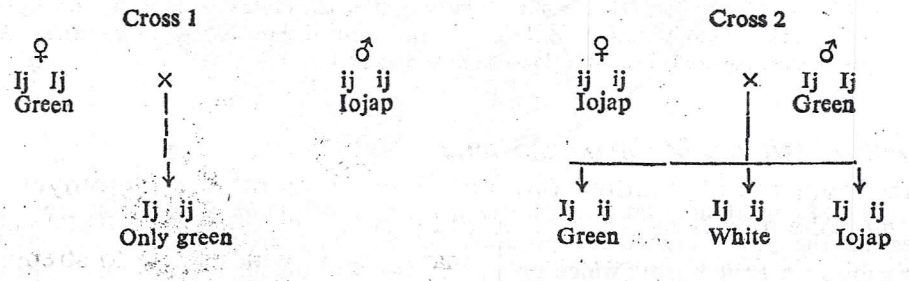


FIG. 20.2 **Differences in reciprocal crosses in maize showing maternal inheritance of *iojap* striping.**

B) Male sterility in plants

Male sterility or failure to produce functional pollen is cytoplasmically inherited in maize, wheat, onion, sugar beets etc. In some cases it is controlled by an inter action of nuclear and cytoplasmic genes and in majority of plants it is controlled by nuclear genes.

Rhoades (1933) discovered extra-chromosomal inheritance of male sterility in maize (Fig.20.3) He noticed male sterile maize, which produced only male sterile progeny when fertilized with pollen from male fertile plants. Male sterile line was repeatedly backcrossed to the male fertile parent until all chromosomes of male sterile variety were replaced by those from male fertile variety and even then male sterility persisted indicating that there is no effect of the nucleus on male sterility and proved to be under the control of some cytoplasmic factor.

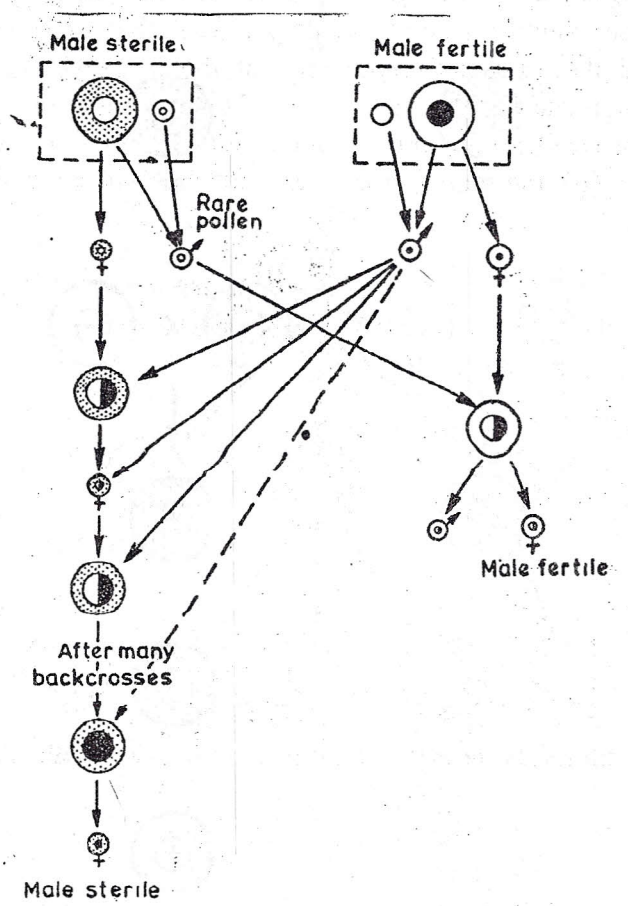


FIG. 20.3 - Maternal inheritance of male sterility in maize. Male sterile plants were repeatedly back-crossed to the male fertile parent until all chromosomes of the male sterile parent were replaced. Even then male sterility persisted.

C) Cytoplasmic inheritance in Chlamydomonas

Ruth Sager (1954) studied the inheritance pattern of streptomycin – resistance in *chlamydomonas*. She found that it is controlled by extra – chromosomal factors and its inheritance was maternal, i.e. the progeny resembled 'mating type' with respect to streptomycin resistance

(fig.20.4). Zygotes in this alga are formed as a result of fusion of gametes derived from + and – mating types. All the progeny were streptomycin – sensitive, when the + mating type was streptomycin sensitive. If the + mating type is streptomycin-resistant, all the progeny were streptomycin - resistant. A number of other characters, like temperature sensitivity, nutritional requirement for acetate, resistance to various antibiotics etc shows uri parental inheritance through + mating type parent. It has been established that during the zygote formation the nuclei of opposit mating types fuse and at the same

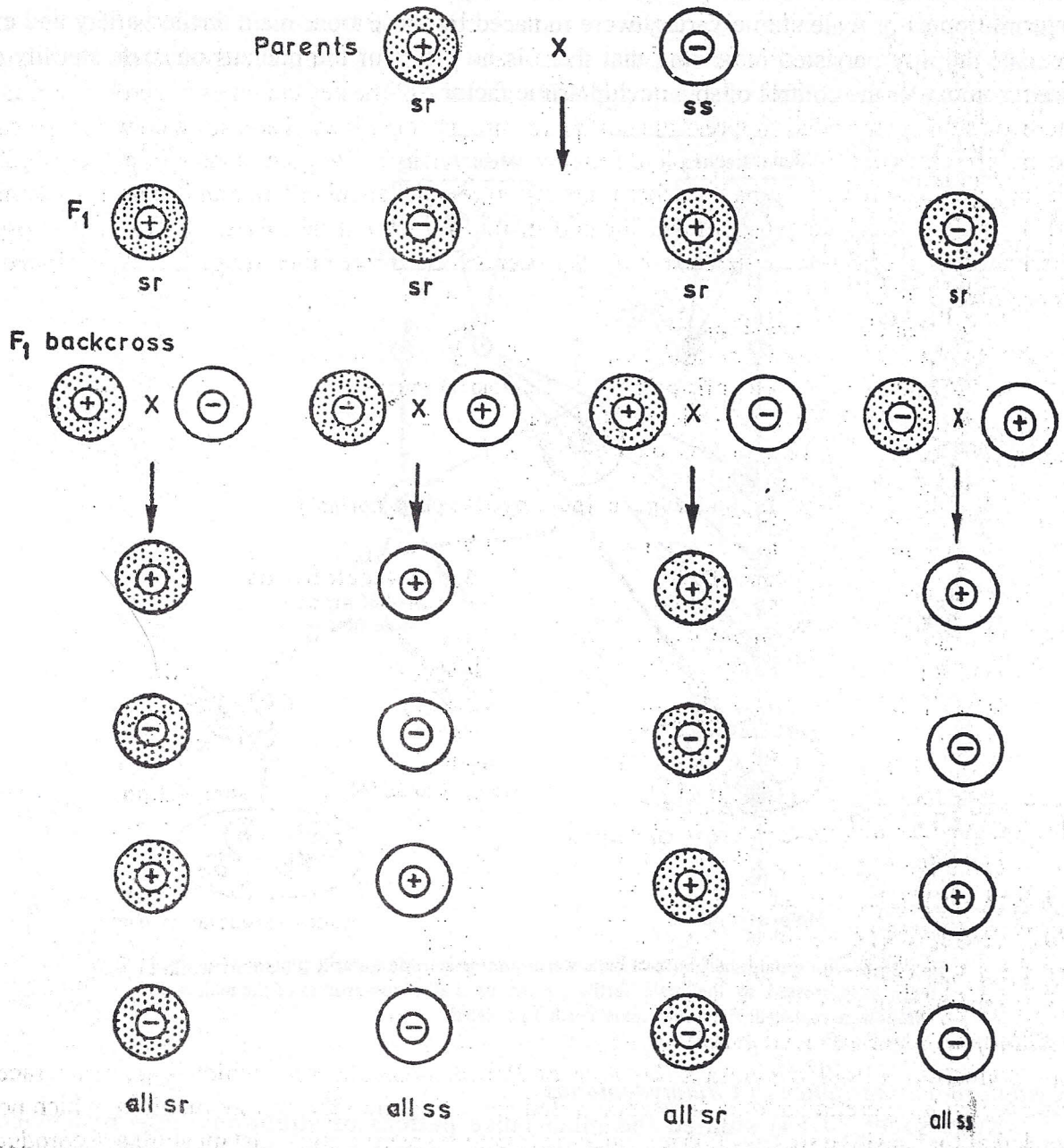


FIG. 20.4 Cytoplasmic inheritance of streptomycin-resistance in *Chlamydomonas*. Mating type (+) is inherited chromosomally. *sr*=streptomycin-resistant and *ss*=streptomycin-sensitive.

time chloroplasts also fuse. But, surprisingly enough, the chloroplast DNA of only + mating type is passed on to the progeny. Hence along with the chloroplast genome, the plastid is inherited to the progeny through + mating type parent only.

D)Petites in fungi

Normal yeast (*Saccharomyces cerevisiae)* cells grow very quickly on glucose medium and form uniform-sized colonies. Some colonies, how ever grow very slowly and are small in size. These small (petite) colonies give rise to only petites on being sub cultured. Petite colonies are unable to respire aerobically and fail to utilize glucose in the medium. Biochemical analyses of petite strians revealed that they are defective in various respiratory enzymes, especially cytochrome oxidase. Cytochrome oxidase is localized in mitochondria and is one of the key enzymes of aerobic respiration. There are some petites that suppress the normal respiratory functions in crosses with wild type strains and in some cases upto 99% of the diploid cells derived from hybrid zygotes consist of petites (fig.20.5). The factor for suppressive petite therefore follows non-mendelian inheritance and behaves as dominant and the biochemical defect has been located in the mitochondrial DNA. Extra –chromosomal inheritance of respiratory deficiency has also been observed in other fungi like *Neurospora and Aspergillus.*

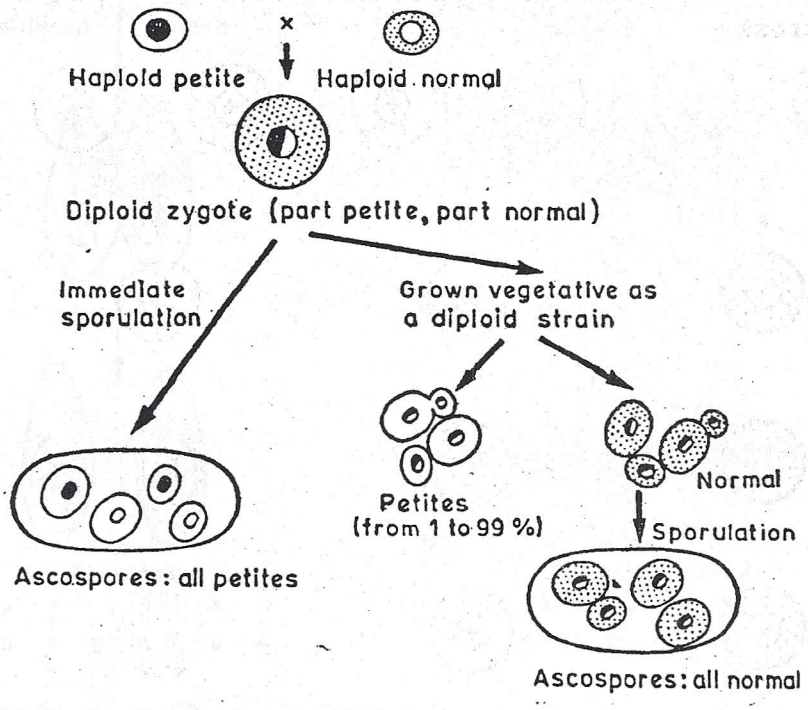


FIG. 20.5 **Results of crosses between a suppressive petite and a normal strain in yeast.**

E) Killer particles in Paramecium

Sonneborn (1949) found a *killer* race of *Paramecium Aurelia,* which kills other races of *Paramecium* by secreting a toxic substance called paramecin into the culture medium, which proves to be lethal for sensitive strains. It is possible to cross the sensitive and resistant strains. Reproduction in paramecium takes place by autogomy through conjugation or by simple cell division. During conjugation haploid micronuclei are exchanged and two genetically different nuclei fuse and individual

Paramecia separate as exconjugants. As a result of nuclear exchanges and fusion, both the exconjugants are genetically identical. Under normal conditions no cytoplasm is exchanged during conjugation. Thus, the exconjugants maintain their original cytoplasm but contain similar nuclei.

Killer and sensitive matings result into killer and sensitive exconjugants. The killer exconjugant produces a clone of killer paramecia and the sensitive exconjugant produces a clone of sensitive paramecia, although Mendelian genes segragate during successive generations after autogamy (fig.20.6). Thus killer and sensitive chracters are not controlled by Mendelian genes. the progeny of a killer-sensitive prolonged mating are killers. This is a proof for the cytoplasmic inheritance of killer

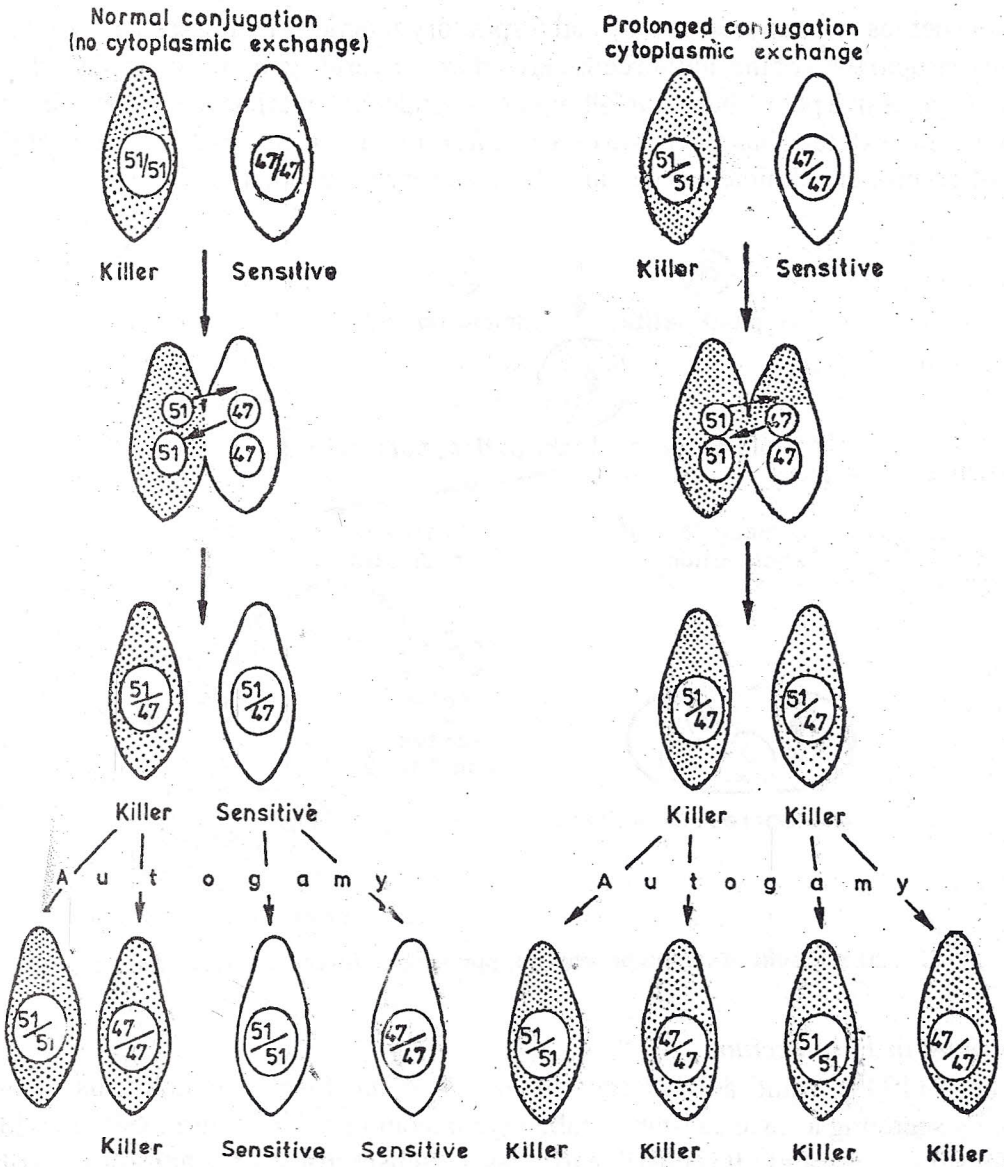


FIG. 20.6 Progeny produced from crosses between killer (No. 51) and sensitive (No. 47) strains of *aecium* with (right) and without (left) prolonged conjugation and cytoplasmic exchange.

character. The extra- chromosomal particle responsible for the killer character has been called *'kappa'* by Sonneborn. Although kappa particle is a cytoplasmic particle its multiplication and existence is dependent on at least one nuclear gene (K). Kappa particles can be maintained in the genotype KK or Kk. Homozygous recessive (kk) Individuals are always sensitive. Extra-chromosomal nature of kappa particles was confirmed by Preer (1950) who observed as many as 1600 particles in each paramecium. Each kappa particle has a diameter of about 0.2 – 0.4μ, almost equal to the size of a large virus and contains its own DNA.

20.3 Chemical basis of cytoplasmic Inheritance

Cytoplasmic inheritance is due to free or organellar DNA. Free DNA is found in the cytoplasm of bacteria. Organelles like chloroplasts and mitochondria of all eukaryotes contain their own DNA. Similarly sigma particles of *Drosophila*, kappa, Lamda, mu and pi particles of *Paramecium* also contain their own DNA that self replicates.

All of the systems detected on the basis of extra chromosomal transmission interact with chromosomal genes or their products. In several cases they are concerned with expression and integration of gene action, in turn with cellular differentiation. Hence, Nanney (1958) suggested that they be called *'epigenetic systems'*. It is clearly understood that transmission of an extra chromosomal hereditary property is independent of the transmission of chromosomes. Further, multiplication of the extra chromosomal property is not synchronized with the multiplication of the chromosomal genetic material. Certain particles endowed with genetic continuity have been called as episomes (Jacob and Wollman 1961) and exist as in integrated or an autonomous state. In an *integrated state* these particles are closely associated with chromosome and multiply in synchrony with chromosomes. The autonomous particles multiply independently.

20.4 Multiple factor OR Polygenic inheritance

Mendel proposed his laws of inheritance based on studies with qualitative characters. In his classical works the parents for crossing had contrasting characters and to his luck each character was governed by one gene. In F_2 generation the segregation was in discrete classes for Eg., red and white or tall and dwarf. There was no doubt as to whether one of his plants was tall or dwarf or its flowers red or white, since the character show distinct classes. Such inheritance is known as qualitative inheritance and the characters are called qualitative characters. They are little affected by the environment. There is another predominant class of characters where individuals show a continuous range of values extending from one extreme to another. Each individual has a unique value which is measured in terms of specific metric like size, weight length etc. to give them name of *Quatitative characters.*

The quantitative traits (eg. yield, maturity, grain size, protein content) on the other hand did not exhibit segregation into discrete classes. The F_2 segregants can not be fitted into any discrete ratios say 3:1; 9:3:3:1 or any modifications thereof. These segregants would show a continuous range of values from low to high showing continuous distribution. The inheritance of quantitative characters was a matter of great controversy for a considerable time. The group led by Francis Galton (1889) considered that the Mendel's laws of inheritance could not explain the continuous variation and speculated blending inheritance for quantitative characters. The controversy was resolved when Yule

(1906) suggested that many genes with small, similar effects could produce continuous variation and many genes effect the same character. Thus quantitative characters like yield of crops, intelligence in man, milk and meat yields in animals etc. are controlled by many genes. The inheritance of quantitative characters is called quantitative inheritance. Since, the character is governed by many genes; it is also known as multi-factor inheritance or polygenic inheritance. The branch of genetics that deals with the study of inheritance of quantitative characters is termed as *Quantitative* or *Biometrical genetics.*

Nilsson – Ehle (1908) presented experimental at evidence for quantitative inheritance by proposing multiple-factor hypothesis, that some characters are governed by several genes with small and cumulative effect. Individual effect of a gene can not be measured or determined. It was also in 1909 Johannsen suggested that heritable and non-heritable (environmental) variations are jointly responsible for continuous variation. The range of such variability can be divided into classes with frequency of segregants in each and distribution is characterized by certain statistics like means, variances and covariances. Fisher (1918) laid the foundation of biometrical genetics for interpreting the above statistics in Mendelian sense. In general one gene with two alleles at a locus give rise two distinct classes and with two genes three distinct classes. Hence, n genes would give n+1 classes where expected number of individuals in each class would be given by the binomial expansion of (˜ + ∫)^n. A few common examples and typical characteristics of polygenic (multiple factor) inheritance are described in this chapter.

Features of Polygenic inheritance:

i) When a character is governed by many genes called polygenes each with small effect, individual effect of a gene can not be measured.

ii) The segregants in F_2 can't be fitted into discrete classes. The segregation is of a continuous type.

iii) Polygenes are more sensitive to environment and thus quantitative characters and their inheritance shows more variation.

iv) In polygenic system F_2 variability is defined in terms of mean, standard deviation and variance.

v) Transgressive segregants namely phenotypes which transgress the maximal and minimal character expressions of the two original parents, appear in F_2 or later generations.

20.5 Polygenic inheritance : Some Examples

A. Seed color in wheat: Nilsson-Ehle (1908) studied the inheritance of seed color in wheat, by crossing red with white. The F_2 generation from various crosses had red and white seeds in ratio of 3:1; 15:1; and 63:1. These results revealed that kernel color in these crosses was governed by one, two or three genes respectively. For further analysis he considered the cross giving 15 red:1 white kernels. A closer study of kernel color revealed that the seeds classified as red differed in the intensity of color. The red seeds could be grouped into four distinct classes viz., dark red, medium-dark red, medium red and light red. The four classes were in the ratio of 1:4: 6:4. Thus the 15:1 ratio was actually 1:4:6:4:1. It can be seen from figure 20.7 that this ratio is obtained if it is assumed that the kernel color is governed by two genes with similar small and additive effects. The dominant alleles of

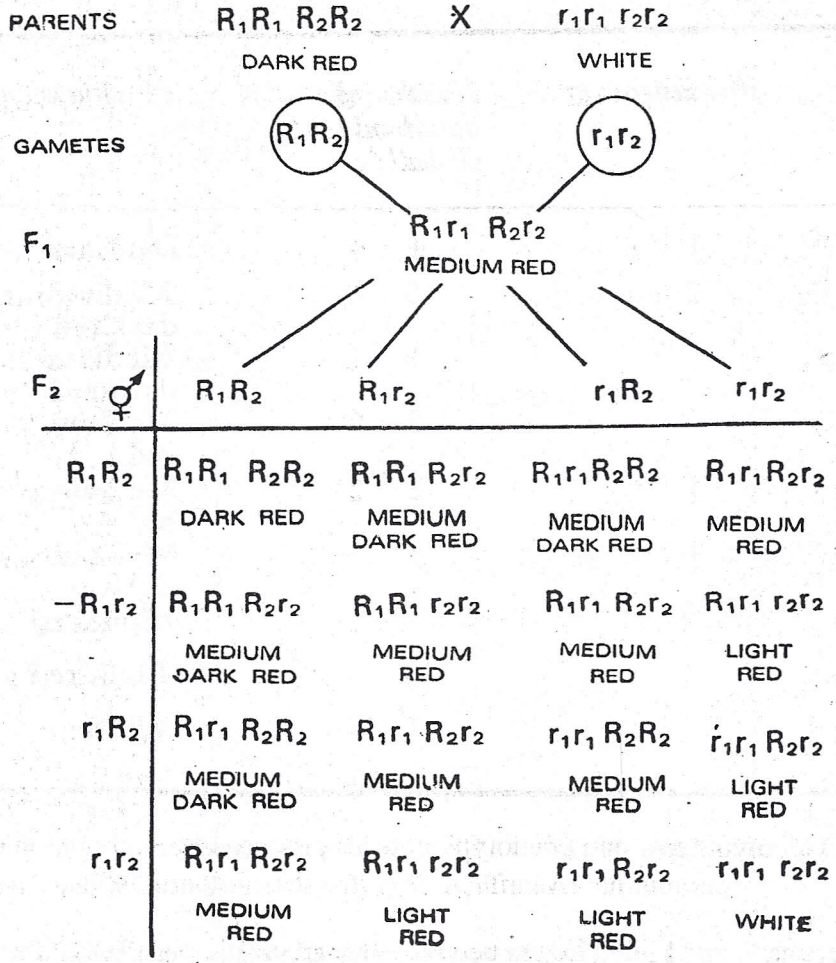


FIG. 20.7 Inheritance of seed colour in wheat.

the two genes produce red color white the recessive alleles produce white color. The intensity of the color depends on the number of dominant alleles present (table 20.1). Thus genotypes $R_1R_1\ r_2r_2$, $r_1r_2R_2R_2$ and $R_1r_1R_2r_2$ will produce the same intensity of color as they have uniformly contained two dominant alleles each. In still other crosses where F_2 progeny produced 63:1 he suggested that three pairs of genes (R_1R_1, R_2R_2, R_3R_3) control graze color. The different shades of red ness and produced depending upon the number of dominant alleles for which parental lines differ. Thus Nilsson Ehle was able to show that certain characters are governed by genes that have small and cumulative effect. This in essence is the multiple factor hypothesis or polygenic inheritance.

B. Corolla length in Nicotiana longiflora: East (1916) presented conclusive evidence that quantitative characters are governed by many genes with small and cumulative effect. He reported the results of classical experiment on the genetic control of corolla length in *Nicotiana longiflora.* The two parents had two contrasting corolla lengths i.e. 40 and 93 mm and the F_1 was intermediate with 63 mm long corolla. The F_2 progeny showed a continuous variation for corolla length, which could not be grouped into separate classes. To analyze his data he used statistical parameters such as frequency distribution, mean and coefficient of variability (CV). The parental lines had significantly different

Genotype	*Frequency*	*Number of dominant alleles*	*Phenotype*	*Frequency*
$R_1R_1\ R_2R_2$	1	4	Dark red	1
$R_1r_1\ R_2R_2$	2	3	Medium-dark red	4
$R_1R_1\ R_2r_2$		3	Medium-dark red	
$R_1r_1\ R_2r_2$		2	Medium red	6
$R_1R_1\ r_2r_2$	1	2	Medium red	
$r_1r_1\ R_2R_2$	1	2	Medium red	
$R_1r_1\ r_2r_2$	2	1	Light red	4
$r_1r_1\ R_2r_2$	2	1	Light red	
$r_1r_1\ r_2r_2$	1	0	White	1

Table : 20.1. Genotypic and phenotypic frequencies produced by segregation of two genes with cumulative effect, R_1r_2, R_2r_2, fro seed colour in wheat.

flower size where as F_1 and F_2 lied in between the parental values (fig. 20.8). The essential features of his study can be summarized below.

- Although two parents were in breeds and almost homozygous, there was variation within the parents for corolla length. This variation therefore assumed to be arising from environmental effects.
- The F_1 mean was intermediate between parents. This would be expected if multiple factor with cumulative effects govern this trait.
- Although all F_1 plants contain the same genotype they exhibited variation, which could be produced by the environment.
- East postulated that the greater variability observed in F_2 result from segregation and recombination of Mendelian genes. Based on the above observations, which corresponded closely to the expectations based on Mendelian inheritance, East proposed that at least 5 genes are involved in the control of corolla size in *Nicotiana.* Although with five genes there would be only 11 classes, the identify of each class would disappear due to the effects of *environment*, as it is evident from the data from parents and F_1 confirmatory evidence for Mendelian inheritance of corolla length obtained form F_3 F_4 and F_5 generations. Based on Mendelian principles it may be predicted that: F_2 plants with marked by different corolla lengths should produce F_3 progenies that differ from each other; Variability in some F_3 progenies may be

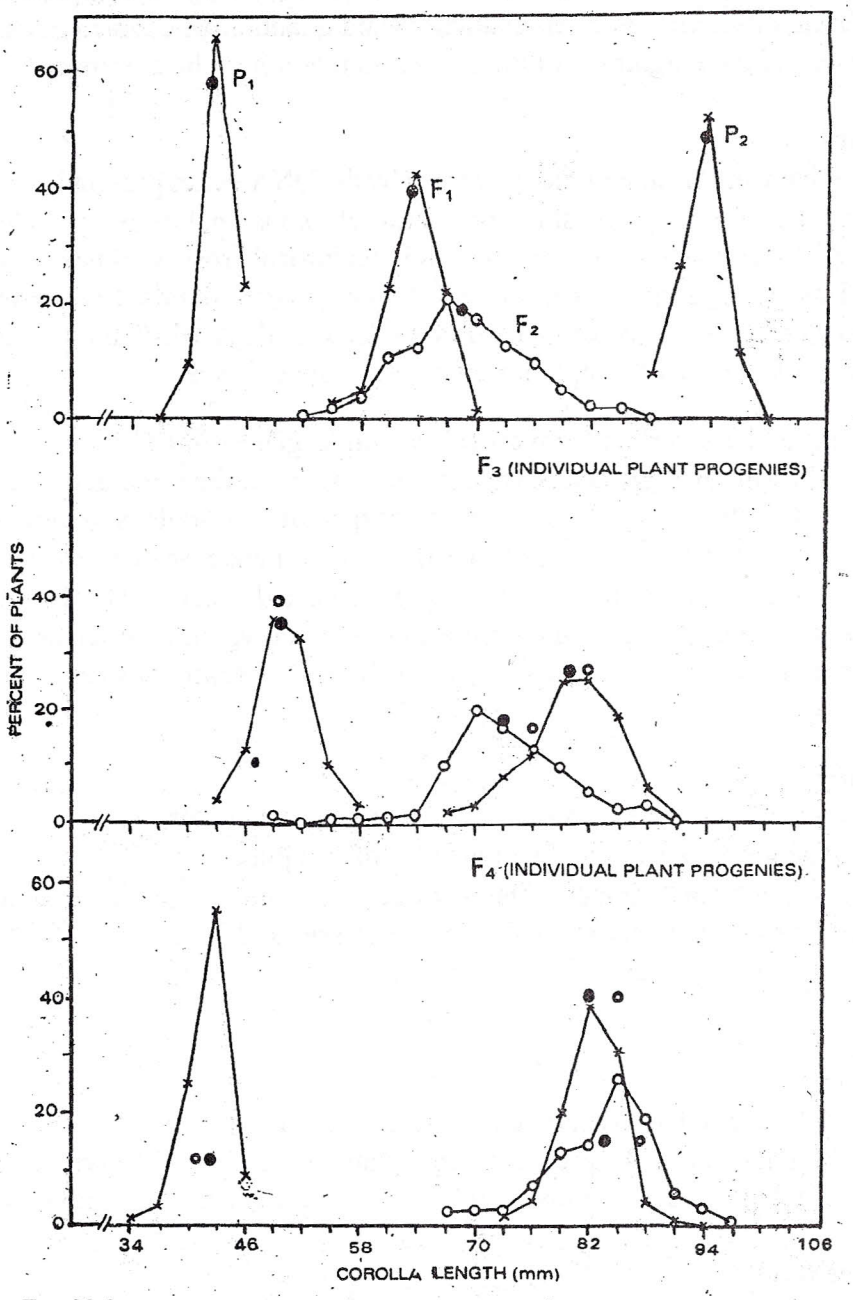


FIG. 20.8 Frequency distribution of the two parents, F_1, F_2 and F_3 and F_4 progenies for corolla length in *Nicotiana longiflora*. The closed circles represent the mean of the various populations. In case of F_3 and F_4 progenies, the open circlesa longside the closed ones denote the corolla length of the parent plant of the previous generation from which the progeny was derived.

comparable to that in parents and one are comparable to $F\leq_1$. In F_3 and succeeding generations the variability of a progeny would be equal or less than that of the progeny from which it arose. But never mere, since selfing causes a decrease in heterozygosity . The data from F_3, F_4 and F_5 generations agreed closely with the above four predictions and the observations of East confirmed that corolla length in *Nicotiana* is governed by a minimum of 5 or more genes.

This basic difference between qualitative and quantitative characters lies only in the number of genes and the magnitude of their effect in relation to the environment.

20.6 Summary

The mechanism of inheritance governed by the DNA/genes present in cytoplasmic organelles/ particles is described as cytoplasmic or extra- chromosomal or extra-nuclear inheritance. This pattern of inheritance produces different results in reciprocal crosses. Since, cytoplasm of the zygote is contributed by the egg rather than pollen, the characters governed by cytoplasmic genes show maternal inheritance. Plastid color in *Mirabilis jalapa*, male sterility in maize and kappa particle in paramecium are some of the example for cytoplasmic inheritance.

Quanlitative characters are governed by single genes and they are not influenced by the environment. Quantitative characters are governed by more than one gene, each having small and similar effect on the character. The inheritance of quantitative traits is governed by poly genes and known as polygenic inheritance or multiple factor inheritance or quantitative inheritance. Yield, maturity, grain size and protein content are polygenic characters. The F_2 progeny of a cross of quantitative trait did not segregate into descrete classes. Polygenes are sensitive to environment and their inheritance shows more variation, which is defined in terms of mean, standard deviation and variance.

20.7 Key terms

Cytoplasmic inheritance: Transmission of characters through cytoplasm
Plasmagenes: The genes present in the organelles of cytoplasm.
Polygenes: Genes with small and cumulative effect that control a quantitative trait.
Qualitative character: Character showing distinct classes and little or no effect of environment.
Quantitative character: Character showing continuous variation and considerable effect of environment.

20.8 Questions:

A. Write a detailed account on extra chromosomal inheritance
B. Explain the nature of qualitative and quantitative traits and give some examples.
C. Give a detailed account on the inheritance mechanism of quantitative traits.

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Author : Dr. Z.Vishnuvardhan

LESSON - 21

MUTATIONS

Objective: A study of the mutations and their role in crop improvement.

21.1 Introduction
21.2 Classification of mutations
21.3 Certain general characteristics of mutations
  21.3.1 Mutagens and their classification
  21.3.2 Physical mutagens
  21.3.3 Chemical mutagens
21.4 How does a mutation act?
21.5 Mechanism of action of mutagens
21.6 Types of mutagen induced changes in DNA
21.7 Genetic suppression
21.8 Selective methods for mutants
21.9 Mutations in crop improvement & mutation breeding
21.10 Summary
21.11 Key terms
21.12 Questions
21.13 Reference Books

21.1 Introduction:

The term mutation (mutare in Latin means 'to change') was introduced by Hugo de Vries in 1900 for describing new types in evening primerose (*Oenothera*) plant. But mutations were known to occur in animals and plants much before this time. *"Mutations are sudden heritable changes in the genotype of an organism"*. Clearly a mutation may be a result of change in gene, a change in chromosome(s) or a change in a plasmagene (genes present in cytoplasm).

Hugo de Vries (1901) gave the idea of producing mutations artificially for use in plant breeding. Mutagenic action of X-rays was discovered by Muller in 1927 on *Drosophila* and in 1927 by Stadler in barley and maize. Muller was awarded Nobel Prize in 1946 in recognition for his work. In 1946, Auerbach and Robson showed that nitrogen mustards produced mutations in *Drosophila.* Subsequently, a number of chemicals with mutagenic action were described. Mutation breeding attracted considerable attention during 1950s and 1960s and several countries took up research projects in mutation breeding. As knowledge accumulated on mutations, it became clear that although mutations have a definite role to play in crop improvement, it is not the magic wand which would create what you want and when you wanted.

21.2 Classification of mutations: Mutations have variously been classified. The mutated individual is called a *mutant.* A chemical or physical agent that induces a mutation is known as *mutagen.* The induction of mutations with the aid of mutagens is called *mutagenesis.* Mutations are classified based on their origin, type of tissue involved, nature of effects on the phenotype and gross changes occurring at chromosome level or in the gene or within the genes.

Spontaneous mutations: Mutations that occur in natural populations without any treatment by man, are known as spontaneous mutations. The rate of such mutations is generally very low and is one in 10 lakhs ($1x10^{-6}$). But different genes have different mutation rates. R locus in maize mutates at a frequency of 4.92×10^{-4}, Su locus at 2.4×10^{-6} and Wx appears to be very stable. Mutations occur much more frequently in certain regions of the gene than in others. The favoured regions are called 'hot spots'.

Induced mutations: These mutations are artificially induced by treatment with certain physical or chemical mutagens.

Macro and Micro mutations: Large mutations are described as macro mutations and mutations with small effects as micro mutations.

Somatic and germinal mutations: Somatic mutation refers to mutants that appear in vegetative part in M_1 generation. Mutations present in germinal cells are known as germinal mutations, and such mutations in germ cells are passed on to the offspring.

Chromosomal Mutations: These include chromosome number change (Aneuploidy and polyploidy), structural alteration of chromosomes (deletion, inversion, duplication and translocation) and mutations that effect centromeric mis-division and chromosome breaks. Some mutations produced by changes in chromosome structure or even in number are termed as *chromosomal mutations.*

Gene Mutations: Mutations produced by changes in *base sequence* of a genes are known as gene mutations (point mutation). When the mutant character shows cytoplasmic or extra nuclear heritance, it is termed as *cytoplasmic mutation.* In some cases, an altered base may not effect codon specificity as a result the codon continues to code for the same amino acid. This type of changes constitute "*silent mutations*". A mutation in which there is deletion or insertion of one or a few mucleotides is called a *frameshift mutation.* The name is derived from the fact that there is a shift in the reading frame backword or farward by one or two nucleotides. Addition or deletion of one or two bases results in a new sequence of codons which may code for entirely different amino acids. A mutation which results in the replacement of one amino acid in a polypeptide chain by another, is known as *missense mutation.* Of the 64 codons, 61 code for amino acids, while three are termination codons, which do no specify any amino acid. The three termination codons are UAA, UAG and UGA. Any mutation resulting in the alteration of a codon specifying an amino acid to a termination codon is called a *nonsense mutation.*

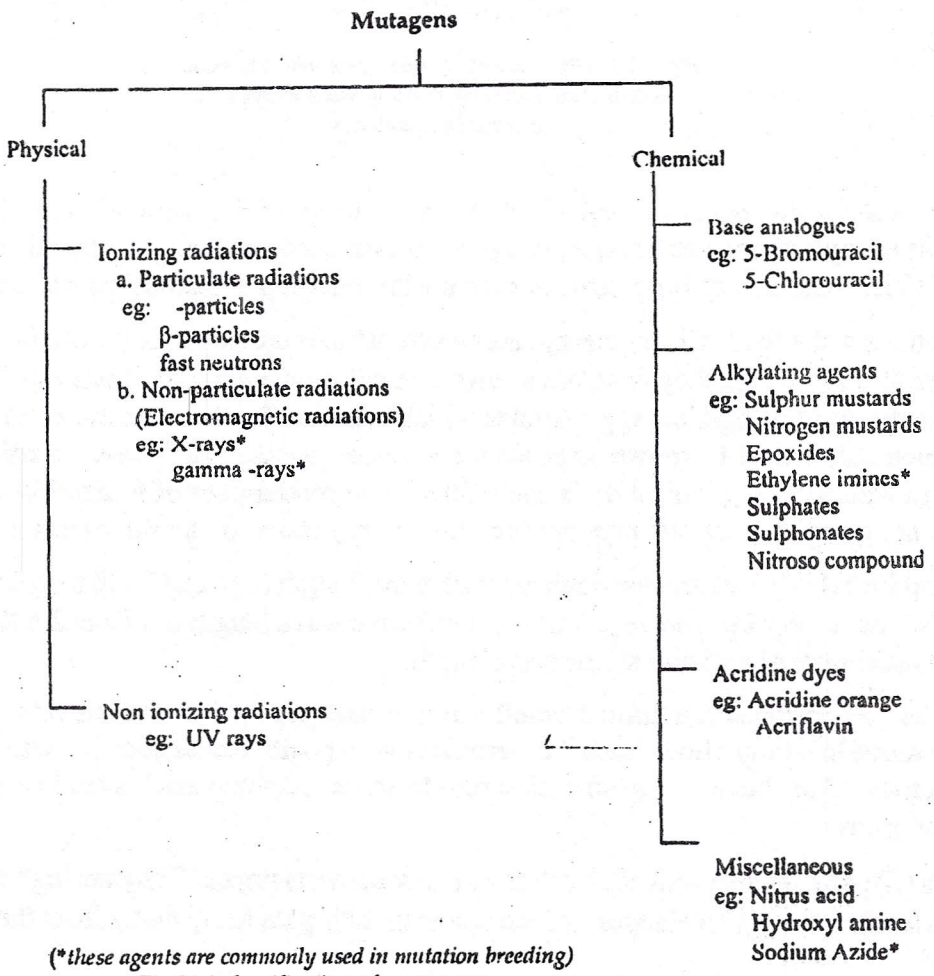
Based on the mutations' effect on the viability of the organism, they are classified as lethal, sub-lethal, sub-vital and vital mutations. *Lethal mutations* result in the death of the cells or organism in which they occur. *Sub-lethal* and *sub-vital mutations* reduce viability but do not kill all the individuals carrying them. *Sub-lethal mutations* kill more than 50 per cent of individuals, while *sub-vitals* kill much less than 50%. *Vital mutations* do not reduce viability of individuals carrying them. Obviously crop improvement can utilize such mutations.

21.3 Certain general characteristics of mutations:

1. *Mutations* are generally recessive, but dominant mutations also occur
2. *Mutations* are generally harmfu[1] to the organism but a small proportion (0.1%) of them are beneficial
3. *Mutations* are random i.e. they may occur in any gene. Some genes show higher mutation rates than the others.
4. *Mutations* are recurrent, that is the same mutation may occur again and again.
5. *Induced mutations* commonly show pleiotropy, often due to mutations in closely linked genes.

21.3.1 Mutagens and their classification:

A variety of agents induce mutations. Such agents are called mutagens. The mutagtens may be different kinds of radiations *(Physical mutagens)* or certain chemicals *(Chemical mutagens)*. The different kinds of these mutagens are given in figure 21.1



*(*these agents are commonly used in mutation breeding)*

Fig 21.1.classification of mutagens

21.3.2 ***Physical mutagen:*** The ultimate source of all physical mutagens is the atom. An atom consists of a nucleus and negatively charged electrons revolving in an orbit. The atomic nucleus consists of positively charged protons and neutral neutrons (Fig.21.02). The entire weight of the atom lies in the nucleus which in a stable atom consists of an equal number of protons and neutrons. The atom contains a high amount of bound energy. An unstable atom tends to give off energy or particles, there by achieving stability. This is known as nuclear decay. Such unstable atoms are known as *radio*

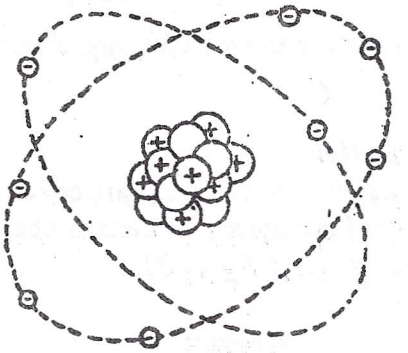


Fig. 21.2 Structure of oxygen atom which consists of 8 protons and 8 neutrons in the nucleus and 8 revolving electrons

isotopes or *radio active isotopes*, and are defined as atoms of the same element having different weights. The energetic particles or electromagnetic waves accompanying nuclear decay are known as '*radiation*'. The treatment of organisms or plants with radiation is called *irradiation.*

Radiations in the form of high energy short waves which cause electric and magnetic disturbances in the matter through which they pass, are known as electromagnetic radiations (eg. X-rays, ?- rays). Radiation in the form of high energy particles which can transfer their kinetic energy to any matter through which they travel is known as *particulate or corpuscular radiation* Particulate and non-particulate radiations cause ionization in the matter. The mechanism of ionization differs from one radiation to the other. Ultraviolet rays produce low energy radiation that does not cause ionization.

Ultraviolet(UV) rays are non-ionising with wave length between 1000 to 4000 A^0 (100 to 400 nm). UV rays have very little energy. The most effective wave length of UV is 2,600 A^0, since DNA bases show maximum absorption at this wavelength.

Alpha (α) particles are emited chiefly by the isotopes of heavy elements. Since they are heavy, they move in straight line. Each " α particle is with positive charge as it contains two neutrons and two protons. They have strong attraction for electrons. Alpha particles are less penetrating than neutrons and x-rays.

Beta (β) particles are produced by decay of radicactive isotopes. They are high energy electrons. x-rays transfer their energy to electrons of the atoms in their path to fly away from their arbit, causing '*ionisation*'.

Neutrons (n) of wide range are obtained from the atomic reactors, where U^{235} is subjected to nuclear fission. They penetrate deep in the tissue as they are neutral and are not stopped by negative and positive charges of tissue.

X-rays were first discovered by Rontgen in 1895. They have a wave length of 0.001-10 A^0. Gamma-rays have shorter waves than X-rays and therefore have more energy and more penetraition. Both rays are non-particulate electromagnetic radiations, consisting of photons i.e. small pockets of energy.

Dosage of Mutagen and Dosimetry:

An optimum dose is one which produces the maximum frequency of mutations and causes minimum killing. Lethal Dose 50 (LD_{50}) is that dose of mutagen which would kill 50 percent of treated individuals. Many researchers feel that a dose close to LD_{50} should be the optimum dose. The dose of X-rays and g-rays is measured in roentgen (r) units. The unit of measuring ionization is REP (Radiation Equivalent physical).

21.3.3 Chemical mutagens

Auerbach (1941) reported that some chemicals have similar mutagenic property as of radiations. Auerbach (1946) classified chemical mutagens into the following four categories.

a. *Base Analogues:*
The chemicals in this group are analogues of four bases of DNA molecule. For example 5-Bromouracil and 5-Bromo-deoxyuridine are analogues of thymine. They pair with Adenine. Similarly 2-Amino purine an analogue of Adenine pairs with Thymine.

b. *Antibiotics:*
A number of antibiotics such as Mitomycin-C and streptonigrin possess chromosome breaking properties.

c. *Alkylating agents:*
This is the most important group of chemical mutagens. They cause alkylation i.e. an alkyl radical replaces hydrogen atoms in a molecule. Methyl Methane Sulphonate (MMS), Ethyl Methane Sulphanoate (EMS), Ethylene amine, Sulphur Mustard gas and epoxides are some alkylating agents.

d. *Miscellaneous:*
Hydroxylamine, Nitrous acid, dyes like acridines and alkaloids constitute this group. These chemicals have mutagenic properties.

21.4 How does a mutation act?:

Genes act by controlling the rate of production of specific proteins (enzymes). The scheme of protein synthesis is as follows. a)The DNA (gene) produces a complementary mRNA strand which has codons consisting of nucleotide triplets. b).The tRNA molecules each forming a complex with a specific amino acid, have three free nucleotides which form *anticodon.* c).The alignment of tRNA molecules on mRNA depends upon complementary *codon-anticodon pairing.* d).Thus the sequence of amino acid molecules in an enzyme depends upon the nucleotide sequence of mRNA. This inturn

depends upon the nucleotide sequence in DNA. It is clear that any change in the sequence of nucleotides of DNA, will result in a corresponding change in the nucleotide sequency of mRNA. This may result in alignment of different tRNA molecules on mRNA. Thus the amino acid sequence, and hence the structure and properties of the enzyme formed will be changed. This may effect the traits controlled by the enzyme.

A change in only one amino acid can have a drastic effect on the phenotype: For example hemoglobin, which is found in RBCs is a protein molecule consisting of four chains, two alpha chains and two beta chains. These chains consist of amino acids arranged in a definite sequence. Normal red blood cells are disc shaped,changes in hemoglobin structure result in certain types of anaemia called sickle cell anaemia and haemoglobin D anaemia. Normal hemoglobin has the amino acid *glutomic* acid in the *sixth position*. In sickle cell hemoglobin it is replaced by *lysine*. In the other type of hemoglobin *valine* is present in the sixth position. As a result of mutation one base of a codon may be substituted by another base. The changed codon may then code for another amino acid. Table 21.2 shows some codon changes and amino acid replacements in hemoglobins.

Table 21.2 Some codon changes and amino acid replacements in haemoglobins.

Amino acid in normal haemoglobin		*Base substitution*	*Amino acid in mutant haemoglobin*	
Lysine	(AAA)	A → G	Glutamic acid	(GAA)
Glycine	(GGU)	G → A	Aspartic acid	(GAU)
Asparagine	(AAU)	U → A	Lysine	(AAA)
Glutamic acid	(GAA)	A → U	Valine	(GUA)

Nonsense mutations

21.5 Mechanism of action of mutagens:

Physical mutagens:

Radiations: The effects of radiations are direct aswell as indirect. The energy content of radiations depends upon their wavelength. In general, the shorter the wave length, the greater the energy value of radiation. In the case of direct effect, energy is transferred to a molecule directly by the radiation. Indirect effect is mediated by free radicals. Hence, the effects of radiation may be grouped into A. *Formation of ion pairs* and B. *Formation of free radicals.*

A. Formation of ion pairs: An electron is ejected from an atom to produce a *positively charged ion.* The ejected electron is captured by another atom to produce *negatively charged ion.* Thus a pair of ions is produced shown as in figure 21.3.

B. Free radicals formation: Free radicals have a very short life of 10 seconds. Free radicals are electrically neutral molecules with an unpaired electron in the outer orbit. Some times when an electron pair may be moved from an inner to an outer orbital shell. This brings about excitation of

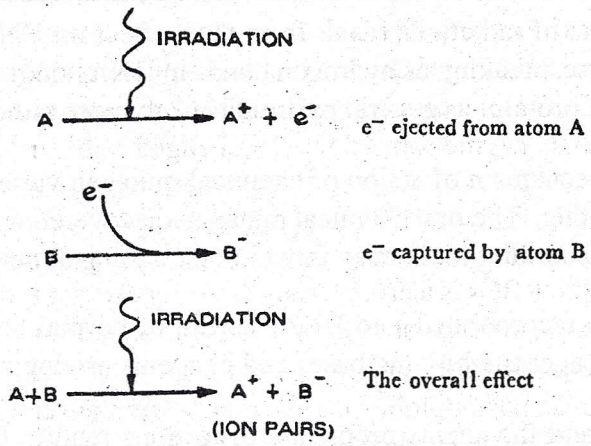


Fig. 20.3 Production of ion-pairs by high energy radiations.

the atom. In this excited state atom is highly reactive and is called a free radical. Production of free radicals from water is shown in (figure 21.4). An electron is ejected from the oxygen atom of H_2O, which is captured by one H atom of another water molecule producing *ion-pair*. In presence of two other water molecules, the ion-pairs dissociate and produce two free radicals. Free radicals produce H_2O_2 (figure 21.5) which is highly reactive and thought to be responsible for a good proportion of biological effects of radiation.

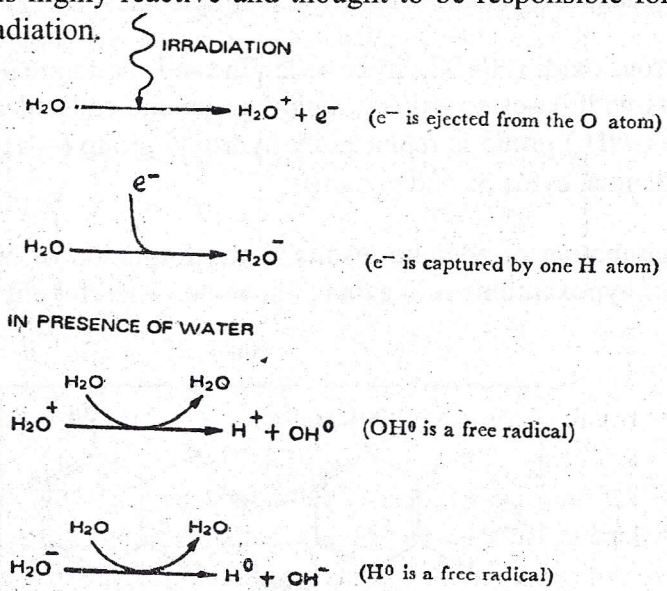


Fig. 21.4 Production of free radicals by high energy radiations, using water molecules as an example.

Free radicals are removed as follows,

$$H^0 + OH^0 \longrightarrow H_2O$$
$$H^0 + H^0 \longrightarrow H_2$$
$$OH^0 + OH^0 \longrightarrow H_2O_2 \quad \text{(The last reaction produces } H_2O_2\text{)}$$

Presence of O_2 increases the yield of H_2O_2,

$$H^0 + O_2 \longrightarrow HO_2^0$$
$$HO_2^0 + HO_2^0 \longrightarrow H_2O_2 + O_2$$

H_2O_2 is destroyed as follows,

$$H_2O_2 + H^0 \longrightarrow H_2O + OH^0$$

Fig. 21.5 Production of H_2O_2 from free radicals

The mutagenic effects of radiations result from their effect on DNA. These effects include change in a base, loss of a base, breaking of hydrogen bonds in DNA, single and double strand breaks in DNA and cross-linking of protein. In general pyrimidines are more radiosensitive.

Chemical mutagens: The mechanism of action of chemical mutagen varies to a greater extent from one group of chemical to another. The first chemical mutagen discovered was *mustard gas*. Chemical mutagens can be classified according to the way in which they bring about mutations:

a)base analogues; which are incorporated into DNA instead of normal bases, b) agents modifying purines and pyrimidines and agents labilizing bases and c) agents producing distortions in DNA.

The base analogues and the agents producing distortions require DNA replication for their action, while the other category of agents can modify even non-replicating DNA.

A chemical substance resembling a base is called *a base analogue.* It may be incorporated into newly synthesized DNA instead of normal base. For example the pyrimidine analogue *5-bromo uracil (5-BU)* is structurally very much similar to thymine. If bacteriophages are grown in the presence of 5-BU, they incorporate the substance as if it were thymine. 2-Aminopurine can be read as either adenine or guanine and pairs with thynine or cytosine.

Nitrous oxide (HNO2), hydroxylamine and alkylating agents modify the DNA bases. Nitrons oxide reacts with bases containing amino group and cause deamination (removal of amino group). The amino ($-NH_2$) group is replaced by hydroxyl group (-OH). The order of frequency of Nitrous Oxide is adenine, cytosine and guanine.

Deamination of adenine results in the formation of hypoxanthine (fig.21.6). The paining behaviour of hypoxanthine is like that of guanine. Therefore hypoxanthine pairs with cytosine, rather

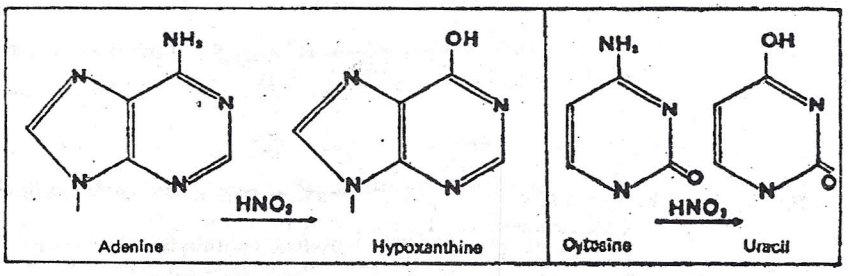


Fig. 21.6 Deamination by nitrous acid.

than with thymine. Thus A-T paining is replaced by G-C pairing. The changes in structure and pairing behaviour of DNA bases as a result of deamination is shown in table 21.3.

Hydroxyl amine (NH_2OH) is very specific in action. It reacts mainly with *cytosine* and *guanine.* It also deaminates the bases. Deaminated cytosine pairs with adenine instead of guanine.

Normal bases of DNA.	*Normal pairing*	*Bases formed by deamination*	*New pairing*
Adenine	A–T	Hypoxanthine	G–C
Cytosine	C–G	Uracil	U–A
Guanine	G–C	Xanthine	X–C

Table : 21.3. Changes in structure and pairing behaviour of DNA bases as a result or deamination by nitrous oxide.

Alkylating agents are widely used as mutagens. They cause alkylation at the N-7 position of guanine residue or at the N-3 position of adenine residiue. Alkylation increases the probability of ionization and introduces pairing error. The base-sugar linkage undergoes hydrolysis and releases the base from the DNA molecule. This creates gap in one chain. During replication the chain without gaps will produce normal DNA. In the chain with gaps, however, any base (A,T,G or C) may be inserted across the gap. This may be the same base or some other base. In the next replication the gap is filled by a base which is complementary to the inserted base. Insertion of the base other than the original one may result in a *transition* or *transversion. Figure* 21.7 explains the effect of alkylating agent EMS on DNA.

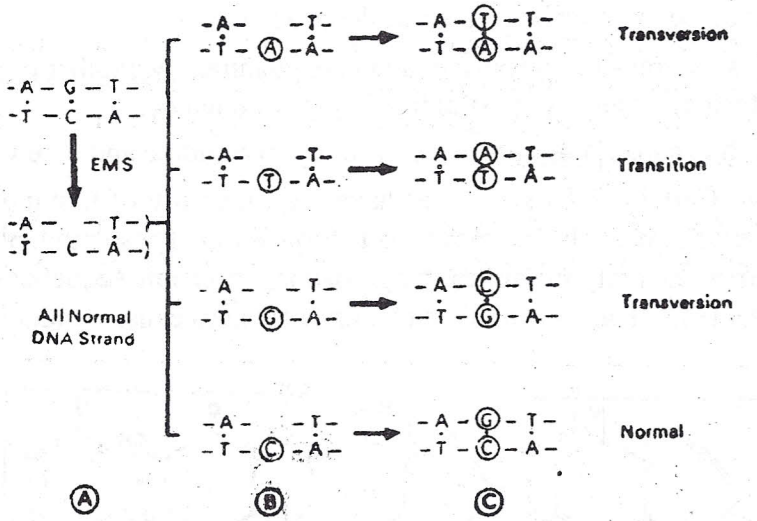


Fig. 21.7 Effect of the alkylating agent ethyl methane sulphonate (EMS) on DNA.
A. EMS removes G and creates a gap in DNA.
B. 1st replication. A/T/G/C inserted across the gap.
C. 2nd replication. Complementary base fills the gap.

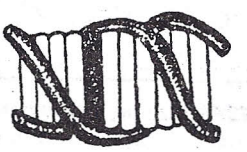


Fig. 21.8 Insertion of acridine dye molecule (black) between bases of DNA.

Certain fluorescent acridine dyes (eg. Proflavine and acridine orange) cause distortions in DNA. The acridines are planer (flat) molecules, like purine bases and can be intercalated between the bases of DNA (fig. 21.8) causing distortion. It can result in deletion or insertion of bases during recombination. Intercalation of the acridine molecule between two bases of the template strand results in the lenghthening of the DNA molecule. During replication a base (X^1) is inserted at random will pair with the opposite the acridine molecule in the new chain. In the next replication a complementary base (X) newly inserted base. Thus the new DNA has an additional base pair. Suppose the acridine molecule is inserted in the new chain during DNA sythesis, it blocks the base in the template and does not permit any base to pair with it. The chain produced is thus deficient in one base, and in the next replication produces DNA with a deficient base pair.

21.6 Types of Mutagen induced changes in DNA that produce mutations

The following types of changes in DNA bring about mutation. i)tautomerism ii)transition iii) transversion iv)transposition v)Inversion vi)Deletion vii)Insertion.

i) ***Tautomerism:*** The ability of a molecule to exist in more than one chemical form is called *tautomerism.* The normal bases of DNA are usually present in *keto* form. As a result of tautomeric rearrangement *they* can be momentarily transformed into the rare *enol* form in which distribution of electrons is slightly different. The tautomeric forms are capable of unusual (forbidden) base pairing (Figure 21.9) like T-G, G-T, C-A and A-C. These result in misreplication of DNA strand and give rise to mutants in some of the progeny.

ii) ***transition:*** It is the transfer one purine (adenine, guanine) by another purine or one pyrimidine (thymine, cytosine) by another pyrimidine in DNA molecule.

iii) ***transversion:*** It is substitution of purine bases by pyrimidine and vice versa.

iv) ***Transposition:*** Certain DNA sequences have the capability of moving from one location to another, in the genome. This is known as *transposition.* These movable elements have been variously named as jumping genes, transposons, Insertion sequences or IS elements and controlling elements. Transpositions of certain sequences cause a mutation.

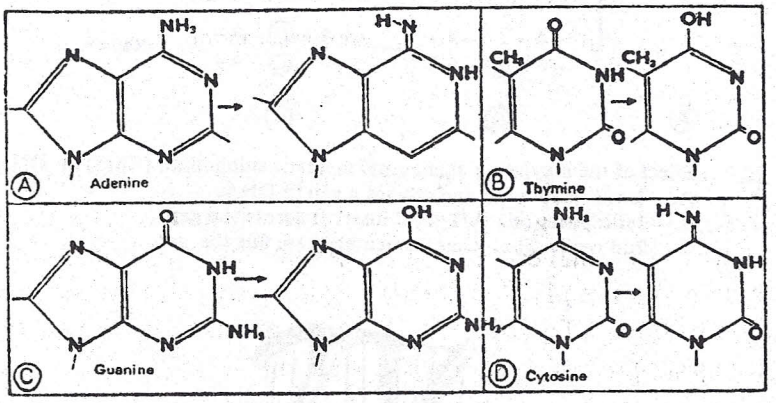


Fig. 21.9 Tautomerism of (A) adenine, (B) thymine, (C) guanine and (D) cytosine. The common state of of each base is shown on the left and the rare state on the right.

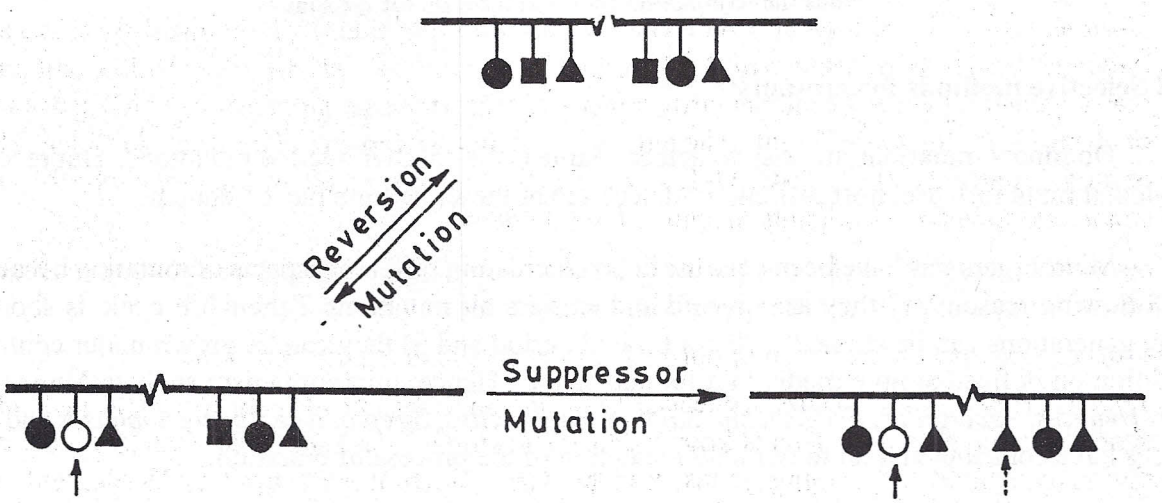
v) ***Inversion:*** If a segment of DNA is removed and reinserted in a reverse direction it results an *inversion.*

vi) **Deletion:** Removal of one or a few bases from a nucleotide chain is called a *deletion.*

vii) ***Insertion:*** Addition of one or a few bases to the nucleotide chain is known as *insertion.* Deletions or insertions result in *'frameshift mutation'* so that there is shift in the reading frame forward or backward by one or two nucleotides.

21.7. Genetic Suppression

The effect of a mutation on the phenotype can be reversed, so that the original wild type phenotype is brought back. This reversal may be due to true reversion (back mutation) or suppression. In a suppression a change at a different site brings about phenotypic correction of the mutation (fig.21.10). Suppressions are of two types: *Intragenic and intergenic.*



[The mutation with a base alteration (↑) leads to an inactive gene product. In true reversion the changed base is altered back to the original. In suppressor mutation, the original mutation remains, but a second base change at a different site (↑) within the gene sequence restores function in the 'pseudorevertant'.]

Fig. 21.10 **The mechanism of suppressor mutation**

In Intragenic suppression, the suppressor mutation is located within the gene sequence. One example is the tryptophan synthetase A gene of *E.coli.* A primary mutation (glycine '! glutamic acid) in this gene resulted in a non-functional enzyme. The effect of this mutation is corrected by a second mutation (tyrosine '! cysteine) taking place ~ 100 base residues away in the same gene. In Intergenic suppression, the deleterious effects of a mutation in a gene are overcome by a mutation in another gene. This is also known as suppressor mutation. (Fig. 21.11)

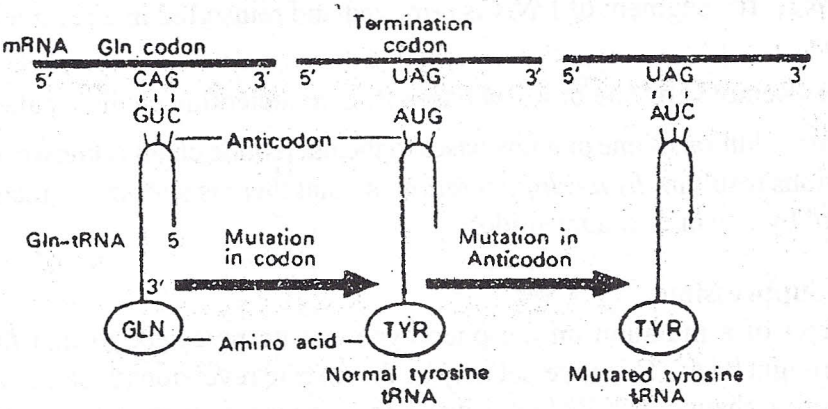


Fig. 21.11 Intergenic suppression. Deleterious effect of mutation of glutamine (Gln) codon (CAG) to termination codon (UAG) is neutralized by mutation of normal tyrosine tRNA anticodon (AUG) to AUC which reads the termination codon as a codon for tyrosine.

21.8 Selective methods for mutants:

Dominant mutations are easy to detect. Same is the case with lethal mutations. But recessive non-lethal mutations are most difficult to detect and at the same time most frequent.

Microorganisms have been very useful in elucidating different aspects of mutation because of the following reasons: 1) they are haploid and express all mutations 2)their life cycle is short and many generations can be studied within a limited period and 3) they can be grown under controlled condition on defined simple media in a limited space. Hence, microorganism such as *Neurospera, Saccaromyces, Aspergillus, Escherichia, Salmonella, Bacillus, Streptomyces, bacteriophases* and other viruses have contributed a lot to our under standing of the process of mutation.

There are a number of selective techniques to isolate rare mutants when they arise in parent organism. Some of them are briefly mentioned below:

i) ***Enrichment:*** It is possible to selectively pick out strains of microorganisms with specific capabilities. Suppose our aim is to isolate a rare bacterial cell present in the culture of a prototroph with the ability to utilize a particular 'normally non-utilizable growth substrate 'X'. For this purpose the bacterial culture is switched to a minimal medium containing X as the only available energy source. Although most of the bacterial cells cannot grow on X, a few rare cells may be able to do so. Consequently only these cells grow and divide slowly, and by repeated sub culturing on medium having 'X', one could enrich the culture with cells that utilize X. Such cells can be conveniently selected by plating method. Replica plating method devised by Lederbergs (1952) is a simple and powerful technique for mutants isolation from bacterial cells.

ii) ***ClB test in Drosophila – to detect X-linked recessive lethals:*** Muller (1928) devised a method to detect X-linked lethal mutations in fruit fly. It makes use of ClB strain which is heterozygous for three genetic markers i)a dominant eye mutation Parr eye gene B; ii)

an inversion involving both genes so that pairing of homologous parts and crossing over between there is suppressed in this region. Such females produce two types of gametes – one with ClB chromosome and another with normal X chromosome. Male flies with ClB chromosome are not viable, because lethal gene is expressed. Thus ClB females with Barr eyes when crossed to irradiate normal males give rise to progeny in which female to male ratio is 2:1 and no Bar-eyed males are recovered in F_1 generation. If a X-linked mutation occurs in the male parent, all the F_1 females will carry it. Bar-eyed F_1 females will carry it. Bar-eyed F_1 females crossed to normal males will give rise to males in which the induced X-linked mutation will be expressed even if it is recessive one. 50 percent of the F_2 males will not be viable because of the presence of the ClB chromosome. If a new mutation is lethal one, no F_2 males will be recovered (fig. 21.12).

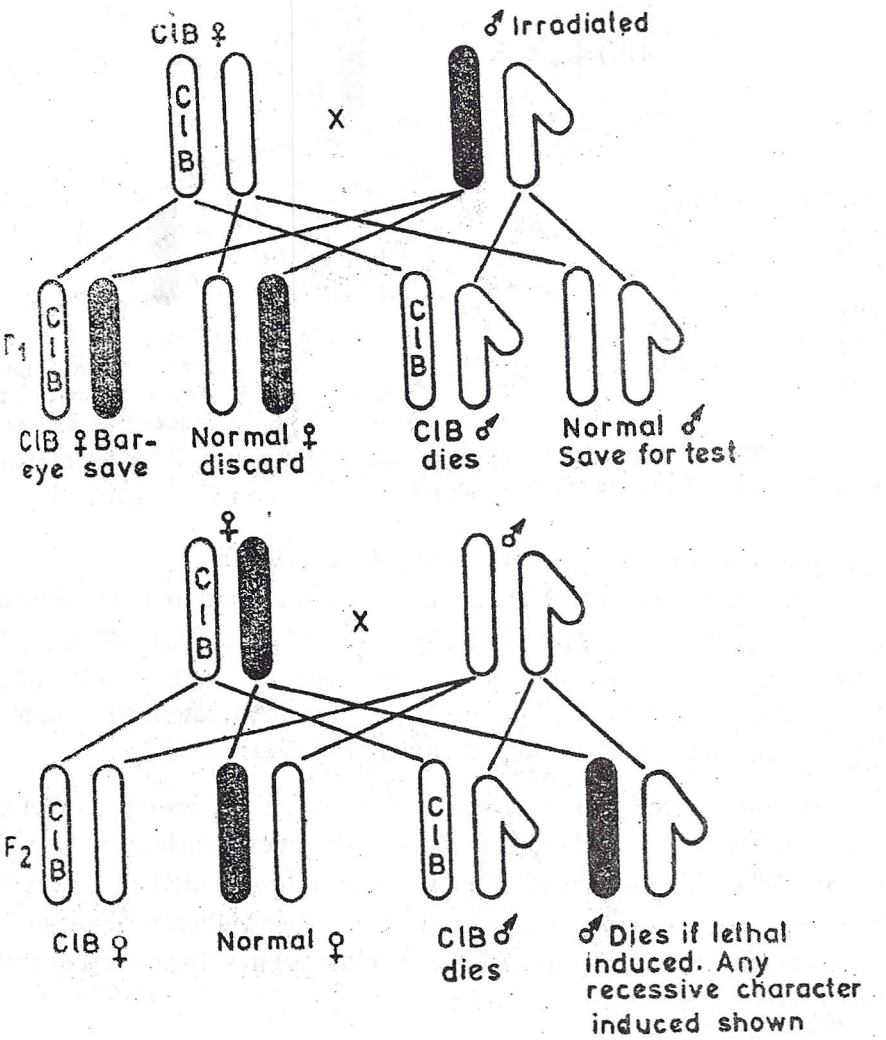


Fig. 21.12 Muller's *ClB* method for detecting mutations in the *X* chromosome of *Droso-*

iii) ***Attached X-method:*** Attached – X females have been very useful in detecting sex-linked recessive mutations in *Drosophila.* In such females there are two X chromosomes which are joined by a single Centro mere and do not separate during gamete formation. Such females have an additional Y chromosome too. A cross between attached – X females and normal males yields four types of off spring, two of which (XXX females and YY males) are in viable or abnormal. The third type is attached X-female (XXY). The fourth type XY male, is the most interesting one because it inherits the Y chromosome from its mother and X chromosome from its father. Thus if a mutation occurs in the X chromosome of the male parent it will be transmitted and expressed in F_1 males (fig. 21.13)). In this method mutation is detected in the first generation itself.

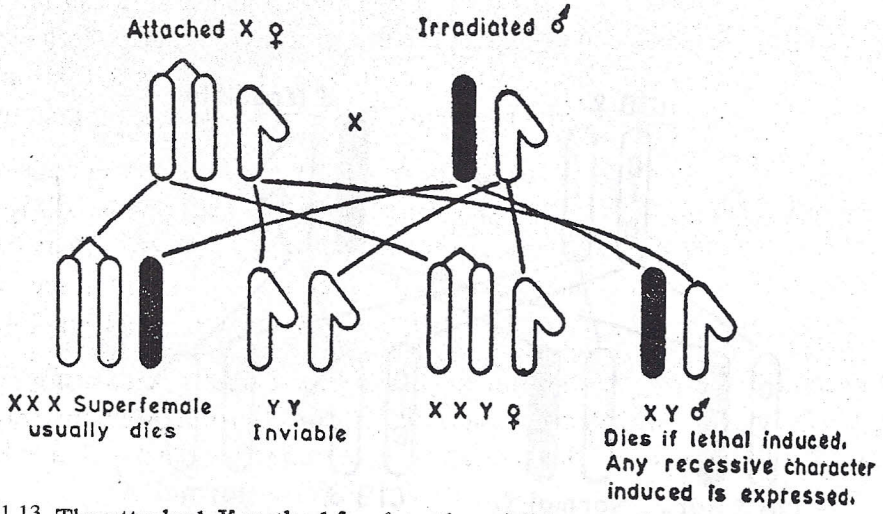


Fig. 21.13 The attached-*X* method for detecting visible as well as lethal mutations in *X* chromosome of *Drosophila melanogaster.*

21.9 Mutations in crop improvement and mutation breeding:

Immediately after the discovery of mutations and their induction by various mutagens, mutation breeding programs were initiated for crop improvement in Sweden, Germany and other countries. The mutation breeding program involves various steps like; a) objective of the program, b) selection of the variety for mutagen treatment, c) part of the plant to be treated, d) dosage of the mutagen e) mutagen treatment and f) handling of mutagen treated populations.

Mutation breeding is usually taken up in a crop variety, if the desired type of variability does not exist naturally in it. Crops pollinated crops have enough genetic variability already in nature, hence have not been subjected to mutation breeding as much as self pollinated ones. Moreover the detection of mutations becomes difficult in open pollinated crops because of their heterozygous and out-breeding nature. A generalized scheme for handling of treated material in self-pollinated crops is given in and briefly discussed here.

M_1 (First treated Generation): Terms like R_1, X_1 etc are also used but the use of M_1 is proper, more common and advocated to designate first treated generation.

Several hundred (500 or more) seeds are treated with a mutagen and are space planted along with untreated. There are many common effects observable in M_1 itself like death of seedlings, growth inhibition, morphological and developmental abnormalities. Also observable some heritable changes in qualitative and quantitative characters. Of all the above effects, only heritable changes (mutations) are of importance to breeders. There are only a few mutants observable in M_1 generation, which are either dominant or double recessive. Therefore only a few M_1 plants are selected as mutants and the majority of them are promoted to M_2 as such by harvesting about 20 seeds from each M_1 plant. These are to be raised separately as M_2 progeny rows.

M_2 Generation: It is the most important generation as maximum number of mutants are recovered due to recombination and segregation. A large population is required for efficient recovery of mutants generally about 2000 progeny rows are grown. Since mutations occur in morphological characters, yield and yield components, biochemical and physiological traits their detection would depend on the efficiency of screening procedure. Hence very careful and regular observations are made on the M_2 rows and later generations. If the mutant is distinct, it is selected for multiplication and testing.

Recovery of mutant in M_2: The embryo is a miniature plant where already some parts are differentiated. Mutations take place usually in a few meristematic cells of the seed; thus seedling developing out of it becomes chimeric. Mutant tissue effecting the formation of vegetative tissue like leaf etc., would be lost. For a mutant tissue of a treated seed to be included in tissue forming seed on M_1 plant, it has to pass through two sieves of selection pressures, namely a) diplontic selection and b)haplontic selection.

Diploid normal tissue and diploid mutant tissue of the treated seed compete with each other during the vegetative stage and during ontogenetic differentiations of reproductive organs. If the mutant tissue is successful it will enter into the formation of reproductive parts. This is known as diploitic selection.

Haploitic selection operates when the M_1 flower is chimeric and produces normal and mutant pollen. These types compete for fertilizing the ovule. If this time again mutant pollen successful, then the mutant would be recovered in M_2 or later generations.

M_3 and later generations: In M_3, plant progenies of selected M_2 plants are grown and evaluated critically. Mostly eyeball selection, that is selection based on appeal to the eyes, is done in M_3. Progenies or single plants thus selected are grown in M_4. Purification evaluation and multiplication of mutants will be made by growing $M \leq_4$ later generations. If in the repeated trails the mutant proves its superiority over standard check, it is released as a variety.

Applications and achievements of mutation breeding: Mutagenesis has been used to improve morphological and physiological characters, disease resistance, and quantitative characters including yielding ability.

Directed mutagenesis: Induction of only desirable mutations is known as directed mutagenesis. It is highly useful in crop improvement. Directed mutagenesis is sought to be achieved by using various mutagens singly or in combination and by changing internal and/or external environments before, during or after the mutagen treatments. The types mutation and their frequently is greatly influenced by these factors. For example low moisture content of seeds increases radio sensitivity and mutation frequency. Similarly pre-soaking of seeds prior to chemical mutagen treatment also produces higher

frequency of mutations. With the availability of a wide spectrum of recombinant DNA techniques it is now possible desired sequence change (may be a single base alteration, an insertion or a deletion) can be engineered in a gene which can be there after put back into an organism to study the consequences. This is known as *'site-directed mutagenesis* or *reverse genetics*. This method usually utilizes plasmids which contain gene sequences of interest.

The utility of mutants: Mutant microorganisms are useful in industry. Mutation breeding helps to generate new varieties of plants with desirable characteristics like resistance, to drought, pests and pesticides. Mutants have been valuable tools for unraveling metabolic pathways. Similarly genes which control developmental pathways have been identified by examination of mutations which abolish or after development.

Mutagenesis has been successfully used for the crop improvement. It is useful in defect elimination and improving specific characteristics of a well adopted high yielding variety. Increased genetic variability and improvement of quantitative characters was also made possible through induced mutagenesis.

Induced chromosomal mutations have also been contributed to crop improvement. For example polyploids have been induced in many crop plants. Translocation stocks have been utilized for transferring characters from related species and genera.

Induced mutations played an important role in special breeding programs which are aimed at achieving the following objectives:

- breaking undesirable linkage
- production of haploids
- increase or decrease of chiasma frequency
- reduction of incompatibility in wide crosses.

Achievements:

The first commercial success with induced mutations was reported in 1934 with the release of a new tobacco cultivar 'chlorina' through X-ray irradiation. Later on varieties have been released through mutation breeding. Eg., NP 836 wheat, Indore 2 cotton, JRO 514 Jute, Jagannadh rice and CO 8152 and CO 8153 sugarcanes.

NP 836 wheat is an awned mutant from the awnless wheat variety NP 799. Jagannath rice is a gamma ray induced dwarf mutant from the tall T 141. CO 8152 is also gamma ray induced sugar cane variety developed from CO 527 and the mutant produces 40% higher cane yield than the parent variety. In the varieties released through mutation breeding almost every character has been improved. Some important varieties developed through mutation breeding are listed in Table 21.4. Micke and Donini (1993) reviewed the progress of mutation breeding and stated that 1363 cultivars have derived valuable characters through mutagenesis. The outstanding contributions of mutant varieties include high yielding rice in China, all malting barley of Czechoslovakia and NIAB-78 variety of cotton produced in Pakistan.

The experience with the mutation breeding has brought out certain limitations of the technique. These limitations are listed as under:

a. The frequency of desirable mutants is very low. Therefore, large M_2 and subsequent populations have to be grown and carefully studied. This involves considerable labor and other facilities.

b. The mutations are unpredictable and hence mutation is still a hit and miss process.

c. Desirable mutations are commonly associated with undesirable side effects.

d. Most of the mutations are recessive. Detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploidy species. Hence, it has been successfully applied to diploid species that reproduce sexually, particularly in self-pollinated crops.

21.10 Summary

Mutations are sudden heritable changes in the characteristics of organisms. Mutations may be chromosomal, cytoplasmic or gene mutations. Mutations can be induced by treatment with physical and chemical mutagens. X-rays and gamma-rays act by causing ionization directly in the DNA or in the medium which produces free radicals, which are highly reactive and act on DNA . Chemical mutagens act primarily with DNA, causing domination and alkylations. The action of mutagens brings about changes in DNA base pair sequences through deletion, insertion and substitution of base pairs in turn resulting gene mutations.

A mutation breeding program should have a clear-cut and well defined objective. The dose of the mutagen should be decided by experimentation; a dose close to LD 50 is generally preferred. The handling of mutagen treated progenies depends on the mode of reproduction of crop species and nature of mutation. Mutation breeding has been used to improve both qualitative and quantitative traits. It has significant contribution for the improvement of several crop plants. Application of mutation breeding is limited by low frequency of desirable mutants, association of undesirable side effects, non-availability of quick and efficient screening techniques and recessive ness of most of the desirable mutations.

21.11 Key Terms:

Base analogues: A chemical substance resembling a DNA base is called base analogue.
Deamination: Removal of the amino group from a base.

Iomization: Loss of gain of an electron by an atom producing charged ions.

Mutagen: A chemical or physical agent that induces mutation

Mutagenesis: Induction of mutations with the aid of mutagens.

Mutation: A sudden heritable change in a gene or chromosome or in characteristics of an organism.

Silent mutation: Any gene mutation that does not result in phenotypic expression is a silent mutation.

Tautomerism: The ability of a molecule to exist in more than one form is called tautomerism.

Transition: Replacement of a purine by a purine or apyrimidine by a pyrimidine in DNA.

Transversion: Substitution of purine bases by pyrimidine and viceversa is called transversion.

21.12 Questions:

1. Define mutation briefly explain different kinds of mutations and their effects on survival of organisms?
2. What are the different kinds of mutagens and explain their properties and mechanism of action?
3. Describe the molecular basis of mutations and explain the different methods of their detection and selection?
4. What is mutation breeding and explain the procedure?
5. Write a detailed account on the applications and achievements of mutation breeding.

21.13 Reference books:

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