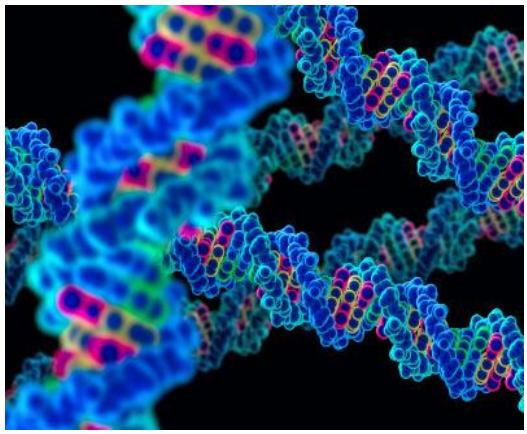
# Principles of Genetics and Cytogenetics

## 8. Principles of Genetics and Cytogenetics (HPG 100) 3(2+1)

Historical background of genetics, theories and hypothesis. Physical basis of heredity, cell reproduction, mitosis, meiosis and its significance. Gametogenesis and syngamy in plants. Mendelian genetics–Mendel's principles of heredity, deviation from Mendelian inheritance, pleiotropy, threshold characters, co-dominance, penetrance and expressivity. Chromosome theory of inheritance, gene interaction. Modification of monohybrid and dihybrid rations. Multiple alleles, quantitative inheritance linkage and crossing over, sex linked inheritance and characters. Cytoplasmic inheritance and maternal effects. Chemical basis of heredity, structure of DNA and its replication. Evidence to prove DNA and RNA – as genetic material. Mutations and their classification. Chromosomal aberrations, changes in chromosome structure and number.

***Practical:*** *Study of fixatives and stains. Squash and smear techniques. Demonstrations of permanent slides and cell division, illustration in plant cells, pollen fertility and viability, determination of gametes. Solving problems of monohybrid, dihybrid, and test cross ratios using chi-square test, gene interactions, estimation of linkages using three point test cross from F2 data and construction of linkage maps. Genetic variation in man.*

## Lecture No. 1

## Introduction

The term genetics was first coined by W. Bateson in 1905, although beginning of the science of genetics was made in 1900 by rediscovery of Mendel's work. The word genetics was derived from the Greek root 'Gen' which means 'to become' or 'to grow into'. Genetics is often described as a biological science, which deals with the principles of heredity and variation. Heredity refers to the transmission of characters from parents to their offspring. The differences among the individuals of a species for a character constitute the variation. The variations are mainly of two types namely (i) heritable (genetic), (ii) nonheritable (environmental). Heredity variation refers to differences in inherited traits. These types of variations are found not only in progenies of parent, but also transmitted from generation to generation. Environmental variation is temporary and not inherited to next generation.

The foundation of this new branch of biology – genetics was laid by Mendel in 1866 when he discovered the basic principles of heredity. However, Mendel's findings came into light only in 1900, when similar results were obtained independently by three scientists, *viz*., Hugo de Vries, Carl Correns and Von Tschermak. Thus, genetics was born in 1900.

### 1. Pre – Mendelian ideas about heredity

In ancient times, there were speculations on the nature of heredity. Early philosophers and workers had forwarded various ideas or theories to explain the phenomenon of inheritance. They are briefly presented below.

### (i) Vapour and fluid theories

Early Greek Philosophers like Pythagoras (500 B.C) proposed the theory that a moist vapour descends from the brain, nerves and other body parts of the male during coitus and from this, similar parts are formed in the uterus of the female forming the embryo.

Empedocles proposed the theory that each parent produces 'a semen' which arises directly from various parts of the body. In embryos the various parts are formed by this semen.

Aristotle thought that the semen of man has some "Vitalizing" effect and he considered it as the highly purified blood. According to him the mother furnishes inert matter and the father gives the life-giving power, "dynamic" to the new life.

**(ii) Preformation Theory**

The theory was proposed by two Dutch biologists, Swammerdam and Bonnet (1720-1793). This theory states that a miniature human called 'homunculus' was already preformed in the egg and sperm. The development of zygote resulted only in the growth of a miniature human who was already present in the egg and sperm. However, this theory was rejected because this could not be proved scientifically.

**(iii) Theory of Epigenesis**

This theory was proposed by Wolf (1738-1794), a German biologist and it states that egg or sperm cells do not contain miniature human but that the gametes contained undifferentiated living substance capable of forming the organized body after fertilization. This proposition was called as epigenetic concept and remained universally accepted.

**(iv) Theory of Acquired characters**

This concept was proposed by Lamarck (1744-1829), a French biologist. This theory states that a new character once acquired by an individual shall pass on to its progeny. This theory was disproved by Weismann. He cut the tail of mice for successive generations and always got the baby mice with tail. Thus, this theory was rejected.

**(V) Theory of Pangenes**

This theory was proposed by Charles Darwin (1809 – 1882), an English naturalist. According to him, each part of the animal body produces a minute copy of its own, called gemmule or pangene. The gemmules are collected in the reproductive organs. The gemmulues were passed on to the gametes. The young one formed from the gametes would be having all the gemmules characteristics of the parents, and will represent a blending of the qualities of its two parents. Thus, theory of pangenesis is also known as the 'theory of blending inheritance'.

**(vi) Germplasm Theory**

This theory was advocated by August Weismann (1834-1914), a German biologist. According to this theory, organism's body contains two types of cells namely somatic cells and reproductive cells. The somatic cells form the body and its various organ systems, while the reproductive cells form sperm and ova. The somatic cells contain the 'somatoplasm' and reproductive cells contain the 'germplasm'. The germplasm can form somatoplasm, but somatoplasm can not form germplasm. The cells of the somatoplasm become differentiated during the formation of the complex organs of the body while cells of the germ cells remain undifferentiated and retain their power to generate new life. The germplasm thus goes on in continuous stream from generation to generation. Changes in the somatic cells (somatoplasm), which were caused by the environment, cannot influence the germplasm and hence acquired characters are not inherited.

**II. Pre- Mendelian experiments**

Knight (1779) conducted experiments on pea much before Mendel but failed o formulate the laws of inheritance because he could not use the mathematics to his results. He crossed pigmented variety with unpigmented variety and F1 was pigmented. When F1 was selfed, F2 showed pigmented and non-pigmented plants. Since he did not keep record on different types, he could not discover the mechanism of inheritance.

J.Kolreuter (1733-1806), a German botanist performed hybridization experiments in tobacco and compared the hybrids with their parents. He demonstrated that the hybrids may resemble one or the other parent or may be intermediate between them. He also showed that both the parents make equal contributions to the hybrids.

Gartner (1772-1850) and Naudin (1815-1899) done experiments similar to Kolreuter and they observed the similar results. However they could not apply mathematics to their results.

## Questions

1. Person who coined the term genetics was

i) Lamarck ii) Weismann iii) Bateson iv) Charles Darwin

**Ans: iii) Bateson**

2. Mendel's finding were rediscovered in the year

i) 1900 ii) 1903 iii) 1904 iv) 1905

**Ans: i) 1900**

3. Theory put forth by Charles Darwin was

i) Germplasm theory ii) Theory of pangenes

iii) Preformation theory iv) Vapour fluid theory

**Ans: ii) Theory of pangenes**

4. Scientist who had done hybridization experiment prior to Mendel was

i) Lamarck ii) Koleruter iii) Weismann iv) Aristotle

**Ans: ii) Koleruter**

5. Who distinguished first between somatoplasm and germplasm

i) Lamarck ii) Weismann iii) Bateson iv) Charles Darwin

**Ans:** ii) Weismann

**Say True or False**

6. According to preformation theory, gamet is contain undifferentiated living substance capable of forming the unorganized body after fertilization.

**Ans :False**

7. Theory of pangenes is a theory of blending inheritance

**Ans: True**

8. Hybridization experiments conducted prior to Mendel could not give any specific pattern of inheritance because mathematics was not applied

**Ans: True**

9. Somatic cells contain the germplasm

**Ans: False**

10. Theory of acquired characters was disproved by cutting the tail of girafee for successive generations

**Ans: False**

## Lecture No. 2

## Historical Developments in Genetics and Cell structure and function

### A. Historical Developments in Genetics

Gregor Johann Mendel (1822-1884) was an Austrian botanist who laid the foundation of the science of genetics. He worked with garden pea (*Pisum sativum*) and formulated two important laws of inheritance, *viz*., (i) law of segregation and (ii) law of independent assortment. For this pioneer work he is called 'the father of genetics'. He presented a paper entitled "Experiments in plant Hybridization" before Natural History Society of Brunn in February 1865 which was published in the proceedings of the society in 1866. However, his results were neglected for 34years. Mendel died in 1884 and his work came into being after 16 years of his death in 1900 when same results were independently discovered by de Vries, Correns and Tschermak.

Today, genetics is a mature but dynamic science. The major developments in genetics from Mendel are given below:

| Development in Genetics | Development in Cytogenetics |
|---|---|
| 1865 - Mendel presented his paper to the Brunn Society for Natural history | 1590- Jansen invented the compound microscope which combines two lenses for greater magnification |
| 1865 - Mendel's paper published in the proceedings of the Brunn Society for Natural History. | 1665-Robert Hooke coined the term cell for hollow space surrounded by walls in the piece of cork. |
| 1900 - Mendel's work discovered by de Vries, Correns, and von Tschermak. | 1831-Robert Brown discovered the nucleus in plant cell. |
| 1903 - Johannson proposed the pureline theory. Pureline is the progeny of a single homozygotes self pollinated plant. | 1838-39- Schleiden (Botanist) and Schwann (Zoologist) formulated cell theory. According to them cell is the basic unit of structure and function in living organism. |

| | |
|---|---|
| 1905 - Bateson named the science genetics.<br><br>1905-Bateson and Punnet reported the modification of dihybrid F2 ratio due to gene interaction. | 1840-Purkinjii, J.E gave the term protoplasm |
| | 1855-Virchow showed that all cells arise from pre-existing cells by cell division |
| | 1879-W.Flemming, introduced the term chromatin. |
| | 1882-W.Flemming introduced the term mitosis. |
| | 1888-Waldeyar introduced the term chromosome.<br>1902- Boveri and Sutton demonstrated the presence of paired chromosomes (homologs) in diploid species. |
| | 1905-Farmer and Moore coined the term meiosis. |

**Developments in genetics and cytogenetics**

1906 **:** Yule advanced the idea of multiple factor hypothesis to explain the inheritance of quantitative characters. According to this hypothesis, quantitative characters are governed by several genes with small effects and the effects are in additive nature.

1908 **:** Hardy and Weinberg formulated the "Hardy Weinberg law" relating genotypic frequencies to gene frequencies in random mating populations.

1909 **:** Johannsen introduced the word gene.

1909 **:** Garrod's book Inborn Errors of Metabolism published.

1909 : Nilson – Ehle provided the genetic explanation for the inheritance of quantitative traits.

1910 : Morgan (Nobel Prize 1933) established the sex liked inheritance of white eyes in *Drosophilla melanogaster.*

1911 : Morgan postulated the chromosomal basis of linkage.

1927 : Muller (Nobel Prize 1946) demonstrated that x rays are mutagenic.

1928 : Griffth's discovery of transformation in *Diplococcus pneumonia*.

1931 : Creighton and McClintock (maize) and Stern (*Drosophila*) papers appeared demonstrating that genetic recombination is correlated with the exchange of morphological markers on chromosomes.

1932 : Knoll and Ruksa produced first electron microscope.

1940 : Oliver's demonstration of recombination within the lozenge functional unit in *Drosphila*.

1941 : Beadle and Tatum's work (Nobel Prize 1958) on Neurospora was published, establishing the one gene-one enzyme concept.

1944 : Avery, Macleod and McCartty demonstrated that the pneumococcal "transforming principle" is DNA.

1946 : Lederberg (Nobel Prize 1958) and Tatum's discovery of conjugation in bacteria.

1946 : Present day Electron microscope became widely used in biology, the ultra structure of the cell was studied.

1950 : McClintock's (Noble Prize 1983) first paper on "transposable elements" in maize.

1952 : Hershey (Nobel Prize 1969) and Chase demonstrated that the genetic material of bacteriophage $T_2$ is DNA.

1952 : Zinder and Lederberg's discovery of phage mediated transduction in bacteria.

1953 : Watson and Crick (Nobel Prize 1962) worked out the - double helix structure of DNA using the X ray diffraction data of Wilkins (Nobel Prize 1962) and the base composition data of Chargaff.

1955 : Benzer's first paper on the fine structure of the phage T4 rII locus.

1956 : Tjio and Levan established that the normal diploid chromosome number in human is 46.

1957 : Fraenkel Conrat and Singer demonstrated that the genetic information of tobacco mosaic virus is stored in RNA.

1958 : Meselson and Stahl demonstrated that DNA replication in semi-conservative.

1959 : Ochoa (Nobel Prize 1959) discovery of the first RNA polymerase.

1961 : Jacob and Monod (Nobel Prize 1965) proposed "operon model" for regulating gene expression.

1964 : Colinearity between genes and polypeptide products established by the work of Yanofsky and colleagues and by Brenner and colleagues.

1964 : Temin (Nobel Prize 1975) proposed the DNA provirus form of RNA tumor viruses.

1965 : Holley (Nobel Prize 1968) worked out the first complete nucleotide sequence of a RNA, a yeast alanine tRNA.

1966 : The complete genetic code was established by the work of Nirenberg and Khorana (Nobel Prize 1968) and co-workers.

1970 : Nathans and Smith (Nobel Prize 1978) isolated the first restriction endonucleases.

1970 : Reverse transcriptase of RNA tumor viruses identified by Baltimore (Nobel Prize 1975).

1972 : First recombinant DNA produced *in vitro* in Berg's (Nobel Prize 1980) laboratory.

1976 : Bishop and Varmus (Nobel Prize 1989) demonstrated the protonocogene to oncogene relationship.

1977 : Demonstration of introns in eukaryotic genes by Breathnach, Mendel and Chambon and by Jeffreys and Flavell.

1977 : Publication of the DNA sequencing techniques of Maxam and Gilbert and of Sanger, Nicklen and Coulson (Sanger and Gilbert, Noble Prize 1980).

1977 : Publication of the complete 5387 nucleotide sequence of phage Φ X 174 by Sanger and colleagues.

1978 : Discovery of splicing of adenovirus RNAs in three different laboratories.

1982 : Publication of the complete 48502 nucleotide pair sequence of phage lambda by Sanger and colleagues.

1983 : Cech and Altman (Nobel Prize 1989) established the existence of catalytic RNAs.

1988 : Watson's acceptance of job as coordinator of the "human genome project".

1989 : NIH Recombinant DNA Advisory Committee recommends approval of first "human gene transplant" experiment.

1990 : Tsui, Collins and colleagues clone the "cystic fibrosis gene' the gene whose mutant alleles account for the majority of the cases of this dreaded disease that affects about one out of every 2000 children in the United States.

**B. Cell structure and function**

The cell could be described as a small unit of living protoplasm always surrounded by cell membrane. The matrix between the nucleus and the cell membrane known as cytoplasm. This contains a variety of organelles. An organelle is a distinct part of a cell which has a particular structure and function. The nucleus is the largest intra cellular cell organelle, which contains deeply staining material known as chromatin, which has a particular structure and function. It contain DNA, the genetic material.

In the case of prokaryotic cell, the DNA lies free in the cytoplasm and the region is known as nucleoid, where as in the case of eukaryotic cells, the DNA is found inside the nucleus, which is surrounded by nuclear envelope. Some of the differences between prokaryotic and eukaryotic cells are give in the Table 2.1.

**Table 2.1 Differences between Eukaryotic and Prokaryotic cells**

| S.No | Eukaryotic cells | Prokaryotic cells |
|---|---|---|
| 1. | Nucleus is surrounded by nuclear envelope | Nuclear envelope is absent |

| | | |
|---|---|---|
| 2. | DNA is associated with histones and non-histone protein to form chromatids fibre | DNA is naked |
| 3. | Cytoplasm contains Endoplamic Reticulum, Golgi bodies etc | Absent |
| 4 | Mitochondria present | Absent. Oxidative phosphorilation associated with plasma lemma. |
| 5 | All green plants possess chloroplast with typical grana | Chloroplast absent. BGA have lamellar photosynthetic structure |
| 6 | Cytoplasm have microtubules and provides stability to cytoplasm | Do not have microtubules |
| 7 | Ribosome 80s (60s & 40s sub units) | Ribosome 70s (50s & 30s sub units) |
| 8 | Movement of chromosome associated with spindle fibres | Spindles fibres absent. Movement of chromosomes accompanied by enlargement of plasma lemma. |
| 9 | Most of Ribosomes attached to EndoplaR | All ribosome are free in cytoplasm |
| 10 | Nucleolus is present in the nucleus | Nucleolus is absent |

Generally the animal cells are similar to plant cells but the former contains centriole which is absent in plant cell. Some of the chief differences which are present in the plant cells are

1. Cell wall which is present outside the cell membrane and pores containing fine threads known as plasmodesmata link the cytoplasm of neighbouring cells through cell walls.
2. Chloroplast-found in photosynthetic plant cells.
3. Large central vacuole.

The function of various cell organelles are given in the Table 2.2

**Table 2.2 Function of Cell Organelles**

| Cell organelle | Function |
|---|---|
| Cell or plasma membrane | Differentially permeable membrane, through which extra cellular substances may be selectively sampled and cell produces may be liberated. |
| Cell wall (plants only) | Thick cellulose wall surrounding the cell membrane giving strength and rigidity to the cell. |
| Nucleus | Regulates growth and reproduction of the cell. |
| Chromosomes | Bearers of hereditary instruction, regulation of cellular processes (seen clearly only during nuclear division) |
| Nucleolus | Synthesizes ribosomal RNA: disappears during cellular replication |
| Nucleoplasm (nuclear sap) | Contains materials for building DNA and messenger molecules which act and intermediates between nucleus and cytoplasm. |
| Nuclear membrane | Provides selective continuity between nuclear and cytoplasmic materials. |
| Cytoplasm | Contains machinery for carrying out the instructions sent from the nucleus. |
| Endoplasmic reticulum | Greatly expanded surface area for biochemical reactions which normally occur at or across membrane surfaces. |
| Ribosomes | Sites of protein synthesis (Shown as black dots lining the endoplamic reticulum) |
| Centrioles | Form poles for the divisional processes; capable of replication |
| Mitochondria | Energy production (Kreb's cycle, electron transport chain, beta oxidation of fatty acids, etc.,) |
| Plastids (plants only) | Structures for storage of starch, pigments, and other cellular products. Photosynthesis occurs in chloroplasts. |

| | |
|---|---|
| Golgi body or apparatus | Production of cellular secretions; sometimes called dictyosomes in plants |
| Lysosome (animals only) | Production of intracellular digestive enzymes which aid in disposal of bacteria and other foreign bodies; may cause cell destruction if ruptured. |
| Vacuoles | Storage depots for excess water, waste products, soluble pigments, etc |
| Hyaloplasm | Contains enzymes for glycolysis and structural materials such as sugars, amino acids, water, vitamins, nucleotides, etc. (nutrient soup or cell sap). |

## Questions

1. The person who coined the term cell was

   i) Robert Hook ii) Jansen iii) Robert brown iv) Johannsen

   **Ans: i) Robert Hook**

2. Mendel's research paper was published in the year

   i) 1900 ii) 1866 iii) 1885 iv) 1905

   **Ans: ii) 1866**

3. Flemming coined the term

   i) Meiosis ii) Mitosis iii) Chromatin iv) Protoplasm

   **Ans: iii) Chromatin**

4. Johannsen introduced the term

   i) Linkage ii) Cell iii) Gene iv) Genetics

   **Ans: iii) Gene**

5. DNA has

   i) Circular structure ii) Double helical structure

   iii) Single helical structure iv) Multiple helical structure

   **Ans: ii) Double helical structure**

**Say True or False**

6. Cytoplasm is the living material present within the nuclear membrane

   **Ans: False**

7. In prokaryotes, nuclear envelope is present

   **Ans: False**

8. Idea of chromosomal basis of linkage was first given by Johannsen

   **Ans: False**

9. Quantitative characters are governed by oligogenes.

   **Ans: False**

10. Ribosomes are the sites of protein synthesis in cells

**Ans: True**

## Lecture No.3

## Cell Division

Cell is a basic unit of structure and function in all living systems. The process of reproduction or formation of new cells from the pre-existing cells is referred to as cell division. In lower organisms like bacteria, cell division takes place by fission of pre-existing cell. But in higher organisms like eukaryotes there are two types cell division *viz.*, Mitosis and Meiosis.

### Mitosis

The term Mitosis was coined by Flemming in 1882. Mitosis is the process by which a cell nucleus divides to produce two daughter nuclei containing identical set of chromosomes to the parent cell. It is usually followed immediately by division of whole cell to form two daughter cells. This process is known as mitotic cell division. (Cell division = Karyokinesis + Cytokinesis)

### Cell cycle

The sequence of events which occur between one cell division and the next is called cell cycle. It has two main stages.

1. Interphase
2. Mitosis or M phase (period of cell division)

### 1. Interphase

It is the period between successive cell divisions consisting of process associated with growth and preparation for mitosis. The period of DNA synthesis during interphase is called the 'S' phase or synthetic phase and it is separated in time from the previous cell division by a gap called '$G_1$' phase. $G_1$ phase is the period between the beginning of interphase and that of DNA synthesis (S phase). After DNA synthesis a further gap called '$G_2$' phase occurs before the next cell division begins, which is the period between termination of DNA synthesis and beginning of prophase of next cell division. The $G_1$ phase shows considerable variation whereas $G_2$ shows more constancy for a given type of cell. During interphase, each DNA molecule replicates an exact copy of itself. This copying process produces a chromosome with two identical functional strands called chromatids, both attached to a common centromere.

## 2. Mitotic phase

The mitotic phase leads to separation of replicated DNA into two daughter nuclei without recombination (Fig 3.2). The M phase consists of two major events viz., division of nucleus (Karyokinesis) followed by division of cytoplasm (cytokinesis). The karyokinesis has got four distinct stages as follows.

### 2.1. Prophase

1. Coiling and condensation of chromosome takes place which make them visible as thread like Structures.
2. Each chromosome has two identical longitudinal splits called identical or sister chromatids, which are attached by common centromere.
3. Migration of centrioles to opposite ends of the cell.
4. Disappearance of nucleolus and beginning of the breakdown of the nuclear membrane.
5. Formation of spindle fibre.

### 2.2. Metaphase

1. Formation of spindle fibres is completed and chromosomes are attached to the spindle fibres at the point of centromere.
2. Movement and arrangement of all chromosomes on metaphase plate or equatorial plate.
3. Sister chromatids of each chromosome are joined together at the point of centromere, but their arms are free.
4. Chromosomes are clearly visible.

### 2.3. Anaphase

1. This is the shortest phase of the mitotic division.
2. This stage begins with splitting of centromere into two, which allow the sister chromatids to separate and move to opposite poles.
3. The separated sister chromatids are called as new chromosomes.
4. The arms of each chromosome drag behind their centromeres giving them characteristic shapes depending up on the location of centromere.

### 2.4. Telophase

1. Chromosomes reach the opposite poles and spindle fibres begin to disintegrate.
2. Nuclear membrane is reestablished.
3. Nucleoli is reformed
4. Chromosomes again become thinner and longer by uncoiling and unfolding.

**Cytokinesis**

It is the division of cytoplasm. This stage normally follows telophase and leads in to $G_1$ phase of interphase. In animals, cytokinesis is accomplished by formation of cleavage furrow which deepens and pinches the cell into two daughter cells. In plants, cytokinesis involves the construction of cell plate at the centre of the cell and spreading laterally to the cell. Later cellulose and strengthening materials are added to the cell plate, converting it into a new cell wall.

**Significance of mitosis**

1. Genetic stability: Mitosis produces two daughter cells which have the same number of chromosomes as that of parent cell. These daughter cells are genetically identical to the parent cell and no genetic variation can be introduced during mitosis.
2. Growth: The number of cells within the organism is increased by mitosis and this is the basis for growth of organisms.
3. Cell replacement: Replacement of old cells and dead cells in an organism is achieved by mitosis.
4. Asexual reproduction: Production of new individuals through asexual reproduction is achieved by mitosis.

**Meiosis**

Meiosis is the process by which a cell nucleus divides to produce four daughter nuclei each containing half the number of chromosomes of the original nucleus or cell. It is also called as reduction division since it reduces the number of chromosomes in the cell from the diploid number (2 n) to the haploid number (n). Like mitosis, it involves DNA replication during interphase in the parent cell, but this

is followed by two cycles of nuclear and cytoplasmic divisions known as Meiosis I (reduction division) and Meiosis II (multiplication division). Thus, a single diploid cell gives rise to four haploid cells.

**First meiotic division (or) reduction division**

It has four stages namely Prophase I, Metaphase I, Anaphase I, and Telophase I.

**Prophase I**

1. It is the longest phase of meiotic division. It has five sub stages namely, Leptotene, Zygotene, Pachytene, Diplotene and Diakinesis.
2. Chromosomes are scattered throughout the nucleus in a random manner.

**Leptotene**

1. Progressive condensation and coiling of chromosome fibres.
2. Chromosomes are scattered throughout the nucleus in a random manner.

**Zygotene**

1. Chromosomes become shorter and thicker.
2. Homologous chromosomes lie side by side and this pairing process is called synapsis.
3. Each synapsed homologue is called bivalent. It consists of four chromatid strands called tetrad.
4. Synaptonemal complex also develops during this stage.

**Pachytene**

1. Exchange of chromosomal segments between non-sister chromatids of the homologous chromosome. This process is called crossing over.
2. The point of exchange of chromatid during crossing over is called chiasma.
3. The homologous chromosomes are attached to each other by chiasmata.
4. Synaptonemal complex can be seen between synapsed chromosomes.

**Diplotene**

1. Separation of homologous chromosomes takes place from one another which begins from the centromere to end of the chromosomes. This process is called terminalisation.
2. Nuclelus decreases in size.
3. Nuclear membrane disappears.

**Diakinesis**

1. This stage begins after the complete terminalization of chiasmata.
2. Chromosomes are in more contracted stage.
3. Due to further contraction and terminalisation, these appear as round bodies evenly scattered throughout the cell.
4. Nucleolus disappears.
5. The spindle fibres begin to be formed at the end of this stage.

**Metaphase I**

1. The homologous chromosomes lie on each side of the equatorial plate and attached with spindle fibres.
2. Due to the contraction of spindle fibres, centromeres of each chromosome are directed towards the opposite poles towards the equator.

**Anaphase I**

1. At first anaphase, the centromeres do not divide, but continue to hold sister chromatids together.
2. The homologues separate and individual chromosome moves to opposite poles.
3. This leads to reduction of number of chromosomes from diploid (2n) to haploid (n) state.

**Telophase I**

1. Chromosomes uncoil and relax and regrouping of chromosomes occur.
2. Nucleolus and nuclear membrane reappear.

3. Two haploid daughter nuclei are formed.
4. Cytokinesis in telophase I divides diploid mother cell into two haploid (n) daughter cells. This ends the first meiotic division.

   The brief period between the first and second meiotic divisions is called interkinesis.

**Second meiotic division (or) multiplication division**

The second meiotic division is equal to miotic division. However meiosis II differs from Mitosis in the following ways.

(i) Interphase (interkinesis) prior to meiosis II is very short. It does not have 'S' period because each chromosome already contains two chromatids.

(ii) The two chromatids in each chromosome are not sisters throughout. In otherwords some chromatids have alternate segments of non-sister chromatids due to recombinations.

(iii) The meiosis II deals with haploid chromosome number, whereas normal mitosis deals with diploid chromosome number.

Meiosis II has four stages. They are prophase II, Metaphase II, Anaphase II and telophase II.

In prophase II, the spindle apparatus reappears. By metaphase II, the centromeres have lined up on the equatorial plane. During anaphase II, the centromere of each chromosome divide, allowing sister chromatids to separate. Cytokinesis followed by telophase II divide the two cells into four meiotic products.

**Synaptonemal complex**

It is a protein framework, which is found between paired chromosomes. It consists of one central and two lateral elements. There are transverse filaments on both sides of the central element. The lateral elements are attached to homologous chromosomes. Synaptonemal complex is considered to be associated with pairing of homologous chromosomes and recombination. However, its origin and exact role in synapsis is still not properly known.

**Significance of meiosis**

- Meiosis enables the chromosome number of a sexually reproducing species to be kept constant from generation to generation.
- Meiosis introduces the genetic variation in the offsprings of sexually reproducing individuals by means of independent assortment and crossing over (recombination).

The comparison between mitosis and meiosis are briefly presented in Table 3.1.

**Table 3.1 Comparison between mitosis and meiosis**

| S.No | Mitosis | Meiosis |
|---|---|---|
| 1. | An equational division which separates sister chromatids. | The first stage is a reduction division which separates homologous chromosomes at first anaphase; sister chromatids separate in an equational division at second anaphase. |
| 2. | One division per cycle, i.e.one cutoplasmic division (cytokinesis) per equational chromosomal division. | Two divisions per cycle i.e. two cytoplasmic divisions, one following reductional chromosomal division and one following equational chromosomal division. |
| 3. | Chromosomes fail to synapse; no chiasmata formation; genetic exchange between homologous chromosomes does not occur. | Chromosomes synapse and form chiasmata; genetic exchange occurs between homologues. |
| 4. | Two products (daughter cells) produced per cycle. | Four cellular products (gametes or spores) produced per cycle. |
| 5. | Genetic content of mitotic products are identical. | Genetic content of meiotic products different; centromeres may be replicas of either maternal or paternal centromeres in varying combinations. |

| | | |
|---|---|---|
| 6. | Chromosome number of daughter cells is the same as that of mother cell. | Chromosome number of meiotic products is half that of the mother cell. |
| 7. | Mitotic products are usually capable of undergoing additional mitotic divisions. | Meiotic products cannot undergo another meiotic division although they may undergo mitotic division. |
| 8. | Normally occurs in most of somatic cells | Occurs only in specialized cells of the germ line. |
| 9. | Begins at the zygote stage and continues through out the life of the organism | Occurs only after a higher organism has begun to mature; occurs in the zygote of many algae and fungi. |

## Questions

1. Zygotene is the stage of cell division that occurs prior to

   i) Leptotene ii) Pachytene iii) Diplotene iv) Diakinesis

   **Ans: ii)Pachytene**

2. Crossing over occurs during

   i) Leptotene ii) Pachytene iii) Diplotene iv) Diakinesis

   **Ans: ii) Pachytene**

3. In telophase

   i) Spliting of centromere occurs

   ii) Chromosomes reach the opposite poles

   iii) Chromosomes get arranged in equatorial plate

   iv) Chromosomes appear as coiled coil

   **Ans: ii) Chromosomes reach the opposite poles**

4. Cell replacement in an organism is achieved by

   i) Mitosis ii) Meiosis iii) $G_1$phase iv) $G_2$ phase

   **Ans: i) Mitosis**

5. Meiosis results in

   i) Two daughter cells ii) Three daughter cells

   iii) Four daughter cell iv) One daughter

   **Ans: iii) Four daughter cell**

**Say True or False**

6. Mitosis involves the separation between homologous chromosomes

   **Ans: False**

7. Synapsis results in the formation of chiasmata

   **Ans:True**

8. Genetic content of meiotic products are always identical

**Ans: False**

9. Chromosome number of daughter cells after mitosis is half of the parental cells

**Ans: False**

10. Meiosis II deals with haploid chromosome no.

**Ans:True**

## Lecture No. 4

## Gametogenesis

Usually, the immediate end products of meiosis are not fully developed into gametes or spores. A period of maturation commonly follows meiosis. In plants, one or more mitotic divisions are required to produce reproductive spores, whereas in animals the meiotic products develop directly into gametes through growth and / or differentiation. The entire process of producing mature gametes or spores, of which meiotic division is the most important part, is called gametogenesis.

### 1. Gametogenesis in animals (mammals)

Gametogenesis in male is called spermatogenesis. Mammalian spermatogenesis originates in the germinal epithelium of the somniferous tubules of the male gonads (testes) from diploid primordial cells. These cells undergo repeated mitotic divisions to form a population of spermatogonia. By growth, a spermatogonium may differentiate into a diploid primary spermatocyte with the capacity to undergo meiosis. The first meiotic division occurs in these primary spermatocytes, producing haploid secondary spermatocytes. From these cells the second meiotic division produces four haploid meiotic products called spermatids. Almost the entire amount of cytoplasm then extrudes into a long whiplike tail during maturation and the cell becomes transformed into a mature male gamete called a sperm cell or spermatozoan.

Gametogenesis in female is called oogenesis. Mammailan oogenesis originates in the germinal epithelium of the female gonads (ovaries) in diploid primordial cells called oogonia. By growth and storage of much cytoplasm or yolk (to be used as food by the early embryo), the oogonium is transformed into a diploid primary oocyte with the capacity to undergo meiosis. The first meiotic division reduces the chromosome number by half and also distributes vastly different amounts of cytoplasm to the two products by a grossly unequal cytokinesis. The larger cell thus produced is called a secondary oocyte and the smaller is a primary polar body. In some cases, the first polar body may undergo the second meiotic division, producing two secondary polar bodies. All polar bodies degenerate, however, and take no part in fertilization. The second meiotic division of the oocyte again involves an unequal cytokinesis, producing a large yolky ootid and a secondary polay body. By additional growth and differentiation the ootid becomes a mature female gamete called an ovum or egg cell.

The union of male female gametes (sperm and egg) is called fertilization and reestablishes the diploid number in the resulting cell called a zygote. The head of the sperm enters the egg, but the tail piece (the bulk of the cytoplasm of the male gamete) remains outside and degenerates. Subsequent mitotic divisions produce the numerous cells of the embryo which become organized into the tissues and organs of the new individual.

**2. Gametogenesis in plants (Angiosperms)**

In plants male gametes are produced in stamen and female gametes are produced in pistil. The production of gametes involves two steps, ie sporogenesis and gametogenesis.

**Sporogenesis**

Production of microspores and megaspores is known as sporogenesis.

**Microsporogenesis**

Production of microspores in the anther is known as microsporogenesis. Pollen sac of the anther contains numerous Pollen Mother Cells (PMC) or microsporocyte. Each PMC or microsporocytes which are diploid in number undergo meiosis to produce four haploid cells or microspores (n). This process is known as microsporogenesis. The microspores mature into pollen grains mainly by thickening of their cell walls.

**Megasporogenesis**

Megasporogenesis occurs in ovules which are present in the ovary. A single cell in each ovule differentiates in to Megaspore Mother Cell (MMC) or megasporocyte. The MMC undergo meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule.

**Gametogenesis**

The production of male and female gametes from the microspore and the megaspore respectively is known as gametogenesis.

**Microgametogenesis**

This refers to the production of male gamete or pollen. During maturation of pollen, the microspore undergoes a mitotic division of the chromosomes without cytoplasmic division to produce one generative nucleus and one vegetative or tube

nucleus. Pollen grains are usually shed in this binucleate stage. Upon germination of the pollen grain on the stigmatic surface, the generative nucleuses divide mitotically without cytokinesis to products two sperm nuclei or male gametes. The pollen, along with the pollen tube, is known as microgametophyte. The pollen tube finally enters the ovule through small pore, micropyle and discharges the two sperm nuclei into the embryo sac.

**Megagametogenesis**

The functional megaspore undergoes three mitotic divisions of the chromosome without intervening cytokinesis producing a large cell with eight haploid nuclei called embryosac. The sac is surrounded by maternal tissues of the ovary called integuments and by the megasporangium (nucellus). At one end of the sac there is opening in the integuments and by the megasporangium (nucellus). At one end of the sac there is opening in the integuments known as micropyle through which the pollen tube will penetrate. Out of eight haploid nuclei three nuclei move to micropylar end and produce central egg cell and two synergid cells. One synergid is situated on either side of the egg cell. Another three nuclei migrate to the opposite end of the sac to give rise to antipodal cells. The two remaining nuclei called polar neclei unite near the center of the sac forming a single diploid fusion nucleus. The mature embryo sac is now ready for fertilization.

**Fertilization**

After germination of the pollen grain on the stigma, pollen tube grows down the style with a direction of tube nucleus and enters into the embryo sac through micropyle. Two sperm nuclei are released into the embryo sac. Pollen degenerate. One sperm nucleus fuses with egg nucleus to form diploid zygote, which will then develop to form a triploid (3n) nucleus. This triploid nucleus undergoes mitotic division to produce endosperm. The outermost layer of the endosperm cells is called aleurone. The embryo either may be surrounded by endosperm tissue or in some cases it is surrounded by diploid maternal tissues called pericarp. In the process of fertilization, since one sperm nucleus fertilizes egg nucleus and the other sperm nuclei fertilizes fusion nucleus, it is termed as double fertilization.

**Alternation of generations**

Life cycle of most plants has two distinctive generations: a haploid gametophytic (gamete bearing plant) generation and a diploid sporophytic (spore

bearing plant) generation. Gametophyte produce gametes which unite to form sporophtes which in turn give rise to spores that develop into gametophtes. This process is referred to as the alternation of generations.

## Questions

1. Chromosomal status of pollen is

i) 3n number ii) n number iii) 2n number iv) 4n number

**Ans: n number**

2. Chromosomal status of endosperm is

i) n number ii) 2n number iii) 3 n number iv) 4n number

**Ans: iii) 3 n number**

3. Name of fertilized egg is

i) Gamete ii) Gametophyte iii) Sporophyte iv) Zygote

**Ans: iv) Zygote**

4. No. of nuclei present in mature embryo sac is

i) 2 ii) 4 iii) 6 iv) 8

**Ans:**

5. No. of gametes formed from individual pollen is

i) One ii) Two iii) Three iv) Four

**Ans: ii) Two**

**Say True or False**

6. Triploid nucleus in the embryo sac divides meiotically to produce endosperm

**Ans: False**

7. Production of microspores in the anther is known as microsporogenesis

**Ans: True**

8. Only one functional megaspore is present in mature ovule.

**Ans: True**

9. Pollen mother cells of the anther undergo meiosis

**Ans: True**

10. Synergids are situated on either side of the egg cell

**Ans: True**

## Lecture No. 5

## Chromosomes

Chromosomes are the darkly stained bodies seen during the metaphase stage of mitosis. Strasburger discovered chromosomes in 1875 and the term chromosome was coined by Waldeyer in 1888. Chromosomes are composed of thin chromatin threads called chromonemata. These chromonemata undergo coiling & super coiling during prophase and it become readily observable by the light microscope. The main features of eukaryotic chromosomes are given below.

1. Chromosomes are clearly visible during mitotic metaphase. Hence, they are studied during metaphase.
2. Chromosomes bear genes in a linear fashion and thus are concerned with transmission of characters, from generation to generation.
3. Chromosomes of eukaryotes are enclosed by a nuclear membrane.
4. Chromosomes vary in shape, size and number in different species of plants and animals.
5. Chromosomes have property of self-duplication, segregation and mutation.
6. Chromosomes are composed of DNA, RNA and histones. DNA is the major genetic constituent of chromosome.

### Chromosome shape

Chromosome shape is usually observed during anaphase. The shape of chromosomes is determined by the position of centromere, a part of chromosome on which spindle fibres are attached during metaphase. Chromosomes have generally three different shapes, viz, rod shape, J shape and V shape. These shape are observed when the centromere occupies terminal, sub-terminal and median (middle) position on the chromosomes, respectively.

### Chromosome size

Chromosome size is measured at mitotic metaphase. It is measured in two ways *viz.*, in length and diameter. Plants usually have longer chromosomes than animals, the maximum length of chromosome is observed during interphase and minimum

during anaphase. Chromosome size varies from species to species. Maize chromosomes have the length of 8-12µ. Giant chromosomes have length upto 300µ.

**Chromosome number**

Each species has definite and constant somatic and gametic chromosome number. Somatic chromosome is the number of chromosomes found in somatic cells and it is represented as diploid number (2n). The somatic cells contain two copies of each chromosome (except sex chromosome) one of which is inherited from father while other is inherited from mother. These two chromosomes are identical in morphology, gene content and gene order and they are known as homologous chromosomes., Gametic cells or gamets contain one half of the somatic chromosome number which is represented by haploid number or (n). The genetic chromosome number of a true diploid species is called basic number. It is the minimum haploid chromosome number of any species, which is denoted by x. For example, in wheat, the basic number is 7, whereas the haploid number is 7, 14 and 21 for diploid, tetraploids and hexaploid species, respectively. Thus, haploid chromosome number differs from basic number. Both are same in case of true diploid species but differ in case of polyploidy species.

Number of chromosomes varies from 2n=4 (n=2) in *Haplopappus gracilis* (Compositae) to 2n=> 12000 in some Pteridophytes. In Plant kingdom, chromosome number is higher in dicots than in monocots.

**Chromosome structure**

The structure of chromosome becomes easily visible during metaphase due to coiling of interphase chromosomes. Each chromosome consists of seven parts, *viz*., (i) centromere, (2) chromatids, (3) seconday constriction and sataellite, (4) telomere, (5) chromomere, (6) Chromonema and (7) matrix. A brief description of these parts is given below:

**1. Centromere (Primary Constriction)**

It is a localized region of the chromosome with which spindle fibres are attaced during metaphase is known as centromeres of primary constriction or kinetochre. Centromere has four important functions, *viz*., (i) orientation of chromosomes at metaspace, (ii) Movement of chromosome during anaphase, (iii) formation of chromatids, and (iv) chromosomes shape. Centromere may occupy

various positions on the chromosome, *viz*., terminal, sub-terminal, median etc. Generally, each chromosome has one centromere, but in some cases, the number of centromere may vary from nil to many. The centromere divides the chromosome in to two arms of varying length.

**2. Chromatid**

One of the two distinct longitudinal subunits of a chromosome is called chromatid. These subunits of a chromosome get separated during anaphase. Chromatids are of two types *viz*., sister chromatids and non-sister chromatids. Sister chromatids originate from homologous chromosomes. Chromatids are formed due to chromosome and DNA replication during interphase. Two chromatids of a chromosome are held together by centromere. After separation at anaphase each chromatid becomes a chromosome.

**3. Secondary Constriction**

Some chromosome exhibits secondary constriction in addition to primary constriction. It may be present either in short or long arm of the chromosome. The chromosomal region between secondary constriction and nearest telomere (end of the chromosome) is called as satellite or trapant. The chromosome having satellite is known as satellite chromosomes. The position of secondary constriction in the chromosome is constant. The number of satellite chromosome in a genome varies from species to species.

**4. Telomere**

The two ends of the chromosomes are known at telomeres. Telomeres are highly stable and they do not fuse or unite with telomeres of other chromosomes. The structural integrity and individuality of the chromosome is maintained by telomeres.

**5. Nucleolar Organizer Region (NOR)**

During interphase, nucleolus of the cell is always associated with secondary construction of satellite chromosome. So the secondary constriction is also called as NOR. The NOR contain several copies of gene coding for ribosomal RNA.

**6. Chromomeres**

The chromosome of some of the species show small bead like structures called as chromomeres. The distribution of chromomeres in the chromosome is constant.

Available evidence indicates that chromomere represents a unit of DNA replication, chromosome coiling, RNA synthesis and RNA processing.

**7. Chromonema**

Under light microscope, thread like coiled structures are found in the chromosomes and chromatids which are called chromonema (plural chromonemata). Chromonema is considered to be associated with three main functions. It controls size of chromosomes, results in duplication of chromosomes and is the gene bearing portion of chromosomes.

**8. Matrix**

A mass of aromatic material in which chromonemata are embedded is called matrix. Matrix is enclosed in a sheath which is known as pellicle. Both matrix and pellicle are non genetic materials.

**Karyotype**

Karyotype is a phenotypic appearance of chromosomes of a particular species. In the study of kayotype, various features of chromosomes are taken into account *viz*., (i) number (ii) position of centromere (iii) size (iv) possibility of satellite (v) degree and distribution of meterchromatin etc. It is represented by arranging the somatic chromosome complements according the somatic chromosome complements according to their length keeping their centromeres in a straight line. Thus, the longest chromosome is placed in the extreme left and smallest in the extreme right.

**Idiogram**

Diagramatic representation of morphological features of haploid chromosome complements of a species is known as ideogram.

**Heterochromatin**

The region of the chromosome, which takes up deep stain during interphase and prophase, is called heterochromatin. It is classified into two types, constitutive heterochromatin and facultative heterochromatin.

i. **Constitutive Heterochromatin:** The regions centromere and telomere of the chromosome remain permanently in the heterochromatin stage. ie., it does not revert to euchromatic stage.

ii. **Facultative Heterochromatin:** It is the region of the chromosome which undergo euchromatin stage.

**Euchromatin**: The region of the chromosome, which takes up little stain during interphase, is called Euchromatin. It is the active region of the chromosome, involved in transcription.

**Classification of chromosomes**

Chromosomes can be classified in different ways. The various criteria which are usually used for classification of chromosomes include, (i) position of centromere, (ii) number of centromere, (iii) shape at anaphase, (iv) structure and appearance, (v) role in heredity essentiality, (vi) role in sex determination, and (vii) structure and function (Table 5.1). A brief classification on the bases of these criteria is presented below:

**Table 5.1. Classification and brief description of chromosomes**

| **Basis of Classification and types of chromosomes** | **Brief Description** |
|---|---|
| **1.Position of centromere** | |
| Metacentric chromosome | A chromosome in which centromere is located in the middle portion, such chromosomes assume V shape at anaphase. |
| Sub-metacentric chromosome | A chromosome in which centromere is located slightly away from the centre point or has sub median position, such chromosomes assume J shape at anaphase. |
| Acrocentric chromosome | A chromosome in which centromere is located very near to one end or has sub terminal position, it is called as sub-terminal chromosome. Such chromosome assumes J shape or rod shape during anaphase. |
| Telocentric chromosome | A chromosome in which centromere is located at one end is called as telocentric. |

| | |
|---|---|
| | Such chromosome assume rod shape during anaphase. |
| Holokinetic chromosome | A chromosome with diffused centromere. Centromere does not occupy a specific part of chromosome. Whole body of such chromosome exhibits centromeric activity. |
| **2. Number of Centromere** | |
| Acentric chromosome | A chromosome without centromere. Such chromosome remains as laggard during cell division and is eventually lost. |
| Monocentric Chromosome | A chromosome with one centromere. It represents normal type of chromosomes. |
| Dicentirc chromosome | A chromosome having two centromeres. Such chromosome makes dicentric bridge at anaphase and are produced due to inversion and translocations. |
| **3.Shape at anaphase** | |
| V shape chromosome | A chromosome which assumes V shape at anaphase. It includes metacentric chromosome. |
| J shape chromosome | A chromosome which assumes J shape at anaphase. It includes sub-metacentric and sub-terminal chromosomes. |
| Rod shape chromosome | A chromosome which assumes rod like shape during anaphase. |
| **4. Structure and appearance** | |
| Linear chromosome | A chromosome with liner structure or having both the ends free. Such chromosomes are found in eukaryotes. |

| | |
|---|---|
| Circular chromosome | A chromosome with circular shape and structure. They are found in bacteria and viruses. |
| **5. Essentially** | |
| A-Chromosome | Normal members of chromosome complements of a species which are essential for normal growth and development. |
| B-Chromosome | Chromosome which are found in addition to normal chromosome complements of a species. They are also called as accessory, supernumerary or extra chromosomes and are not essential for normal growth and development. |
| **6. Role in Sex Determiantion** | |
| Allosomes | Chromosomes which differ in morphology and number in male and female sex and contain sex determining genes. They are generally of two types, viz., X and Y or Z and W types (for details see under sex determination) |
| Autosomes | Chromosomes which do not differ in morphology and number in male and female sex and rarely contain sex determining genes. |
| **7. Structure and function** | |
| Normal Chromosome | Chromosomes with normal structure (shape and size) and function |
| Special Chromosome | Chromosome which significantly differ in structure and function from normal |

| | |
|---|---|
| | chromosomes. Such chromosomes include lamp brush chromosomes, polytene chromosomes and B-chromosomes. |

**Chromosome models**

Chromosome fibres are the basic units of chromosome structure. Chromosome model refers to organization of chromatin fibres in a chromosome. Two models namely folded fibre model and nucleosome solenoid model are widely accepted to explain chromosome structure and organization if chromatic fibre in a chromosome. These models are briefly described below:

**1. Folded fibre model**

This model was proposed by DuPraw in 1965. According to this mode, chromatin fibres are about 230° A in diameter. Each chromatid consists of single chromatin fibre, which is made up of single chromatin fibre, which is made up of single DNA double helix. The folding and super coiling of very long chromatin fibre causes reduction in length and increase in thickness of the chromosome.

**2. Nucleosome – solenoid model**

This model was proposed by Kornberg and Thomas in 1974. Chromatin is composed of DNA, RNA, histones and other proteins. Chromatin fibres are 300°A in diameter. The nucleosomes are sub units of chromatin and have bead like appearance. Each nucleosome is composed of a histone octamer and 146 base pairs (bp) of DNA. Each nuclesome consists of a core particle and linker or spacer DNA. The core particle has two copies each of H2B, H3 and H4 hsitone molecules. Thus, it has a histone octamer. The core particle is about 100°A in a diameter and 60°A in height. A duplex DNA strand is tightly wound around this core particle making two circles. Spacer of liner DNA has four base pairs. One molecule of histone H1 is connected with linker DNA. The super coiled nuclesome fibre is known as solenoid.

According to this theory, a very long molecule of DNA (146 bp) is packed into a single unit of nucleosome and several units of nucleosome constitute chromatin fibre. The chromatin fibre of 300°A which is visible under electron microscrope at

metaphase develops from the nucleosome fibres as a consequence of super coiling of latter. This model is universally accepted as a model of chromatin fibre organization.

**3. Special types of chromosome**

Some tissues of certain organisms contain chromosomes, which differ significantly from normal chromosomes in terms of either morphology or function. Such chromosomes are referred to as special chromosomes.

**1. Lambrush chromosome**

These are the special type of chromosomes found in primary oocyte nuclei in amphibians. Lambrush chromosomes are up to 1mm length. Each lambrush chromosome contains a central axial region where the two chromatids are highly condensed and numerous pairs of lateral loops give them a characteristics lambrush appearance. The loops are the transcriptionally active region of the single chromatids.

**2. Salivary gland chromosome / polytene chromosome / Giant chromosome**

The polytene chromosomes occure in the tissues of salivary glands, guts epithelium and malpighain tubules of many insects of the order Diptera.

In salivary gland cells of dipteral species giant chromosomes were observed by E.G.Balblani for first time in 1881. The chromosomes may reach a size of 20 times or more than the normal chromosomes. These salivary gland chromosomes have characterstics of somatic pairing as a result, the number of giant chromosomes in the salivary gland cells always appears to half that in the normal somatic cells. Giant chromosomes have distinct pattern of transverse banding, which consists of alternate chromatic and achromatic regions. The band occasionally forms reversible puffs known as chromosome puffs or Balbiani rings which are associated with differential generation.

Giant chromosomes represented by bundle of fibres, which arise by repeated cycle of endo reduplication of single chromatids (Endo – reduplication means chromatids replicates without cell division as a result of which number of chromonemata keep on increasing). That is why these chromosomes are also called as polytene chromosomes and the condition is refered to as polytene. The number of chromonemata per chromosome may be upto 2000 and in some cases it maybe aroung 16,000.

**Iso-chromosome**

A chromosome with two identical arms and identical genes is called as isochromosome. The arms are mirror images of each other. It is thought to arise when a centromere divides in the wrong plane yielding two daughter chromosomes, each of which carries the information of one arm only but present twice. At meiosis isochromosomes pair in three different ways. (i) Internal pairing (ii) Fraternal pairing (iii) Normal pairing

In internal pairing, the two arms of the isochromosomes pair with each other. In fraternal pairing, one or both of the arms of the isochromosomes pair with a homologous arm of another chromosome. In normal pairing, the isochromosome pairs with another one just like it.

**'B' chromosome**

It is a particular kind of supernumery chromosome that may or maynot be found in organisms as extra chromosomes over and above the standard diploid or polyploidy chromosome complements. The standard complements are called 'A' chromosome. The 'B' chromosomes found in natural population are recognized on the basis of following characteristics.

i. They are dispensable ( not found is all the individuals of the species or all the cells of the organisms)
ii. They are not homologous with any of the basic 'A' chromosomes.
iii. Their inheritance is non Mendalian.
iv. They are usually smaller than the 'A' chromosomes.
v. Generally they are genetically inert rarely organize nucleoli.
vi. When it present in higher number they suppress the vigour and fertility.
vii. Their origin and functions are largely unknown.

The most significant effect of 'B' chromosome is on seed and pollen fertility. Flowering time is generally delayed by 'B' chromosomes and has negative consequences for the organism as they have deleterious effect because of abnormal crossing over during meiosis.

**Ring chromosome**

The chromosomes of higher organisms usually have two ends and do not form a continuous ring. However, the chromosomes of lower organisms such as prokaryotes. (*E.coli*) normally have ring shaped chromosomes. Often such chromosomes are referred to as genophores, which are more than 1 mm in length and consists of a single DNA molecule.

Chromosomes in higher organisms are not naturally ring shaped. However ring chromosomes have been detected in humans, *Drosphila* and certain plant species. Ring chromosomes were most thoroughly studied in maize by Mc Clintock.

Normal chromosomes do not form rings because they are believed to have telomeres on each end. Telomeres prevent the union of chromosome arms into ring formation. A chromosome can form a ring chromosome by fusion of the raw ends only if it has two terminal deletions producing centric segment with two raw ends and two acentric fragements.

A ring chromosome lacks the genetic information that was carried by the terminally deleted fragments. Ring chromosomes are meiotically unstable and they are associated with several syndromes.

## Questions

1. Major genetic constituent of chromosome is

i) DNA ii) RNA iii)Histone proteins iv)Non histone proteins

**Ans: i) DNA**

2. Chromosome size is measured at

i) Mitotic prophase ii) Mitotic metaphase

iii) Miotic Anephase iv) Mitotic telephase

**Ans: ii) Mitotic metaphase**

3. Localized region of the chromosome with -------spindle fibres are attached during metaphase is called as

i) Telomere ii) Centomere

iii) Secondary construction iv) Nudeolar organizing region

**Ans: ii) Centomere**

4. Phenotypic appearance of chromosome is called as

i) Karyo type ii) Ideograms iii) Heterotype iv) Homotype

**Ans: i) Karyo type**

5. V shape chromosome is normally a

i) Telocentric ii) Holokinetic iii)Metacentric iv)Acrocentric

**Ans: iii)Metacentric**

**Say True or False**

6. Circular shapes chromosomes are not present in living organism

**Ans:False**

7. B Chromosome are supernumerary chromosomes and are dispensable

**Ans:True**

8. Polytene Chromosomes are which significantly differ in structure and function from normal chromosomes

**Ans:True**

9. Telomere prevent the fusion of one chromosome with other chromosome

**Ans:Ture**

10. Gene bearing portion of chromosome is called nucleosome

**Ans: False**

## Lecture No. 6

## Work of Mendel and his laws

Gregor Johann Mendel, father of Genetics was borne in 1822 to a family of poor farmer near Brunn in Austria, now it is part of Czechoslovakia. During young age, his education was seriously hampered by poverty and in order to continue his education he entered the Augustinian monastery at Brunn and was ordained a priest in 1847. A few years later, he was sent to the University of Vienna for training in physics, mathematics and the natural sciences. Although his performance at the University was not outstanding, his training provided him with many technical and mathematical skills that were of value in performing his later experiments. After completing his studies, he returned to Brunn in 1854 and he became a teacher and in 1857 began his famous experiments on peas in the monastery garden. After seven years experimentation he presented his findings before the Natural History Society of Brunn during 1865. This paper was published in the annual proceedings of the society in 1866 entitled "Experiment s in plant Hybridization" and he died in 1884.

### Rediscovery of Mendel's findings

Mendel's work was not recognized until 1900. In 1900, his finding was discovered independently by three scientists **Hugo De Vries** of Holland, **Carl Correns** of Germany and **Eric Von Tschermak** of Austria. Then only the significance of Mendel's work was realized.

*Pisum sativum*, Mendel's choice of materials

Mendel choose the garden pea *Pisum sativum* as his experimental material because,

1. Pea plants have constant clear-cut alternatives of characters.
2. These are annual plants. They could be grown and crossed easily.
3. They are normally self- fertilizing. But cross-fertilization can be done easily.
4. Hybrids are fully fertile.

**Merits of Mendel's method**

Mendel was able to discover the law of inheritance because of his intelligent methods of study and by the application of mathematics.

(i) Mendel selected garden pea which is a self fertilized short duration crop.

(ii) He always used pure breeding parents and did crossing experiments with in the same species.

(iii) He confined his study only on one character at a time.

(iv) After finding the inheritance of characters separately, he studied two characters together.

(v) He counted the number of each type of the progeny and he analyzed his numerical results in the form of ratios *i.e*., he applied mathematics to his findings.

**Characters studied by Mendel in pea plants**

Mendel studied seven pairs of contrasting characters. They are

| | | | |
|---|---|---|---|
| 1. | Color of unripe pods | : | Green or Yellow |
| 2. | Color of the seed coat | : | White or Grey |
| 3. | Colour of the cotyledons | : | Yellow or Green |
| 4. | Form of ripe pods | : | Inflated (full) or Constricted |
| 5. | Form of ripe seeds | : | Round or Wrinkled |
| 6. | Position of flowers | : | Axial or Terminal |
| 7. | Length of the stem | : | Tall or Dwarf |

**Law of segregation**

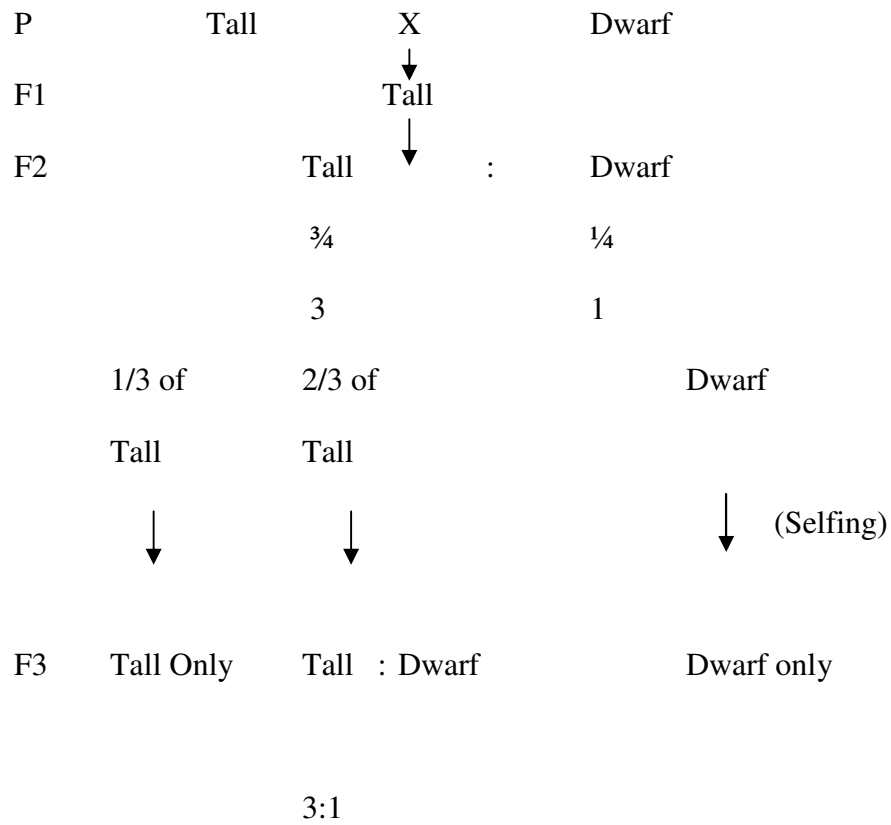
**Mendel's experiment**

Mendel tested the seven characters individually, by crossing a variety carrying a particular trait of a character (eg.Tall) with another variety carrying a different trait of the same character (eg.Dwarf). When he crossed tall variety with dwarf, he obtained

only tall plants in $F_1$ generation. When the $F_1$ plants were selfed the tall and dwarf plants were segregated in 3: 1 ratio in $F_2$ generation.

He made crosses for all seven characters and results appeared to fit the following pattern.

1. When crosses were made between the parents having contrasting characters, the $F_1$ always showed one of the parental traits and not the other.
2. The traits that had disappeared (or) hidden in the $F_1$ was reappeared in the $F_2$ generation, but only in the frequency of one-quarter of the total number.
3. Irrespective of which parent used as male or female, both direct and reciprocal crosses gave the same results.



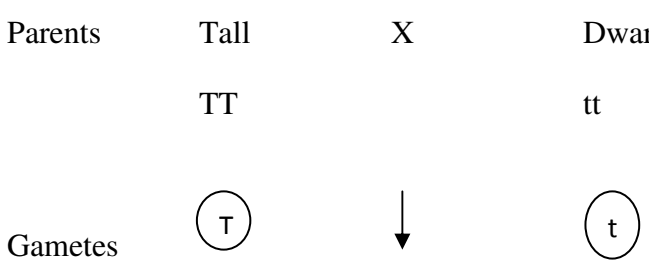

4. Mendel called the determining agent responsible for each trait 'a factor'. From the evidence of $F_1$ and $F_2$ generation, the 'Factor' that determine each trait could be hidden, but not destroyed.
5. Though the $F_1$ hybrids contain factor for both Tall and Dwarf traits, all the $F_1$ were tall. The phenomenon of the suppression of expression of one trait by another is called as dominance. The trait whose expression is suppressed in $F_1$

is called recessive. In Mendel's experiment tall is a dominant character designated capital letter T and dwarf by corresponding small letter't'.

6. The regular reappearance of hidden recessive trait revealed that the character is not blended together or not diluted in the hybrid offspring and factors determining each trait did not change throughout several generations of mating.
7. According to the symbols used, the Tall hybrid '$F_1$' contains factor T (since it is Tall) and't' (since it produces some dwarf plants in $F_2$). Since T is dominant over t, the Mendel's dwarf plant contains't'.
8. To find out the number of factor controlling each trait Mendel selfed the plants in $F_2$ generation. The 'dwarf' plants upon self fertilization always gave rise (breed true) to dwarf in all the generations. Whereas tall plants in $F_2$ did not always breed true i.e., 1/3 of the tall plants breed true and 2/3 of the tall $F_2$ plants upon self fertilization produce Tall and Dwarf plants in 3:1 ratio.
9. From the above results, it is clear that the hybrid plant for tallness contains two factors 'T' and't'. The true breeding tall and dwarf plants also contains TT and it respectively. So the true breeding line produce only one kind of gamete, whereas, the hybrid tall plants contain two kinds of factors which can separate or segregate from each other during gametogenesis and produce two kinds of gametes in equal proportion. The random combination of these gametes leads to the production of tall and dwarf progeny in the (phenotypic) ratio of 3:1 and with the genotype ratio of 1:2:1

**Law of segregation (law of purity of gametes)**

Allelic genes in a hybrid do not blend or contaminate each other, but segregate and pass in to different gametes during gametogenesis.



| | | | |
|---|---|---|---|
| $F_1$ | Tall | x | Tall |
| | Tt | | Tt |
| Gametes | T t | | T t |

| | (T) | (t) |
|---|---|---|
| (T) | TT (Pure Tall) | Tt (Hybrid Tall) |
| (t) | Tt (Hybrid Tall) | Tt (Pure Darf) |

Phenotypic ratio : 3:1 (Tall: Dwarf)

\ Genotypic ratio : 1:2:1 (TT, Tt,tt)

For example, the $F_1$ hybrid (Tt) of a monohybrid cross between tall (TT) and dwarf (tt) pea plant have one dominant allele (T) for tallness and one recessive allele (t) for dwarfness.

Though the tall and dwarf alleles remain together but does not contaminate or mix with any one. Both the alleles segregate to produce gametes either having dominant allele 'T' or recessive allele't'. The law of segregation is universal in its application and it has been found to occur in both plants and animals.

**Chromosomal basis of segregation**

In the anaphase stage of meiosis, the members of homologous chromosome pair segregate or separate from each other and move to opposite poles. As a result the allelic genes present in the homologous chromosome are also separated and carried to opposite poles.

**Incomplete dominance**

Dominance does not occur in all organisms always. Some gene may neither be dominant nor be a recessive. In this condition the hybrids are intermediate in phenotype. This is called incomplete dominance. For example when red flowered snapdragon is crossed with white flowered snapdragon, the $F_1$ hybrid is pink flowered.

| | | | |
|---|---|---|---|
| P | Red | X | White |
| | RR | ↓ | rr |
| G | (R) | | (r ) |
| $F_1$ | | Rr | |
| | | Pink | |

**F2 Generation**

| | **(R )** | **(r )** |
|---|---|---|
| **(R )** | RR<br>Red | Rr<br>Pink |
| **(r )** | Rr<br>Pink | Rr<br>White |

Phenotypic and Genotypic ratio 1 : 2 : 1

Red Pink White

The self fertilization of $F_1$ hybrid produces three types of offspring in $F_2$ generation in the ratio of 1 Red: 2 Pink: 1 White. This ratio is equal to that of genotypic ratio and of Mendel in $F_2$ plants.

**Law of independent assortment**

**Mendel's Experiment**

After studying the characters independently, Mendel dealt two characters, such as colour and shape of the seeds together. He crossed a pure line of yellow round seeds with a pure line of green wrinkled seeds. The $F_1$ dihybrids received genes for yellow and round characters from one parent and genes for green and wrinkled characters from another parent. Since yellow is dominant over green and round is dominant over wrinkled characters from another parent, all $F_1$ offspring were uniformly yellow round. When $F_1$ offspring were self fertilized, they produced four kinds of offspring in $F_2$ in 9:3:3:1 ratio.

| Types of offspring | Number | Ratio |
|---|---|---|
| Yellow Round | 315 | 9 |
| Yellow Wrinkled | 101 | 3 |
| Green Round | 108 | 3 |
| Green Wrinkled | 35 | 1 |

The double dominants are more frequent and the double recessives are least frequent.

P Yellow Round x Green wrinkled

YYRR yyrr

G (YR) (yr )

$F_1$ YyRr x YyRr

(Yellow Round)

G (YR) (Yr) (YR) (Yr)

(yR) (yr) (yR) (yr)

Gamete formation in $F_1$

Y → R = (YR)

Y → r = (Yr)

y → R = (yR)

y → r = (yr)

**$F_2$ generation**

| | **(YR)** | **(Yr)** | **(yR)** | **(yr)** |
|---|---|---|---|---|
| **(YR)** | YY RR<br>Yellow<br>Round | YY Rr<br>Yellow<br>Round | Yy RR<br>Yellow<br>Round | Yy Rr<br>Yellow<br>Round |
| **(Yr)** | YY Rr<br>Yellow<br>Round | YY rr<br>Yellow<br>Wrinkled | Yy Rr<br>Yellow<br>Round | Yy rr<br>Yellow<br>Wrinkled |
| **(yR)** | Yy RR<br>Yellow<br>Round | Yy Rr<br>Yellow<br>Round | yy RR<br>Green<br>Round | yy Rr<br>Green<br>Round |
| **(yr)** | Yy Rr<br>Yellow<br>Round | Yy rr<br>Yellow<br>Wrinkled | yy Rr<br>Green<br>Round | yy rr<br>Green<br>Wrinkled |

Phenotypic ratio 9 : 3 : 3 : 1

Yellow Round | Yellow wrinkled | Green Round | Green wrinkled

**Mendel's Interpretation**

The factor for yellow color many be represented by Y and the green by y; the round seed by R and wrinkled by r. Thus, a pure line yellow round is YYRR and a pure line green wrinkled is yy rr. The gametes produced by these parents are YR and yr respectively. The $F_1$ offspring formed by the union of these gametes have the genotype of Yy Rr.

The phenotype is yellow round. Since the parents differ in two characters, this offspring is called dihybrid.

During gametogenesis, the $F_1$ dihybrid produces four kinds of gametes YR, Yr, yR and yr because, the segregation of the seed color alleles occurs independently of the segregation of the seed shape alleles. This is called law of the independent assortment.

**Law of independent assortment**

"The segregation of one pair of allele is independent of the segregation in any other pair of allele".

In other words, when two or more independent characters are considered together, the factors responsible for them assort themselves freely and at random when gametes are formed.

## Questions

1. Suppression of expression of one trait by another is called as

   i) Dominance ii) Codominance

   iii) Incomplete dominance iv) Segregation

   **Ans: i) Dominance**

2. When Mendel crossed a pure breeding dwarf with pure breeding tall, he obtained

   i) Any one type in $F_1$ ii) obtained both tall and dwarf in $F_1$

   iii) Obtained only tall plants iv) Obtained only dwarf plants

   **Ans: iii) Obtained only tall plants**

3. Phenotypic expression of monohybrid cross in case of incomplete dominance is

   i) 3:1 ii) 9:3:3:1 iii) 1:2:1 iv) 1:1

   **Ans: ii) 9:3:3:1**

4. Phenotypic $F_2$ ratio of a normal dihybrid cross is

   i) 3:1 ii) 9:3:3:1 iii) 1:2:1 iv) 1:1:1:1

   **Ans: ii) 9:3:3:1**

5. Mendel belongs to the country

   i) Austria ii) Australia iii) Germany iv) Hollana

   **Ans: i) Austria**

**Say True or False**

1. Mendel called the present day ‘gene’ as ‘factor’

   **Ans: True**

2. Mendel carried out his hybridization experiments in his college

   **Ans: False**

3. Pea plants are perennial and continuously flowering. Hence hybridization experiments can be carried out throughout the year

   **Ans: False**

4. Pea is a cross pollinated crop

   **Ans:False**

5. Mendal totally studied seven characters

   **Ans:True**

## Lecture No.7

## Chromosomal basis of independent assortment

Among two pairs of homologous chromosomes, the members of one pair move to opposite poles independently of the members of the other pair. As a result of this independent assortment of chromosomes, the genes present in the non-homologous chromosomes undergo independent assortment.

The four kinds of gametes, YR, Yr,yR and yr produced by $F_1$ dihybrid unite at random and produce 16 types of offspring in $F_2$ generation in the ratio of 9 yellow Round 3 Yellow Wrinkled, 3 Green Round and 1 Green Wrinkeld.

This ratio shows that each pair of alleles behaves independently and bears no permanent association with other pair of alleles. The allele Y is found along with the allele R in the parent. But it does not always remain associated with it. The allele Y becomes associated with the allele r also. Thus, the independent assortment of alleles forms the basis for the 9:3:3:1 ratio.

**Dihybrid Test Cross**

A dihybrid Yy Rr is crossed with the double recessive parent yyrr. The dihybrid produces four kinds of gametes namely YR, Yr,yR and yr in equal proportions. The green wrinkled produces only one kind of gamete. The expected result is Yellow round, yellow wrinkled, green round and green wrinkled in 1:1:1:1 ratio. In actual experiment, the name ratio was obtained.

| P | Yellow Round | X | Green Wrinkled | |
|---|---|---|---|---|
| | Yy Rr | | yy rr | |
| G | (YR) (Yr) (yR) (yr) | | (yr) | |
| $BC_1F_1$ | Yy Rr | Yy rr | yy Rr | yy rr |
| | 1 | 1 | 1 | 1 |
| | Yellow : | Yellow : | Green : | Green : |
| | Round | Wrinkled | Round | Wrinkled |

**Table 7.1 Number of different kinds of gametes produced by $F_1$ and number of individuals in perfect $F_2$ population**

| Number of genes segregating | Number of different kinds of gametes produced by $F_1$ | Number of individuals in the perfect $F_2$ | Number of different homozygous genotype in $F_2$ | Number of different phenotype in $F_2$ (complete dominance) |
|---|---|---|---|---|
| 1 | 2 | 4 | 2 | 2 |
| 2 | 4 | 16 | 4 | 4 |
| 3 | 8 | 64 | 8 | 8 |
| N | $2^n$ | $4^n$ | $2^n$ | $2^n$ |

**Reasons for over looking of Mendel's Results**

The important reasons for the neglect of Mendels findings related to mechanism of inheritance for a long time are given below.

i. Mendel generalized his results based on his studies on garden pea. Later on he worked on hawkweed (Hieraceum) on the advice of C.V.Nagali. Mendel could not prove his results on this plant as the embryo is formed from the ovule without fertilization.

ii. Mendel explained his results with help of mathematics. The scientists at that time did not appreciate this approach.

iii. Mendel could not support his findings through cytological studies.

iv. After his failure to demonstrate the results on hawkweed, he did not give proper publicity to his work and kept quite.

**Dominance**

The suppression of the expressions of one trait of a character by another trait of the same character is called dominance.

**Types of dominance**

**i. Complete dominance**

If the phenotypes of the heterozygotes as well as homozygote dominant individuals are identical then the concerned dominant allele is said to have complete dominance.

(eg.,) In garden pea, the homozygote (YY) and heterozygote (Yy) individuals produce only yellow colour seeds. (Yy=YY)

**ii. Incomplete dominance**

Some alleles are neither dominant nor recessive. In this condition, the hybrids are intermediate in phenotype. This is called incomplete dominance. (Rr≠RR)

(eg.) Flower colour in 4 'O' clock plant.

a. Homosygous dominant genotype (RR) produce Red colour flower.
b. Homozygous recessive genotypes (rr) produce White colour flower.
c. Heterozygous genotype (Rr) produce Pink colour flower.

**iii. Co-domiance**

Expression of phenotypic trait of both homozygotes in the heterozygote condition is called co-dominance. In Co-dominance, both alleles of gene have the full expression in heterozygous individuals.

(eg.) Coat color in shorthorn cattle or Blood group in human beings.

In shorthorn cattle, a pair of gene controls, red and white coat color. Crosses between red ($C^RC^R$) and white ($C^W$ $C^w$) cattle produce $F_1$ offspring of reddish gray or roan. Superficially this would seem to be a case of incomplete dominance, but close examination of roan animal reveals that the coat colour is composed of a mixture of red hairs and white hairs. The coat color of the roan is not intermediate between red and white but due to the phenotypic expression of both homozygotes.

Genotypic and phenotypic ratios are identical in incomplete dominance and Co-dominance. The difference lies in the operating ways of the gene.

**iv. Over dominance or Hetero dominance or Super dominance**

The phenomenon of expression of phenotype in heterogygote in greater intensity than in the two concerned homozygotes is called over dominance.

The over dominance is not the property of an allele and it is due to the heterozygous state (inter allelic interaction) of the concerned gene.

(eg.) Eye colour in fruit fly

Dominant allele WW, Ww-Red eye

Recessive allele ww-White eye

Eye pigments seprateridine and himmelblaus are present in low concentrations in ww types. WW have relatively higher concentrations of these pigments. However, files heterozygous for this gene Ww have an appreciably higher concentrations of these two pigments than the two homozygotes.

## Questions

1. Formula for finding out the no.of different kinds of gametes produced by $F_1$ is where n is the no.of segregating genes.

   i) n2 ii) 2n-1 iii) $2^n$ iv) $4^n$

   **Ans: iii) $2^n$**

2. Formula to find out the no.of different kind of individuals in perfect $F_2$ is

   i) $n^2$ ii) 2n-1 iii) $2^n$ iv) $4^n$

   **Ans: iv)$4^n$**

3. Expression of phenotypic trait of both homozygotes in the heterozygote condition is called as

   i) Dominance ii) Codominance

   iii) Incomplete dominance iv) Over dominance

   **Ans: ii) Codominance**

4. Progenies obtained by crossing YyRr with yyrr are in the ratio of

   i) 9:3:3:1 ii) 1:2:1 iii) 3:1 iv) 1:1:1:1

   **Ans: iv) 1:1:1:1**

5. Flower colour character is controlled by the alleles showing

   i) Incomplete dominance ii) Codominane

   iii) Over dominate iv) Complete dominance

   **Ans: i) Incomplete dominance**

**Say True or False**

1. The plant in which Mendel could not prove his findings was *Hieraceum sp.*

   **Ans: True**

2. Independent assortment of alleles forms the basis for 9:3:3:1 ratio

   **Ans: True**

3. Crossing a double heterozygote with another double recessive parent is called test cross

   **Ans: True**

4. Use of mathematics in hybridization experiments by Mendel was well appreciated in Mendel's period

   **Ans: False**

5. Phenotypic expression of a heterozygote in greater intensity than that of the two concerned homozygotes in called heterodominance.

   **Ans: True**

## Lecture No.8

## Genetic terminologies

### Gene

An inherited factor that determines biological characteristics of an organism is called gene. In modern term, gene may be a segment of DNA which code for single polypeptide chain.

### Allele

Alternative form of a gene occupying the same locus of the homologous chromosome.

### Locus

The position of gene on the chromosome.

### Dominance

The suppression of expression one allele by another allele of the same gene is called dominance.

### Recessive

The characters which lack the ability to express in $F_1$ generation is called recessive.

### Genotype

Genetic constitution (make up) of an individual is called genotype. The genotype of tall plant is TT/Tt and dwarf plant is tt.

### Phenotype

The appearance of an individual produced by the genotype in co-operation with the environment is called phenotype.

### Homozygous

1. Individual having identical alleles for a character is known as homozygous genotype.

2. The homozygous genotypes are produced by the union of gametes carrying identical alleles.

3. A heterozygote produces different kinds of gametes.

**Pure line**

A pure line is a plant which breeds true on selfing, i.e., produces only one type of offspring on self pollination.

**Monohybrid**

The $F_1$ offspring produced by crossing two true breeding parents, which differ in one character only. The monohybrid individuals are heterozygous at one locus i.e, Monohybrid tall individual has 'Tt' genotype.

**Dihybrid**

The $F_1$ offspring produced by crossing two true breeding parents, which differ in two characters. Dihybrid individuals are heterozygous at two loci. The genotype of yellow round is Yy Rr.

**Polyhybrid**

The $F_1$ offspring produced by crossing two true breeding parents, which differ for more than three characters, is known as polyhybrid.

**Back cross**

If the $F_1$ progeny is mated back to one of their parents, the mating is termed as Back cross.

| | Tall x Dwarf | (or) | Tall x Dwarf |
|---|---|---|---|
| P | TT ↓ tt | | TT ↓ tt |
| G | (T) (t) | | (T) (t) |
| $F_1$ | Tt (Tall) | | Tt (Tall) |
| | TtxTT | | Ttxtt |
| $BC_1F_1$ | Tt:TT | $BC_1F_1$ | Tt:tt |

**Test cross**

It is a cross between the $F_1$ hybrid and its recessive parent. The purpose of test cross is to discover how many different kind of gametes are being produced by the individual whose genotype is in question.

Heterozygous Recessive parent

Tt x tt

(Tall) (Dwarf)

Back cross progeny Tall: Dwarf

Tt tt

**Uses of test cross**

**1. Test cross verifies the Mendel's factorial hypothesis**

According to Mendel, a monohybrid tall (Tt) produce two kinds of gametes in equal proportion and recessive parent produce only one kind of gamete't' .Hence this back cross should give Tall and dwarf plants in 1 : 1 ratio. In actual experiment also we get tall and dwarf in '1:1' ratio. Thus Mendel's factorial hypothesis is verified.

**2. Test cross is used for identifying the genotype of an unknown parent**

A tall pea plant may be either homozygous (TT) or heterozygous (Tt). Its genotype may be determined by test cross. If the test cross progeny were tall, then the unknown tall genotype is 'homozygous'. If that test cross progeny have tall and dwarf plants in equal proportion, then the unknown genotype is heterozygous.

## Questions

1. Position of gene on the chromosome is called as

   i) Allele ii) Gene iii) Locus iv) Genotypes

   **Ans: iii) Locus**

2. Individual having identical alleles for a character is called as

   i) Heterozygous genotype ii) Homozygous genotype

   iii) Hemizygous genotype iv) Incompletely dominant genotype

   **Ans: ii) Homozygous genotype**

3. Cross between $F_1$ hybrid and its recessive parent is called as

   i) Mono hybrid cross ii) Dihybrid cross iii) Test cross iv) Pure cross

   **Ans: iii) Test cross**

4. A plant producing only one type of offspring on self pollination is called as

   i) Dominant genotype ii) Co-dominant genotype

   iii) Pureline iv) Incompletely dominant genotype

   **Ans: iii) Pureline**

5. The trait which is not expressed in $F_1$ generation is considered to be

   i) Dominant ii) Codominant iii) Recessive iv) Incompletely dominant

   **Ans: iii) Recessive**

**Say True or False**

1. Genetic constitution of an individual is called as genotype

   **Ans: True**

2. $F_1$ offspring produced by crossing two true breeding parents which differ for more than three characters is known as multihybrid

   **Ans:False**

3. Phenotype is produced by the genotype in cooperation with the environment

   **Ans: True**

4. Union of gametes carrying identical alleles results in heterozygous genotypes.

   **Ans:False**

5. In test cross, the phenotypic and genotypic ratios are same.

   **Ans: True**

# Lecture No. 9

## Deviation from Mendelian ratios

In Mendel's dihybrid cross, each pair of allelic gene influences one character. Two or more pairs of genes may influence some times a single character. Depending upon the form of interaction the 9:3:3:1 ratio is modified in various ways. The phenomenon of two or more genes affecting the expression of each other in various ways in the development of a single character of an organism is shown as gene interaction.

**Inheritance of comb pattern in fowls (without modification of 9:3:3:1 ratio)**

This was reported by W.Bateson and R.C. Punnett. Domestic breeds of chickens have different comb shapes. Rose comb is found in Wyandotte breed, pea comb in Brahmas and single comb in leghorns. Each of these types breeds true. When Rose comb fowl is crossed with Pea comb, the $F_1$ walnut combed birds are crossed together, four kinds of combs appear in the $F_2$ generation in the ratio of 9 walnut:3 rose: 3 Pea:1 single. In this cross neither single comb nor walnut was expressed in the original parent lines. These two phenotypes were explained as the result of gene product interaction.

The $F_2$ ratio of 9:3:3:1 is expected only in dihybrid cross. The number of Walnut in $F_2$ generation (9) indicates that they are double dominants. The number of single comb in $F_2$ generation (1) indicates that they are double recessives. The Walnut comb depends on the presence of two dominant genes R and P, R alone produces rose comb and P alone produces pea cob. The absence of both R and P produces single comb.

| | | | |
|---|---|---|---|
| Parents | Rose | x | Pea |
| | RRpp | ↓ | rrPP |
| Gametes | (Rp) | | (rP) |
| $F_1$ | RrPp x Rr Pp<br>Walnut | | |
| Gametes | (RP) (Rp) (rP) (rp) | | |

**F2 generation**

| | **(RP)** | **(Rp)** | **(rP)** | **(rp)** |
|---|---|---|---|---|
| (RP) | RR PP<br>Walnut | RR Pp<br>Walnut | Rr PP<br>Walnut | Rr Pp<br>Walnut |
| (Rp) | RR Pp<br>Walnut | RR pp<br>Rose | Rr Pp<br>Walnut | Rr pp<br>Rose |
| (rP) | Rr PP<br>Walnut | Rr Pp<br>Walnut | rr PP<br>Pea | rr Pp<br>Pea |
| (rp) | Rr Pp<br>Walnut | Rr pp<br>Rose | rr Pp<br>Pea | rr pp<br>Single |

Although the usual 9:3:3:1 ratio was obtained, the result from this cross was unusual in three important respects.

i. The $F_1$ resembles neither parent-a new character appears in the $F_1$ – Walnut.

ii. Two phenotypes (Walnut and single) not expressed in the original parents appeared in $F_2$

iii. The genes 'R' and 'P' were non-allelic and the comb pattern is influenced by two different genes.

**Epistasis**

Epitstasis is a phenomenon in which the expression of one gene is masked or prevented by another non-allelic gene. The gene which prevents the expression of another gene is called epistatic gene, the gene whose expression is masked is called hypostatic gene. Epistasis should not be confused with dominance. Epistasis is the interaction between different genes (non-alleles) where as dominance is the interaction between different alleles of the same gene.

**Epistatic interactions (Modification of 9:3:3:1 ratio)**

When epistasis is operative between two gene loci, the number of phenotypes appearing in the offspring from dihybrid parents will be less than four. There are six

types of epistatic ratios commonly recognized, three of which have 3 phenotypes and the other three having only 2 phenotypes.

**(i) Dominant epistasis (12:3:1) or Epistatic gene interaction**

In dominant epistasis, a dominant allele at one locus can mask the expression of both alleles (dominant and recessive) at another locus, it is known as dominant epistasis.

When the dominant allele at one locus, for example A allele, produces a certain phenotype regardless of the allelic condition of the other locus, then the 'A' locus is said to be epistatic to the B-locus. Furhter more, the dominant allele A is able to express itself in the presence of either B or b, then this epistasis is said to be dominant epistasis. Only when the genotype of the individual is homozygous recessive at the epistatic locus (aa) can the alleles of the hypostatic locus (B or b) be expressed. Thus the genotypes A-B- and A-bb produces the same phenotype, where as aaB- and aabb produce two additional phenotypes. The classical 9:3:3:1 ratio becomes modified into a 12:3:1 ratio.

In sorghum, the nature of the grain is either pearly or chalky. When a plant with pearly grains and another with chalky grains are crossed the $F_1$ is pearly. In the $F_2$ there is segregation of 3 pearly: 1 chalky. Similarly, the colour of the grain either red or white. When a plant with red grains in crossed with white grains the $F_1$ in red and the $F_2$ shows a segregation of 3 red: 1 white.

Red colour of the grain masks another character ie., the pearliness or chalkiness of grain. When the colour of the grain is white, it is possible to say whether it is pearly or chalky but when the colour is red it is not possible to find out whether it is pearly or chalky.

## Questions

1. The gene which prevents the expression of another gene is called as

   i) Epistatic gene  ii) Hypostatic gene  iii) Abnormal gene  iv) Negative gene

   **Ans: i) Epistatic gene**

2. When epistasis operates between two gene loci, the no. of the phenotypes appearing in the offspring from dihybrid parents is

   i) More then four  ii) Four  iii) Less than four  iv) Four or less than four.

   **Ans: iv) Four or less than four.**

3. Gene interaction in which a dominant allele of one locus mask the expression of both the alleles of the other locus is called as

   i) Dominant epistasis (12:3:1)  ii) Recessive epistasis(9:3:4)

   iii) Additive gene interaction (9:6:1)  iv) Duplicate dominant interaction (15:1)

   **Ans: i) Dominant epistasis (12:3:1)**

4. Regarding sorghum grain colour, red colour trait is

   i) Epistatic over white colour trait  ii) Hypostatic over white colour trait

   iii) Dominant over white colour trait  iv) Recessive over white colour trait

   **Ans: i) Epistatic over white colour trait**

5. Regarding comb shape in fowl when a true breeding rose combed fowl is crossed with a true breeding pea comb, $F_1$ has

   i) Rose comb  ii) Pea comb  iii) Walnut comb  iv) Single comb

   **Ans: iii) Walnut comb**

**Say True or False**

1. Regarding the inheritance of comb pattern in fowl, the phenotypic ratio in a cross involving true breeding rose comb with the true breeding pea combed fowl, is 9:6:1.

   **Ans:** False

2. Regarding the inheritance of comb pattern in fowl, a new phenotype appears in $F_2$ which was not seen in $F_{2,}$ which was not seen in $F_1$ or in parents, when true breeding rose combed on true breeding pea combed fowl are used as parents.

   **Ans:** True

3. In dominant epistatic interaction, interaction between two alleles of a gene is responsible for obtaining $F_2$ ratio as 12:3:1

   **Ans**: False

4. Regarding the inheritance of grain colour in sorghum, white colour is dominant over red colour

   **Ans:** False

5. Regarding the inheritance of grain colour in sorghum, pearliness or chalkiness of grain is expressed only when the grain is white

   **Ans:** True

## Lecture No.10

### (i)Recessive epistasis (9:3:4) or Supplementary gene interaction

In recessive epistasis the recessive allele of ne locus masks the expression of both dominant and recessive alleles at another locus. It is known as recessive epistasis.

**Supplementary gene**

Gene which by itself has no effect but qualitatively alters the effect of another gene is the supplementary gene.

If the recessive genotype at one locus (aa) suppress the expression of alleles at the B-locus, the A-locus is said to exhibit recessive epistasis over the B locus. Only if the dominant allele is present at the 'A' locus can the alleles of the hypostatic B-locus be expressed. The genotypes A-B- and A-bb produce two additional phenotypes. The 9:3:3:1 ratio becomes a 9:3:4 ratio.

Eg. Coat colour in mice is either agouti, black or albino.

Agouti Colour is commonly occurring one (wild type) and is characterized by colour banded hairs. The hair near the body is gray followed by yellow band and finally the distal part is either black or brown.

The agouti and black coat colour in mice is controlled by 'A' and 'a' alleles respectively. Another non allelic dominant gene 'C' controls the production of an enzyme which converts a colorless precursor into melanin pigment and is required for the production of any pigment. The homozygous recessive'cc' lacks the enzyme, no melanin is produced and the animal is white coated.

### iii. Duplicate genes with cumulative effects (9: 6: 1) or Additive gene interaction

Two non-allelic genes have similar effect when they are separate, but produced enhanced effect when they come together. Such gene interaction is known as duplicate genes with cumulative effect.

If the dominant condition (either homozygous or heterozygous) at either locus (but not both) produces the same phenotype, the $F_2$ ratio becomes 9: 6: 1. For example, where the epistatic genes are involved in producing various amounts of

substance such as pigment, the dominant genotypes of each locus may be considered to produce one unit of pigment independently. Thus genotypes A-bb and aaB- produce one unit of pigment each and therefore have the same phenotype. The genotype aabb produces no pigment, but in the genotype. A-B- the effect is cumulative and two units of pigments are produced. The 9 : 3 :3 :1 ratio is modified into 9 : 6 : 1 ratio.

In a Cross between two light purple grains *ie*., $P_1$ and $P_2$ the $F_1$ was with dark purple grains. The $F_2$ segregated for 9 dark purple: 6 light purple: 1 white. Light purple of the grains is evidently due to the presence of a dominant gene $P_1$ or another dominant gene $P_2$. The two non-allelic dominant genes $P_1$ and $P_2$ possess an additive effect and the colour of the grain is dark purple when the genes $P_1$ and $P_2$ are present together. When both the dominant genes are absent, the color of the grain is white.

**iv. Duplicate Dominant genes (15:1) or Duplicate gene interaction**

Duplicate genes are two pairs of alleles either alone or together produce the same effect. They are identical genes but are situated on two different pairs of chromosomes. Each gene is dominant to its allele but does not add to the effect of the other. Eg. Floating habit in rice.

When a non floating rice strain is crossed with a floating strain, the $F_1$ is non floating. The $F_2$ segregates for 15 non floating and 1 floating habit. The presence of a single dominant allele of any one of the two genes governing the trait produces the dominant phenotype ie., non floating habit while recessive phenotype ie., floating habit is produced only when both the genes are in the homozygous recessive state.

The 9: 3: 3: 1 ratio is modified in to a 15:1 ratio if the dominant alleles of both loci each produce the same phenotype without cumulative effect.

## Questions

1. In recessive epistatic interaction, the phenotypic $F_2$ ratio obtained is

   i) 9:3:4 ii) 9:6:1 iii) 15:1 iv) 9:3:3:1

   **Ans: i) 9:3:4**

2. In additive gene interaction, the phenotypic $F_2$ ratio obtained is

   i) 9:3:4 ii) 9:6:1 iii) 15:1 iv) 9:3:3:1

   **Ans: ii) 9:6:1**

3. In duplicate dominant gene interaction, the phenotypic $F_2$ obtained is

   i) 9:3:4 ii) 9:6:1 iii) 15:1 iv) 9:3:3:1

   **Ans: iii) 15:1**

4. Coat colour in mice is controlled by the gene interaction of

   i) Additive gene interaction ii) Duplicate gene interactive

   iii) Supplementary gene interaction iv) Dominant epistatic interaction

   **Ans: iii) Supplementary gene interaction**

5. Example for duplicate dominant gene interaction is

   i) Grain colour in sorghum ii) Floating habit in rice

   iii) Comb pattern in fowl iv) Node colour in sorghum

   **Ans: ii) Floating habit in rice**

**Say True or False**

1. In additive gene interaction dominant alleles of both the two loci produce same phenotype without cumulative effect

   **Ans:** True

2. Additive gene interaction in also called as duplicate gene with non-cumulative effects.

   **Ans:** False

3. Supplementary gene interaction is also called as dominant epistasis

   **Ans:** False

4. Duplicate gene interaction is also called as duplicate dominant gene interaction

   **Ans:** True

5. In supplementary gene interaction, dominant genotype at one locus suppresses the expression of alleles in the other locus.

   **Ans:** False

# Lecture No.11

## (i) Duplicate Recessive Genes (9:7) or Complementary Gene Interaction

**Complementary genes**

Non allelic genes that act together to produce a phenotype different from that produced by either alone.

In the case where identical phenotypes are produced by both homozygous recessive genotypes, the $F_2$ ratio becomes 9:7. The genotypes aa B-, A-bb and aabb produce one phenotype. Both dominant alleles, when present together, complement each other and produce a different phenotype.

In sweet pea, the development of purple flowers requires the presence of two dominant genes $P_1$ and $P_2$. When either $P_1$ and $P_2$ or both the genes in homozygous recessive condition produce white flowers. Since both the dominant alleles P1 and P2 when present together, they complement each other and produce a new phenotype and hence called complementary genes.

**i. Dominant and Recessive interaction (13:3) or Inhibitory gene interaction**

In this type a dominant allele at one locus can mask the expression of both alleles at second locus.

Only two F2 phenotypes result when a dominant genotype at one locus (eg.A-) and the recessive genotype at the other (bb) locus produce the same phenotypic effect. Thus A-B-, A-bb and aabb produce one phenotype and aaB- produces another in the ratio of 13:3. Eg Node colour in sorghum.

In sorghum, when crosses were made between plants with purple node and green node, the $F_1$ was with purple node. The $F_2$ segregated for 3 purple and 1 Green. In certain other crosses between plants with purple node and green node, the $F_1$ was with green node. Since purple is dominant over green, the $F_1$ is expected to be purple, but it is observed to be green, the gene for purple node is unable to express itself probably because of the presence of another gene. This gene is called inhibitory gene. It is capable of inhibiting the production of purple colour. Plants are purple, only if

they possess the gene for purple colour, in the absence of the inhibitory gene. In the presence of the inhibitory gene, plants with the gene for purple are unable to exhibit the purple colour and are only green. Plants, which do not have the gene for purple colour, are also green whether they have the inhibitory gene or not. The summary of all six epistatic ratios are given in the Table 11.1.

| | **A-B-** | **A-bb** | **aaB-** | **aabb** |
|---|---|---|---|---|
| Classical ratio | 9 | 3 | 3 | 1 |
| Dominant epistasis/Epistatic gene interaction | 12 | | | 1 |
| Recessive epistasis / Supplementary gene interaction | | | | |
| Duplicate genes with cumulative effect / Additive gene interaction | | | | 1 |
| Duplicate dominant genes / Duplicate gene interaction | 15 | | | |
| Duplicate recessive genes / Complementary gene action | 9 | | | |
| Dominant and recessive interaction / Inhibitory gene action | 13 | | | |

## Questions

1. Phenotypic $F_2$ ratio obtained with two genes due to duplicate recessive genes is

   i) 9:7 ii) 9:3:3:1 iii) 13:3 iv) 9:6:1

   **Ans: i) 9:7**

2. Two genes controlled phenotypic F2 ratio due to inhibitory gene interaction is

   i) 9:7 ii) 9:3:3:1 iii) 13:3 iv) 9:6:1

   **Ans: iii) 13:3**

3. An example for complementary gene interaction

   i) Node colour in sorghum ii) Flower colour in sweet pea

   iii) Coat colour in mice iv) Floating habit in rice

   **Ans: ii) Flower colour in sweet pea**

4. In which gene interaction and aabb produces one phenotype and aaB-produces another phenotype

   i) Inhibitory gene interaction ii) Supplementary gene interaction

   iii) Duplicate gene interaction iv) Complementary gene interaction

   **Ans: i) Inhibitory gene interaction**

5. Number of phenotypic classes produced by complementary gene interaction in $F_2$

   i) 1 ii) 2 iii) 3 iv) 4

   **Ans: ii) 2**

**True or False**

1. The genes that are involved in complementary gene interaction are non-allelic

   **Ans:True**

2. In complementary gene interaction, two dominant genes are required to produce a phenotype

   **Ans: True**

3. Node colour in sorghum is produced by complementary gene interaction

**Ans: False**

4. Inhibitory gene interactions produce three phenotypic classes in $F_2$

   **Ans: False**

5. Inhibitory interaction is also known as dominant and recessive interaction

   **Ans: True**

## Lecture No.12

## Lethal genes, Pleiotrophy, Penetrance, Expressivity, Phenocopy, Multiple alleles, Pseudo alleles and Isoalleles.

The percentage of expression of a gene in called penetrance. When a gene is expressed in 100 percentages of cases, the gene is said to have complete penetrance. Sometimes environmental factors may suppress completely the expression of a gene in some cases. This gene has a reduced penetrance.

For example, a dominant gene causes blue sclera in human eye. This dominant gene is not expressed in about 10 per cent of the people. Hence these peoples have normal white sclera, even though they carry the dominant gene. These people can transmit the gene to their children. The children may express this dominant gene. Thus this dominant gene for blue sclera has reduced penetrance of 90%.

### Expressivity

The degree of variation in the expression of a gene is called expressivity. The genes for blue sclera also illustrate variation in expressivity. Among the 90 percent of the people who carry the gene and express it, there will be a considerable variation in the shade of the blue of the sclera. In some persons, the sclera will be pale blue. In others, the sclera appears almost black.

The reduced penetrance and variable expressivity may be due to modifying genes or due to external environmental factors.

### Phenocopy

An environmentally induced change with resembles the effect a gene mutation is called phenocopy. The term “phenocopy” was first proposed by Richard Goldschmidth. He subjected pupae of Drosophila to a high temperature (35°C) for a short time at different periods in their development. Several phenotypes appeared which were similar to the phenotypes produced by certain mutant genes. Goldschmidth found that the genes had not been changed by the heat treatment, and the descendants of the phenocopies were normal in their phenotypes, when they were grown at normal temperature.

Rappaport (1939) found that when the larvae of the normal brown bodies fruit flies were reared on food with sliver salts, the emerging adults had yellow body. They were genotypically brown but phenotypically yellow because of the changed environment. These phenocopies when their larvae are fed with food without silver salts produce only brown-bodied adults, as their genotype is that of brown body. Thus a Phenocopy can last only for that generation in which the environment that induces the change is present.

**Pleiotropism**

The phenomenon of multiple phenotypic expression of a single gene is called pleiotropism. For example, the tomato mutant gene *'Is'* suppresses the growth of

a) The axillary shoot

b) The development of petals in flower

c) It produces apocarpous pistil and dilatory anthers. According to Williams this gene suppresses the growth of meristematic tissue at the apex regardless of its position. For this reason, a single gene produces many fold effects.

In human, the gene for disease ***phenylketonuria*** has pleiotropic effect and produces various abnormal phenotypic traits, collectively called syndrome. For example, the affected individuals have excess quantity of amino acid ***phenylalanine*** in their urine, cerebrospinal fluid and blood. They have short stature, mental retardation, widely spaced incisors, pigmented patches on skin, excessive sweating and non-pigmented hairs and eyes.

**Modifying genes**

A modifying gene is one that alters the expression of a major gene but has no effect on the allele of the major gene. The modifiers have similar but individually small effects and are usually present in large members that they cannot be individually identified.

In, Guernsey breed of cattle the solid color (light yellowish brown) of the coat is due to dominant gene 'S' and the spotted coat (white spouting) is due to its recessive allele 's'. A number of modifying genes influence the intensity of spotting. If a large no. of modifying genes are present in animals with 'ss' the animals are highly spotted. If only a small number of modifying genes are present, they are

medium spotted. If the modifying genes are absent, animals will have only few spots. These modifying genes have no effect in the presence of the gene for solid colour and animals with SS or Ss have solid coats irrespective of the number of modifying genes present.

**Lethal genes**

A lethal gene causes the death of all the individuals carrying it before these individuals reach the adulthood.

The lethal genes are classified into following types

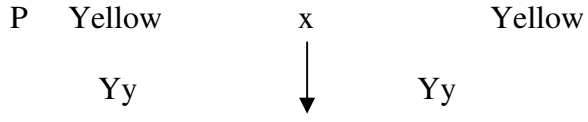
**1. Recessive lethal**

The lethal effect is expressed in the individuals only when the alleles are in homozygous state. This condition is known as recessive lethal.

French geneticist Cuenot (1905) discovered a classic example for recessive lethal affecting coat colour in mouse. He found that yellow coat colour in mouse is produced by dominant gene Y, while its recessive allele y produces gray / agouti color and yellow is dominant over gray. Further he also observed that all the mouse with yellow coat colour were heterozygous (Yy) and no mouse was there in the population with homozygous for Y allele. Cuenot supposed that the sperm carrying yellow (Y allele) could not penetrate egg carrying Y allele.

But later Castle and Little gave the explanation that yellow homozygotes were formed but died in embryonic stage. According to them, yellow had a dominant phenotypic effect on coat color but had at the same time a recessive effect on lethality, so that homozygotes for yellow were in viable. So a cross of yellow x yellow therefore always produced offspring in the ration 2/3 yellow: 1/3 agouti instead of ¾ yellow: ¼ agouti.

From the above experiment it was evident that whether the lethal gene has a dominant or recessive phenotypic effect, it is called recessive lethal as long as its lethality depends upon its presence in homozygous condition.



**Progenies**

| | **(Y)** | **(y)** |
|---|---|---|
| **(Y)** | YY | Yy |
| **(y)** | Yy<br>(yellow) | Yy<br>(agouti) |

Phenotypic ratio 2 : 1

Yellow grey / agouti

**ii. Dominant lethal**

The lethal gene whose lethal effects occur in heterozygous individuals is known as dominant lethal. An example of a dominant lethal is the epiloia gene in human beings, this gene causes abnormal skin growth, severe mental defects and multiple tumors in the heterozygotes so that they die before reaching adulthood. Therefore, dominant lethal cannot be maintained in the population, while recessive lethal are maintained in heterozygous state. Thus the dominant lethal have to be produced in every generation through mutation.

**Multiple alleles**

When more than two allelic forms of a gene occupy the same locus of the homologous chromosome, they are said to be multiple alleles. In other words all the mutant form of a single gene constitutes a series of multiple alleles.

**Characteristic features of multiple alleles**

1. The members of a multiple alleleic series occupy the same locus of homologous chromosomes.
2. Only two members of such alleles are present at a time in a diploid organism.
3. There is no crossing over in the multiple allelic series. If two alleles are involved in the cross the same two alleles are recovered in $F_2$ or test cross progeny.

4. In a series of multiple alleles, wild type is always dominant. Rest of the alleles in the series may exhibit dominance or intermediate phenotypic expression.
5. The cross between two mutant alleles will always produce mutant phenotype (intermediate). Such cross will never produce wild phenotype. That is multiple alleles do not show complementation.
6. Multiple alleles always control the same character of an individual. However, the expression of the character will differ depending on the allele present.

**Examples for multiple alleles**

Several cases of multiple alleles are known both in animals and plants. Some of the examples are given below.

**1. Coat color in rabbits**

A classical example of multiple allele is found in coat color of rabbits. The coat colour of the rabbit is controlled by four allelic form of a gene. The allele C produces full color or wild type; $c^{ch}$ produces silver gray appearance due to mixture of black and gray hairs called Chinchilla, $c^{h}$ produces white coat color rabbit with black tips on the ear, nose, feet and tail called Himalayan ; c produce no pigment resulting albino rabbits. Inheritance studies revealed that alleles for coat colour show a gradation of dominance in the order of C> $c^{ch}$>$c^{h}$ >c (Table 6.1)

**Table 12.1 Inheritance for colour in different crosses of rabbits**

| S.No | Parents | $F_1$ | $F_2$ |
|---|---|---|---|
| 1. | Coloured x Albino | Coloured | 3Coloured:<br>1 Albino |
| 2. | Coloured x Chinchilla | Coloured | 3Coloured:<br>1 Chinchilla |
| 3. | Coloured x Himalayan | Coloured | 3Coloured:<br>1Himalayan |
| 4. | Chinchilla x Himalayan | Chinchilla | 3 Chinchilla : |

| | | | |
|---|---|---|---|
| | | | 1 Himalayan |
| 5. | Chinchilla x Albino | Chinchilla | 3 Chinchilla :<br>1 Albino |
| 6. | Himalayan x Albino | Himalayan | 3Himalayan :<br>1 Albino |

**All these experiments clearly indicate that**

1. The coat colour of the rabbit is controlled by a series of multiple alleles viz., C, $C^{ch}$, $C^{h}$ and c.
2. The allele C is dominant over all other alleles.
3. The allele c is recessive to all other alleles.
4. The allele $c^{h}$ is recessive to C but dominant over $c^{h}$ and c.
5. The allele $c^{h}$ is recessive to C and $c^{ch}$ but dominant over c.
6. Thus the alleles C, $c^{ch}$, $c^{ch}$, c forms a series of multiple alleles.

| **Phenotypes** | **Genotypes** |
|---|---|
| Coloured | CC,$Cc^{ch}$,$Cc^{h}$,Cc |
| Chinchilla | $c^{ch}c^{ch}$, $c^{ch}c^{h}$, $c^{ch}$c. |
| Himalayan | $c^{h}c^{h}$, $c^{h}$c |
| Albino | cc |

**2. Blood groups in human beings**

One of the most firmly established series of multiple alleles in humans involve the genetic locus controlling the blood types A,B AB and O.

In 1900, Landsteiner discovered blood groups A, B, AB and O in human beings. He found that agglutination may occur during transfusion of blood from one person to another. This agglutination occurs due to antigen-antibody reaction'.

**Antigen** is a specific protein found on the surface of the RBCs. **Antibody** is another kind of specific protein found in the plasma. There are two kinds of antigens

*viz*., antingen A and antigen B and two kinds of antibodies viz., **A antibody'** and '**B antibody'**. The agglutination which is '**antigen antibody reaction'** is a highly specific one. '**A antigen'** can react with 'Aantibody' alone and 'B antigen' can react with 'B antibody' alone. When there are 'A antigen' and 'B antibody', there will be no reaction and agglutination will not occur. Among human beings, the blood group is classified on the basis of the antigen present.

Persons with A antigen belong to A group. They have 'A antigen' on their RBCs and 'B antibody' in their plasma. Persons with B antigen belong to B group. They have 'B antigen on their RBCs and 'A antibody' in their plasma.

Persons with both A and B antigens belong to AB group. They have A and B antigens on their RBCs and no antibodies in their plasma. Persons with no antigens belong to O group. Their RBCs are without A and B antigens. But they have both A and B antibodies in their plasma (Table 6.2).

**Table 12.2 Blood groups in human beings**

| Antigen | Antibody | Blood group |
|---|---|---|
| A antigen | B antibody | A group |
| B antigen | A antibody | B group |
| A and B antigen | No antibody | AB group |
| No antigen | A and B antibodies | O group |

**Inheritance of blood group**

Specific alleles control the production of specific antigens. Antibody is produced by immunological mechanism. The blood group is determined by a series of three alleles *viz*., $I^A$, $I^B$ and i.

**12.3. Blood group and their possible genotypes**

| Blood group | Possible genotypes |
|---|---|
| A | $I^AI^A$, $I^Ai$. |
| B | $I^BI^B$, $I^Bi$ |
| AB | $I^AI^B$ |
| O | ii |

The $I^A$ controls the production of 'A antigen'. The allele $I^B$ controls the production of 'B antigen'. Both $I^A$ and $I^B$ are dominant over the recessive allele i. $I^A$ and $I^B$ lack dominance over each other. The heterozygote $I^A$ and $I^B$ is not intermediate between the homozygote's $I^A$ $I^A$ and $I^B$ $I^B$. It shows characteristics of both homozygotes. That is, both the alleles are expressed. This is called codominance (Table 6.3)

**Medical applications of blood group inheritance**

It is necessary to match the donor and recipient before a blood transfusion is made. If A group blood is transfused into a B group man, the 'A antigen' of the door reacts with 'A antibody' of the recipient and agglutination occurs. This agglutination reaction may be severe or even fatal. In blood transfusion, the antigen of the donor and antibody of the recipient must be considered and matched.

Since O group contains no antigen, it can be given to any blood group. Hence, it is called universal donor. AB group contains no antibody. So, it can receive any type of blood group. Hence, AB group is called universal recipient.

**Self-incompatibility system in plants**

**Self-incompatibility**

The inability of the pollen grains to fertilize the same flower or other flowers of the same plant is known as self-incompatibility.

Self-incompatibility system is controlled by a series of multiple alleles. In sporophytic self incompatibility system, the self incompatibility reaction of the pollen grain is determined by the genotype of the plant in which it is produced.

S1>S2>S3>S4

| Genotypes of the plants | $S_1$ $S_2$ | $S_1$ $S_3$ | $S_1$ $S_4$ | $S_2$ $S_3$ | $S_2$ $S_4$ | $S_3$ $S_4$ |
|---|---|---|---|---|---|---|
| Genotype of the gametes | ($S_1$) ($S_2$) | ($S_1$) ($S_3$) | ($S_1$) ($S_4$) | ($S_2$) ($S_3$) | ($S_2$) ($S_4$) | ($S_3$) ($S_4$) |
| Incompatibility reaction of the pollen grain | All $S_1$ | All $S_1$ | All $S_1$ | All $S_2$ | All $S_2$ | All $S_3$ |
| Incompatability reaction of the style | $S_1$ | $S_1$ | $S_1$ | $S_2$ | $S_2$ | $S_3$ |
| Complete incompatability (S1S2 selfed) | $S_1$ | $S_1$ | $S_1$ | $S_2$ | $S_2$ | $S_3$ |

**Pseudo alleles**

Pseudo alleles refer to closely linked and functionally related genes. A cluster of pseudo alleles is known as pseudo allelic series or complex locus or a complex region.

**Characteristics of pseudo alleles**

1. Pseudo alleles govern different expressions of the same character.
2. Pseudo alleles occupy a complex locus, which is divided into sub loci.
3. They exhibit low frequency of genetic recombination by crossing over.
4. They exhibit cis-trans position effect.

**Iso alleles**

An allele that is similar in its phenotypic expression to that of other independently occurring allele is known as isoallele. Isoalleles are two types.

a. **Mutant isoalleless**: Such alleles act within the phenotypic range of a mutant character.

b. **Normal isoalleles**: such alleles act within the phenotypic range of a wild character.

## Questions

1. Percentage of expression of a gene is called as

   i. Expressivity ii. Degree of Dominance

   iii.Penetrance iv. Recessiveness

   **Ans: iii.Penetrance**

2. Degree of variation in the expression of a penetrant gene is called as

   i. Expressivity ii. Penetrance iii. Phenocopy iv. Pleiotropism

   **Ans: i. Expressivity**

3. The reduced penetrance is variable expressivity may be due to

   i. Modifying genes ii. Environmental factors & modifying genes

   iii. Environmental factors is rethal genes iv. Lethal genes

   **Ans: ii. Environmental factors & modifying genes**

4. The phenomenon of multiple phenotypic expression of a single gene is called as

   i. Penetrance ii. Phenocopy iii. Pleiotropism iv. Multiple allels

   **Ans: iii. Pleiotropism**

5. A gene which alters the expression of a major gene is called as

   i. Modifying gene ii. Lehtal gene iii. Polygene iv. Pseudogene

   **Ans: i. Modifying gene**

**True or False**

1. More than two allelic forms of a gene is called as multiple gene

   **Ans: False**

2. Multiple alleles do not show complementation

   **Ans: True**

3. Phenocopy defines environmentally induced change

   **Ans: True**

4. Recessive lethal genes are expressed in heterozygous state

**Ans: False**

5. An allele which is identical to the expression of another independent allele in Pseudo allele

   **Ans: False**

# Lecture No.13

# Multiple factor inheritance

The Inheritance of many different genes influencing the same phenotype in a cumulative fashion is called multiple factor inheritance.

**Features of polygenic traits**

The term polygene was introduced by Mather in 1941. This term has found wide usage in quantitative genetics replacing the older term multiple gene. Main features of polygenic characters are briefly presented below:

(i) Each polygenic character is controlled by several genes and has cumulative effect.

(ii) Polygenic characters exhibit continuous variation rather than a discontinuous variation. Hence, they cannot be classified into clear cut groups.

(iii) Effect of individual gene is not easily detectable in case of polygenic character and, therefore, such traits are also known as minor gene characters.

(iv) The statistical analysis of polygenic variation is based on means, variance and co-variances, whereas the discontinuous variation is analysed with the help of frequencies and ratios. Thus, polygenic characters are studies in quantitative genetics and oligogenic characters in Mendelian genetics.

(v) Polygenic traits are highly sensitive to environmental changes, whereas oligogenic characters are little influenced by environmental variation.

(vi) Classification of polygenic characters into different clear cut groups is not possible because of continuous variation from one extreme to the other. In case of qualitative characters, such grouping is possible because of discrete or discontinuous variation.

(vii) Generally, the expression of polygenic characters is governed by additive gene action, but now cases are known where polygenic characters are governed by dominance and epistatic gene action. In case of oligogenic characters, the gene action is primarily of non-additive type (dominance and epistasis).

(viii) In case of polygenic characters, bio-metric measurements like size, weight, duration, strength, etc, are possible, whereas in case of oligogenic characters only the counting of plants with regards to various kinds like colour and shape is possible. Thus, metric measurement is not possible in case of oligogenic characters.

(ix) Transgressive segregants are possible from the crosses between two parents for a polygenic character. Such segregants are not possible in case of qualitative or oligogenic traits.

(x) The transmission of polygenic characters is generally low because of high amount of environmental variation. On the other hand, oligogenic characters exhibit high transmission because there is little difference between the genotype and phenotype of such character. Thus, polygenic characters differ from oligogenic ones in several aspects.

Yule (1906) gave the theoretical explanation for the multiple factor hypotheses. According to him quantitative characters are controlled by many genes with cumulative effect without dominance and would produces continuous variation.

The experimental evidence for multiple factor hypothesis was provided by Nilsson &Ehle (1908) in studies on the inheritance of seed colour in wheat and oats. They obtained 3:1, 15:1 and 63:1 ratios between coloured and white seeds from different crosses and revealed that seed colour in wheat and oats is produced by one, two or three genes. The seed colour genes interact in duplicate manner, so that white colour seed is produced only when all the genes are present in the recessive state. Further the coloured seeds showed a varied intensity for colouring pattern and they obtained in the ratio of 1 dark red : 4 medium dark red : 6 medium red : 4 light red : 1 white. This suggested that the seed odour in wheat is controlled by genes which show lack of dominance and have small and cumulative effects (Table 8.1).

In order to explain the 1:4:6:4:1 ratio in kernel colour in wheat, Nilson – Ehle made the following assumptions.

i. In crosses showing 15:1 ratio in the $F_2$ seed colour is governed by two genes.

ii. One of the alleles of each colour gene produces seed colour and is called positive allele denoted by capital letter (eg.) $R_1$,$R_2$ etc. The other

allele of each colour gene does not produce any colour and is known as negative allele denoted by corresponding small letter (eg.) $r_1$, $r_2$ etc.

iii. These genes do not show dominance and each of the gene (positive allele) has a small, equal effect on seed colour.

iv. The positive alleles of different coloured genes are additive in phenotypic effect.

**Inheritance of kernel colour in wheat**

| | Dark red | x | White |
|---|---|---|---|
| Parents | $R_1R_1R_2R_2$ | | $r_1r_1r_2r_2$ |
| Gametes | $(R_1R_2)$ | | $(r_1r_2)$ |

$R_1r_1R_2r_2$

Medium Red

**F2 generation**

| | **$(R_1R_2)$** | **$(R_1r_2)$** | **$(r_1R_2)$** | **$(r_1r_2)$** |
|---|---|---|---|---|
| $(R_1R_2)$ | $R_1R_1R_2R_2$ DR | $R_1R_1R_2r_2$ MDR | $R_1r_1R_2R_2$ MDR | $R_1r_1R_2r_2$ MR |
| $(R_1r_2)$ | $R_1R_1R_2r_2$ MDR | $R_1R_1r_2r_2$ MR | $R_1r_1R_2r_2$ MR | $R_1r_1r_2r_2$ LR |
| $(r_1R_2)$ | $R_1r_1R_2R_2$ MDR | $R_1r_1R_2r_2$ MR | $r_1r_1R_2R_2$ MR | $r_1r_1R_2r_2$ LR |
| $(r_1r_2)$ | $R_1r_1R_2r_2$ MR | $R_1r_1r_2r_2$ LR | $r_1r_1R_2r_2$ LR | $R_1r_1r_2r_2$ white |

**Table.13.1 Genotype and phenotype frequencies produced by two genes with cumulative effect on seed colour in wheat**

| Genotype | Frequency | No. of positive allele | Phenotype | Frequency |
|---|---|---|---|---|
| $R_1R_1R_2R_2$ | 1 | 4 | Dark Red | 1 |
| $R_1r_1R_2R_2$ | 2 | 3 | Medium Dark Red | 4 |
| $R_1R_1R_2r_2$ | 2 | 3 | | |
| $R_1r_1R_2r_2$ | 4 | 2 | Medium Red | 6 |
| $R_1R_1r_2r_2$ | 1 | 2 | | |
| $r_1r_1R_2R_2$ | 1 | 2 | | |
| $R_1r_1r_2r_2$ | 2 | 1 | Light Red | 4 |
| $r_1r_1R_2r_2$ | 2 | 1 | | |
| $r_1r_1r_2r_2$ | 1 | 0 | White | 1 |

## Questions

1. The term polygene was coined by

   i. Mather ii. Johanssen iii. Tinks iv. Jones

   **Ans: i. Mather**

2. Multiple factor hypothesis was given by

   i.Mather & Jinks ii. Nilsson & Ehle iii. Flour iv. Bateson & Punnet

   **Ans: ii. Nilsson & Ehle**

3. Quantitative genetics deals with

   i. Oliogenes ii. Polygenes iii. Multiple genes iv. Multiple alleles

   **Ans: ii. Polygenes**

4. Inheritance of many different genes influencing the same phenotype in a cumulative fashion is known as

   i. Polygenic inheritance ii. Mendalion inheritance

   iii. Multiple factor inheritance iv. Oligogenic inheritance

   **Ans: iii. Multiple factor inheritance**

5. The expression of polygenes is governed by

   i. Additive gene action ii. Dominance gene action

   iii. Epistasis iv. Interaction

   **Ans: i. Additive gene action**

**True or False**

1. Polygenic characters show discontinuous variation

   **Ans: False**

2. Oligogenic characters are greatly influenced by environment

   **Ans:False**

3. Analysis of polygenic variation is based on frequencies & ratios

   **Ans:False**

4. Crossing between two parents for a polygenic character produces transgressive segments

   **Ans:True**

5. Kernel colour in wheat is governed by polygenes

   **Ans: True**

## Lecture No.14

## Multiple factor inheritance

### Oligogenes

Genes having larger effect on the characters they govern and shows discontinuous variation are called oligogenes or major genes.

### Polygenes

Genes individually having small cumulative effect but jointly responsible for continuous variation, specific to a metric trait are called polygenes or minor genes.

**Table 13.2 Differences between qualitative and quantitative characters**

| S.No | Qualitative characters | Quantitative characters |
|---|---|---|
| 1. | Controlled by oligogenes (one or two genes) | Controlled by polygene (each) With small cumulative effect |
| 2. | Shows discontinuous variation | Shows continuous variation |
| 3. | Less influenced by environment | Much influenced by environment |
| 4. | Concerned with individuals mating and their progeny | Concerned with population of organisms consisting of all possible kinds of mating. |
| 5. | Exhibit high heritability | Exhibit low heritability |
| 6. | Analysis by making counts and ratios | Analysis by mean, variance and covariance |

### Transgressive segregation

The appearance in F2 individuals falling out side the parental range in respect to some character is called transgressive segregation. Transgresive segregation results due to fixation of dominant and recessive genes in separate individuals. Such segregation occurs when the parents are intermediate to the extreme values of the segregating populations. The superior plants are produced by an accumulation of plus

or favorable genes from both the parents as a consequence of recombination. The example for transgressive segregation is given below.

Parents AA BB cc dd ee X aa bb CC DD EE

$F_1$ AaBb Cc Dd Ee

$F_2$ aa bb cc dd ee and AA BB CC DD EE are the transgressive segregants.

## Questions

1. Genes showing larger effect on the characters they govern are

   i. Polygenes  ii. Oligogenes  iii. Minor genes  iv. Multiple genes

   **Ans: ii. Oligogenes**

2. The appearance of $F_2$ individuals falling outside the parental range is called as

   i. Transgressive segregants  ii. Dominance

   **Ans: i. Transgressive segregants**

3. The genes specific to metric traits are

   i. Polygenes  ii. Oligogenes  iii. Multiple genes  iv. Dominant genes

   **Ans: i. Polygenes**

4. Analysis of oligogenic characters is done by

   i. Mean & Variance  ii. Variance & Co-variance

   iii.Counts & Ratios  iv. Ratios & Co-variance

   **Ans: iii. Counts & Ratios**

5. Genes having small, cumulative effect on the characters they govern is called as

   i. Minor genes  ii. Major genes  iii. Polygenes  iv. Both 1& 3

   **Ans: iv. Both 1& 3**

**True or False**

1. Oligogenic characters exhibit high heritability

   **Ans: True**

2. Analysis of quantitative characters is done by mean, variance and co-variance

   **Ans: True**

3. Transgressive segregants results due to fixation of dominant and recessive genes in the same individual

   **Ans: True**

4. Qualitative characters show discontinuous variation

**Ans: True**

5. Quantitative characters are controlled by polygenes

**Ans: True**

# Lecture No.15

## Linkage

When two or more genes present on the same chromosome and they do not exhibit independent assortment, they are said to be linked and the phenomenon of transmission of linked genes is called linkage.

Large deviations from a 1:1:1:1 in the testcross progeny of dihybrid experiment is used as a first evidence for linkage. This effect of linkage was first reported in 1906 by Bateson and Punnet in sweet peas. Among sweet peas they crossed a variety having blue flower (B) and long pollen grains (L) with another variety having red flower (b) and round pollen grains (I). The F1 hybrids had blue flowers and long pollen grains. A dihybrid test cross was carried out. The F1 blue long (Bb L1) was crossed with the double recessive parent, red-round (bb II).

According to Mendel four kinds are expected in the ratio of 1 blue long:1 Blue round : 1 Red round : 1 Red long : 1 Red round. But in Bateson and Punnet's experiment the four kinds are produced in different ratio i.e.7 Blue long: 1 blue round: 1 Red long: 7 Red round.

| | | | |
|---|---|---|---|
| Parents | Blue long | X | Red round |
| | BB LL | | bb ll |
| F1 | Bb Ll | X | bb ll |
| | Blue long | | Red round |
| Gametes | (BL) Bl) (bL) (bl) (bl) | | |

**Test cross progenies**

| Offspring | Expected ratio | Actual ratio |
|---|---|---|
| Blue long | 1 | 7 |
| Blue round | 1 | 1 |
| Red long | 1 | 1 |
| Red round | 1 | 7 |

Bateson and Punnet suggested that the F1 hybrid blue long (Bb Ll) produced the gametes (BL) and (bl) about seven times than the gametes (Bl) and (bl).

They carried out another experiment. They crossed a variety of blue round with another variety of red long. The $F_1$ hybrid was blue long. It was test crossed.

| | | | |
|---|---|---|---|
| Parents | Blue round | X | Red long |
| | BB ll | ↓ | bb LL |
| F1 | Bb Ll | X | bb ll |
| | Blue long | | Red round |
| Gametes | (BL) Bl) (bL) (bl) (bl) (ll) | | |

| Test cross progeny | Expected ratio | Actual ratio |
|---|---|---|
| BbLl Blue long | 1 | 1 |
| Bbll Blue round | 1 | 7 |
| bbLl Red long | 1 | 7 |
| bbll Red round | 1 | 1 |

Bateson and Punnet suggested that the F1 hybrid blue long (Bb Ll) produced gametes (Bl) and (bL) about seven times than the gametes (BL) and (bl).

Bateson and Punnet put forward the following points.

1. The alleles, which come from the same parents, tend to enter the same gametes. The alleles which come from different parents tend to enter different gametes.
2. When the genes are linked, greater than 50 % of progeny with parental phenotypes and less than 50% of progeny with recombinant phenotypes will occur.

Though Bateson and Punnet (1906) explained the effect of linkage in sweet pea they did not interpret their results in terms of the behavior of genes located on the

same chromosome and the occurrence of crossing over between homologous chromosomes during meiosis.

**T.H.Morgan's Experiments in *Drosphila***

T.H. Morgan (1911) was first to relate linkage to the segregation of homologous chromosomes and the occurrence of crossing over between homologous chromosomes during meiosis in the fruit fly *drosophila melanogaster*.

T.H. Morgan explained the effect of linkage by considering the result of two crosses involving pairs of alleles of two genes located on the second chromosomes of *D.melanogaster*. One gene affect the body colour (gray body (b*) [which is dominant over black body (b)]. The second gene affects the phenotype of the wing (long wing ($s^+$) [which is dominant over vestigial wing or short wing (s)].

In the first cross (cross I) he made crosses between true breeding long wing and grey bodied with true breeding short wing and black bodied flies. This cross produced heterozygous F1 files with long wings and grey bodies. Among the progeny of this test cross, 82 per cent exhibited one or the other (41 per cent each) of the parental combination of traits. The other 18 per cent of the progeny had new or recombinant combinations.

Next, consider a different cross (cross II) one between homozygous flies with long wing and black bodies and homozygous flies with short wings and gray bodies. Again in cross II, 82 per cent of the test cross progeny have parental phenotypes (phenotypes identical to one or the other of the original parents) and 18 per cent have new or recombinant phenotypes.

Although the $F_1$ flies have the same phenotype (long wing, gray bodies) in both crosses, the test cross progeny of the F1 female contain very different frequencies of the four phenotypic classes in the two cases. For example 41 per cent of the test cross progenies in cross I are wild type (have long wing and gray bodies); in cross II only 9 per cent are wild type. Clearly, this shows that the allelic forms of the two genes that are present together on the homologous chromosomes of the parent tend to remain together on the chromosome of the progeny.

In cross I, the $F_1$ files carried the wild type forms ($s^+b^+$) of the two genes on one homologue and the mutant forms (s b) on the other homologue. The genotype of a heterozygote of this type is frequently written as $s^+b^+$ / sb. This arrangement of mutant

and wild type form of two genes in a heterozygote is called the coupling state or cis configurations. The alternative arrangement, illustrated in cross II where each homologue contains one mutant gene and one wild type gene ($s^+$ b / $sb^{+)}$ is called the repulsion state or trans configuration.

Morgan formulated his theory of linkage from this experiment. According to this theory.

1. Genes located on the same chromosome are inherited together. They are said to be linked.
2. Genes are present in the chromosomes in a linear order.
3. The distance between the linked genes determines the strength of linkage. Closely located genes show strong linkage and widely located genes show weak linkage.
4. Only those genes located in different chromosome show independent assortment. But now we came to know that genes that are located far apart on the same chromosome would also assort independently.

**Types of linkage**

Linkage is classified on the basis of following three criteria.

**I. Based on crossing over**

(i) **Complete linkage**

Linkage in which crossing over does not occur is known as complete linkage or absolute linkage. In complete linkage test cross progenies posses only parental types.

**(ii) Incomplete linkage**

In some cases, frequency of crossing over occurs between linked genes, it is known as incomplete linkage. In incomplete linkage, the test cross yields some recombinants besides parental combinations.

**II. Based on status of genes involved**

**(i) Coupling linkage**

It refers to linkage either between dominant genes or between recessive genes.

(ii) **Repulsion linkage**

It refers to linkage of some dominant genes with some recessive genes.

**III. Based on chromosomes involved**

**(i) Autosomal linkage**

It refers to linkage of such genes, which are located in other than sex chromosomes.

**(ii) X-chromosomal linkage**

It refers to the linkage of genes, which are located in sex chromosomes.

## Questions

1. The phenomenon of two or more genes present on same chromosome which do not show independent assortment is called as

i. Pleiotropism ii. Linkage iii. Dominance iv. Lethal

**Ans: ii. Linkage**

2. Linkage was first reported by

i.Bateson & Punnet ii. Mendel iii. T.H.Morgan iv. Bridges

**Ans: i. Bateson & Punnet**

3. Who linked linkage to crossing over and segregation of homologous chromosomes during meiosis

i. Bateson & Punnet ii. Mendal iii. T.H.Morgan iv. Bridges

**Ans: iii. T.H.Morgan**

4. Linkage which do not allow any crossing over

i. Complete linkage ii. Incomplete linkage iii. Repulsion iv. Coupling

**Ans: i. complete linkage**

5. Dihybrid test cross ratio

i. 1:1 ii. 1:1:1:1 iii. 1:2:1 iv. 3:1

**Ans: ii. 1:1:1:1**

**True or False**

1. Genes present on chromosome in a linear order

   **Ans: True**

2. Linkage between two dominant genes is known as coupling linkage

   **Ans: True**

3. Incomplete linkage allows some frequency of crossing over between linked genes

   **Ans: True**

4. Linkage of genes located in sex chromosomes is called as x-chromosomes linkage

   **Ans: True**

5. Strength of linkage is determined by distance between the linked genes

   **Ans: True**

## Lecture No.16

## Crossing over

Crossing over may be defined as "interchange of chromosomal segments between non-sister chromatids of a homologous chromosome pair". The term crossing over was first used by Morgan and Cattell in 1912.

**The main features of crossing over are given below**

i. Crossing over takes place at four strand stage during pachytene stage of Miosis I.

ii. Crossing over occurs between non-sister chromatids of the homologous chromosomes.

iii. Crossing over produces new combination of genes between linked genes.

iv. The value of crossing over or recombination may vary from 0-50%.

v. The frequency of recombinants can be worked out from the test cross progeny. It is expected as the percentage ratio of recombinants to the total population (parental types +recombinants). Thus,

$$\text{Crossing over frequency (\%)} = \frac{\text{No. of recombinants}}{\text{Total progeny}} \times 100$$

**Cytological basis of crossing over**

Morgan first proposed crossing over to explain the formation of recombinant genes that were linked by gametic data. F. Janssens first correlated the chiasma frequencies with recombination frequencies, and showed that a direct relationship between crossing over and chaistmata.

Direct cytological evidence for crossing over was first given by Curt Stern (1931) in *Drosphila* and in maize by H.B.Creighton and B McClintock.

**Curt Stern Experiment**

Curt stern found aberrant x chromosomes in a variety of Drosophila and one was an x chromosome to which a portion of Y chromosome was attached. This is

inverted ‘L’ shaped. The other was a broken ‘X’ chromosome. Under microscope these two kinds of chromosomes can be easily distinguished from each other as well as from the normal.

He produced female flies heterozygous for these two morphologically distinguishable X chromosomes. These female flies were also heterozygous for alleles of two genes that are located on the X chromosome. One gene affect the eye shape, the partially dominant mutant allele B results in bar- shaped eyes and its wild type allele $B^+$ produces round eyes in homozygous condition. The second gene affect the eye colour, the mutant allele ear results in carnation coloured eye and its dominant wild-type allele car produce red eyes. The females used in stern’s study carried the allele pairs in the cis-configuration as shown below.

Stern crossed such heterozygous females (car B / $Car^+$ $B^+$) with males having carnation coloured, normal shaped eyes (car $B^+$ / Y males) and studied the offspring of the next generation. The cross and results obtained by him are diagramed in Fig 9.2.

In the absence of crossing over only two kinds of eggs were expected, one with broken X chromosome carrying the genes car and B and other with attached ‘X’ chromosome carrying the genes car $^+$ and $B^+$. Thus the heterozygous females (car B/$Car^+B^+$) have produced all four kinds of eggs in which, the X chromosomes were expected to be different not only genetically but also structurally.

After fertilization, these four kinds of eggs produced the four types of phenotypes in $F_1$ offspring in both sexes.

i. Carnation barred eye

ii. Red colour and round eye (wild type)

iii. Carnation round eye

iv. Red bar shaped eye

These four kinds of offsprings were expected to have different X chromosomes. The shape of ‘X’ chromosome observed in the progeny agreed with their observation. That is, if crossing over involved the breakage and exchange of parts of homologus chromosomes, then the recombinant male progeny with bar shaped red eyes (Car $^+$ B/Y) were found to carry the short X chromosome, but

translocated piece of the Y chromosome is attached to X chromosomes at one end. Thus Curt Stern's experiment was unique demonstration of the hypothesis that the genetic crossing over is accompanied by physical exchange between the homologous chromosomes.

**Factors controlling crossing over**

i. High and low temperatures increase the frequency of crossing over.

ii. X-rays and other irradiations increase the crossing over frequency.

iii. The age of the individual also affects the crossing over frequency. It was found that crossing over frequency is higher in older ages.

iv. Gene mutations affected the frequency. Some mutations are known to decrease the frequency.

v. Crossing over at one point of the chromosome tends to prevent other crossing over in nearby places. This phenomenon is called interference.

vi. Crossing over does not take place in Drosophila male; and silk worm females. Thus, sex also affects the crossing over.

vii. Crossing over is less frequent near centromeres and the tips of chromosomes.

viii. Inversions of chromosome segments suppress the crossing over.

**Significance of Crossing Over**

i. Crossing over provides a direct evidence for the linear arrangement of genes in the chromosome.

ii. Since crossing over results in recombination of genes variations are produced.

iii. Crossing over helps in the construction of chromosome maps.

## Questions

1. Interchange of chromosomal segments between non sister chromatids of homologous chromosome pair is called as

i. Modification ii. Crossing over iii. Interaction iv. Hybridity

**Ans: ii. Crossing over**

2. The term crossing over was coined by

i. Bateson & Punnet ii. Murgan iii. Morgan & Cattell iv. Mendel

**Ans: iii. Morgan & Cattell**

3. Crossing over takes place during which stage of meiosis I.

i. Diplotene ii. Leptotene iii. Pachytane iv. Metaphase

**Ans: iii. Pachytane**

4. The direct cytological evidence for crossing over was given by

i. Stern in *Drosophila* ii. Morgan in Drosphila

iii. Creighton &Meclintock in maize iv. Both 1 & 3

**Ans: iv.Both 1 & 3**

5. The value of crossing over may vary from

i. 0-50% ii.1-30% iii.0-60% iv.0-100%

**Ans: i.0-50%**

**True or False**

1. Crossing over frequency = $\frac{\text{No.of recombinants}}{\text{Total progency}} \times 100$

**Ans:True**

2. Crossing over at one point tends to prevent other crossing over in nearby places is called as interference

**Ans: True**

3. Sex of the organism do not influence crossing over

**Ans:False**

4. Variations are created due to crossing over & recombination

**Ans: True**

5. High or low temperature and X-rays decreases the frequency of crossing over.

**Ans: False**

# Lecture No.18

## Linkage, map distance, two point and three point test cross

Crossing over between particular linked genes occurs at constant frequencies. The percentage of crossing over is directly proportional to the distance between the two genes. Thus, the percentage of crossing over between any two genes indicates the relative distance between them. Percentages of crossing overs between various genes of a chromosome can be calculated experimentally. From this data, the relative distances between the various genes can be worked out. From the relative distance of various genes, their exact locations in the chromosome can be determined. Sturtevant, a student of T.H.Morgan formulated this idea of chromosome mapping. Construction of a linkage or genetic map or chromosome mapping.

**Determination of linkage groups**

Before starting the genetic mapping of the chromosomes of a species, one has to know the exact number of chromosomes of that species and then, he has to determine the total number of genes of that species by undergoing hybridization experiments in between wild and mutant strains.

**Determination of map distance**

After knowing total number of genes in each linkage group of a species, the relative distance between each linked gene have to be determined. The distance between two given genes is calculated according to the percentage of crossing over, because, cross over frequency is directly proportional to distance between the genes. For example, if the percentage of crossing over between two linked genes is 1 per cent means, the map distance between two linked genes is one unit of map distance, which is known as centimorgan. If the mean number of chiasmata is known for a chromosome pair, the total length of the map for that linkage group may be predicted:

Total length = Mean number of chiasmata x 50

**Two point test cross**

The percentage of crossing over between two linked genes is calculated by test crosses in which a $F_1$ dihybrid is crossed with a double recessive parent. In such crosses, crossing overs occur at two points, hence it is called two point test cross. For example, a dihybrid having the genotype AC/ac is test crossed with a double recessive parent ac/ac and test cross produces 74% parental combinations and 26% cross over types. Then, the distance between the loci A and C is estimated to be 26 centimorgans. If the distance between the two loci is large in two point test cross, double cross over types are undetected and it would appear as parental types. Hence, we underestimate the true map distance (crossover percentage).

**Three point test cross**

Double crossovers usually do not occur between genes less than 5 map units apart. For genes further apart, it is advisable to use a third marker between the other two in order to detect any double crossovers. A thee point test cross or thihybrid test cross gives us information regarding relative distance between these genes, and also shows us the linear order in which these genes should be present on chromosome.

## Questions

1. Who gave the idea of construction of linkage map?

i. T.H.Morgan ii. Sturtevant iii. Bateson

**Ans: ii. Sturtevant**

2. The unit of map distance

i. Centimeter ii. Millimeter iii. Centimorgan iv. Feet

**Ans: iii. Centimorgan**

3. Douple cross over do not occur between genes less than ---------- map units apart

i. 3 ii.5 iii.7 iv. 9

**Ans: ii. 5**

4. The linear order of the genes on a chromosome is given by

i. Three point test cross ii. Two point test cross

iii. Bivalent study iv. Univalent study

**Ans: i. Three point test cross**

5. Total length of the chromosome map is predicted by---------

i. Mean number of chiasmata x 30 ii. Mean number of chiasmata x 50

iii. Total number of chiasmata x 50 iv. Total number of chiasmata x 30

**Ans: ii.Mean number of chiasmata x 50**

**True or False**

1. Number of linkage groups in a species is equal to its haploid chromosome number

   **Ans: True**

2. Distance between two genes on a chromosome is measured by two point test cross

   **Ans: True**

3. A test cross, which involves two crossing overs is called as three point test cross

   **Ans:False**

4. Trihybrid test cross gives information regarding distance between genes and their order on chromosome

   **Ans: True**

5. The idea of chromosome mapping was given by T.H.Morgan

   **True: False**

# Lecture No.19
# Construction of genetic map

The method of construction of genetic maps of different chromosomes is called genetic mapping. The genetic mapping includes the following.

**Determination of gene order**

After determining the relative distances between the genes of a linkage group, it becomes easy to place genes in their proper linear order. For example, if the linear order of three genes ABC is to be determined, then these three genes may be in any one of the three different orders depending upon that which gene in the middle. If we suppose that the distance between the genes A-B =12, B-C=7, A-C=5, we can determine the order of genes correctly in the following manner. Let us assume that gene A is in the middle, the distances between B-C are not equitable, gene A cannot be in the middle.

In case II, let us assume that gene B is in the middle (A-B-C) the distances between A-C is not equitable; therefore, gene B cannot be in the middle. In case III, let us assume that gene C is in the middle (A-C-B), the distance between A-B are equitable, therefore, gene C must be in the middle.

**Combination map segments**

The different segments of map of a complete chromosome are combined to form a complete genetic map of a long chromosome.

**Interference and coincidence**

In most higher organisms it has been found that one chiasma formation reduces the probability of another chiasma formation in an immediately adjacent region of the chromosome probably because of physical inability of the chromatids to bend back upon themselves within certain minimum distances. The tendency of one crossover to interfere with the other crossover is called interference. The net result of this interference is the observation of fewer double crossover types than would be expected according to map distances. The strength of interference varies in different segments of the chromosome and is usually expressed in terms of a coefficient of coincidence, the ratio between the observed and the expected double crossovers.

$$\text{Coefficient of coincidence} = \frac{\%\text{ of observed double crossovers}}{\%\text{ of expected double crossovers}}$$

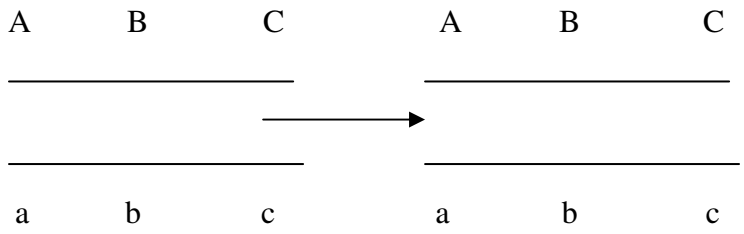
The coincidence is the complement of interference, so Coincidence + Interference = 1.0.

When the interference is complete (1.0), no double crossovers will be observed and coincidence becomes zero. When we observe all the double crossovers expected, coincidence is unity and interference becomes zero.

**Determination of map distance using three point test cross**

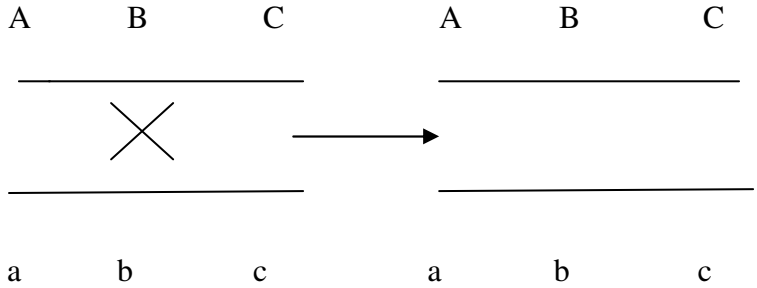
In a three point test cross, eight different phenotypic classes are obtained. These eight classes are identified in two different ways, viz., (1) by phenotypic frequencies, and (2) by alternation of gene sequence in the genotype as a result of single crossing over or double crossing over between three linked genes. Parental types have the maximum phenotypic frequencies, and the single crossovers have phenotypic frequencies between these two classes. Suppose, ABC/abc are three linked genes located on two classes. Suppose, ABC/abc are three linked genes located on two different chromosomes in $F_1$ of a cross between AABBCC and aabbcc parents.

1. Single crossover between A and B will alter the position of two genes ,*viz*., B and C
2. Single crossover between B and C will alter the position of only one gene, *i.e*., C.
3. Double crossover between A and C will alter the position of only middle gene, *i.e*.(Fig 19.1)

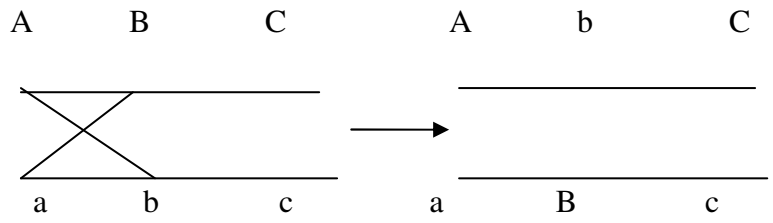


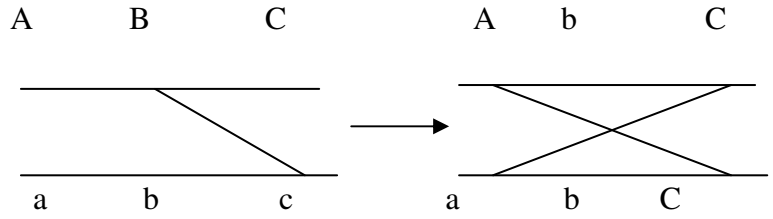

Parental types

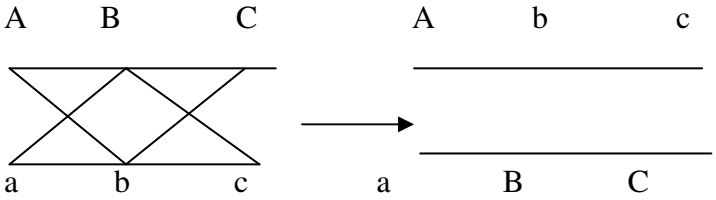
(a) No crossing over



(b) Single crossing over between A and B




Thus eight types of gametes are produced by $F_1$ and only one type of gamete is produced by homozygous recessive parent. Union of male and female gametes will produce eight different phenotypic classes (Table 19.1)

Table 19.1 Summary of the results obtained from a three point test cross between ABC/abc x abc /abc

| **Genotypic classes** | **Phenotypic classes** | **Assumed frequencies** | **Remarks** |
|---|---|---|---|
| ABC / abc<br>abc / abc | ABC<br>abc | 349<br>360 | Parental type |
| Abc / abc<br>aBC / abc | abc<br>aBC | 114<br>116 | Single crossover<br>Between A and B |
| ABc / abc<br>abC / abc | ABc<br>abC | 128<br>124 | Single crossover<br>Between B and C |
| AbC / abc<br>aBc / abc | AbC<br>aBc | 5<br>4 | Double crossover<br>Between A and C |
| **Total** | | **1200** | |

**Calculation**

The recombination percentage or unit distance between genes worked out by calculating the crossing over percentage between genes A and B is P, between genes B and C is Q, between genes A and C is R, and total progeny is T, Then,

Recombination (%)

1. Between genes A and B = $\frac{P + R}{T}$ x10

$$= \frac{230+9}{1200} \times 100 = 19.92$$

P = 114+116 = 230

R = 5+4 = 9

2. Between genes B and C = $\frac{Q+R}{T}$ x100

$$= \frac{252+9}{1200} \times 100 = 21.75$$

Q = 128+124 =252

R = 5+4 = 9

3. Between genes A and C = $\frac{P+Q}{T}$ x 100 = 40.30

**Gene Sequence**

The gene sequence is determined with the help of crossing over percentage between two genes. Greater the recombination percentage between two genes, more is the distance between them and vice versa. In this case, the maximum crossing over % is between gene A and c (40.3%). This indicates that B is located between A and C as given below.

| A | B | C |
|---|---|---|
| 19.72 | | 21.75 |
| | 41.67% | |

Coefficient of Coincidence

It is calculated with the help of following formula:

$$\text{Coefficient of coincidence} = \frac{\text{Observed double crossover}}{\text{Expected double crossovers}} \times 100$$

$$\text{Observed double cross over's} = \frac{9}{1200} \times 100 = 0.75$$

Expected double cross overs = Product of two single recombination values

= 19.92x21.75 / 100 = 4.33%

$$\text{Coefficient of coincidence} = \frac{0.75}{4.33} \times 100 = 17.32\%$$

Coefficient of interference = 1-0.1732 = 0.1732 = 0.8268

or 82.68%

**Questions**

1. If the distance between the genes A-C=5, A-b=12, B-C=7 then the correct order of genes on the chromosome is

i. A-B-C ii. B-A-C iii. C-B-A iv. A-C-B

**Ans: iv. A-C-B**

2. Coincidence + Interference =

i. 1.0 ii. 0.5 iii. >1 iv. <0.5

**Ans: i. 1.0**

3. Tendency of one cross over to interfere with other cross overs of a same linkage group

i. Interaction ii. Wincidence iii. Interference iv. Epistasis

**Ans: iii. Interference**

4. Strength of interference is expressed in terms of

i. Percentage of coincidence ii. Coefficient of coincidence

iii. Percentage of double cross overs iv. Percentage of single cross overs

**Ans: ii.Coefficient of coincidence**

5. When we observe all the double cross overs expected, the coincidence is

i. 0 ii. 1 iii. 0.5 iv. >1

**Ans: ii.1**

**True or False**

1. The strength of interference is equal at all regions of chromosome

**Ans: False**

2. When the interference is complete, double cross overs will be observed

**Ans:False**

3. Coincidence + Interference = 2

**Ans:False**

4. Coefficient of coincidence = $\frac{\%\text{ of observed double cross overs}}{\%\text{ of expected double cross overs}}$

**Ans: True**

5. Three point test cross produces, eight different phenotypic classes

**Ans: True**

# Lecture No.20
# Sex determination

Nature contains a vast array of diverse mechanisms of sex determination. In lower organisms, the two sexes are phenotypic ally indistinguishable except for the reproductive organs and in some lower eukaryotes, the two genetically distinct type of gametes are some time morphologically indistinguishable and called as isogamy (iso – means 'same' gametes) eg. Green alga ***Chlamydomonas reinhardtii.***

In higher form, there are many distinct morphological differences between male and female sexes. This phenomenon is called sexual dimorphism. Basically the reproductive organs and sex cells are different between males and females. This forms primary sexual character. The male and female sexes differ from each other in many somatic characters. For example mammary glands in females and beard in males are secondary sexual characters.

**Two kinds of chromosomes**

In dioecious organisms, chromosomes are two kinds. They are autosomes and allosomes.

**Autosomes**

Chromosomes containing gene, which determine the various somatic characters.

**Allosomes**

H.Henking (1891) first identified the chromosome involved in sex-determination. Allosomes are otherwise called as sex-chromosomes. These are the chromosomes responsible for the determination of sex. The allosomes are of two types viz., X and Y.

Modern geneticists have reported many different mechanisms of determination of sex in living organisms. Some important and common mechanisms of 'sex' determination are the following.

**Homogametic and Heterogametic sexes**

The individuals carrying the same type of sex chromosomes namely XX are called homogametic. They give only one kind of gametes (X). The individuals having dissimilar sex chromosome namely XY are called heterogametric. They give two kinds of gametes (X) and (Y). Among human being and Drosophila the female is homogametic. In birds, moths and butter flies, the females are heterogametic and males are homogametic.

**XX-XY type of sex determination**

In insect like Drosophila and in human beings, the male have dissimilar sex chromosomes – XY chromosomes. In female, two similar sex chromosomes represent – XX chromosomes. The female produces only one kind of egg (22 autosomes + one X chromosome) and hence homogametic. The male produces two kinds of sperms one with 22 autosome + one X chromosome and other with 22 autosome + one Y chromosome and hence heterogametic. The egg (X) fertilized by (X) sperm produces female offspring XX. The egg (X) fertilized by (Y) sperm produces male offspring XY.

In many species including most birds, moths and some fish the sex determination is identical to that of XX-XY mechanism but female is heteogametic (usually designed as ZW) and males being homogametic (usually designated as ZZ). This mechanism of sex determination is sometimes called ZZ – ZW. However, mechanically this system is identical to the XX – XY system but with the relationship between sex – chromosomes and sex phenotypes reversed. Stated differently in birds, the chromosomes composition of the egg determines the sex of the offspring , where as in humans and fruit flies, the chromosomes composition of the sperm determines the sex of the offspring.

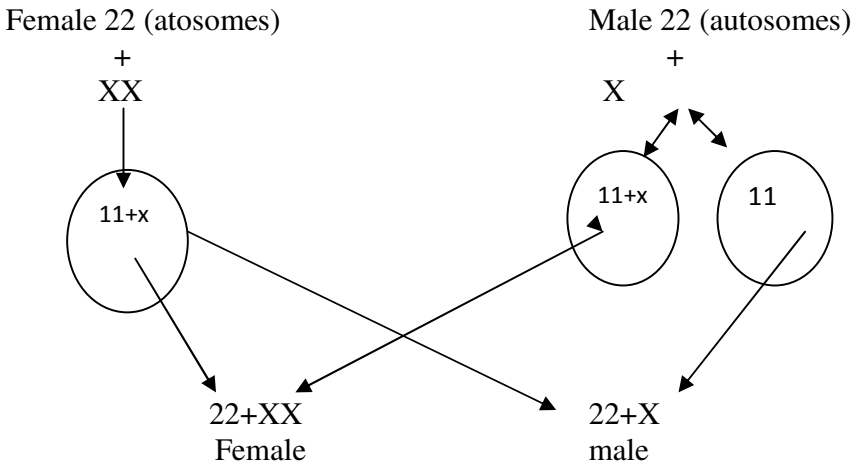
**The 'Y' chromosome and sex determination in mammals**

In both Drosophila and humans, normal females have XX sex chromosome composition and normal males have XY sex chromosome composition. Thus it might be tempting to assume that in both species the genes for maleness is on Y chromosome. In mammals the presence of 'Y' chromosome is required for the development of male sex phenotype.

In contrast recent evidence shows that Y chromosome plays no significant role in sex determination in Drosphila. In mammals surprisingly X chromosomes present in any number (eg.XXX) in the absence of Y chromosome give rise to female sex phenotype. So Y chromosome in human that is responsible for the development of testis is called TDF (for Testis Determining Factor). The TDF gene exhibits very dominant effect on the development of the sex phenotype.

**XX-XO type of sex determination**

In some of the insects like grass hoppers, all the eggs carry an X chromosome, but it was included in only half of the cells forming sperm. All the sperm however had the usual complement of other chromosomes (autosomes). Eggs fertilized by sperm containing the X chromosome produced zygote with two X chromosomes produced zygotes with one 'X' which become males. Males are referred as hemizygous for the X chromosomes or for genes located on the X chromosome.




**Bridges genetic balance theory in Drosphila**

Soon after the identification of X chromosome, the sex determination in Drosphila was more complicated than the preliminary observation. C.B.Bridges showed that female determining genes were located on the X chromosome and male determining genes were on the autosomes of Drosphila. The genetic balance theory of sex

determination was devised to explain the mechanics of sex determination in *D.melanogaster*.

Bridges experimentally produced various combinations of X chromosomes and autosomes in Drosophila. A triploid female was crossed with a diploid male. The triploid female produces four types of eggs.

(i)X2n (A) (ii) XX2n(A) (iii)X n (A) (iv)XX n (A)

The diploid male produces two types of sperms

(i)Xn (A) (ii) Yn(A)

When the four types of eggs are fertilized by the two types of sperm at random eight kinds of offspring are produced (Table 10.01)

**Table 20.1. Sex expression in Drosophila in relation to X/A rules**

| | Xn (A) | Yn(A) |
|---|---|---|
| X2n (A) | XX3n (A) = 2X/3 = 0.66 Inter sex | XY3n (A) = 1X/3 = 0.33<br>Super male |
| Xn (A) | XX2n (A) = 2X/2 = 1.00 Female | XY2n (A) = 1X/2 = 0.50 Male |
| XX2n (A) | XXX3n (A) = 3X/3 = 1.00 Triploid female | XXY3n (A) = 2X/3= 0.66 Inter sex |
| XXn (A) | XXX2n (A) = 3X/2 1.50 Super female | XXY2n (A) = 2X/2 = 1.00<br>Female |

**Eight kinds of offspring are produced as follows**

1. Triploid female with three x chromosomes and three sets of autosome.

2. Normal diploid female with two chromosomes and two sets of autosomes.

3. Diploid XXY female with two X chromosomes and one Y chromosome and two sets of autosome.

4. Intersexes with two X chromosomes and three sets of autosomes.

5. Intersexes with two X chromosomes and one Y chromosome and three sets of autosomes.

6. Normal males with one x and one Y chromosome and two sets of autosome.

7. Super females with three X chromosomes and two sets of autosomes.

8. Super males with one X and one Y chromosome and three sets of autosomes.

First, it was supposed that the XX individual is female and XY male. After finding of non-disjunction, this early formulation was altered slightly that the XX is female and X male. **The importance of Y chromosome in the sex determination was removed.**

In Bridge's experiment, there is an individual with two X chromosomes. Yet it is not female. It is shifted out of the female class by the addition of one set of autosomes and it becomes an intersex. So, autosomes also play a positive role in the determination of sex. The intersexes lead to the conclusion that in Drosphila, sex is determined by the X chromosomes as well as by the autosomes.

The intersex differes from female by the assumption of certain male characters. This occurs due to "the internal preponderance of male tendency genes" present in the autosomes, which are added as an additional set.

Every individual has both male and female potentialities, because X chromosomes have female tendency genes and the autosomes have male tendency genes. The sex is decided by the balance that is, by preponderance of either male tendency genes or the female tendency genes. The deciding factor is the ratio between the number of X chromosome and number of the sets of autosomes in the zygote. This is called **'sex index'** by Bridges.

$$\text{Sex index} = \frac{\text{Number of X chromosomes}}{\text{Number of sets of autosomes}}$$

If the ratio is 1.0, the individual will be female and if it is 0.5 male will result. The ratio between 0.5 and 1.0 result in intersex. The ratio 1.5 leads to super female and 0.33 leads to super males.

**Haplo-diploidy sex determination**

In several species of Hymenoptera such as honey bees, ants, wasp and saw flies males develop parthenogenetically (from unfertilized eggs) and have a haploid chromosome number (16 in the drone / male honey bees). The queen honey bee and the workers, which arise from fertilized eggs carry the diploid chromosome number (32). So, in the honey bees, the sex is determined by the haploid and diploid chromosome numbers. It is some times said that a drone honey bee has no father but has a grand father. This is possible by the haploid diploidy mechanism of sex determination.

Similarly in the parasitoid wasp *Bracon hebetor* (formerly Habrobracon), the females are diploid with 20 chromosomes and males are haploids with 10 chromosomes. Female originate from fertilized eggs and male from unfertilized eggs. This mechanism of sex determination is often referred to as haplo-diploidy.

Results of the experiments by Whiting showed that the sex determination depends upon the genetic composition of the certain region of the chromosome ie, homozygous, heterozygous or hemizygous status of certain chromosome segments and not on diploidy versus haploidy per se. If Xa, Xb, Xc are different chromosomal segments, then female sex is produced by heterozygous of the certain chromosomal segments (Xa, xb / xa, Xc) and male phenotype is due to hemizygous or homozygous condition of chromosomal segements (Xa, xb, Xc/Xaxa, Xbxb, Xcxc).

**Role of environment and sex determination**

In some lower animals, sex determination is non-genetic and depends on factors in the external environments. Males and females have similar genotypes, but stimuli from environmental sources initiate development towards one sex or the other.

In the case of Bonellia, for example, females are free living form with an ovoid body and long proboscis. The male are small, parasitic and lives in the reproductive tracts

of the larger female. Larvae of Bonellia are potentially capable of developing either into males or females. If the larvae are isolated, they will become females. If they are grown near the females, they will become males. Sex determination is non-genetic and depends on the external environment. The hormone like substances secreted by the female has an effect to turn the larvae into males. So the presence or absence of this hormone like substance in the environment determines the sex in Bonellia.

**Gynandromorphism**

Gynandromorph is an individual in which one half is male and other half is female. The mosaic condition of sex chromosomes leads to phenotypic sex mosaic.The Gynandromorphism is best studied in Drosophila, where there is no dilutions of the characteristics i.e. the male side is fully male and the female side is fully female. There are three kinds of gynandromorphism.

**(i) Bilateral gynandromorphs**

It is found in Drosphila. One lateral side of the fly is male and other lateral side is female. This is due to abnormal mitosis during early cleavage of the zygote.

(ii) **Anterior-Posterior gynandromorphs**

Some gynanders posses' male characters on the anterior side and female character on the posterior side of the body or vice-versa. These are called anterior – posterior gynanders.

(iii) **Sex – piebalds**

In some gynanders, the individual is predominantly a male or female with patches of opposite sex scattered on it. They are known as sex-pie balds.

**Sex – mosaics**

Mosaicism refers to a condition in which a person's cell consists of two or more populations, each with different chromosome complements. Murry Barr found a girl in whom both buccal and vaginal smears showed two barr bodies, thus indicating the XXX

chromosome complements. Blood cells showed no barr body indicating a XO chromosome complements. These mosaics arise as results of errors in mitosis in early stages of embryonic development.

**Questions**

1. Chromosomes containing genes, which determine various somatic characters

   i. Autosomes ii. Allosomes iii. X-chromosomes iv. Sex chromosomes

   **Ans: i. Autosomes**

2. Sex determination by chromosome was first reported by

   i. T.H.Morgan ii. Bridges iii. Henking

   **Ans: iii. Henking**

3. Sex determination in drosophila & human beings by

   i. XX-XO type ii. XX-XY type iii. XXXY type iv. Haplo-diploids

   **Ans: ii. XX-XY type**

4. Genic balance theory was proposed by

   i. T.H.Morgan ii. Bridges iii. Henking

   **Ans: ii. Bridges**

5. Sex index

i. $\frac{\text{No.of x Chromosomes}}{\text{No.of sets of autosomes}}$ ii. $\frac{\text{No.of x Chromosomes}}{\text{No.of y chromosoes}}$

iii. $\frac{\text{No.of allosomes}}{\text{No.of autosomes}}$ iv. $\frac{\text{No.of sets of autosomes}}{\text{No.of sets of allosomes}}$

**Ans: i.** $\frac{\textbf{No.of x Chromosomes}}{\textbf{No.of sets of autosomes}}$

**True or False**

1. Haplo –diploidy sex determination is observed in hymenopteran insects

**Ans: True**

2. In Bonellia, the free living forms with an ovoid body and long proboscis are females

**Ans: True**

3. The sex index of 1.5 will produce super males

**Ans: False**

4. In honey bees, the males have haploid chromosome number

**Ans: True**

5. Sex index between 0.5 and 1.0 result in intersex

**Ans: True**

# Lecture No.21

## Sex linked inheritance

Inheritance through X chromosome is called sex linked inheritance. It was discovered by T.H. Morgan in 1910. Eye color and bar eye in Drosophila, color blindness and haemophilia in human and barred plumage in fowls are inherited through X chromosome.

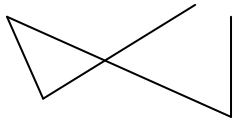
**Characteristic features of sex linked genes**

1. Sex linked genes are located on 'X' chromosome only.
2. In diploid, homogametic sex contains two copies of sex linked alleles where as heterogametic sex contains only one sex linked allele.
3. A recessive gene, in a homogametic sex can express only when it is homozygous state, whereas in heterogametic sex a recessive allele express in hemizygous condition.
4. Sex linked genes follow the criss cross inheritance.
5. Sex linked gene exhibit several deviations from the normal segregation pattern.

**Criss-cross inheritance of X-chromosome in *Drosophila***

Female is produced when an X egg is fertilized by X sperm. Male is produced when Y sperm fertilizes an X egg.

| | | |
|---|---|---|
| P | Female | Male |
| | XX | XY |
| Gametes | (X) | (X) (Y) |
| Progeny | XX | XY |



A male receives an X-chromosome only from the mother and never from father. The male receives Y chromosome only from his father, never front his mother. Thus the inheritance of X chromosome in ***Drosophila*** follows specific pattern. The male transmits his X chromosome to grandson only through his daughter. This is called criss- cross inheritance. The female transmits the X-chromosome both to her son and daughter.

The criss – cross pattern of inheritance is characteristics of sex-linked genes. This distinctive criss-cross pattern, from father through daughter to grandson replacing the usual pattern for the $F_1$ and $F_2$ segregation is now interpreted as evidence of sex-linkage.

**Criss-cross inheritance of eye colour in Drosophila**

A cross was made between white-eyed male Drosophila and red-eyed female Drosophila by T.H.Morgan in 1910. The $F_1$ flies were red eyed. When $F_1$ files were intercrossed, three fourth of the $F_2$ flies possessed red eyes and one fourth white eyes. From this familiar 3:1 ratio, it is clear that this is a monohybrid inheritance where red is dominant over white. But, when the $F_2$ flies were classified for both eye color and sex.

**It was found that**

(i) All the $F_2$ females were red eyed.
(ii) Half of the $F_2$ males were red eyed.
(iii)Half of the $F_2$ males were white eyed.

When reciprocal cross was made between white eyed female and red eyed male the $F_1$ was composed of two different phenotypes *ie*., red eyed females and white eyed males. When these $F_1$ flies were intercrossed, the $F_2$ consisted of flies in the ratio-2 red eyed: w white eyed. When these $F_2$ files were classified for both eye color and sex, it was found that

(i) Of two red-eyed flies, one is male and another is female.
(ii) Of two white-eyed flies, one is male and another is female.

**Direct crosses**

P $X^wX^w$ X $X^wY$

Red eyed female ↓ White eyed male

G $(X^w)$ $(X^w)(y)$

↓

$F_1$ $X^wX^w$ $X^wy$

Red eyed female Red eyed male

G $(X^w)$ X $(^w)$ $X^wY$

$F_2$ $X^wX^w$ $X^wX^w$ $X^wy$ $X^wy$

Red eyed Red eyed Red eyed White

Female female male eyed male

**Reciprocal cross**

P $X^wX^w$ X $XW^Y$

White eyed female ↓ Red eyed male

G $(X^w)$ $(X^w)$ $(y)$

$F_1$ $X^WX^w$ ↓ $X^wy$

Red eyed female White eyed male

G $(X^W)$ X $(^w)$ $X^wY$

$F_2$ $X^WX^w$ $X^wX^w$ $X^wy$ $X^wy$

Red eyed White eyed Red eyed White

Female female male eyed male

In the normal Mendelian inheritance, the $F_2$ ratio does not differ from that of reciprocal cross. But in the inheritance of eye color in Drosophila, the $F_2$ ratio depends on the sex of the parent by which eye color is introduced.

In *Drosophila*, the white eye color follows a criss cross inheritance. The kind of inheritance from father to grandson only through daughter is called criss-cross inheritance. The male transmits his red eye color to his grand sons through his daughters, never to or through his sons. Thus, the transmissions of eye color and X chromosome are similar. Hence, it is assumed that the gene for eye color is located in the X chromosome and y chromosome carries no allele for eye color.

**Holandric genes**

Most sex-linked genes in male heterogametic animals are on the X chromosome. However, Y chromosome also contains few genes that produce visible effects on the phenotype of the organism. Such genes are called Y linked or holandric genes. Holandric genes would be transmitted directly from father to son and never appear in females.

**Sex-influential dominance/Sex influenced character**

The condition in which the same gene acts as dominant in one sex and recessive in other sex is called as sex influenced dominance. That is, the sex influences the gene either to be dominant or recessive. The sex influenced genes are present in autosomes. This differential behaviour of the gene is due to female and male sex hormones.

For example, in human being baldness is due to sex influenced gene. This trait is dominant in men and recessive in women. A man is bald in homozygous recessive as well on heterozygous condition for baldness. Whereas women exhibit baldness only in homozygous recessive condition for baldness and heterozygous condition for baldness in female sex produce normal phenotype.

$H^NH^N$ -Normal female and normal male

$H^NH^B$ -Normal female and bald male

$H^BH^B$ -bald female and male

**Sex-limited gene expression/sex limited characters**

Sex – limited genes are those which produce characteristics that are expressed in only one fo the sexes. Sex limited genes may be located in any of the chromosomes. The sex hormone is found to be limiting factor in the expression of sex limited gene. Sex limited genes are responsible for secondary sexual characteristics.

For example beard in man and breast in women are produced by sex-limited genes. A women does not have a beard, though she carries all the genes necessary for beard. Similarly man does not have breasts though he carries all the genes necessary for breast. The expression of sex-limited characteristics depends upon the presence or absence of sex hormones.

## Questions

11. Sex linked inheritance was discovered by

i. T.H.Morgan ii. Bridges iii. Henking iv. De vries

**Ans: i. T.H.Morgan**

2. Sex linked genes are located on

i. X and Y chromosomes ii. Y Chromosomes only

iii. X chromosomes only iv. Autosomes

**Ans: iii. X chromosomes only**

3. In diploid, homegametic sex contains --------- copies of sex linked alleles

i. 1 ii. 2 iii. 3 iv. 4

**Ans: ii. 2**

4. Y linked genes are called as

i. Holandric genes ii. Sex linked genes iii. X linked genes

**Ans: i. Holandric genes**

5. Baldness in human beings is due to

i. Sex linked genes ii. Y linked genes

iii. Sex limited genes iv. Sex influenced genes

**Ans: iv. Sex influenced genes**

**True or False**

6. Sex linked genes do not follow criss-cross inheritance

**Ans:False**

7. In a heterogametic sex, a recessive allele express in a hemizygous condirion.

**Ans: True**

8. Holandric genes are transmitted directly from father to son and never appear in females

**Ans: True**

9. $H^NH^B$ genotype in human produces normal female and bald male

**Ans: True**

10. Sex limited genes are responsible for secondary sexual characteristics.

**Ans:True**

## Lecture No.22

## Sex determination in plants

### Sex-determination in *Melandrium album*

In *M.album* which follows the XY mode of sex determination. The Y chromosome of *M.album* has three district regions influencing sex-determination and male fertility has been localized on the differential part of the Y chromosome (Which does not have a homologous part on the X). The region I suppress the femaleness. In the absence of this region, plants are bisexual. Region II promotes male development. When this region (with or without Region I) is missing a female plant is produced. Region III carries male fertility genes, loss of this region result in male sterility.

### Sex determination in papaya

A single gene controls sex-determination in papaya. In this plant single gene with three alleles (m, $M_1$, $M_2$) control the sex-differentiation. Female plants are homozygous with a genotype of mm, while male plants are heterozygous with a genotype of $M_1$ m; the heterozygote condition of $M_1$ $M_2$ are recessive lethal. The mating between female (mm) and male ($M_1$m) produce 50% male ($M_1$m) and 50% female (mm) progeny. Similarly, a cross between a female (mm) and a hermaphrodite ($M_2$m) plant would yield 50% hermaphrodite ($M_2$m) plant and 50% female (mm) plants.

**Table 22.1 Postulated sex determination in papaya**

| Genotype | Survival | Sex-expression |
|---|---|---|
| Mm | Vital | Female |
| $M_1$m | Vital | Male |
| $M_2$m | Vital | Hermaphrodite |
| $M_1M_1M_2M_2$ | Lethal | - |

**Sex - determination in maize**

Maize plants are generally monoecious, i.e. male and female flowers are produced on the same plant. A single recessive gene, *ba* in homozygous condition (**baba**) interferes in the cob development and making these plants functionally male. Another recessive gene, **ts** converts the male flowers in tassels of ***ts ts*** plants into female flowers and plants do not produce pollen grains. In plants homozygous for both *ba* and *ts* are functionally female. So the two recessive genes (***ba*** and ***ts)*** have converted a naturally monoecious plant into a dioeciously one.

**Table 22.2 Sex expression in maize**

| **Genotype** | **Female flower** | **Male flower** | **Sex expression** |
|---|---|---|---|
| BaBa TsTs | Normal | Normal | Monoecious |
| baba TsTs | Rudimentary | Normal | Male |
| BaBa tsts | Normal | Develop into female flower | Female |
| Baba tsts | Rudimentary | Develop into female flower | Female |

## Questions

1. Region II of Y-chromosome of *Meladrium olbum*

   i. Promotes male development

   ii. Promotes female development

   iii. Promotoes bisexuality

   iv. Suppresses femaleness

   **Ans: i. Promotes male development**

2. In papaya, sex determination is influenced by

   i. Two allele's ii. Three allele's iii. Four alleles iv. Multiple allels

   **Ans: ii. Three alleles**

3. Mating between individual with mm condition and individual with $M_1m$ condition in papaya gives

   i.50% male and 50% female ii. All males

   iii. All females iv. All hermaphrodites

   **Ans: i. 50% male and 50% female**

4. In papaya $M_2m$ condition produces

   i. Male ii. Female iii. Hermaphrodite iv. Sterile

   **Ans: iii. Hermaphrodite**

5. Genotype of sex expression in male maize is

   i. BaBa TsTs ii. Babatsts iii. BaBatsts iv. babaTsTs

   **Ans: i. BaBa TsTs**

**True or False**

6. $M_2m$ condition in papaya cause lethality

   **Ans: False**

7. Region I of Y chromosome of *Melanidrium album* suppresses femaleness.

**Ans: True**

8. Region III of Y chromosome of *Melandrium album* carries male sterility genes.

**Ans: False**

9. In maize, sex determination is controlled by two genes

**Ans: True**

# Lecture No.23

## Cytoplasmic inheritance

Besides chromosomes, various organelles of cytoplasm also contain DNA. The mitochondria and plastids have their own DNA and carry their genetic characters themselves. The mechanism in which cytoplasmic inclusions (e.g., alpha, beta, sigma and kappa particles) and organelles (plastids, mitochondria, centriole, etc) take part in transmission of characters from generation to generation is called cytoplasmic inheritance. Since cytoplasmic inheritance is based on cytoplasmic DNA molecules, it is also called extra chromosomal inheritance.

The smaller inheritable extra chromosomal unit is called as **plasma gene** and all the plasmagenes of a cell constitute the **Plasmon** (like the genome).

Cytoplasmic inheritance is due to the plasmagenes located in cell organelles that are integral constituents of normal cells. The characteristic features of this inheritance are summarized below.

**1. Different in reciprocal crosses**

In Mendelian inheritance, the results of reciprocal crosses are identical (one exceptional – sex linked inheritance). If the character is transmitted through cytoplasm, the reciprocal cross results will be different.

**2. Somatic segregation**

Plasma genes generally show somatic segregation during mitosis, a feature of rare occurrence in the case of nuclear genes.

**3. Non-mappability**

Gene controlled characters shows linkages and hence they are mappable. But the characters transmitted through cytoplasm show no linkage. Hence, they are not mappable.

**4. Non-Segregation**

Segregation is typical of Mendalian heredity. The cytoplasmic heredity fails to show segregation. Sometimes, segregation may occur in cytoplasmic heredity also. But it will not be consistent with the segregation of chromosomes.

**5. Indifference to nuclear substitution**

When the nucleus is transplanted, no change is found in the cytoplasmic inheritance.

**6. Infection like transmission**

Cytoplasmic inheritance seems like infection through some agents.

**Maternal inheritance**

Maternal effects are produced due to the influence of mother's nuclear genotype on the phenotype of its progeny and last for one generation. Characters showing the maternal effect exhibit clear-cut differences in $F_1$ for reciprocal crosses. One of the examples for maternal effect is coiling pattern of shell in snail limnaea. In this snail, the direction of coiling of its shell is controlled by single nuclear gene D/d; the dominant allele D produces right-handed coiling, while its recessive allele d produces left-handed coiling. The direction of shell coiling in an individual is governed by the genotype of its female parent and not by its own genotype. As a result, reciprocal crosses show differences in coiling in $F_1$ and there is no phenotypic segregation in $F_2$ the phenotypic effect of segregation is observable in $F_3$ only.

Crosses between females with left-handed coil (dd) and males having right handed coil (DD) produce $F_1$ progeny (Dd) with left-handed coil, since the genotype of the female parent is dd. In $F_2$ segregation of Dd produces three genotypes (DD,Dd,dd) in the ratio of 1:2:1. But the $F_2$ snails with DD, Dd as well as dd genotypes exhibit right handed coiling since their female parent has the genotype Dd which determines right-handed coiling in the progeny (irrespective of the genotypes of the progeny). The $F_3$ progeny from the $F_2$ individuals with the genotypes DD and Dd will show right handed coiling, while those from dd $F_2$ individuals will exhibit left-handed coiling of their shells; thus produces the typical 3:1 ratio in $F_3$.

The reciprocal cross (DDxdd), on the other hand, yields right-handed coiling in the $F_1$ (Dd) as well as in the three genotypes, 1DD:2Dd:1dd, obtained in the $F_2$. But in $F_3$ 2/3 of the progenies show right-handed coiling since they are derived from $F_2$ individuals having the genotypes DD and Dd. The remaining 1/3 of the $F_3$ progenies exhibit left handed coiling since their female parents had the genotype dd; this yield the typical monohybrid ratio of 3:1 in the $F_3$.

The direction of coiling in this snail is determined by the plane or the direction of the first mitotic division of the zygote. The plane of the first division, of the other hand, is determined by some substances already present in the egg cell. Obviously, these substances are produced by the female parent; as a result, they would produce the phenotype appropriate for the maternal genotype. Further, genotype of the zygote itself has no effect of the plane of first division and consequently, on the direction coiling since its gene products are not involved in determining this trait. As a result, the direction of coiling in an individual is governed by the genotype of its female parent. Therefore, phenotypes appear one generation later than the appearance of the concerned genotypes, producing delayed segregation in $F_3$.

**Inheritance of kappa particles in Paramecium**

There are two types of strains in ***Paramecium***. One has kappa particles in its cytoplasm and other does not have such particles in its cytoplasm. The presence of kappa particles in the cytoplasm leads to production of a toxin known as paramecin. This toxin can kill the strain Paramecium that lacks kappa particle. Thus the strain with kappa particle is known as killer strain and that without kappa particle is called as sensitive strain.

The production of kappa particles is dependent on a dominant allele K, so that the killer strains are KK or Kk and sensitive strains are ordinally kk. In the absence of dominant allele K, kappa particles can not multiply and in the absence of kappa particles, dominant allele K cannot produce them de novo.

If the killer (KK) and sensitive (kk) strains are allowed to conjugate, all exconjugants (the cells separating after conjugation) will have the same genotype Kk. The phenotypes of these exconjugants will however depend upon duration for which conjugation is allowed. If conjugation does not persist long enough for exchange of cytoplasm, heterozygote (Kk) exconjugants will only have parental phenotypes. It means that killers will remain as killers and sensitive will remain as sensitive after conjugation. If conjugation persists, sensitive stain will receive kappa particles and will become killer, so that exconjugants will be killers having genotype Kk.

**Plastid inheritance**

Plastids are minute cytoplasmic organelles in plant cells. Most important are the chloroplastids, which carry chlorophyll. Plastids arise from smaller cytoplasmic

particles (plastid primordial) that contain DNA. They duplicate themselves independently. They are transmitted through the cytoplasm of the egg.

The Four-O'clock plant, *mirabilis jalapa*, has branches that produce either green, white or mixed green-white (variegated) leaves. In crosses between flowers of these branches, the offspring are all green if the maternal parent is a flower from a green branch. Such offspring remain green throughout subsequent generations as long as maternal plant is green. Similarly, as long as the maternal parent is from a white branch, the offspring are all white. When variegated branches are used as female source, both green and plastids are present in cells of female parent. Therefore, female gametes may carry either green or pale plastids or both. Consequently, three kinds of plants namely green, pale and variegated plants would be obtained.

**Inheritance of leaf colour in *Mirabilis jalapa***

| **Egg source** | **Pollen Source** | **Progeny** |
|---|---|---|
| White | Green<br>White<br>Variegated | White |
| Green | Green<br>White<br>Variegated | Green |
| Variegated | Green<br>White<br>Variegated | Green<br>White<br>Variegated |

**Plasmids**

Plasmids are called episomes. They are extra chromosomal, circular, covalently closed double stranded DNA molecules found in bacteria. In effect, plasmids are accessory chromosomes. Plasmids can replicate autonomously of the host chromosome. The size of plasmid ranges from two to several hundred kilobases.

Plasmids carry genes for the inactivation of antibiotics, metabolism of natural products and production of toxins. The F factors and R factors are important plasmids of *Escherichia coli.*

**Mitochondria (mt DNA)**

Mitochondria are present in living organisms arise from pre existing mitochondria. They are small cytoplasmic organelles present in animal and plant cells but not present in bacteria and viruses. Mitochondria provide cellular energy through oxidative phosphorylation. Mitochondria contain a small circular DNA molecule codes for limited number of structures and functions. The size of mtDNA ranges from about 16 kb in mammals upto several hundred kilo base pairs in higher plants (eg 570 kb in maize) and mt DNA usually found in multiple copies per organelle. The mtDNA play a significant role in crop improvement. Recent evidences showed that the cytoplasmic genetic male sterility system in crop plants is due to the interaction of mitochondrial genome to the nuclear genome.

**Chloroplast DNA**

Chloroplast of the plant cell contain circular DNA molecule which are self-replicating in nature. The isolated chloroplast found to be capable of protein synthesis in the presence of light. The DNA analysis revealed that 30-60 copies of the chloroplast genome are found in each chloroplast of higher plants. The chloroplast genome contains herbicidal resistant and streptomycin resistant genes.

## Questions

1. Cytoplasmic inheritance is based on

i. Cytoplasmic inclusions ii. Organelle DNA

iii. Nuclear DNA iv. Both 1&2

**Ans: iv.Both 1&2**

2. Plasmagenes are not mappable, because they show

i. Complete linkage ii. Incomplete linkage

iii. Coupling linkage iv. No linkage

**Ans: i. Complete linkage**

3. Cytoplasmic male sterility in crop plants is governed by

i. Chloroplast genes ii. Mitochondrial genes

iii.Nuclear genes iv. Kappa particles

**Ans: ii. Mitochondrial genes**

4. Influence of mother's nuclear genotype on the phenotype of its progeny is called as

i. Maternal effect ii. Paternal effect

iii. Nuclear effect iv. Both 1&2

**Ans: i. Maternal effect**

5. The genes for herbicidal resistance and sterptomycin resistance are located on

i. Mitochondria ii. Chloroplast

iii. Nuclear iv. Alpha particles

**Ans: ii. Chloroplast**

**True or False**

6. Reciprocal differences are observed in cytoplasmic inheritance

**Ans: True**

7. In paramecium, the strain with kappa particles is known as sensitivesrain

**Ans: False**

8. Plasmids/episomes are extra chromosomal, circular, double stranded DNA molecule

**Ans: True**

9. Plasmagenes do not show somatic segregation

**Ans: False**

10. Prokaryotes have cytoplasmic organelles and plasmagene

**Ans: False**

## Lecture No.24

## DNA as genetic material

### Griffith experiment

The phenomenon of transformation *in pneumococcus* bacterium was discovered by Fredrick Griffith in 1928. There are two types of *pneumococcus* bacteria – *virulent (pathogenic)* and Avirulent (non-pathogenic). Virulent strains have polysaccharide capsules and give smooth colonies. Avirulent strains have no capsules and give rough colonies. The virulent strain has the antigenic property with serotype III and Avirulent has the serotype II.

| **Virulence** | **Colony morphology** | **Serotype** | **Designation** |
|---|---|---|---|
| Virulent | Smooth | III | III S |
| Avirulent | Rough | II | II R |

Live II R and heat killed IIIS are not lethal, when injected into the mice separately. But a mixture of live II R and heat killed IIIS was lethal to the mice. The blood of dead mice contained live IIIS. The heat killed IIIS had transformed live II R into live IIIS. Griffith (1928) called this phenomenon as transformation. Thus the transformation of non virulent Type II R cells to virulent Type IIIS cells cannot be explained by mutation, rather some component of the dead Type IIIS cells must convert living Type II R cells to Type IIIS.

The same phenomenon occurred in the test tube when live Type II R cells were grown in the presence of dead type IIIS or extract of Type IIIS was hereditary and it set the stage for determining the chemical basis of hereditary in *Pneumococcus*.

### Proof that the DNA is the genetic material

The first direct evidence showing that the genetic material is DNA rather than protein or RNA was published by **O.T. Avery, C.M. Macleod and M. Mc Carty in 1944.** The most definite experiments conducted by them proved that the **DNA was the transforming principle** (DNA is the genetic material) involved the use of enzymes that degrade DNA, RNA, or protein. In separate experiments highly purified DNA from Type IIIS cells was treated with (1) Deoxyribonuclease (DN ase which

degrades DNA) (2) Ribo nuclease (RN ase which degrades RNA) or (3) Proteases (which degrade proteins) and then tested for its ability to transform Type II R cells to Type IIIS. Only Deoxyribonuclease had any effect on the transforming activity of the DNA preparation. It totally eliminated all transforming activity. The results obtained by Avery and co-workers clearly established that the genetic information in *pneumococcus* was present in DNA. We now know that the segment of DNA in the chromosome of pneumococcus that carries the genetic information specifying the synthesis of a Type III capsules is physically integrated into the chromosome of the Type II R recipient cell by a specific recombination process occurring transformation.

**The Hershey-Chase Experiment**

The additional direct evidence indicating that DNA is the genetic material was published in 1952 by A.D Hershey (1969 Nobel Prize Winner) and M.Chase. These experiments showed that the genetic information of a particular bacterial virus (bacteriophage $T_2$) was present in DNA.

Viruses are the smallest living organisms. They never as such enters the cell; only the tail contacts the host and enzymatically cuts a small hole through the membrane and then the nucleic acid of the virus head flows to the cell. This idea was tested by Hershey and Chase in the following way. Phage DNA was labelled with radio-isotope $^{32}P$ in place of normal isotope where as protein coat was labelled with $^{35}S$ in the place of normal isotope $^{32}S$. These labels are highly specific, because DNA does not contain sulphur and the protein coat is devoid of phosphorus. A sample of an *E.coli* culture was infected with labelled $T_2$ phage. After a short incubation period, the suspension was spun for a few minutes in warring Blender at 10,000 rpm. This treatment served the connections between the viruses and bacteria. The resulting suspension was centrifuged. The pellet contained infected bacteria, where as supernatant contained smaller particles. These fractions were analysed for $^{32}P$ and $^{35}S$ to determine the location of the phage DNA and the protein coat. The results of the experiment were:

1. Most of the phage DNA was found in the bacteria.
2. Most of the phage protein was found in the supernatant.
3. The blender treatment did not prevent the infection.
4. The progeny of $T_2$ phage contained the parental $^{32}P$ and not the parental $^{35}S$.

This led to the conclusion that the phage has a genetic part-DNA and non-genetic protective part-protein. The protein coat serves as a vehicle. Only DNA carry necessary information for the new generation of phages.

**RNA as genetic material in small viruses**

In viruses the genetic information are present either in DNA or RNA. Tobacco_mosaic virus (TMV) is an RNA virus. It consists of a single molecule of RNA surrounded by a protein coat. By using the appropriate chemical treatments, one can separate the protein coats of TMV from RNA. Moreover, this process is reversible; by mixing the proteins and RNA under appropriate conditions "reconstitution" will occur producing complete infective TMV particles.

Frankel-conrat and Singer (1957) took two different strains of TMV, separated the RNA from the protein coats and reconstituted mixed virus by mixing the proteins of one strain with the RNA of second strain and vice-versa. When these mixed virus were used to infect tobacco leaves, the progeny viruses produced were always found to be phenotypically and genotypically identical to the parent strain from which the RNA had been obtained. Thus the genetic information of TMV is stored in RNA and, not in protein.

## Questions

1. The phenomenon of transformation was discovered by

   i. Avery  ii. McCarty  iii. Griffith  iv. Mccleod

   **Ans: iii. Griffith**

2. Evidence for DNA as the genetic material was given by

   i. Avery  ii. Mccarty  iii. McCleod  iv. All the three

   **Ans: iv. All the three**

3. Ribonuclease enzyme degrades

   i. Protein  ii. RNA  iii.DNA  iv. Both RNA&DNA

   **Ans: ii. RNA**

4. The genetic material in tobacco mosaic virus (TMV) is

   i. RNA  ii.DNA  iii. Plasmagenes  iv. Betaparticles

   **Ans: i. RNA**

5. Hershey and Chase conducted their experiments on

   i. Pneumococcus  ii. $T_4$ Bacteriophage  iii. $T_2$ bacteriophage  iv. TMV

   **Ans: iii. $T_2$ bacteriophage**

**True or False**

6. Proteases degrades proteins

   **Ans: True**

7. Genetic information of TMV is stored in protein

   **Ans: False**

8. RNA as the genetic material in virus was proved by Conrat & Singer

   **Ans: True**

9. Deoxyribonucleases degrades both DNA & RNA

   **Ans: False**

10. The phenomenon of transformation was confirmed by Griffith in *Pneumoccocus*

**Ans: True**

## Lecture No.25

## Structure of DNA

### Deoxy ribonucleic acid

DNA is a long thread like unbranched polymeric molecule of heredity. DNA molecule composed of repeating sub units called nucleotides. Each nucleotide composed of (i) a phosphate group (ii) a five carbon deoxyribose sugar (iii) cyclic nitrogen containing compound called nitrogenous base.

### Structure of DNA molecule (Watson and Crick's DNA double helix model)

The correct structure of DNA was proposed by J.D.Watson and F.H.C Crick (1953). The double helix model proposed by them is based on two evidence.

### 1. Chargaff's chemical analysis

Chargaff (1950) found that a specific quantitative relationship is present between purines and pyrimidines of DNA molecule. In DNA molecule, the ratio of adenine to thymine and guanine to cytosine are 1:1 that is

| | | |
|---|---|---|
| Amount of purine | = | Amount of pyrimidine |
| A+G | = | T+C |
| A | = | T |
| G | = | C |
| AT | = | GC |

It is called as Chargaff rule.

### 2. Crystallographic studies by Wilkins and Franklins

The X-ray diffraction patterns and crystallographic data on DNA structure from studies of M.H.F. Wilkins and R. Franklins showed that DNA is highly ordered multiple strand structure with repeating sub unit structures spaced in every 3.4 A° along the axis of the molecule

### Structure of DNA

On the basis of Chargaff's chemical data and crystallographic data by Wilkins and Franklins, Watson and Crick proposed the structure of DNA. The important features of their model are

1. DNA exists in double helix in which two polynucleotide chain coiled about one another in a spiral way.
2. Each polynucleotide chain consists of sequence of nucleotides linked together by **phosphodiester bonds** and two polynucleotide chains held together **by hydrogen bonding** between bases.
3. The base pairs are stacked between two chains perpendicular to the axis of the molecule similar to the steps of a spiral staircase. The base pairs in DNA stacked 3.4 A° apart with 10 base pairs per turn (360°) of the double helix.
4. The base pairing in DNA molecule is specific ie Adenine pairs with Thymine (A=T) and Cytosine pair with Guanine (C=G). So each base pair consists one purine and one pyrimidine.
5. The two strands of the DNA are complementary in nature (non-identical) ie once the sequences of bases is one strand is known, the sequences of bases in the other strand is also known because of specific base pairing. The complementary nature is very important for storing and transmitting the genetic information.
6. The purine and pyrimidine bases are on the inside of the helix where as the phosphate and deoxyribose unit are on the outside. The sugar phosphate backbones of the two complementary strands are antiparalle. Among two strand of DNA, one strand go from 3' carbon of one nucleotide to 5' carbon of the adjacent nucleotide. Whereas the complementary strand go from 5' to 3' carbon. This mechanism is very important in considering the mechanism of replication of DNA.
7. The high degree of stability of DNA is due to more number of hydrogen bonds.

## Questions

1. A nucleotide is composed of

   i. Ribose sugar + Phosphate group

   ii. Deoxyribose sugar + 'N' bases

   iii. Phosphate group + Deoxyibose sugar + Nitrogenous bases

   iv. Phosphate group + Ribose sugar + Nitrogenous bases

   **Ans: iii. Phosphate group + Deoxyibose sugar + Nitrogenous bases**

2. The structure of DNA proposed by Watson & crick is

   i. Doble helix

   ii. Triple helix

   iii. Double helix with right handed coiling

   iv. Double helix with left handed coiling

   **Ans: iii. Double helix with right handed coiling**

3. Chargaff rule says

   i. A+G: T+C  ii. AT: GC  iii. A: T  iv. All the three

   **Ans: iv. All the three**

4. How many polycuelectides are present in DNA

   i.1  ii. 2  iii. 3  iv. 4

   **Ans: ii. 2**

5. The bond existing between DNA bases is

   i. Phosphodiester bond  ii. Nitrogen bond  iii. Hydrogen bond  iv. Oxygen bond

   **Ans: iii. Hydrogen bond**

**True or False**

6. The base pairs in DNA are stacked 3.4A° apart

   **Ans: True**

7. The bond existing between two nucleotides is phosphodiesler bond

**Ans:True**

8. A≡T, G=C

**Ans: False**

9. Higher the number of hydrogen bonds, higher the stability of DNA

**Ans: True**

10. The complementary base for adenine in DNA is Guanine

**Ans: False**

## Lecture No.26

## DNA replication

Watson and Crick proposed that during DNA replication, the two strands of DNA molecule separate after the breakage of hydrogen bonds and each strand acts as a template for the synthesis of a new companion strand. Thus, resulting daughter DNA molecules each containing an old strand derived from parent DNA molecule and another strand newly synthesized. This type of distribution of parental strands is called as semi-conservative. However in considering possible mechanisms of DNA replication, three different hypothetical modes are apparent in addition to semi conservative method of replication.

**1. Semi – conservative method**

In this method two strands of the parental DNA molecule separates and each strand act as a template for the synthesis of new complementary strand. Thus resulting daughter DNA molecules each contain one old strand derived from parental DNA molecule and another strand newly synthesized.

**2. Conservative method**

In this method, two parental DNA strand separates and each strand act as template for synthesizing a complementary strand. The resulting progeny DNA molecule composed of two newly synthesized strand and parental DNA strands remain intact (totally conserved after replication)

**3. Dispersive method**

In this method a segments of parental strands and progeny or nascent strands become interspersed through some kind of fragmentation, synthesis and rejoining process.

**Proof for semi-conservation method of DNA replication Meselson and Stahl's Experiment**

*E.coli* was grown for many generations in a medium that contained $^{15}N$, the heavy isotope as the sole nitrogen source (added as $^{15}$ $NH_4Cl$.) Their DNA thus labelled with $^{15}N$, was denser than ordinary DNA. This density difference can be distinguished by 'cesium chloride density gradient equilibrium sedimentation

technique'. Then these bacterial cells were transferred to $^{14}N$ medium. The bacterial cells were sampled at various times to ascertain the density of DNA. The sample time corresponded with the doubling of the cells. After one generation, the DNA of daughter bacteria had neither original $^{15}N$ density nor the pure $^{14}N$ density. Instead, this DNA had an intermediate (or) hybrid density (precisely between $^{15}N$ – $^{14}N$ densities). The absence of $^{15}N$ DNA indicated that the parental DNA was not conserved as an intact unit on replication. The absence of $^{14}N$ DNA indicated that the daughter DNA molecule was not synthesized entirely *denovo* (afresh).Of two strands of the daughter DNA molecule, one strand was derived from the parent $^{15}N$ DNA and the other strand was newly synthesized from $^{14}N$ source. Hence, the daughter DNA molecule, being hybrid in nature ($^{15}N^{14}N$) gives an intermediate (or hybrid) density.

After two generations of bacteria in $^{14}N$ medium there were equal amounts of hybrid DNA ($^{15}N^{14}N$) and ordinary DNA ($^{14}N$).

Meselson and Stahl concluded that during DNA duplications, each daughter molecule receives one strand from the parent molecule. This strand is conserved through much duplication. Their results agreed perfectly with the Watson-Crick hypothesis of DNA replication.

**Mechanism of DNA replication**

**1. Relaxation of packed DNA**

The highly packed super twisted DNA molecules are relaxed by the enzyme, DNA gyrase.

**2. Unwinding of DNA strands**

An enzymes rep protein (helicase) unwinds the parental DNA helix. The unwinding is powered by the hydrolysis of ATP. About two ATP are consumed for each base pair separation. The separated strands are stabilized by S-S binding proteins.

**3. DNA polymerase**

New DNA strand is synthesized by the enzyme DNA polymerase, with the old DNA strand acting as the template. There are three kinds of DNA polymerases-I, II and III. DNA polymerase I was discovered by Kornberg, hence called Kornberg enzyme. DNA polymerases II and III were later discovered by De Lucia and Cairns.

The catalytic rates of the three enzymes differ: 10 nucleotides per second are added by polymerase. It adds deoxyribonucleotide to the 3' –hydroxyl terminus of a pre existing DNA (or RNA) strand. That is a primer chain with a free 3'-OH group is required for the action of DNA polymerase I. In other words, DNA polymerase cannot initiate the DNA chain but only elongates the chain.

**4. DNA synthesis is primed by RNA**

DNA polymerase requires a primer with a free 3' – OH group for the initiation of DNA synthesis. Hence, DNA polymerase cannot initiate the DNA synthesis. Since RNA polymerase can start chains *denova*, RNA primes the synthesis of DNA.

**The initiation of DNA synthesis takes place in the following steps**

(i) A particular protein "d na B" protein acts as a recognition signal for the initiation of DNA synthesis. It enables a specific RNA polymerase (Primase) to initiate.

(ii) This RNA polymerase (primase) synthesizes a short stretch of RNA (10 nucleotides) that is complementary to the DNA template.

(iii) The 3' – hydroxyl group of the terminal ribonucleotide of this RNA chain serves as the primer for the synthesis of new DNA by DNA polymerase III.

(iv) The RNA portion of this RNA-DNA hybrid is then hydrolysed by DNA polymerase.

(v) The gap resulting from the removal of RNA is filled up through DNA polymerase I.

**5. Elongations of new DNA strand**

DNA polymerase III begins to add deoxyribonucleotides to the 3' – hydroxyl end of the RNA primer. The chain elongation reaction occurs by means of a nucleophilic attack of the 3' – OH terminus of the primer on the inner most phosphorus atom (phosphorus atom) of the incoming deoxyribonucleoside triphosphate. A phosphor diester bridge is formed and pyrophosphate is released. The subsequent hydrolysis of pyrophosphate drives the polymerization forward. The elongation of the DNA chain proceeds in the 5" ⟶ 3" direction. The polymerization is processive – that is, many nucleotides are added without the release of the enzyme from the template. DNA polymerase catalyses the formation of a phosphor-diester

bond only if the base on the incoming nucleotide is complementary to the base on the template strands. Thus DNA polymerase is a template directed strand.

**6. Proof reading by DNA polymerase I**

DNA polymerase I have (i) 5' ⟶ 3' polymerase activity (ii) 3' ⟶5' exonuclease activity and (iii) 5'⟶ 3' exonuclease activity.

i.Function of 5' ⟶3' polymerase activity

-step by step addition of nucleotides in the chain elongation

ii.Function of 5'⟶3' exonuclease activity

-DNA repair: Removal of DNA segment damaged by UV – rays

-Removal of RNA primer: Which are used in DNA synthesis

iii.Function 3'⟶ 5' exonuclease activity

Used for proof reading and editing function.

**7. Contain and discontinuous synthesis of DNA strands**

Both strands of parental DNA serve as templates for the synthesis of new DNA. The parental strands are antiparallel. Hence, the overall direction of DNA synthesis must be 5' ⟶ 3' for the other. But the DNA polymerase can synthesize DNA only in 5'⟶ 3' directions. These fragments are called Okazaki fragments. These fragments become covalently joined DNA ligase to form a continuous DNA strand. This strand formed from Okazaki fragments is termed the lagging strand.

## Questions

1. DNA replication is

   i. Conservative ii. Semi-conservative iii. Dispersive iv.Both2&3

   **Ans: ii.semi-conservative**

2. Which among the following is known as Kornberg enzyme

   i. DNA polymerase I ii. DNA polymerase II

   iii. DNA polymerase III iv. All the three

   **Ans: i. DNA polymerase I**

3. Which is the enzyme that relaxes the packed DNA during DNA replication

   i. Helicase ii. Gyrase iii. Polymerase iv. Primase

   **Ans: ii. Gyrase**

4. The enzyme that joins the Okazaki fragments is

   i. Helicase ii. Gyrase iii. Ligase iv. Endonucleare

   **Ans: iii. Ligase**

5. The DNA replication method in which parental and progeny strands become interspersed

   i.Conservative ii. Semiconservative iii. Dispersive iv. Both 1&2

   **Ans: iii. Dispersive**

**True or False**

6. DNA polymerase initiates DNA replication

   **Ans: False**

7. RNA polymerase is otherwise known as primase

   **Ans: True**

8. Semiconservative mode of DNA replication was proved by Meselson & Stahl

   **Ans: True**

9. Replication of the leading strand (3'-5') generates Okazaki fragments

**Ans: False**

10. DNA helicase unwinds DNA during replication

**Ans: True**

# Lecture No.27

# RNA

**Ribonucleic acid**

RNA is found in cells of all living organisms. It is found both in chromosomes in nucleus and ribosomes in cytoplasm. It contains ribose sugar, nitrogen bases and phosphate group. The nitrogen bases include adenine, guanine, cytosine and uracil and pairing occurs between AU and GC. The function of RNA is transfer of genetic message from nucleus to the cytoplasm and synthesis of protein in the ribosomes. In some viruses, RNA acts as the genetic material and regulates the gene action.

Flow of genetic information

DNA $\xrightarrow[\text{(Transcription)}]{}$ RNA $\xrightarrow[\text{(Translation)}]{}$ Protein

**Structure of RNA**

RNA is a long unbranched polymer consists of nucleiotides joined by phosphor-diester bonds. RNA differs from DNA in two ways.

- RNA is single stranded; DNA is double stranded.
- RNA contains ribose sugars where as DNA contains deoxy ribose sugar.
- RNA contains the uracil in place of thymine. Uracil lack methyl group present in thymine.
- RNA molecules are generally much shorter than DNA molecules.

**Three types of RNA**

**1. Messenger RNA or mRNA**

- It is a kind of single strand RNA molecule which is complementary to sense strand of DNA molecule.
- Produced by transcription of structural genes in the DNA sequence.
- mRNA carries the genetic message from the chromosome to the site of protein synthesis ie.ribosome
- mRNA molecule corresponds to each gene that is expressed.

**2. Transfer RNA or tRNA**

- tRNA is a kind of RNA molecule consists of 75 mucleotides and it becomes smallest of all RNA molecules.
- They carry the activated aminoacids to the ribosome for protein synthesis.
- There is a specific tRNA for each of the twenty aminoacids.
- The 5' end of tRNA have poly G and it is phosphorilated.
- tRNA contains many unusual bases between 7 and 15 per molecule.
- tRNA is folded into a clover leaf pattern. It has five following special region as follows.

**i.CCA end**

The base sequence in 3' end of all tRNA is CCA. The activated amino acid is attached to 3' hydroxyl group of the terminal adenosine.

**ii.TΨC arm**

Involved in the binding of the tRNA to ribosome.

**iii. Anticodon loop**

It consists of sevenbases with following sequences.

*Pyrimidine - pyrimidine -X-Y-Z -Purine variable modified*
Anticodn (codon Recognition site)

Codon recognition site is complementary to codons of mRNA.

**iv.DHU arm**

It is the site for the recognition of amino acid activated enzymes

**v. Extra arm**

Some tRNA have extra arm.

**3. Ribosomal RNA or rRNA**

i. It is a kind of RNA molecule serving as a major component of ribosomes.

ii. *E .Coli* has three kind of rRNA *i.e*., 23s, 16s, 5s

iii. rRNA is transcript of rRNA genes.

As it becomes evident that the genes controlled the structure of polypeptides, attention focused on how the sequence of four base pairs in DNA control the sequence of 20 amino acids found in proteins.

**Definition of genetic code**

The genetic code is the relationship between the sequences of bases in DNA (on its mRNA) and the sequence of amino acid in the protein. The sequence of three nucleotides in DNA (or its mRNA) that specifies a particular amino acid in the protein synthesis is called genetic code.

**Co-linearity between Gene and protein**

Benzer revealed that there is a linear correspondence between a gene and its polypeptide products. This co-linearity gives the clue that specific arrangement of nitrogenous bases in DNA determines the specific sequence of amino acid in protein. So the genetic information is written by four-letter language of DNA nitrogen base.

**Characteristic features of genetic code**

**1. Triple code**

There are 20 kinds of amino acids in the cytoplasm but only four kinds of nitrogenous bases. A singlet code is inadequate because it codes for only 4 amino acids and also a doublet code is also inadequate since it codes for 4x4 =16 amino acids only. A triplet code is only adequate since it codes for 64 (4x4x4) amino acids.

A group of three bases that codes for one amino acid is called codon.

Amino acids coded by the 64 possible codons of the triplet code

| **First base position S end** | **Second base position** | | | | **Third base position 3 end** |
|---|---|---|---|---|---|
| | **U** | **C** | **A** | **G** | |
| U | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | U |
| | UUU Phe | UCU Ser | UAU Tyr | UGU Cys | C |
| | UUA Leu | UCA Ser | UAA Stop | UGA Stop | A |
| | UUG Leu | UCG Ser | UAG Stop | UGC Trp | G |
| C | CUU Leu | CCU Pro | CAU His | COU Arg | U |
| | CUC Leu | CCC Pro | CAC His | CGC Arg | C |
| | CUA Leu | CCA Pro | CAA Glu | CGA Arg | A |
| | CUG Leu | CCG Pro | CAG Glu | CGG Arg | G |
| A | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUU Ile | ACU Thr | AAU Asn | AGU Ser | U |
| | AUA Ile | ACA Thr | AAA Lys | AGA Arg | C |
| | AUG Met | ACG Thr | AAG Lys | AGG Arg | A |

| | | | | | |
|---|---|---|---|---|---|
| G | GUU Val<br>GUC Val<br>GUA Val<br>GUG Val | GCU Ala<br>GCC Ala<br>GCA Ala<br>GCG Ala | GAU Asp<br>GAC Asp<br>GAA Glu<br>GAG Glu | GGU Gly<br>GGC Gly<br>GGA Gly<br>GGA Gly | U<br>C<br>A<br>G |

**2. Codons have degeneracy**

The occurrence of more than one codon per amino acid called degeneracy. The amino acids like methionine and tryptophan has only one codon each. Where as the amino acids leucine, serine and arginine have six codon each. Where as the amino acids leucine, serine and arginine have six codon each. The codons that specify the same amino acid are called synonyms. For example the codons CAU and CAC code for histidine and most of the synonyms differ only in the last base of the triplet. The degeneracy helps for minimizing the deleterious effect of mutation.

**3. Codons have wobbling**

The hydrogen bonding between the bases in the anticodon of tRNA and the codon of mRNA appears to follow strict base pairing rules only for the first two bases of the codon. The base pairing involving third base of the codon is apparently less stringent, allowing wobbling at this site.

| **5' base in anticodon** | **3' base in codon in the mRNA** |
|---|---|
| G | U or C |
| C | G |
| A | U |
| U | A or G |
| I (inosine) | A, U, C |

**4. Genetic code in non-overlapping**

It means that no single base can take part in the formation of more than one codan.

**5. Genetic code is commaless**

The genetic code is commaless, which means that no codon is reserved for punctuations.

**6. Start and stop codon (initiation and termination codon)**

UAG (Ochre), UAA (Amber) and UGA (opal) are only the three codons that donot specify aminoacid. They designate chain termination. These codons are not read by tRNA

molecules but by specific proteins called release factors. The codon AUG (methioine) and GUG (valine) act as starting codon for translation.

**7. Genetic code is universal**

With one or two exceptions the genetic code is same or nearly same in all the organisms. The codon UGA code for tryptophan in human mitochondrial system. Whereas UGA is a termination codon for non-mitochondrial system. Mutation that produces chain – termination triplets within genes is called as non-sense mutation whereas mis-sense mutation cause change a triplet to another triplet specifying a different aminoacids.

**Questions**

1. Flow of genetic information

i.RNA ⟶ DNA ⟶ Protein ii.DNA ⟶ RNA ⟶ Protein

Iii.DNA ⟶ Protein ⟶ RNA iv.Protein ⟶ RNA ⟶ DNA

**Ans: ii.DNA ⟶ RNA ⟶ Protein**

2. Transcription of structural genes in a DNA sequence generates

i. mRNA ii. rRNA iii. tRNA iv. miRNA

**Ans: i. mRNA**

3. Clover leaf pattern is associated with

i. mRNA ii. rRNA iii. tRNA iv. miRNA

**Ans: iii. tRNA**

4. Stop codon

i.UAA ii.UAG iii.AUG iv. Both 1&2

**Ans: iv. Both 1&2**

5. How many letters are present in genetic code

i.1 ii.2 iii.3 iv.4

**Ans: iii.3**

**True or False**

1. Codons have degeneracy

**Ans: True**

2. AUG & GUG act as starting codons for translation

**Ans: True**

3. Synthesize of protein from mRNA is called as transcription

**Ans: False**

4. Codon recognition site in tRNA is complementary to codons of mRNA

**Ans: True**

5. RNA contains uracil in place of cytosine

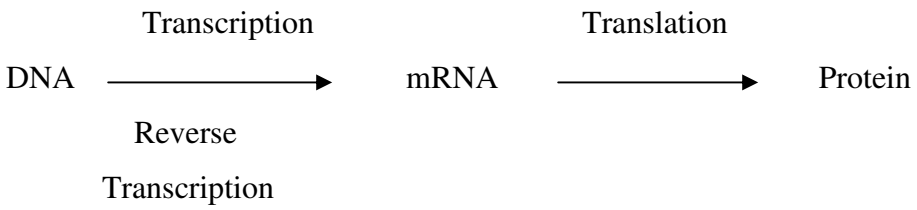
**Ans:False**

# Lecture No.28

# Gene expression and protein synthesis

## 1. The central dogma

The sequence of amino acids in a protein is determined by the base sequence of DNA, through the m RNA. The flow of genetic information is




## II. Transcription

### 1. Only one strand of DNA is transcribed

RNA polymerase synthesizes m RNA with DNA acting as template. Synthesis of m RNA is called transcription. Only one strand of DNA is transcribed. The other strand is never used for transcription.

### 2. RNA polymerase

RNA polymerase is a holoenzyme, made up of six polypeptides and two of these polypeptides are identical and thus enzymes consists of five distinct sub unit 2α, β,β',ω and σ. RNA polymerase without the sigma sub unit causes binding with DNA template and the β sub unit helps in the binding of ribonucleoside triphosphate. The σ sub unit participates in the selection of initiation sites for transcription. The synthesis of m RNA by RNA polymerase takes place in three stages (i) initiation (ii) elongation and (iii) termination.

### i. Initiation of transcription

The transcription starts at specific sites, called promoters on the DNA template. The promoter sites consist of about 40 base pairs (about 140 A°). The 2α, β, β', ω core of RNA polymerase is unable to start transcription at promoter sites. The 2α, β, β', ω holoenzyme is essential for specific initiation. In addition, sigma activates RNA polymerase to recognize promoter sequences. The sigma subunit dissociates from the holoenzyme after the new RNA chain is started. The core polymerase continues to transcribe the DNA template. The role of the holoenzyme is selection and initation, whereas that of the core enzyme is elongation. Repressors block the transcription by interfering with the binding of RNA polymerase. RNA chains start

with pppA or pppG. That is, the new RNA chain has a triphosphate group at its 5' terminus and a free hydroxyl group at its 3' terminus. In contrast with DNA synthesis, primer is not needed. RNA chains can be formed *de novo*.

**ii. Elongation of transcription**

As in DNA synthesis, the growth of an RNA chain is in the 5' 3' direction. The RNA polymerase moves along the DNA template strand in the 3' 5' direction because the template strand is antiparallel to the newly synthesized RNA strand. The same RNA polymerase molecule synthesized RNA strand. The same RNA polymerase molecule synthesizes an entire transcript that is, transcription is processive. The transcribed region of DNA remains its double – helical conformation as the next section of DNA unwinds. The maximum rate of elongation is about 50 nucleotides per second.

In contrast with DNA polymerase, RNA polymerase does not edit the nascent polynucleotide chain. Hence, the fidelity of transcription is much lower than that of replication. The error rate of RNA synthesis is of the order of one mistake per $10^4$ or $10^5$. The lower fidelity of RNA synthesis can be tolerated because, a cell synthesizes many RNA transcripts of a gene.

**iii. Termination of transcription**

The DNA template contains stop signals for transcription. Before the termination site, GC rich region is followed by an AT rich sequence. The most distinctive feature of termination sequences is the two fold symmetry of their GC rich region. Hence, the RNA transcript of this region is self-complementary and so, it can base-pair to form a hairpin structure. In addition, the nascent RNA chain ends with several U residues, which are specified by a series of A bases in the AT-rich region of the DNA template. These structural features cause RNA polymerase to pause when it encounters such a signal. The rho protein participates in the termination of transcription.

**III. Translation**

Synthesis of polypeptide chain from mRNA molecule is called translation.

**1. Activation and linkage of amino acids to tRNA**

The amino acids are activated by ATP. In the first step, the carboxyl group of the amino react with ATP, forming amino acyl adenylate and releasing pyrophosphate. This reaction is catalyzed by amino acyl synthetase in the presence of magnesium. The amino acyl synthetase is amino acid specific.

In the second step, the enzyme bound amino acyl adenylate reacts with tRNA and forms amino acyl- tRNA. The same enzyme acts to transfer the amino acid to tRNA. When amino acyl tRNA product is formed, adenosine monophosphate and the amino acyl synthetase are released.

The attachment of an amino acid to a tRNA is important because

(i) Amino acids themselves cannot recognize the codons on mRNA.

(ii) Amino acids are carried to the ribosomes by specific tRNAs which recognize codons on mRNA.

(iii) Thus, the specific tRNAs acts as adaptor molecules.

**1. Specificity of aminoacyl – tRNA synthetases**

The correct translation of the genetic message depends on the high degree of specificity of amino acyl – tRNA synthetases. Teh amino-acyl tRNA synthetase contains two sites synthetic site and hydrolytic site. These sites function as rejects amino acids that are larger than the correct one whereas the hydrologic site destroys activated intermediates that are smaller than the correct species. In fact, the synthetase corrects its own errors.

**2. Wobble hypothesis**

A codon of mRNA is recognized by the anticodon of a tRNA base of codon forms a Watson – Crick type of base pair with a complementary base on the anticodon. The codon and anticodon are antiparallel in base pairing. Some tRNA molecules can recognize more than one codon. For example, the yeast alanine tRNA binds to three codons: GCU, GCC and GCA. This is explained by Wooble hypothesis.

**3. Ribosomes – the site of protein synthesis**

The *E.coli* ribosomes has a sedimentation coefficient of 70 S. It can be dissociated into a large subunit (50S) and a small submit (30 S). These sub-units can be further split into their constituent proteins and RNAs. The 30 S subunit contains twenty one proteins and a 16 S RNA molecule. The 50 S subunit contains about thirty four proteins and two RNA molecules, a 23 S species and a 5 S species. The formation of a ribosome ***in vitro is a*** self- assembly process. That is, non-ribosomal factors are not needed.

The ribosome of eukaryotic cells is larger. It has sedimentation coefficient of 80 S. It dissociates into 60 S and 40 S sub units.

Many ribosomes can simultaneously translate an mRNA. This increase the efficiency of utilization of the mRNA. The group of ribosomes bound to an mRNA is

called a polyribosome or a polysome. In this unit, the ribosomes operate independently, each synthesizing a complete polypeptide chain. The maximum density of ribosome operates independently, each synthesizing a complete polypeptide chain. The maximum density of ribosomes on mRNA is about one ribosome per eighty nucleotides. Ribosomes near the 5' end of the mRNA have shortest polypeptide chains, whereas those near the 3' end have almost finished chains. Ribosomes dissociate into 30 S and 50 S subunits after the polypeptide product is released.

**4. Initiation of translation**

Protein synthesis is initiated by formyl methoinine tRNA

N-formyl methionine (f Met) is a modified methionine which has a formyl group attached to its terminal amino group. A blocked amino acid like N-formyl methonine can be used only to start protein synthesis.

Protein synthesis starts with the association of mRNA, a 30 S ribosomal subunit, and formylmethionyl – tRNA to form a 30 S initiation complex. The formation of this complex requires GTP and three protein factors *viz*., 1F-1, 1F-2, 1F-3. Which mediates the binding of mRNA to a 30 S subunits from coming together to form a dead end 70 S complex devoid of m RNA, 1F1, and 1F-2 enhance the binding of initiator tRNA to the mRNA – 30S subunit complex.

A 50 S ribosomal subunit then joins a 30 S initiation complex to form a 70S initiation complex. The bound GTP is hydrolyzed in this step. The 70S initiation complex is ready for the elongation phase of protein synthesis. Ribosome contains two sites A and P. The f Met – t RNA molecule occupies the P (peptidyl) site on the ribosome. The other site for a tRNA molecule on the ribosome, the A (aminoacyl) site is empty. The anticodon of f Met – tRNA pairs with the initiating AUG (or GUA) codon on mRNA. Thus, the reading frame is defined by specific interactions of the ribosome and of Met RNA within RNA.

**5. Elongation of Translation**

The elongation cycle in protein synthesis consists of three steps:

i. Binding of aminoacyl – tRNA (codon recognition)
ii. Peptide bond formation
iii. Translocation

**(i) Binding of amino acyl tRNA in "A site"**

The cycle begins with the insertion of an aminoacyl tRNA into the empty "A site" on the ribosome. The particular species inserted depends on the mRNA codon that is positioned in the A site. The complementary amino acyl tRNA is delivered to the A site by a protein elongation factors called EF-Tu and EF-T. GTP bound to Ef-Tu is hydroloyzed as the amino acyl –tRNA is precisely positioned on the ribosome "A" site.

**(ii) Formation of a peptide bond**

When amino acyl-tRNA occupies the A site and fMet RNA occupies the P site, the stage is set for the formation of a peptide bond. This reaction is catalyzed by peptidyl transferase an enzyme that is integral part of the 50S subunit. The activated formyl methionine unit of fMet- tRNA (in the P site) is transferred to the amino group of the aminocayl RNA (in the A site) to form a dipeptidyl – tRNA.

The discharged tRNA occupies the "P site", whereas a dipepetidyl tRNA occupies the "A" site, following the formation of a peptide bond.

**(iii) Translocation**

Three movements occur: the discharged tRNA leaves the "P site" and mRNA moves a distance of three nucleotides. The result is that the next codon is positioned in the "A site" for reading by the incoming aminoacyl –tRNA. Translocation requires a third elongation factor, EF-G (translocase). The GTP bound to EF-G is hydrolysed during translocation. After translocation, the "A site" is empty, ready to bind an aminoacyl – tRNA to start another round of elongation.

Normally at each translocation step, the mRNA template advances three nucleotides precisely. The movement of peptidyl tRNA from the "A site" to "P site" plus the mRNA three nucleotides distance correctly. That is, the movement of a triplet codon is a consequence of its binding to an anticodon in tRNA.

**6. Termination of translation**

Protein synthesis is terminated by release factors. Aminoacyl –tRNA does not bind to the "A site" of a ribosome if the codon is UAA, UAG or UGA. In normal cells, tRNAs with anticodons complementary to these stop signals are recognized by release factors, which are proteins. Release factor RF1 recognizes UAA or UAG, RF2 recognizes UAA or UGA. Thus, proteins can recognize trinucletoide sequences with high specificity. The release factors are bound to GTP. Termination requires energy that is supplied by the splitting of GTP into GDP and phosphate.

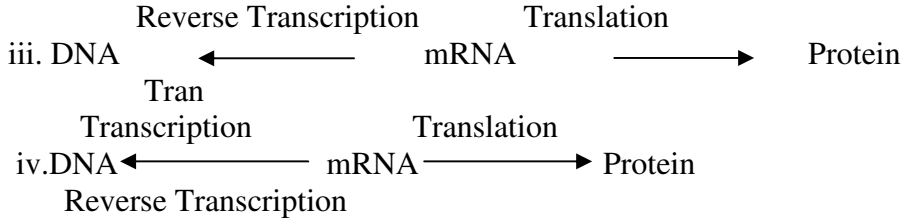
The binding of release factor to a termination codon in the “A site” activates peptidyl transferase in such a way to hydorlyze the bond between the polypeptide and the tRNA in the “P site”. The polypeptide chain then leaves the ribosome. The 70 s ribosome then dissociates into 30 S and 50S subunits as the prelude to the synthesis of another protein molecule.

Each cell of the living organisms contains thousands of genes. But all genes do not function at a time. Genes control the phenotypic expression of characters through the production of specific enzymes. The synthesis of particular enzyme is depending upon the requirement of the cell. Thus, there exists an on-off system which regulates protein synthesis in all living cells. The study of this on-off mechanism is called regulation of gene expression.

## Questions

1. The central dogma of life






**Ans: iv.DNA** ← **Transcription** / **Reverse Transcription** → **mRNA** → **Translation** → **Protein**

2. A holoenzyme consists of the following subunits

i. $2\alpha,\beta,\beta^1,\omega$ ii. $2\alpha,\beta,\beta^1,\sigma$ iii. $2\alpha,\beta,\beta^1$ , $\omega$, $\sigma$ iv. $2,2\alpha,\beta,\beta^1,\omega$, $\sigma$

**Ans: iii.$2\alpha,\beta,\beta^1$ , $\omega$, $\sigma$**

3. Aminoaryl tRNA binds with --------- site on ribosome

i.A ii. P iii. T iv. AP

**Ans: i. A**

4. Discharged tRNA occupies which position on ribosome

i. A ii. P iii. T iv. AP

**Ans: ii. P**

5. Sedimentation coefficient of E.coli ribosomes

i.30 s ii.50 s iii.70 s iv.80 s

**Ans: iii.70 s**

**True or False**

6. Both the strands of a DNA is used for transcription

**Ans: False**

7. Group of ribosomes bound to an mRNA is called as polysome

**Ans: True**

8. Synthesis of mRNA from a DNA is called as transcription

**Ans: True**

9. Hydrolytic site on amino-acyl tRNA synthetase destroys activated smaller intermediates during translation

**Ans: True**

10. The codon GUG encodes for methionine

**Ans: False**

## Lecture No.29

## Regulation of gene expression

**The gene expression is regulated at many different levels. They are**

i. Transcriptional level
ii. mRNA processing
iii. mRNA turn over
iv. translation level
v. enzyme function

Most of the data indicate that regulation of transcription is the most important mode of control of gene expression.

Synthesis of enzyme depends mainly on two factors. In degradative process (catabolic pathway) the synthesis of enzyme depends on the availability of the molecule to be degraded. In biosynthetic pathway the synthesis of an enzyme governed by end product. There are two types of gene regulation *viz*., (1) negative regulation and (2) positive regulation.

**The operon model**

F.Jacob and J.Monad (1961) proposed the operon model to explain the regulation of genes coding the enzymes required for lactose utilization in *E.coli*. The operon is a co-ordinated unit of the gene expression. The operon consists of structural genes, the operator and promoter.

**Structural genes**

The lac operon of *E.coli* consists of three structural genes namely z,y and a . These structural genes transcribe a single polycistronic mRNA molecule. This mRNA molecule controls the synthesis of three different enzymes *viz.,* β-galactosidase, galactosidase permease and galactosidase transacetylease. All the above three enzymes are involved in breakdown of lactose. The function of all the structural genes is regulated by two controlling elements namely regulator and operator. Thus, the main function of structural genes is to control synthesis of protein through mRNA.

**Operator gene**

The operator is usually located between the promoter and the structural genes. In lac oeron of *E.coli*, operator is located contiguous to the structural genes. It is the binding site for the protein called repressor. When the repressor is bound to the operator, transcription of the structural genes cannot occur. Because the binding of the repressor to the operator strictly prevents RNA polymerase from binding at the promoter site.

The promoter gene is always located contiguous with or even overlapping with operator sequence or operator. The promoter segment is a place where mRNA polymerase enzyme binds with DNA. The main function of promoter gene is to initiate mRNA transcription. The promoter starts mRNA transcription only when operator is free or when repressor is not bound to the operator. The binding of repressor with operator inactivates the promoter gene and prevents transcription.

**Regulator gene**

The regulator gene is located either on one end of the operon or away from the operon. The function of the regulator gene is to synthesis a protein called repressor. The repressor may be either active or inactive.

In the case of an inducible operon, the free repressor binds to the operator and turning off the transcription. When the effectors molecule (the inducer) is present, it binds to the repressor and becomes repressor-inducer complex, which cannot bind the operator. There by the regulator gene turn on the transcription of structural genes in the operon. In the case of reversible operon, the repressor is inactive and cannot bind to the operon there by transcription of structural genes in the operon is turned on. The only repressor effectors molecule (co-repressor) is active in binding to the operator and turn off the transcription of structural genes in the operon.

**Mechanism of gene regulation in lac operon**

In the absence of lactose in the medium, the regulator gene produces the active repressor molecule. These repressor molecules will bound to the operator and it strictly prevents the RNA polymerase from binding to the adjoining promoter. Thereby synthesis of enzymes by structural genes involved in lactose metabolism *viz.*, β galactosidase, galactosidase permease and galactosidase transcetylase were switched off. When the lactose (effectors molecule) is added to the medium, which act as inducer, will bind to the repressor and become repressor-inducer complex. This

complex is inactive in nature, which cannot bind to the operator. The operator is now free from repressor and RNA polymerase will bind with promoter region and start the transcription of structural genes involved in lactose metabolism.

Traditionally, the gene has been defined as the unit of genetic material controlling the inheritance of one phenotypic characteristic or one trait and also believed that gene was not to be sub divisible by mutation or recombination. To day the gene is precisely defined as the unit of genetic material coding for one polypeptide.

The functional allelism of the gene is operationally defined by the cis-trans or complementation test. Alleles may be arranged in two ways ***viz*** when two wild alleles are located in one homolog and their corresponding mutant allele in another member of the homologous chromosomes (++ / $m_1$ $m_2$), it known as cis-arrangement and the organism is called as cis-heterozygotes. On the other hand when one wild and one mutant type alleles are located in each member of a given homologous chromosome (+ m2 / m1 +), it is known as transposition and the organism is called as transheterozygote. Complementation test is used to determine whether two mutant alleles (mutations) belong to the same gene or two different genes.

The two mutations are considered to be in the same gene if their cis-heterozygotes produce wild type phenotype and transheterozygote produce mutant phenotype (because no functional gene product will be synthesized). If both the cis-heterozygotes and transheterozygote lead to the development of wild type phenotype, then the two mutations are in two different genes (both gene products will be synthesized in the common protoplasm).

**Modern concept of gene**

Now the gene can be defined as the unit of genetic material coding for one polypeptide. So the gene can also be called as cistern.

**Cistron**: The portion of DNA specifying a single polypeptide chain.

**Muton**: Muton is defined as the smallest unit of genetic material which when changed to produce different phenotypes.

**Recon:** It is smallest unit of DNA capable of recombination.

The cistron contains so many mutons and recons, the smallest unit of mutation or recombination. It's a single nucleiotide pair. So the unit of genetic material not sub divisible by mutation or recombination is known to be single nucleotide – pair.

**Split Genes**

In prokaryotes, polypeptide chains are encoded by continuous array of triplet in DNA. In eukaryotes, the genes are discontinuous. For example, the gene for β chain of haemoglobin interrupted by a long non coding sequence of 550 base pairs and a short one of 120 base pairs. Thus, the β-globin chain is split into three coding sequences. The coding sequences are called **exons** (regions which are expressed) and the intervening sequences are called introns**.**

All avian and mammalian genes mapped so far are split genes, except the histone gene. In general, the intervening sequences of split genes are longer than their expressed sequences.

## Questions

1. Operon concept was proposed by

   i.Watson & Crick ii.Meselson & Stahi

   iii.Jacob & Monad iv. Morgan

   **Ans: iii.Jacob & Monad**

2. The operon consist of

   i. Structural genes ii. Operator iii. Promoter iv. All the above

   **Ans: iv.All the above**

3. The structural genes of lac operons are

   i. x,y,z ii. z,y,a iii. a,x,y iv. z,y,b

   **Ans: ii.z,y,a**

4. Smallest portion of DNA specifying a single polypeptide chain

   i. Cistron ii. Recon iii. Muton iv. Exons

   **Ans: i.Cistron**

5. Smallest unit of DNA capable of undergoing mutation

   i. Cistron ii. Recon iii. Muton iv. Intron

   **Ans: iii. Muton**

**True or False**

6. Regulator gene is located between promoter and structural genes

   **Ans:False**

7. Introns are the coding sequence of a DNA

   **Ans: False**

8. Smallest unit of DNA capable of recombination is Recon

   **Ans: True**

9. Operators are the binding site for repressor proteins

**Ans:True**

10. Prokaryotes have no introns

**Ans: True**

## Lecture No.30

## Special types of chromosome

Some tissues of certain organisms contain chromosomes, which differ significantly from normal chromosomes in terms of either morphology or function. Such chromosomes are referred to as special chromosomes.

### 1. Lambraush chromosome

These are the special type of chromosomes found in primary oocyte nuclei in amphibians. Lambrush chromosomes are up to 1mm length. Each lambrush chromosome contains a central axial region where the two chromatids are highly condensed and numerous pairs of lateral loops give them a characteristics lambrush appearance. The loops are the transcriptionally active region of the single chromatids.

### 2. Salivary gland chromosome / polytene chromosome / Giant chromosome

The polytene chromosomes occur in the tissues of salivary glands, guts epithelium and malpigian tubules of many insects of the order Diptera.

In salivary gland cells of dipteral species giant chromosomes were observed by E.G.Balblani for first time in 1881. The chromosomes may reach a size of 20 times or more than the normal chromosomes. These salivary gland chromosomes have characterstics of somatic pairing as a result, the number of giant chromosomes in the salivary gland cells always appears to half that in the normal somatic cells. Giant chromosomes have distinct pattern of transverse banding, which consists of alternate chromatic and achromatic regions. The band occasionally forms reversible puffs known as chromosome puffs or Balbiani rings which are associated with differential generation.

Giant chromosomes represented by bundle of fibres, which arise by repeated cycle of endo reduplication of single chromatids (Endo – reduplication means chromatids replicates without cell division as a result of which number of chromonemata keep on increasing). That is why these chromosomes are also called as polytene chromosomes and the condition is refered to as polytene. The number of chromonemata per chromosome may be upto 2000 and in some cases it maybe aroung 16,000.

**Iso-chromosome**

A chromosome with two identical arms and identical genes is called as isochromosome. The arms are mirror images of each other. IT is thought to arise when a centromere divides in the wrong plane yielding two daughter chromosomes, each of which carries the information of one arm only but present twice. At meiosis isochromosomes pair in three different ways. (i) Internal pairing (ii) Fraternal pairing (iii) Normal pairing

In internal pairing, the two arms of the isochromosmes pair with each other. In fraternal pairing, one or both of the arms of the isochromosomes pair with a homologous arm of another chromosome. In normal pairing, the isochromosome pairs with another one just like it.

**'B' chromosome**

It is a particular kind of supernumerary chromosome that may or may not be found in organisms as extra chromosomes over and above the standard diploid or polyploidy chromosome complements. The standard complements are called 'A' chromosome. The 'B' chromosomes found in natural population are recognized on the basis of following characteristics.

i. They are dispensable ( not found is all the individuals of the species or all the cells of the organisms)
ii. They are not homologous with any of the basic 'A' chromosomes.
iii. Their inheritance is non Mendalian.
iv. They are usually smaller than the 'A' chromosomes.
v. Generally they are genetically inert
vi. When it present in higher number they suppress the vigour and fertility.
vii. Their origin and functions are largely unknown.

The most significant effect of 'B' chromosome is on seed and pollen fertility. Flowering time is generally delayed by 'B' chromosomes and has negative

consequences for the organism as they have deleterious effect because of abnormal crossing over during meiosis.

**Ring chromosome**

The chromosomes of higher organisms usually have two ends and do not form a continuous ring. However, the chromosomes of lower organisms such as prokaryotes. (E.,coli) normally have ring shaped chromosomes. Often such chromosomes are referred to as genophores. Which are more than 1 mm in length and consists of a single DNA molecule.

Chromosomes in higher organisms are not naturally ring shaped. However ring chromosomes have been detected in humans, Drosphila and certain plant species. Ring chromosomes were most thoroughly studied in maize by Mc Clintock.

Normal chromosomes do not form rings because they are believed to have telomeres on each end. Telomeres prevent the union of chromosome arms into ring formation. A chromosome can form a ring chromosome by fusion of the raw ends only if it has two terminal deletions producing centric segment with two raw ends and two acentric fragements.

A ring chromosome lacks the genetic information that was carried by the terminally deleted fragments. Ring chromosomes are meiotically unstable and they are associated with several syndromes.

## Questions

1. Following types are called as special types of chromosomes

   i. X chromosomes ii. Lambrush & Giant chromosome

   iii. B chromosomes iv. Both 2 & 3

   **Ans: iv. Both 2 & 3**

2. Salivary gland chromosomes are found in

   i. Diptera ii. Lepidoptera iii. Hymenoptera

   **Ans: i. Diptera**

3. Balbiani rings are associated with

   i. Lambrush chromosomes ii. Polytene chromosomes

   iii. Iso chromosomes iv. B chromosomes

   **Ans: ii. Polytene chromosomes**

4. Super numerary chromosome in addition to the standard complement is called as

   i.A chromosome ii. B chromosome iii. Extra chromosome iv. Iso-chromosome

   **Ans: ii. B chromosome**

5. Number of giant chromosomes in a cell is equal to

   i. n ii. 2n iii.3n iv.4n

   **Ans: i. n**

**True or False**

6. Loops on a lambrush chromosome are transcriptionally active.

   **Ans: True**

7. Isochromosomes are represented by two identical arms

   **Ans: True**

8. B chromosomes show Mendalian inheritance

**Ans: False**

9. Ring chromosomes are produced when telomere is lost

**Ans: True**

10. Bands on a giant chromosome is transcriptionally inactive

**Ans: False**

## Lecture No.31

## Chromosomal aberrations – i: structural aberrations

### Two major kinds of chromosomal aberrations

Chromosomes may undergo changes. This is called chromosomal mutation or chromosomal aberration. The change may occur either in structure of the chromosomes or in the number of chromosomes. Based on these, the chromosomal aberrations are grouped into two major kinds- variation in structure and variation in number.

### Variation in chromosome structure

These are four kinds of variations in the structure of chromosomes.

1. Deletion
2. Duplication
3. Inversion
4. Translocation

### 1. Deletion

**Definition:** It is an "intra-chromosomal aberration" in which, an interstitial or terminal chromosomal segment is lost. That is, some genes are deleted. Based on which it is called inter calary or terminal deficiency.

**Cytological effect**: In deletion heterozygotes, "deletion loop" occurs during pairing of homologous chromosomes. The portion of the normal chromosome homologous to the deficient segment bulges out.

**Genetic effect**: When a segment of a chromosome is absent, some genes are also absent. If these lost genes are physiologically important, deletion leads to death of the organism.

Deficiencies produce unique phenotypic effects in Drosophila. The characters such as bended, delta, gull, minute and notch are associated with some deletions in chromosomes.

In human beings, Cri-du-chat' syndrome is characterized by a mewing cat like cry during infancy, widely spaced eyes physical and mental retardations. This 'Cri-du-chat' syndrome is caused by a deletion in the short arm of $5^{th}$ chromosome.

## 2. Duplication

**Definition:** It is an 'intrachromosomal aberration' in which a segment is represented two or more times in a chromosome.

**Cytological effect**: During meiotic pairing of heterozygotes, the chromosome with duplicated segment forms a loop to maximize the juxtaposition of similar segments of homologous chromosomes.

**Genetic effect**: The duplications are not lethal. The unusual dosage of genes can be investigated. Duplications are useful in evolution of new characters without loss of original traits. Relocations of chromosomal materials, due to duplication, results in an altered phenotype. This is called position effect.

### Position effect

The position effect is an altered phenotype due to relocation of chromosomal material. A fly homozygous for Bar eye has four 16 A segments, two in each chromosome. A fly heterozygous for ultra Bar has also four 16 A segments – one in the normal chromosome and three in the duplicated number of 16A segments, they are expected to be similar in phenotype. But the flies homozygous for ultra Bar (BB/+) produced smaller size eyes.

## 3. Inversion

**Definition:** It is an intrachromosomal aberration. Inversions occur when a part of chromosome become detached, turn through 180° and reinserted in such a way that the genes are in reverse order.

### Inversions are of two kinds

(i) Pericentric inversion: The inverted segment includes centromere.

(ii) Paracentric inversion: The inverted segment does not include centromere. Centromere lies outside the inverted portion.

**Origin of inversion**: A chromosome may form a loop. Breakages occur at the point of intersection. When the sticky ends unite with new partners, inversion results.

**Cytological effect:** In inversion heterozygote the part of the uninverted chromosome corresponding to the inversion forms a loop. A similar loop is formed by the inverted section of the homologous chromosome but in reverse direction.

**Genetic effects:** Paracentric inversion produce dicentric and acentric chromosomes. Pericentric inversion produce duplications and deficiencies. Inversion acts as cross over suppressor and inversion maintains heterozygosity from generation to generation.

**4. Translocation**

**Definition**: It is an inter-chromosomal aberration where in exchange of chromosomal a segment occurs between non-homologous chromosomes.

**Cytological effect**: In the translocation heterzygote, pairing of homologous chromosomal segments is effected by a cross-shaped configuration. This cross opens out into a ring as chiasma terminalizes. The meiotic products are of three kinds (i) normal, (ii) balanced and (iii) unbalanced.

**Genetic effect**: Translocation gives three kinds of genetic effects.

*i.* Translocations alter the linkage relationships of genes.

*ii.* Heterozygotic translocation causes semi sterility because most of the gametes fail to receive full, balanced complement of genes required for viable development.

*iii.* The phenotypic expression of a gene may be modified when it is translocated to a new position.

## Questions

1. Intra-chromosomal aberrations are

   i. Deletion  ii. Inversion  iii. Duplication  iv. All the above

   **Ans: iv. All the above**

2. Cri-du-chat syndrome is caused by

   i. Deletion in the short arm of $5^{th}$ chromosome

   ii. Delection in the long arm of $5^{th}$ chromosome

   iii. Duplication in the short arm of $5^{th}$ chromosome

   iv. Duplication in the long arm of $5^{th}$ chromosome

   **Ans: i. Deletion in the short arm of $5^{th}$ chromosome**

3. Exchange of chromosomal segments between non-homologous chromosomes is called

   i. Recombination  ii. Transversion  iii. Translocation  iv. Inversion

   **Ans: iii.Translocation**

4. Types of deletions

   i.Intercalary  ii.Terminal  iii.Both 1&2  iv.Pericentric

   **Ans: iii.Both 1&2**

5. Paracentric inversions produce

   i. Dicentric chromosomes  ii. Duplications

   iii. Acentric chromosomes  iv. Both 1&3

   **Ans: iv. Both 1&3**

**True or False**

6. Duplications are not lethal

   **Ans: True**

7. Pericentric inversions do not include centromere

   **Ans:False**

8. Duplications are useful in evolution of new characters

**Ans: True**

9. Translocation is an intrachromosomal observation

**Ans: false**

10. Inversion act as cross over suppressor

**Ans: True**

## Lecture No.32

## Chromosomal aberrations – numerical aberrations

### Variation in Chromosome number

Variation in number of chromosomes is called **ploidy.** A set of chromosome present in an organism is called genome. In a genome, each type of chromosome is represented only once. Most of the sexually reproducing plant species are diploids i.e., have two set of chromosomes. Any change in the chromosome number from the diploid condition is referred to as heteroploidy. The heteroploidy is of two types namely, aneuploidy and euploidy. The variation in number may involve any particular chromosome or in entire sets.

### Aneuploidy

Loss or gain of one or more particular chromosomes occur within a set is called aneuploidy. The aneuploidy organism bears irregular number of chromosomes. Aneuploidy arises due to non-disjunction. Aneuploidies are of three types.

### Types of Aneuploids

| **Types** | **Genomic constitution** |
|---|---|
| Monosomic | 2n-1 |
| Double monosomic | 2n-1-1 |
| Nullisomic | 2n-2 |
| Trisomic | 2n+1 |
| Double trisomic | 2n+1+1 |
| Tetrasomic | 2n+2 |
| Pentasomic | 2n+3 |

### 1. Monosomics

A monosomic is an individual that lacks one chromosome of the normal complement of somatic cells (2n-1). If the lost chromosome is one that is not absolutely essential for the organism, it may survive but if the lost chromosome is very important, the organism may not survive.

**2. Nullisomics**

A nullisomic is an individual that lacks both members of one specific pair of chromosomes (2n-2). A nullisomic diploid does not survive. However a nullisomic polyploidy (hexaploid wheat 6x-2) may survive but exhibit reduced vigour and fertility. Nullisomic analysis helps to identify genes with specific chromosomes in a polyploidy species.

**3. Polysomics**

An individual having either single or one pair of extra chromosome in the diploid complement is known as polysomics. Polysomics are called as hyperploids. Polysomics are of two types (i) trisomics and (ii) tetrasomics.

**(i) Trisomics**

A trisomic is an individual with one chromosome more than the normal complement of the somatic cells (2n+1). In general the extra chromosome does not produced so striking effect as a missing one. In wheat, trisomics (2n=43) are nearly indistinguishable from normal plants. Trisomics give rise to two kinds of gamets *i.e*., one kind with 'n' chromosomes and other with 'n+1' chromosomes. Trisomics are more stable genetically than monosomics.

**(ii) Tetrasomics**

Addition of two chromosomes of one pair or two different pairs is known as tetras my and such individuals are called as tetrasomics.

**Use of aneuploidy**

i. Aneploids are extremely useful in several genetic studies.
ii. They are useful to determine the phenotypic effects of loss or gain of different chromosomes.
iii. Aneuploids have been used to produce chromosome substitution lines which give information on the effects of different chromosomes of a variety.
iv. They are used to produce alien addition and alien substitution lines which are useful in gene transfers from one species into another.
v. Aneuploid analysis permits the location of a gene as well as of a linkage group of a specific chromosome.

**Aneuploids in Human beings**

**i. Down's syndrome**

It is due to trisomic condition of 21$^{st}$ chromosome. It is also called Mongolian idiocy. Affected individuals are mentally deficit and physically retarded, broad face and flat stubby nose.

**ii. Kline felters syndrome (44+XXY)**

It is due to trisomic condition of sex chromosome. The individual is male with XXY Chromosome. The individuals with this syndrome have defective dev elopement of testis, famine character like Enlarged breast, under- developed body hair, presence of one barr body in the body cells.

**iii. Turners syndrome**

It is due to monosomic condition of sex chromosome. The individual is female with 44 autosomes and one'X' chromosome. The female individual is without menstrual cycle. No barr body is present in body cells.

**The origin of aneuploids**

i. Spontaneous
ii. Meiotic irregularities
iii. Triploid individuals
iv. Translocation heterozygote

**Use of aneuploids in crop improvement**

a. Aneuploids are useful tools for locating the genes on a specific chromosome. Monosomics and nullisomics are used for this prupose.
b. Monosomics are also used in interspecific gene transfer *ie* monosomics are used in transferring chromosomes with a desirable genes from one species to another.
c. Aneuploids are used for developing alien addition and alien substitution lines in various crops.
d. Primary trisomics are useful in identification of chromosomes involved in translocations.

## Questions

1. An individual that lacks one chromosome of the normal complement is

   i. Monosomic ii. Trisomic iii. Nullisomic iv. Double monosomic

   **Ans: i.Monosomic**

2. Nullisomic has the chromosome status

   i. 2n-1 ii. 2n-1-1 iii. 2n-2 iv.2n+2

   **Ans: iii.2n-2**

3. An individual with one extra chromosome is called as

   i. Monosomic ii. Nullisomic iii. Trisomic iv. Tetrasomic

   **Ans: iii. Trisomic**

4. Trisomic condition of the sex chromosome results in

   i. Down syndrome ii. Kline felters syndrome

   iii. Turner syndrome iv. Cri-du-chal syndrome

   **Ans: ii. Kline felters syndrome**

5. The gametes produced by trisomics are

   i. n type ii.n+1 type iii.Both n & n+1 iv.n+2 type

   **Ans: iii. Both n & n+1**

**True or false**

6. Mangolian idiocy is due to trisomic condition of 21$^{st}$ chromosome

   **Ans: True**

7. Individuals with Kline felter syndrome are females

   **Ans: False**

8. Individuals having one or pair of extra chromosomes are called as polysomics

   **Ans: True**

9. Change in chromosome number from diploid condition is known as euploidy

   **Ans:False**

10. Monosomic condition of sex chromosome results in Turner syndrome

**Ans:True**

## Lecture No.33

## Polyploidy

These are variation that involves entire set of chromosomes. In Euploids the chromosome number is an exact multiple of the basic or genomic number. Euploids are differ in multiple of n or x.

| **Types** | **Genomic formula** |
|---|---|
| Monoploids | n |
| Diploid | 2n |
| Triploid | 3n |
| Tetraploid | 4n |
| Pentaploid | 5n |
| Hexaploid | 6n |

### Monoploid

The monoploid organisms have one set of chromosomes or one genome (n) in the nuclei of their body cells. The monoploids are often weak and sterile. Monploids differ from haloids which carry half or gametic chromosome number (n). In true diploid species, both monoploid and haploid chromosome number is the same (n =x) thus a monoploid can be a haploid but all haploids cannot be monoploids.

### Diploids

Normal diploids are known as disomics. They have regular bivalent pairing during meiosis. Diploids also have disomic genetics with two alleles at each locus.

### Polyploids

Polyploids refer to any organism in which the number of chromosome sets exceeds two *i.e*., an organism with more than two set of chromosomes or genome.

They have larger cells than diploids. These larger cell sizes contribute to larger plant size and higher yield. Polyploids have generally larger, thicker and darker green leaves bigger flowrf, fruits than the diploids. In each genus, there is an optimum level of polyploidy beyond which growth may be depressed with increasing number of chromosomes. (eg) triploid (3n).

There are two types of Polyploids.

1. **Autopolyploid**

In autoployploids the multiple sets of chromosomes are identical (eg). Genome is identical with each other.

Autopolyploids arise by abnormal mitosis and meiosis and induced artificially by colchicines.

Auto triploid - 3x

Auto tetraploid- 4x

Auto hexaploid- 6x

(eg). Banana 2n=3 x=33, Groundnut 2n=4x=40; Sweet potato 2n=6x=90 and Potato 2n=4x=48

**Autotriploid**

The triploid organisms have three sets of chromosomes. A triploid may originate by the union of a monoploid gamete (n) with a diploid gamete (2n). Since an autotriploid remains sterile and cannot produce seeds, it has great commercial value in producing seedless varieties of economic plantgs. Eg. Seedless water melon.

**Autotetraploid:**

The organisms with four genomes (4n) in the nuclei of their somatic cells are called tetraploids. They arise due to somatic doubling of chromosome number. Doubling is accomplished by either spontaneously or it can be induced by chemicals such as colchicines.

2. **Allopolyploid**

A species or types of plant derived from doubling the chromosomes in the $F_1$ hybrid of two species, is called an amphidiploids. In amphidiploids the two species are knonw.

Eg.

| | | |
|---|---|---|
| Gossyplum hirsutum | - | 2n=4x=52 |
| G.barbadense | - | 2n=4x=52 |
| Nicotiana tabacum | - | 2n=4x=48 |
| Triticum aestivum | - | 2n=6x=42 |

Saccharum officinarum - 2n=8x=80

**Morphological and Cytological features of polyploids are**

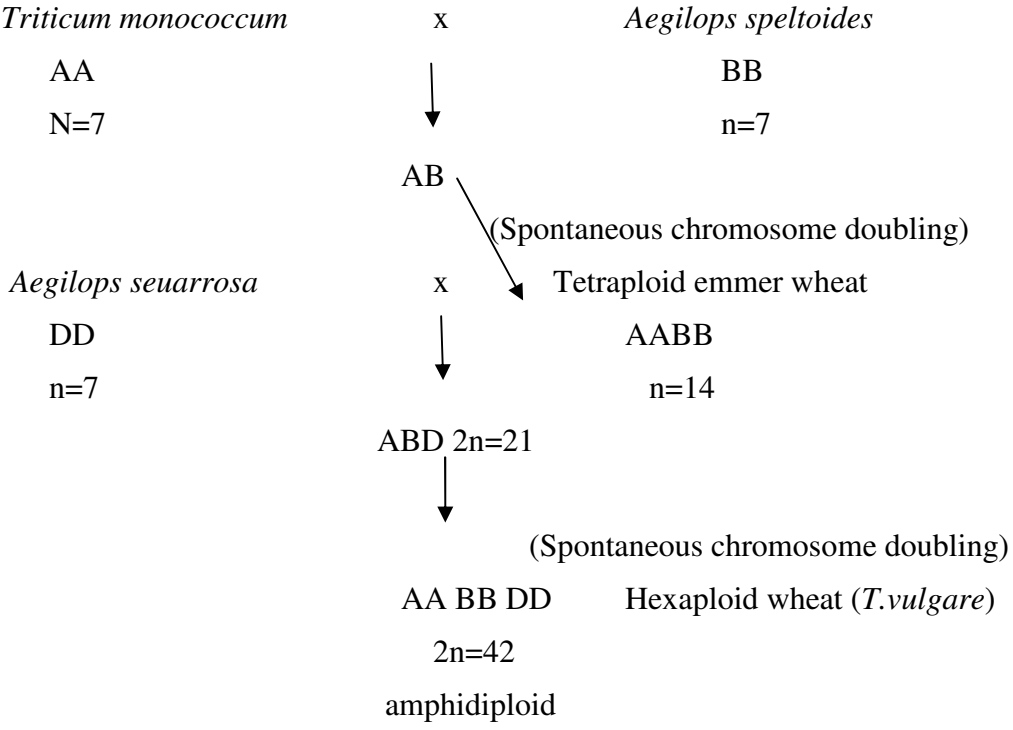
i. Larger in size than diploids
ii. Generally more vigorous than diploids
iii. Slower in growth and late in flowering
iv. Polyploids may have reduced fertility than diploids

**Role of polyploids and their evoluation**

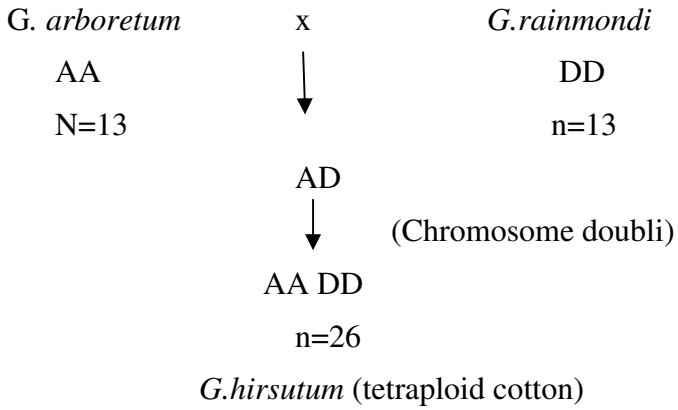
i. About 1/3 of angiosperms are polyploids. These suggest that polyploids have significant role in the evolution of crop species.
ii. Allopolyploids have contributed great extent in the evolution of plants than auto polyploids.
iii. The identification of diploid parental species is primarily based on paising between the chromosome of diploid and the allotetraploid species.
iv. Allopolyploids combine the genome of different species, hence the resulting individuals differ from progenitor.
v. Evolution is a slow process; but due to allopolyploids new species originate very quickly.
vi. Polyploids have wider adaptation to different environmental condition than diploids.

**Role of polyplodis in evolution of crops**

**Wheat**



**2. *Gossypium hirsutum***



## Questions

1. The organism which have one set of chromosome

i. Monoploid ii. Diploid iii. Euploid iv. Polyploid

**Ans: i.Monoploid**

2. Individuals with more than two sets of same genome

i. Heteroploid ii. Autopolyploid iii. Allopolyploid iv. Diploid

**Ans: ii.Autopolyploid**

3. Autotetraploids

i. AA ii. AAA iii. AAAA iv. AABB

**Ans: iii. AAAA**

4. An allopolyploid species

i. Banana ii. Groundnut iii. Potato iv. Wheat

**Ans: iv. Wheat**

5. Genemic formula for Allohexploid

i. AAAAAA ii. AABB iii. AABBDD iv. ABAB

**Ans: iii. AABBDD**

**True or false**

6. Evolution is a slow process

**Ans:True**

7. Autotriploids are sterile

**Ans: True**

8. Banana is an autotetraploid species

**Ans: False**

9. *Gossypium hirsutum* is an allotetraploid species

**Ans: True**

10. Polyploids have narrow adaptation

**Ans: False**

## Lecture No.34

## Mutation

### Mutation

Mutation is the sudden heritable change in the phenotype of an organism. In molecular term, mutation is the permanent and relatively rare change in the number or sequence of nucleotides which results due to change in in DNA bases in nuclear or cytoplasmic DNA or due to chromosomal aberrations.

The term mutation was introduced by Hugo devries in 1900. Mutagenic action of X –rays was discovered by Muller in 1927 on *Drosophilla* and of gamma rays and X-rays in 1928 by Stadler in barley.

### General characteristics of mutation

(i) Mutations are generally recessive and dominant mutations also occur.

(ii) Mutations are generally harmful to the organism. A small proportion of them (0.1%) are beneficial

(iii) Mutations occur at random (*i.e*) they may occur in any gene or chromosome. But some genes show higher mutation rates than others. Highly mutable sites are called mutational hot spots.

(iv) Mutations are recurrent (*i.e*) same gene may undergo mutations repeatedly.

(v) Induced mutations often show pleiotropy due to close linkage of mutated gene with other genes.

(vi) Macro mutations which occur in oligogenes are easily identifiable and micro mutations which occur in poly genes are not easily identifiable.

### Classification of mutations

1. Depending upon the size of genetic material involved in the mutation, mutations are classified as

**(i) Point mutation**

If the mutation affects a single gene causing a change in the allele status from dominant to recessive or vice versa, the mutation is called as point mutation. It is also called as intra genic mutation. Change in allelic status may be from wild type to a new type called as forward mutation or may be from new type to wild type called as reverse mutation.

Change in allelic status is also classifies as below based on efficient of the function of the mutated allelomorph.

a) Hypomorph- allele functioning less efficient
b) Hypermorph – allele functioning more efficient
c) Amorph- allele completely losing the function
d) Neomorph allele acquiring a new function
e) Antimorph – allele impacting the auction of normal allele

**(ii) Chromosomal mutation**

a. If the mutation induces structural aberration in the chromosomes such as deletion, duplication, inversion and translocation (transversion) or number aberrations, the mutation is called as chromosomal mutation.

2. Depending upon the level of detectability of mutations / the magnitude of phenotypic effect produced.

**(i) Macro mutation**

If the mutation result is easily identifiable distinct morphological changes in the phenotype, the mutation is called as macro mutation. It is called as oligogenic mutation, as it is observed generally in qualitative characters which are controlled by oligogenes. Since, the phenotypic change is easily recognizable on individual plant basis; selection in $M_2$ generation is easy.

**(ii) Micro mutation**

If the mutation result is not easily identifiable and not clearly distinguishable morphological changes in the phenotype, the mutation is called as micro mutation.

It is called as polygenic mutation as it is observed in quantitative characters which are controlled by polygenes. Micro mutations are of most economic value in plant breeding than macro mutation as most of the economic characters are governed by polygenes. In micro mutation, since the small phenotypic change is not recognized on individual plant basis and detected only in a group of plants, selection is carried out on $M_3$ or later generations only.

3. Depending upon the survivability level of mutated individuals or the mutation effect on the survival level of individuals.

(i) **Lethal:** if the mutation kills all the individuals carrying the mutated gene, the mutation is called as lethal mutation. Lethal mutation reduces the viability of the individuals completely and hence all the individuals are killed. Dominant lethals (AA) do not survive in homozygous or heterozygous condition and hence cannot be studied. Recessive lethal (aa) do not survive in homozygous condition alone. E.g Allina chlorophyll mutation.

(ii) **Sub-lethal**: If the mutation kills more than 50% of the individuals carrying the mutated gene, the mutation is called as sub-lethal. Sub lethal mutation reduces the viability of the individuals to a greater extant but not completely. Hence, most of the individuals are killed.

(iii) **Sub-vital:** If the mutation kills less than 50% of the individuals carrying the mutated gene, the mutation is called as sub- vital mutation. Sub-vital mutation reduces the viability of the individuals to a limited extent and hence lesser no of individuals are killed.

(iv)**Vital**: If the mutation does not kill any of the individuals carrying the mutated gene, mutation is called as vital mutation. Vital mutations do not reduce the viability of the individuals and hence all the individuals survive. Vital mutations occur in a much lower frequency (0.1% of all the mutations) as compared to other three types.

Vast majority of mutations are lethal, sub-lethals, and sub-vital and are of little value in crop improvement and sometimes have considerable academic interest. Vital mutations are of high value in crop improvement.

**Molecular basis of mutations**

In any organism, triplet codon consisting of three nitrogenous bases in the DNA codes for an amino acid and a specific combination of triplet codons produce several aminoacids which unite through peptide linkage to produce a protein. Proteins (which include enzymes) decide the phenotype of the organism. Hence, any change in triplet codon (a single or no of bases) causes a change in the sequence of aminoacids in a protein regularity in the altered phenotype. A heritable change in N base is the ultimate cause of mutation. Chromosomal aberrations (change in no. or segment) occurring due to mutation also result in the change in the sequence of aminoacids or in the absence of entire protein.

4. Depending upon the nature of change in the nitrogenous base of nucleotide DNA, molecular basis of mutation can be classified as below,

A) Single base change at the particular point

(i)Base substitution → a) Transition
(i)Base substitution → b) Transversion

(iii)Base addition

(iv)Base deletion

B) Multiple base change at a particular point

(i) Triplet base addition or deletion

(ii) Non triplet base addition or deletion – Frame shift mutation

**A) Single base change at a particular point**

**(i) Base substitution**

When one base in a DNA molecule is replaced by another one, it is called as base substitution. It is of two types

**(a) Transition**

When a purine is replaced by another purine (replacement of adenine by guanine and vice versa) or a pyrimidine by another pyrimidine (replacement of thymine by cytosine and vice versa) the base substitution is called as transition.

Four changes are possible in A ↔ G and T ↔ C at a nucleotide level

**(b) Transversion**

When a purine (adenine or guanine) is replaced by a pyrimidine ( Thymine or cytozine or vice versa), the base substitution is called as transversion.

A ⟷ T or C

G ⟷ T or G

**(ii) Base addition**

When one or more nitrogenous bases in a DNA molecule is lost it is called as base addition.

**(iii) Base deletion**

When one or more nitrogenous bases in a DNA molecule is lost, it is called as base deletion.

**B. Multiple base changes at a particular point**

**(i) Triplet base addition or deletion**

If the no. of nitrogenous bases added or lost (base addition or deletion) is a multiple of three, one to several amino acids are added or deleted from the concerned protein. In such cases, the function of concerned protein does not generally change.

**(ii) Non triplet base addition or deletion-Frame shift mutation**

If the no. of bases added or lost is not a multiple of three, the sequences of all the triplet codons beyond the point of insertion or deletion are altered and all the codons code for a different amino acid. Thus the reading frame of the subsequent codons is shifted in such mutations. This type of mutation is called as frame shift mutation. A frame shift mutation, changes all the amino acids of the concerned protein, located subsequent to the addition or deletion of bases. In such cases, the concerned protein becomes non functioned. Hence, such mutations are much more deleterious than those produced by base substitution except non sense mutations.

**C. Change in a base at atom level.**

**Tautomerization**

When the hydrogen atoms in a DNA molecule gets shifted from one position to another in a purine or in a pyrimidine, the process is called as tautomerization and the new product is called as tautomer.

**5. Depending upon the location of the genetic material of the cell involved in mutation.**

**(i) Nuclear mutation**

If the mutation occurs in the nuclear genetic material, the mutation is called as nuclear mutation.

**(ii) Cytoplasm mutation**

If the mutation occurs in extra nuclear genetic material, the mutation is called as cytoplasmic or plasma gene mutation. Cytoplasmic genetic material

is present in mitochondria and chloroplast, which also controls the expression of certain characters of the organism like cytoplasmic male sterility.

**6. Depending upon the type of cells involved in the mutation**

**(i) Germinal mutation**

If the mutation occurs in the generative cells (reproductive cells) of an organism, the mutation is called as gametic mutation and the mutation is passed on to the next generation, via the gametes.

**(ii) Somatic mutation**

If the mutation occurs in the somatic cells of an organism, the mutation is called as somatic mutation.

**(a) Non heritable somatic mutation**

In sexually reproducing organisms, if the somatic mutation occurs, the mutation lasts up to the end of the generation and is not inherited to the next generation.

**(b) Heritable somatic mutation (Bud mutation)**

In asexually reproducing organisms, If the somatic mutation occurs, the propagating part of the somatic cells of the mutation is called as heritable somatic mutation and the mutation is inherited to the subsequent generation through vegetative propagation. Such somatic mutations occurring in vegetative propagation is called as bud mutations. Since, in general buds are the core vegetative propagating part.

**7) Depending upon the presence or absence of artificial causal factors**

**(i) Spontaneous mutation**

If the mutation occurs naturally without the artificial treatment by physical or chemical agents, the mutation is called as spontaneous mutation. Frequency of spontaneous mutation is generally very with range of one in 10 lakhs. i.e $10^{-6}$. However, some loci undergo spontaneous mutations at high frequency. E.g R locus in maize with a frequency of $4.92 \times 10^{-4}$, while Wx locus is highly stable. Spontaneous mutation of certain genes are influenced by the genetic background in which it is present.

Certain intrinsic and extrinsic factors produce spontaneous mutation. Genetic instability due to hybridity or polyploidy or due to specific genes promoting chromosomal sickness in mitosis or meiosis and physiological conditions are the intrinsic factors involved on spontaneous mutation.

Extrinsic (external) factors causing spontaneous mutation are nutrition, temperature, naturally occurring radiations are chemicals and very high oxygen pressure.

**ii) Induced mutation**

If the mutation occurs due to the artificial treatment by physical or chemical mutagenic agents, the mutation is called as induced mutation. Induced mutation produces new allele similar to spontaneous mutations. But in contrast to spontaneous mutation,very high frequency of mutation can be induced through the mutagenic agents at desired level. Hence induced mutations are more useful in crop improvement.

The method of crop improvement utilizing the induced mutation is known as mutation breeding. The agents that induce mutation are known as mutagens.

**Mutagens**

Mutagens may be physical or chemical.

**I. Physical mutagens**

Physical mutagens are radiations. Radiation causing mutations are of two types (1) Ionizing radiation (2) Non-ionizing radiation.

**1. Ionizing radiation**

Ionizing radiations are those which when pass through matter, transfer energy to the matter rendering it to lose electrons. Ionizing radiation affected atoms of the matter becomes positively charged particles called ions. Because of these, the molecule containing the positively charged particles undergoes chemical change. When this change occurs in DNA molecule, the result is a heritable change-mutation. There are different types of ionizing radiations. They are,

**A. Non particulate (Electromagnetic and sparsely ionizing)**

(i) X-rays- They are produced by X-ray machines. They are sparsely ionizing, non particulate and penetrating. Hard X-rays have wave lengths of 0.1 to 0.01A° and soft x-rays have 1 to 10 A° wave lengths.

(ii) Gamma rays– They are produced by $^{60}$Co and other radioactive isotopes.They are shorter in wave length than x-rays (0.01 A°) and more penetrating than X rays.

**B. Particulate**

**(iii) Alpha particles:** They are produced by radioisotopes of heavier elements. They have two protons and two neutrons. They are positively charged and less penetrating than neutrons and beta rays; Densly ionizing.

**(iv) Beta particles** : They are high energy electrons produced by decay of radioactive isotopes like $^{32}$P, $^{35}$S, $^{3}$H. They are more penetrating than $\alpha$ particles, but less penetrating than X rays; Sparsely ionizing.

**(v) Fast and thermal neutrons**: They are neutral in charge and produced by cyclotron of atomic reactors by radioactive decay of heavier elements. They are highly penetrating.

(Isotopes are chemically identical substances having the same no. of protons but different no. of neutrons. Radioisotopes or radioactive isotopes spontaneously disintegrate Eg. $^{32}$P in to an element with less no. of neutrons).

**2. Non ionizing radiations**

Non ionizing radiations are those which when pass through matter transfer energy to the matter rendering it's electrons to move to higher energy levels (higher orbits). Incase of ionizing radiations, electrons are lost by the matter, but incase of nonionizing radiaons, electrons are raised to higher energy levels (Excitation). The atoms in excited shows increased activity.

Eg. UV rays: They are produced by mercury vapour lamp and the weave length is 100-3900°A. They are lower in energy and less penetrating. Hence, thin tissues like pollen of plants or eggs of *Drosophila* are irradiated using UV rays to induce mutation. Since, DNA bases show maximum absorption at 2540°A. The wavelength

of UV is suitable for mutation. Dimer formation and deamination occur due to UV radiation.

**II. Chemical mutagens:** Oehlkers in 1943 found that mixtures of ethylurethane and potassium chloride induced translocations in *Oenothera.* Auerbach and Robson reported the mutagenic action of mustard gas in 1946. Different categories of chemical mutagenic agents are given below:

1. **Alkaylating agents**

   (i) Sulphur mustards: Eg. Dichloro Diethyl Sulphide (mustard gas)

   (ii) Nitrogen mustards

   Eg. 2 –Chloro ethyl dimethyl amine,

   Di (2-chloro ethryl) amine

   (iii) Epoxides

   Eg. Ethyl oxide, Glycidol

   (iv) Ethylene imines

   Eg. Ethylne imine (EI), Acetyl ethylene imine

   (v) Sulphate, Sulphonates, Sultones and lactones

   Eg. Dimethyl sulphate (DMS)

   Diethyl sulphate (DES)

   Methyl methane sulphonate (MMS)

   Ethyl methane sulphonate (EMS) – $CH_3$ $SO_2OC_2H5$)

   (vi) Diazo alkanes and nitroso compounds

   Eg.Diazomethane, Diagoethane, Nitroso ethyl urea.

2. **Acridine dyes**

   Eg. Acridine orange, Ethidium bromide.

3. **Base analogues**

   Eg. 5 bromo uracil, 5 Chloro uracil, 2 Amino purine

Among these some of the alkylating agents viz, EMS, MMS, DES are frequently used for induced mutagenesis.

**Mechanisms of chemical mutagenesis**

**i. Tautomeric shift**: If the chemical mutagens change the amino group ($-NH_2$) into imino group (- NH) in purines, A (amino adenine) = T pairing in DNA is converted to A (imino adenine) = C pairing. Similarly C=G pairing is to converted to C = A pairing. This is called as tautomeric shift.

In case of pyrimidines, keto group (C=O) is converted to enol group (COH). Because of this A = T (keto) pairing in converted to G=T (enol) pairing.

(ii) **Substitution**: Base analogues substitute for purine and pyrimidines during nucleotide and DNA synthesis.

(iii) **Deamination**: Amino group ($NH_2$) is converted to keto group(C=O). Adenine is converted to hypoxanthine and cytosine is converted to uracil.

**Questions**

1. The term mutation was introduced by

i. Mullor ii. Hugo de Vries iii. Statler iv. Mendel

**Ans: ii.Hugo de Vries**

2. Change in the allele states from dominant to recessive is called as

i. Lethal mutation ii. Transition iii. Point mutation iv. Reverse mutation

**Ans: iii.Point mutation**

3. In subvital mutation

i. 50% of the individuals carrying mutated gene are killed

ii. None of the individuals are killed

iii. More than 50% of the individuals carrying the gene are killed

iv. all the individuals carrying the mutated gene are killed

**Ans: i. 50% of the individuals carrying mutated gene are killed**

4. In one of the transition types of mutation

i. Adenine in replaced by cytosine

ii. Advise is replaced by Guanine

iii. Thymine is replaced by odenine

iv. Uracil is replaced by Guanine

**Ans: i. Adenine in replaced by cytosine**

5. Gamma rays are

i. shorter in wavelength then X-rays ii. Longer in wavelength then X-rays

iii. Equal in wavelength as that of X-rays iv. Nor radiations

**Ans: i. shorter in wavelength then X-rays**

**True or False**

6. X-rays are not used as a physical mutagen and used only in medical field

**Ans:False**

7. Fast and thermal neutrons are produced by gamma chamber

**Ans: False**

8. UV rays is an ionizing radiation

**Ans: False**

9. Ethylene imine is an alkylating agent

**Ans: True**

10. Deamination is due to the conversion of keto group to enol group

**Ans: False**

## Ex. No: 1 Use of microscopes and study of cell shapes

The term microscopy refers to the study of microscopes. Microscope is a Greek word meaning (Mikron$^{G}$ – small, Skopein$^{G}$ – to see) to see small things. It is an instrument used to produce an enlarged image of a small object, which otherwise would be invisible to naked eyes. This is achieved by a combination of several lenses and a light source to illuminate the object. According to the source of light employed for illumination, microscopes can be classified as

1. Light microscope
2. X ray microscope and
3. Electron microscope.

**1. Light microscopes**

These microscopes use ordinary light as source of illumination. These can be of several types.

**i) Simple microscope**

These are similar to magnifying glasses and consist of one or several lenses. These microscopes produce erect virtual image on the retina. Eg. Hand lenses, Spectacles and dissecting microscopes.

**ii) Compound microscope**

A compound microscope consists of three types of lens systems *viz*., Condenser lens system, Objective lens system and Eyepiece lens system. The condenser lens system occurs beneath the specimen and its function is the collection and focusing of the light rays on the object or specimen, which is placed on the stage of the microscope. The objective lens system remains near and above the specimen. It produces and magnifies the image of the specimen. The eyepiece or ocular lens system remains near the eyes of the observer and it magnifies and forms the secondary image of the primary image previously produced by the objective.

Apart from these lens systems, the compound microscope consists of the following components

a) The body of the microscope is called stout having a stout base, which is horseshoe shaped and a slightly curved limb. By adjusting the hinge joint of the limb, the stout can be tilted backwards slightly
b) A mirror with a plain side and a concave side is attached to the base of the stout. It is provided with a cymbal adjustment and can be manipulated in any way depending on the light source so as to reflect the source light on to the object through the condenser lens.
c) The platform on which objects are placed is called stage of the microscope. It consists of a central aperture and clips. The stage is attached to the stout.
d) The body tube, which is vertical or slanting, is attached to the limb. The body tube can be lowered or lifted up.
e) Fixed to the lower end of the body tube is the revolving nosepiece which carriers the objectives.

**iii) Ultra Violet microscope**

In UV microscopes, instead of ordinary light, invisible UV rays of short wave lengths are employed for the illumination of objects. The glass lenses are replaced with lenses of fused quartz. These microscopes are efficient in qualitative and quantitative determinations of different cellular components.

**iv) Phase contrast microscope**

These microscopes are employed to study materials showing little or no contrast in their properties like colour, density etc. This is achieved by passing the light rays through glass of two thicknesses so that the rays passing through thickest glass travel slowly. These microscopes are used mainly for scanning of unstained cells for division.

**v) Fluorescence microscope**

Fluorescent microscopes substitutes' fluorescence in UV light for the colour of the specimen. Mercury lamps are used as UV source. These microscopes are used in microbiological and histological studies and to some extent in chromosome banding technique.

**2. X-Ray microscope**

This microscope is used for analysis of the three dimensional structures of micro molecules and macromolecules of the cell. The molecules to be investigated must be present in crystalline form. In X ray microscopes, electromagnetic lenses or reflecting curved mirrors focus the x-ray beams and image are formed on a film.

**3. Electron microscope**

Things that are too small to be seen with a light microscope can be seen with an electron microscope. Instead of using lens to bend beams of light, an electron microscope uses a magnetic field to bend beams of electrons. The electrons are released from the cathode in an electron gun. These beams travel in straight line in vacuum. They can be concentrated and can be deflected in an electrostatic or magnetic field. This microscope can magnify objects 3,00,000 times. The magnified images are projected on a fluorescent screen where they are visible and are photographed. This microscope is classified into two types.

i) Transmission Electron Microscope (TEM): In this type of microscopes, the electrons emitted by the cathode ray tube passes through the object and are focused on to the fluorescent screen by an electromagnetic objective lens. This is used to study parts inside the cell.
ii) Scanning electron Microscope (SEM): This microscope is used to scan the surface of the objects. It produces three-dimensional images of the surface architecture.

**Method of Measurement of cells**

The cells are the fundamental units of life forms and are of microscopic size. The cells and their components are usually measured in micrometer (μm) or microns (μ) and Angstroms ($A^{O}$). In light microscope the cells are measured by an ocular micrometer disc and a stage micrometer. The ocular micrometer disc is usually a glass plate, which is placed on the ocular diaphragm. The micrometer disc must be calibrated before measurements are made. A calibration is made for each ocular lens, length of body tube and objective to be used. The stage micrometer is usually a glass slide on which a scale of known intervals has been marked. When the ocular micrometer disc and the stage

micrometer are graduated in the same units, the calibration of the ocular micrometer disc can be done easily. This is done by focusing on the stage micrometer, setting the zero line on it coincident with the zero line on the ocular micrometer disc, and reading across the scale to the next two lines that are coincident with each other. The magnification factor is obtained by dividing the known value on the stage micrometer into the ocular value. Then the size of any object viewed in the light microscope will be the dimension shown on the ocular disc scale divided by the magnification factor.

**Exercise**

i) Draw the diagram of a compound microscope and label its parts.
ii) Draw the optical path ways of light and electron microscope.

## Ex.No:2 Principles of killing and fixing, preparation of stains and preservatives

**Killing and Fixing**

The process of killing and fixing brings about the sudden death of cells or tissues in such a manner that the morphological organization and chemical composition of the cells remain unaltered greatly. Though both are distinct processes, both are usually obtained by means of a single fluid, which in turn is commonly a mixture of various chemical reagents, which are compatible.

Killing means the sudden and permanent termination of the life processes. Killing invariably precedes fixation.

Fixation is a process by which the cellular and structural elements are preserved in nearly the natural living condition.

**Properties of good fixative:**

i) It should penetrate rapidly
ii) It should change the soluble cell contents to insoluble substances that will remain in their original position.
iii) It should protect tissues against shrinkage and distortion during subsequent treatment.
iv) It should make the cell constituents stainable.

**Type of fixation:**

Fixation images obtained by the action of fixing agents are of two types

| | | |
|---|---|---|
| i) Acid fixation image | : | By this method of fixation, chromatin, nucleoli and spindle fibres are preserved. Cytoplasm is fixed strongly while nucleoplasm and mitochondria are dissolved. |
| ii) Basic fixation image | : | By this method, resting chromatin and spindle fibres are dissolved. Nucleoplasm and mitochondria are preserved. Cytoplasm is fixed as hyaloplasm and vacuoles are more or less preserved. |

***Types of fixatives***

Fixatives are broadly classified into two types.

| | | |
|---|---|---|
| i) Non metallic | : | Ethanol, Chloroform, Formalin, Glacial acetic acid, Methanol and Propionic acid. |
| ii) Metallic fixatives | : | Chromic acid, Mercuric chloride and Uranium nitrate. |

Apart from the above solution, combinations of several of the above chemicals are also employed. Among the solution, Carnoys fluid I and II finds wide application for cytogenetical studies.

Carnoys fluid I:

It consists of Glacial acetic acid 1 part and

Absolute ethanol 3 parts

It is effective for fixing all plant, animal and human materials. The period of fixation varies from 15 minutes to 24 hours. After fixation, the specimen should be washed in 95% ethanol 2-3 times to remove the acetic acid since it may interfere with staining process. The constituents of Farmer's solution are same as carnoy's fluid I.

Carnoys fluid II:

It is composed of

Glacial acetic acid 1 part

Chloroform 3 parts and

Absolute ethanol 6 parts

Formalin – Aceto – Alcohol (FAA) fixative:

This fixative is widely employed for botanical studies. The standard proportion of chemicals for this fixative is

70% Ethanol - 90 ml

Glacial acetic acid - 5 ml

Formation - 5 ml

***Staining***

***Preparation of stains***

The process of colouring the cells by employing certain inorganic or organic dyes is known as staining. The selection of dye or stain for a particular material depends on its chemical nature, the pH value of the fixative used and the chemical reactivity of stain to the material. Most cytological stains are solutions of dyes of aromatic organic compounds which have two kinds of active chemical groups *viz*., chromophoric and auxochromic groups. The chromophoric group gives colour to the dye while the auxochromic group gives to the dye the ability to adhere to the tissue or material.

Dyes are broadly classified into three groups

i) The natural dyes ii) Coal tar dyes and iii) Other compounds.

Natural Dyes

Three natural dyes are used. One is derived from Cochineal insects while the other two are derived from plants belonging to Caesalpiniaceae.

i) Brazillin: The dye is obtained from *Caesalpinia crista* and *C. echinata*. It is employed as 0.5% solution in 70% alcohol, which is allowed to ripen for a week in a dark place in a tightly stopped bottle. It imparts colour only after mordenting with Ferric ammonium sulphate.
ii) Hematoxylin: Hematoxylin is derived from log wood (*Hematoxylin campuchianum*). The stain is made up in combination with metallic salts of iron (always in the ferric form) Aluminum and Copper. In presence of acid medium, it produce red colour and in presence of alkaline medium it produce blue colour.
iii) Cochineal and derivatives : Cochineal is a yellowish- red powder obtained by grinding the dried bodies of the female cochineal insects and extracting the colouring matter. These are not employed for botanical studies to grate extent.
iv) Carmine: It is a bright red colour stain obtained by adding alum to cochineal. It is extensively used in iron acetocarmine smear technique by botanists.

**The coal tar dyes**

Coal tar dyes have been classified into six groups consisting of several dyes. The important dyes are Aniline blue, Congo red, Picric acid and Safranin.

**Other substances acting as stains**

Iodine**:**

Iodine is well known as a specific colour indicator for starch when made upon combination with potassium iodide. It produces blue colour with starch, brown with cellulose and yellow with pectin and callose.

Some of the important stains employed for cytological studies are Aceto carmine, Propiono carmine, Aceto orcein and Feulgen stain.

**Preparation of Aceto carmine**

Aceto carmine stain is prepared by dissolving carmine stain in Acetic acid. Forty five percent acetic acid (45ml glacial acetic acid + 55 ml distilled water) is boiled gently. A quantity of 1g of carmine powder is added to 45% acetic acid at about boiling point and allowed to boil for a few minutes. After boiling, the solution is removed from the flame and allowed to cool at room temperature. The solution is then filtered with a Whatman No.1 filter paper in to a clear bottle. The filtrate is light red coloured. If necessary, ferric chloride and ferric acetate may be added for deep staining and preservation.

**Preparation of Propino carmine:**

One gram of carmine powder is dissolved in boiling 45% Propionic acid and the procedure is similar to the preparation of aceto carmine. Propionic acid gives better fixation and staining properties than Glacial acetic acid. It also dissolves more carmine and gives more intensive reaction together with great clarity.

**Preparation of Aceto orcein**

The procedure for preparation of Aceto orcein is the same as Aceto carmine. One gram of pure orcein is devolved in 45% of Glacial acetic acid and heated to boiling point

for a few minutes. The contents are allowed to cool. Later this solution is filtered through Whatman No.1 double filter paper and stored in a clean bottle.

**Preparation of crystal violet**

It is also referred as Gentian violet. The disadvantage with this stain is over staining of cytoplasm. Distilled water (100ml) is heated to boiling point in a clean beaker. Gentian violet (1g) is added to boiling water and cooled down to room temperature. Then the solution is filtered to give 1% crystal violet solution.

***Feulgen stain***

Feulgen stain is employed for biological studies because of its specificity to DNA. It is prepared by dissolving 0.5g of basic Fuchsin in 100 ml boiling distilled water. The contents are shaken thoroughly, cooled to $50^{O}C$ and filtered. To the filtrate about 10ml of 1N HCL and 0.5g potassium meta sulphate are added, shaken thoroughly in a tightly closed container and keep in a dark cupboard for about 18 hours. The colorless solution obtained is stored in a coloured bottle in a cool place away from sunlight.

(1N Hcl can be obtained by adding 1 part of concentrate Hcl to 11 parts of distilled water).

## Ex.No:3 and 4. Study of mitotic phases in the root tips of onion and *Aloe sp*. *Arabidopsis*

One of the most important characteristics of all living cells is their power to grow and divide. Cell division is a process by which the cell duplicates itself for growth and reproduction of the organism. It is a complex process by which cellular materials are equally divided between daughter cells. Cell division in plants and animals are of three kinds. They are i) Amitosis ii) Mitosis and iii) Meiosis.

**Amitosis**

This is also called as direct cell division and is a means of asexual reproduction in unicellular organisms like Bacteria and Protozoa. During amitosis the nucleus elongates first and assumes dumb-bell shape. The constriction in the centre further deepens and divides the nucleus into two. This is followed by the constriction of cytoplasm, which divides the cell into two equal or approximately similar halves. Thus without the occurrence of any nuclear event, two daughter cells are formed.

**Mitosis**

Mitosis is a common type of cell division usually occurring in somatic cells. Mitosis was discovered by W. Flemming in 1880. During mitosis, the dividing cell passes through two main phases that constitutes the mitotic cycle. They are interphase and Mitotic phase. The mitotic phase is of four stages namely Prophase, Metaphase, Anaphase and Telophase.

Interphase

The resting phase or stage between the two mitotic divisions called inter mitotic phase or interphase. In interphase no division of chromosomes or cytoplasm occurs, but the nucleus and cytoplasm remain metabolically active and due to that an increase in volume of nuclear as well as cytoplasmic substances takes place. It is the longest phase during cell cycle and includes 3 sub stages.

- Pre DNA synthesis phase ($\mathbf{G_1}$) is the preparatory phase for the replication of DNA. Enzymes and substrates required for DNA replication are synthesized during this phase.

- Synthetic phase (**S**) includes the replication of DNA molecules that is duplication of the genomic DNA
- Post DNA synthesis phase ($\mathbf{G_2}$) includes all the activities concerned with the growth of cytoplasmic organelles and other cytoplasmic macromolecules. At the completion of these sub phases, the cell enters the mitotic phase.

**MITOSIS**

Prophase

Prophase ($Pro^G$ – before; $Phases^G$ – appearance) is the beginning of mitotic phase. During prophase, the chromatin reticulam condenses into shorter and thicker structures called chromosomes. The chromatin reticulam appears as a definite number of chromosomes, which is a constant for an organism under normal conditions. Each chromosome splits into two chromatids. The chromatids are in very close association with each other all along their length. The chromatids are held together by the centromere or kinetochore. The nucleolus and nuclear membrane disintegrate. The spindle appears in the cytoplasm at opposite poles of the cell. From this numerous microtubules that radiates towards the centre of the cell are formed.

Metaphase

The transition phase between prophase and metaphase is called prometaphase. This is a very short period in which nuclear envelope disintegrates and the chromosomes are in apparent disorder. During metaphase, chromosomes are arranged in the equatorial plane in the middle of the cell. Each chromosome gets attached to the spindle at the centromere region. The chromosomes with their centromere are clearly visible. It is a very short stage.

**<u>Anaphase</u> ($Ana^G$ – back)**

It starts with centromere division. The two chromatids, move towards their respective poles. This movement is caused by contraction of the spindle fibres. Thus, during anaphase, two identical sets of chromatids or daughter chromosomes move and group at opposite poles of the cell. The original diploid number is thus maintained in developing daughter nuclei.

**Telophase**

This is more or less reversal of prophase. The spindle disappears. The chromatids which have moved to the opposite poles uncoil and lengthen into slender threads called chromatin fibres once again. The nuclear membrane and nucleolus reappear. Thus two daughter nuclei are formed one at each pole.

**Cytokinesis**

The division of the cytoplasm is called cytokinesis. It follows nuclear division by formation of cell wall between the two daughter nuclei. This results in two daughter nuclei.

The best method to study of mitosis is by smear method. The smear method gives a chance to study the chromosomes in a detailed manner. The essential idea underlying in all smear methods is to spread the cells out into a single layer in order that they may be killed instantly and fixed evenly and uniformly without distortion or the production of artifacts.

Onion is a convenient and popular specimen for studying mitosis because the chromosomes are very large, diploid number is relatively very low and cell materials are easily prepared.

**Protocol**

1. Fix the root tips of onion (5 mm size) in Farmer's solution for 1 to 5 hours.
2. Hydrolyze for 30 minutes in 1 N HCl at $60^{O}$C.
3. Wash in 95% ethanol for 30 minutes with at least four changes.
4. Transfer the root tips to a Petri dish containing hemotoxylin stain for 20 minutes.
5. Rinse the tips briefly in glacial acetic acid to wash off any excess hemotoxylin.
6. Place the root tip on a glass slide and it is overlaid with a small drop of glycerin.
7. A cover slip is placed on the root tip and squash.
8. The cover slip is gently pressed with thumb.
9. The slide is examined under a microscope

**Exercise**

**Identify and draw diagrams to bring out the stages of mitosis in onion.**

## Ex.No:5 and 6. Study of different meiotic phases in the inflorescence of maize and pearl millet/sorghum

**MEIOSIS**

In the higher plants, meiosis takes place in germ cells *viz*., the Ovaries and the Anthers. It consists of two divisions of the nucleus but only one division of chromosomes. The first division (or Meiosis I) is called reduction division because of the reduction in chromosome number and the second division (or Meiosis II) is called equational division because it brings about equal separation of sister chromatids in to daughter nuclei.

**Meiosis I**

Meiosis I involves Prophase I, Metaphase I, Anaphase I and Telophase I.

***Prophase I***

- Chromosome remains enclosed in a nuclear membrane
- Nucleolus is also distinct.

This has five sub stages.

Leptotene:

Chromosomes appear as very long slender threads with bead like structures called chromomeres. These beads are constant in number and structure. The nucleus has diploid number of chromosomes - one set having been contributed by the female parent and the other set by the male parent. Hence the nucleus has pairs of similar homologous chromosomes.

Zygotene:

Pairs of chromosomes begin to come together by a force of mutual attraction. The pairing of homologues chromosomes is called synapsis and it begins at one or more points and gradually proceeds along their length in a zipper like fashion.

Pachytene:

The chromosomes become shorter and thicker as synapsis continues. Pairing of chromosomes is complete. The two homologues chromosomes are called bivalents. There is a reciprocal exchange of segments between non-sister chromatids of the homologues

chromosomes. This phenomenon is called crossing over. The point of attachment of non-sister chromatids is called as chiasma.

Diplotene:

The chromosomes further contract and thickens. The chiasma is clearly visible. The homologous chromosomes move apart in repulsion. The number of chiasma per homologus chromosomes depends on the species and length of chromosomes. Longer the chromosome more the chiasma. During late diplotene, the chiasmata move away from the centromere and diminish in number. This movement of chiasmata is called terminalisation.

**Diakinesis:**

Chromosomes become highly condensed. Bivalents are evenly distributed throughout the nuceleus. The nuclear envelope breaks down. Chromosomes are easily countable in this stage.

***Metaphase I***

- A spindle is formed and the bivalents more towards the equator.
- The bivalents are assembled at the equatorial plate.
- The bivalent consisting of two chromosomes has two centromere, which are oriented on the spindle in such a way that one is on either side of the equator.
- The arrangement of the bivalents is completely at random and does not necessarily result in all the centromeres of the maternal set of chromosomes lying on one side of the equatorial plane and all the centromers of the paternal set of chromosomes lying on the other.

***Anaphase I***

- The chiasmata that have been holding the homologues chromosomes loose their retentivity and so the chromosomes of each bivalent separate and move to opposite poles.
- At each pole, only half the numbers of chromosomes are received.
- Each chromosome consists of one of its original chromatids and the other has a mixture of segments of its own and a segment of chromatid from its homologue.

***Telophase I***

- ❖ Reorganization of the chromosomes at poles occurs to form two haploid nuclei.
- ❖ The spindle disappears and nucleolus appears.
- ❖ Telophase I brings to an end the first meiotic cell division.
- ❖ After telophase I, no cytokinesis takes place. After a short interphase, second meiotic division is initiated.

***Meiosis II***

- ❖ Cell division process in meiosis II is similar to mitosis.
- ❖ It involves separation of sister chromatids to daughter cells.
- ❖ The sub stages of meiosis II are prophase II, metaphase II, anaphase II and telophase II

***Prophase II***

The nucleolus and nuclear membrane disappears. The spindle fibres are formed.

***Metaphase II***

The chromosomes move to the equilateral plane. Spindle fibres are attached to the centromere.

***Anaphase II***

The sister chromatids separate from one another. They are drawn to opposite polls by contractions of spindle fibre.

***Telophase II***

The chromosomes begin to uncoil and become thin. Nucleolus and nuclear membrane reappear. Cytokinesis follows telophase and four haploid daughter cells are formed.

**Significance of Meiosis:**

- ❖ Meiosis results in the reduction of chromosome number by one half after each division. Thus haploid gametes or sex cells are produced.
- ❖ After fertilization, the original chromosome number of every species is maintained which in turn serves to maintain the character of a species.

- ❖ Crossing over results in a new combination of genetic materials, which is the basis of variation and has great evolutionary significance.

Meiosis is a basic feature in the life cycle of all sexually reproducing eukaryotes. In a series of two cell divisions, the diploid number of chromosomes is reduced to haploid number. This cell division results in formation of pollen grains and eggs. Squash preparation of the anthers gives the details of meiosis in plant species.

**Protocol**

1. Young flower buds are fixed in Farmer's solution between 8 and 11 a.m.
2. After 5 hours the flower buds are transferred to 75% ethanol for preservation.
3. The preserved buds are washed with distilled water.
4. Anthers are removed from the flower buds by viewing under a simple microscope.
5. Anthers are placed in a drop of acetocarmine on a clean glass slide.
6. After a few minutes, the fluid are withdrawn with absorbent paper and replaced with a drop of fresh dye.
7. The anthers are cut transversely and the microsporocytes are squeezed out by pressing the anther with a scalpel.
8. Anther walls and debris are removed using a clean needle.
9. A cover slip is placed on the squash without any air bubble.
10. The slide is heated over a slide warming plate or a sprit lamp flame for 2 seconds to have good spread of cells.
11. The slide is placed between folds of a blotting paper and is give a gentle press to remove the excess stain.
12. The slides under are observed under a microscope and necessary drawings are made.
13. Good slide preparations can be preserved temporarily by placing molten wax on the border of the cover slip on all sides so as to block the entry of air beneath the cover slip. Appropriate labels are affixed on the slides

***Exercise***

*1. Identify and draw the stages of meiosis in the specimen given to you.*

2. Write the differences between Mitosis and Meiosis.

3. Define Chromosome, Chromatid, Chromonemata, Chaisma, Tetrad, Dyad and Synapsis.

## Ex.No:7 Procedure for making temporary slide to permanent

**Protocol**

1. The exact placement of the cover slip over the glass slide is carefully marked with the help of a diamond headed knife.
2. The temporarily waxed slide are warmed to remove the wax with help of a needle

- Invert the slide in a mixture of 3:1 glacial acetic acid: Butyl alcohol until the cover slip fallen away
- The cover slip and slide are washed gently in the following order for one or two minutes

  1:1 of glacial acetic acid and normal butyl alcohol

  3: 1 of butyl alcohol and % glacial acetic acid.

  Pure normal butyl alcohol

- Place a drop of Canada balsam on the slide where the cover slip was previously lying.
- Gently replace the cover slip with the slide in such a manner that the original position under the surface of cover slip not changed.
- Keeps the slide under ordinary room temperature or in a warm place maintaining $35^{O}$– $36^{O}$ until the balsam is completely dried. Remove the excess Canada balsam on the side of the slide with xylol.
- Carefully inscribe the particulars of the slide with the help of diamond pencil label and preserve the slide in slide box.

**Cleaning the slides and cover slips.**

The materials required for cleaning are potassium dichromate (28g), distilled water (250ml) and conc. Sulphuric acid (28 g or 15-22 ml).

# Potassium dichromate is dissolved in water in a trough and kept it in another trough containing water.

# Add the acid in small quantities and the mixture is cooled for every addition of acid.

# The mixture is stored in glass containers.

# Use the mixture until the colour of solution becomes black.

# The slides kept in the mixture for 5-8 hours and then they are washed and rinsed I distilled water.

## Ex.No:8 Principles of dominance, recessive, back cross, test cross, incomplete, co-dominance and lethal factors; Principles of chi-square test

### Dominance

This is the appearance of a parental character in the first filial generation ($F_1$ generation). The character that appears in the $F_1$ progeny is called as dominant character.

Example: In a cross between long stemmed plant and a short stemmed plant, the progeny was long stemmed. Here, long stemmed trait is said to be dominant.

### Recessivity

The character that is not expressed in the first filial generation is called as the recessive character. In the above example, short stemmed character is called as the recessive character.

### Test cross

Because homozygous dominant genotype has the same phenotype as the heterozygous genotype, a test cross is required to distinguish between them. The test cross parent is always recessive for all of the genes under consideration. The outcome of this would be the number of genotypes produced by the individual whose genotype is in question.

- A homozygous dominant individual produces one kind of gamete (genotype)
- A heterozygous (at one locus) individual produces two kinds of gametes at equal frequency.
- Test cross is the cross between $F_1$ and its recessive parent.

### Back cross

If the $F_1$ progeny is crossed to any one of their parents, it is called back cross.

**Incomplete dominance**

This is the case wherein the heterozygotes of a monohybrid cross would produce an intermediate phenotype due to absence of complete dominance of either traits of parents.

Lethal genes

These are the genes, mostly in the recessive condition which does not allow the organism carrying it to survive.

**Co dominance**

This is the simultaneous occurrence of both the parental traits in the offspring. For example, when the cow with black spotted coat was crossed to the bull of brown spots, the resulting offspring had roan colour of the coat with both the parental characters.

**Chi square test**

The chi square test is a significance test. It establishes how closely the observed data fit the predicted ratio. It is made by ascertaining the 'probability that the deviation of the observed ratio from the predicted ratio is due to chance and not due to some other factor such as experimental condition, biased sampling or even a wrong hypothesis.

**Procedure for Chi square test**

Chi square test involves

**1) Formulation of null hypothesis (No)**

The null hypothesis states that the observed data are in agreement with the expected ratio. In other words, deviation, if any, of the observed data from the expected ratio is not real *i e.,* it is due to chance only.

**2) Computation of $X^2$ from observed data**

It is obtained by finding the actual deviations of the observed frequency for each term of the ratio, squaring them, dividing them by the expected value, summing up these values.

Chi square would be zero, if the total observed number in each class is the same as expected. Thus the value of chi square will increase as the observed numbers deviate in increasing amounts from the expected. Conversely the value of chi square will decrease, as the observed numbers approach the expected.

**3) Determination of tabular $X^2$ value**

The values of chi square obtained entirely due to chance are given in the table. This table helps to decide, whether a calculated value of $X^2$ is due to real or chance deviations. The value of $X^2$ depends on (a) degrees of freedom and (b) Probability.

**Degree of freedom (df)**

Number of classes of observed data ……1

(If no. of classes 2, then df. = 1

If no. of classes 3, the df. = 2 and so on)

**Probability**

In most biological experiments 0.05 probability is accepted as the standard probability level for decision making.

**4) Drawing conclusion**

The value of $\mathbf{X}^2$ at 0.05 probability against appropriate degree of freedom is obtained from the $X^2$ table. If the calculated value of $\mathbf{X}^2$ is less than the table value, the deviations of observed data from the expected frequencies are purely due to chance. Therefore, the null hypothesis is accepted, and it is concluded that the observed data are in accordance with the expected ratio. If the calculated $X^2$ value is equal or greater than

the table value of $\mathbf{X}^2$, the deviations of observed data from the expected frequencies are accepted to be real. In such a case, the null hypothesis is rejected and it is concluded that the observed data are not according to the expected ratio.

**Chi square ($X^2$) values**

| **P =** | **0.99** | **0.98** | **0.95** | **0.90** | **0.80** | **0.70** | **0.50** | **0.30** | **0.20** | **0.10** | **0.05** | **0.02** | **0.01** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | | | | | | | | | | | | | |
| 1. | 0.000157 | 0.00628 | 0.00393 | 0.0158 | 0.062 | 0.148 | 0.455 | 1.074 | 1.642 | 2.706 | 3.841 | 5.412 | 5.635 |
| 2. | 0.0201 | 0.0404 | 0.103 | 0.211 | 0.446 | 0.713 | 1.386 | 2.408 | 3.219 | 4.605 | 5.991 | 7.824 | 9.210 |
| 3. | 0.115 | 0.185 | 0.352 | 0.584 | 1.005 | 1.424 | 2.306 | 3.665 | 4.642 | 6.251 | 7.816 | 9.837 | 11.345 |
| 4. | 0.297 | 0.429 | 0.711 | 1.064 | 1.649 | 2.195 | 3.357 | 4.878 | 5.989 | 7.779 | 9.488 | 11.668 | 13.277 |
| 5. | 0.554 | 0.752 | 1.145 | 1.610 | 2.343 | 3.000 | 4.354 | 6.064 | 7.289 | 9.236 | 11.070 | 13.388 | 15.086 |
| 6. | 0.872 | 1.134 | 1.635 | 2.204 | 3.070 | 3.828 | 5.348 | 7.321 | 8.558 | 10.645 | 12.592 | 15.033 | 16.812 |
| 7. | 1.239 | 1.564 | 2.167 | 2.833 | 3.822 | 4.671 | 6.346 | 8.383 | 9.803 | 12.017 | 14.067 | 16.622 | 18.475 |
| 8. | 1.616 | 2.032 | 2.733 | 3.490 | 4.594 | 5.527 | 9.344 | 9.524 | 11.030 | 13.362 | 15.507 | 18.168 | 20.090 |
| 9. | 2.088 | 2.532 | 3.325 | 4.188 | 5.380 | 6.393 | 8.343 | 10.656 | 12.242 | 14.684 | 16.919 | 19.679 | 21.666 |
| 10. | 2.588 | 3.059 | 3.940 | 1.885 | 6.179 | 7.267 | 9.342 | 11.781 | 13.442 | 15.987 | 18.307 | 21.161 | 23.209 |

P = Probability

n = Degrees of freedom

**Monohybrid**

A hybrid between two individuals differing in one pair of contrasting character.

**Single gene (monofactorial) crosses:**

**Six basic types of Matings:**

A pair of alleles governs pelage colour in the guinea pig. A dominant allele B produces black and its recessive allele b produces white. There are six types of matings possible among the three genotypes.

Summary of six basic types of matings

| S. No. | Matings | Expected $F_1$ ratios | |
|---|---|---|---|
| | | Genotypes | Phenotypes |
| 1. | BB x BB | all BB | all black |
| 2. | BB x Bb | ½ BB: ½ Bb | all black |
| 3. | BB x bb | all Bb | all black |
| 4. | Bb x Bb | ¼ BB: ½ Bb : ¼ bb | ¾ black : ¼ white |
| 5. | Bb x bb | ½ Bb : ½ bb | ½ black : ½ white |
| 6. | bb x bb | all white | all white |

**Test cross**

This is the cross between hybrid ad the homozygous recessive parent.

**Example:**

Test cross of a black female always produced a black progeny

Parents B- x bb

Black female white male

(genotype incompletely known) (test cross parent)

◯ ◯ ⓑ

Gametes B ? b

Bb

All offsprings are black

**Conclusion**

The female parent must be producing only one kind of gamete and therefore, she is a homozygous dominant (BB)

**Case 2**

Test cross of a black male with white female (test cross parent)

Parent B- x bb

Black male white female

Gametes B b

$F_1$ Bb bb

Black white

**Conclusion**

The male parent must be producing two kinds of gametes and therefore is heterozygous Bb.

**Back cross**

If the $F_1$ progeny is mated to any one of their parents, the mating is called back cross.

Parents BB x bb

Black female ↓ white male

$F_1$ Bb

Black

Parents Bb x BB

Black son black mother

½ BB: ½ Bb

All blacks

Note: All test crosses are back crosses but all back crosses need not be necessarily test crosses.

Steps in working out a problem in genetics

1. First find out the type of inheritance in the question *ie*.,
   - ❖ Whether it is a monohybrid or dihybrid cross and
   - ❖ Whether it exhibits complete dominance or incomplete dominance.
2. Determine the genotype of the individuals involved in the cross.
   - ❖ Genotypes of recessive individuals are represented by small letters. Both the alleles for recessive characters will be in homozygous state in recessive individual.
   - ❖ Individuals with dominant characters may be homozygous or heterozygous.
3. Determine the types of gametes produced by either parent.
   - ❖ Homozygous individuals produce only one type of gamete.
   - ❖ Heterozygous individuals produce two types of gametes.
4. Draw the Punnet square of checker board and place the gametes on the checker board and fill up the chambers of the checker board to show the union of different types of gametes or do the schematic or branching method.
5. Work out the result in form of genotype and genotypic ratios.
6. Calculate phenotype as a result of genotype. Calculate the ratio between dominant and recessive characters considered in question.

## Ex.No:9 Problems on monohybrid genetic ratio with dominance and with incomplete dominanace

**Model 1**

1. Gene A is dominant over gene a. What will be the phenotypic ratio in the offsprings obtained from the following matings?
   a) Aa X aa b) AA X aa c) Aa X Aa d) Aa X AA

**Determination of type of inheritance**:

This is a typical case of monohybrid cross and presents a simple case of complete dominance. Gene A is dominant over gene a.

**Determination of genotype**

One parent has Aa and other aa.

**Types of gametes**

Parent Aa produce 2 types of gametes *i.e*., one with 'A'gene and another with 'a' gene.

Parent aa produces only 1 type of gamete - a.

Arrangement of gametes in the checker board.

| | a | a |
|---|---|---|
| A | Aa | Aa |
| a | aa | aa |

Aa - 2 aa – 2

(50 %) (50 %)

**Conclusion**: This is an example of a test cross.

**Note**: Solve the other crosses in the same fashion.

**Model 2**

Two (*Pisum sativum*) plants were crossed and the following progenies were obtained. What will be the most likely genotypes of the parents and offsprings ?

a) Tall x Tall gene 86 Tall, 29 dwarf

b) Tall x Tall gene 124 tall

c) Tall x dwarf gene 14 tall, 6 dwarf

d) Tall x dwarf gene 5 tall, 4 dwarf

**Solution**

Data: a) Tall x Tall

↓

86 Tall, 29 dwarf

b) Tall x Tall

↓

124 Tall

c) Tall x dwarf

↓

14 Tall, 6 dwarf

d) Tall x dwarf

↓

5 tall, 4 dwarf

**Inference**

**a) Cross a**

i) Since there is segregation in the immediate progeny both the parents should be heterozygous (case a, c, d).

ii) Segregation is approximately in the ratio of 3 : 1.

iii) Since this a monohybrid type of inheritance, tall is preponderant over dwarf. (Tall is dominant over dwarf).

**b) Cross b**

When phenotypically similar parents are crossed and there is no segregation in the immediate progeny, both the parents are homozygous or one of them is homozygous tall while the other is heterozygous tall.

**c) Cross c**

i) Since there is segregation in the immediate progeny, there could be a possibility of one of the parent being heterozygous. Here, since tall is dominant over dwarf, tall parent is heterozygous.

ii) If a heterozygous parent for one trait is crossed with recessive parent (test cross), the expected segregation is in 1: 1 ratio. However, in this case, the observations made differ, which could be due to the low number of population and other causes, which might have affected the results (However, we can do the statistical analysis to prove our segregation ratio).

**d) Cross d**

The progeny shows segregation in 1 : 1 ratio, which is quite a classical case of a test cross and it is clear that the tall parent should be heterozygous.

Proof

**Cross a**

**Chi square test**

| Sl.No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation (d) (O-E) | $d^2$ / E |
|---|---|---|---|---|---|---|
| 1. | Tall | 3 | 86 | 86.2 | -0.2 | 0.0004 |
| 2. | Dwarf | 1 | 29 | 28.8 | 0.2 | 0.0013 |
| | Total | 4 | 115 | 115.0 | 0 | 0.0017 |

The calculated $\chi^2$ value is (0.0017) less than the tabular $\chi^2$ value for df: 1 at P: 0.09% (3.84). The deviation of observed data is purely due to chance. The observed data agree with the expected ratio of 3: 1.

**Cross C**

| Sl.No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation (d) (O-E) | $d^2$ / E |
|---|---|---|---|---|---|---|
| 1. | Tall | 1 | 14 | 10 | 4 | 1.6 |
| 2. | Dwarf | 1 | 6 | 10 | -4 | 1.6 |
| | Total | 2 | 20 | 20 | 0 | 3.2 |

At d.f-1 and at 0.09% probability level observed data agreed with the expected ratio of 1: 1.

**Cross d**

| Sl.No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation (d) (O-E) | $d^2$ / E |
|---|---|---|---|---|---|---|
| 1. | Tall | 1 | 5 | 4.5 | 0.5 | 0.05 |
| 2. | Dwarf | 1 | 4 | 4.5 | -0.5 | 0.05 |
| | Total | 2 | 9 | 9.0 | 0 | 0.10 |

Table $X^2$ =3.84 at 0.05 probability and 1df.

Observed data agree with expected ratio of 1: 1 since the calculated chi square value was found to be lesser than the table chi square value.

**Conclusion**

a) (i) Genotype of the parents

Tall: Tt

(ii) Offsprings : Phenotypic Frequency Genotype

Tall 1 TT

Tall 2 Tt

Dwarf 1 tt

b) (i) Genotype of parents and offspring : TT

(or)

(ii) Genotype of 1 parent: TT

Genotype of other parent: Tt

c) Parent Tall : Tt

Dwarf : tt

Offsprings : Phenotype Genotype Frequency

Tall Tt 1

Dwarf tt 1

d) (i) Parent Tall : Tt

Dwarf : tt

Offsprings Phenotype genotype frequency

Tall Tt 1

Dwarf tt 1

(ii) Tall is dominant over dwarf

**Incomplete dominance**

This is the case wherein the heterozygotes of a monohybrid cross would produce an intermediate phenotype due to absence of complete dominance of either traits of parents.

**Worked out problem**

In cotton the $F_1$ of a cross between a plant with complete petaloidy of anthers and normal one was intermediate. The $F_2$ gave 106 plants with complete petaloidy, 203 intermediate and 90 normal ones. Comment on the inheritance and probability of segregation.

**Data**

Complete petaloidy of anthers x Normal

↓

**$F_1$** intermediate

| | | |
|---|---|---|
| **$F_2$:** | Complete petaloidy | - 106 |
| | **Intermediate** | **- 203** |
| | Normal | - 90 |

**Inference**

(i) Since there is new character, *viz.,* intermediate type in $F_1$, there is no dominance of character or the dominance is incomplete.

(ii) Since the segregation is in a 1 : 2 : 1 ratio, and half of it are new combinations, this is a simple monohybrid ratio in which the heterozygous forms are intermediate.

(iii)There is no dominance of character involved.

**Proof:**

**Schematic representation**:

Parents Complete Petaloidy x Normal

PP ↓ pp

$F_1$ Intermediate

Pp

**Offsprings :**

| Phenotype | Genotype | Frequency |
|---|---|---|
| Complete petaloidy | PP | 1 |
| **Intermediate** | **Pp** | **2** |
| Normal | pp | 1 |

**Chi Square test**

| Sl. No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation (d) (O-E) | $d^2$ / E |
|---|---|---|---|---|---|---|
| 1. | Complete petaloidy | 1 | 106 | 99.75 | 6.25 | 0.39 |
| 2. | Intermediate | 2 | 203 | 199.50 | 3.50 | 0.06 |
| | Normal | 1 | 90 | 99.75 | 9.75 | 1.05 |
| | Total | 4 | 399 | 389 | 0 | 1.50 |

The calculated $\chi^2$ value for df. 2 is 1.50 which is less than the tabular $\chi^2$ value (5.991). So the observed data agrees with the expected ratio of 1 : 2 : 1.

**Conclusion**

(i) The segregation is in a simple monohybrid ratio with incomplete dominance.

(ii) The ratio is 1 : 2 : 1.

(iii) The genotypes of the parents are PP for complete petaloidy and pp for normal.

(iv) The $F_1$ is intermediate, a heterozygous form with genotype Pp.

**Do it yourself**

1) In one strain of sesame, coloured lip of the flower is dominant over colourless (white). If a pure breeding strain of each one is crossed, what will be the fruit colour of $F_1$. How would you distinguish a heterozygous coloured lip flower from a homozygous coloured lip flower?
2) Mendel crossed pea plans producing round seeds and those producing wrinkled seeds. From a total of 7324 $F_2$ seeds, 5474 were round and 1850 were wrinkled. Using your own notation for these traits, symbolize the original parental cross, $F_1$ progeny, $F_2$ obtained by selfing and summarize the expected $F_2$ results for phenotypes, genotypes, genotypic frequency and phenotypic frequency ratios.
3) a) Diagram a cross between a homozygous pea plant that produces yellow seed, and the one that produced green seeds. Carry to $F_2$ and summarize the expected results under the following heads: phenotypes, genotypes and phenotypic ratio.

b) From a total of 8023 $F_2$ seeds, Mendel classified 6022 yellow and 2001 green. Interpret the segregation and prove by chi-square test.

4) A woman has a rare abnormality of the eyelids called ptosis which makes it impossible for her to open her eyes completely. This condition has been found to depend on a single dominant gene (P). The woman's father had ptosis, but her mother had normal eyelids. Her father's mother had normal eyelids. a) What are the probable genotypes of the woman, her father and mother? b) What proportion of her children would be expected to have ptosis if she marries a man with normal eyelids?
5) On *Mirabilis jalapa*, a plant hybrid for red (R) and White (r) flower bears pink flowers (Rr). A plant with pink flowers is crossed with one having red flowers and other having white flowers. Give the genotypic and phenotypic ratios, expected in the progenies of the two crosses.
6) In rice, there are varieties wit short and long outer glumes. When 2 pure breeding lines with short and long glumes were crossed, $F_1$ was intermediate. On selfing, the progenies segregated as short glume (436), long glume (412) and intermediate (808). Interpret the segregation and prove by chi square test.
7) In cattle, hornless condition (P) is dominant over Horned (p). A certain bull is bred to three cows. With cow A, which is horned, a hornless calf is produced. With cow B also horned, a horned calf is produced. With cow C which is hornless, a horned calf is produced. What are the genotypes of the four parents?

## Ex.No: 10 Dihybrid ratio – dominance, incomplete dominance and test cros ratios and combination of one or two of the above

Mendel in his experiment with sweet peas, considering two characters at a time found that segregation of one character is independent of the segregation in the other.

**DIHYBRID CROSS**

This is a cross which involves parents differing for two pairs of contrasting characters. Mendel formulated the law of independent assortment from the results of a dihybrid cross.

**Model:**

In a cross between pure breeding, round yellow seeded plant with wrinkled, green seeded plant, he observed that all the $F_1$s were round yellow seeded and on selfing the $F_2$ progenies segregated as 304 round yellow, 102 round green, 95 wrinkled yellow and 35 wrinkled green. Depict the cross diagrammatically and give proof for your inference.

**Data**

| | | | | |
|---|---|---|---|---|
| Pure breed | Round Yellow | x | wrinkled green | |
| $F_1$ | Round Yellow | | | |
| $F_2$ | Round Yellow | Round green | wrinkled Yellow | wrinkled green |
| | 304 | 102 | 95 | 35 |

**Inference**

i) The given data was reclassified as follows:

| | | | | |
|---|---|---|---|---|
| Shape : | round | 304 + 102 | : | 406 |
| | Wrinkled | 95 + 35 | : | 130 |
| Colour: | Yellow | 304 + 95 | : | 399 |
| | Green | 102 + 35 | : | 137 |

The segregation of individual character is approximately 3: 1.

ii) Since round and yellow are dominant characters and the $F_1$ is heterozygous for round and yellow seed, a 3: 1 ratio in $F_2$ is expected for individual characters.

iii) When both the characters are considered together, as per the law of independent assortment, it is expected that of round will segregate for colour in the proportion of $3/4^{th}$ yellow and $1/4^{th}$ green. Likewise $1/4^{th}$ wrinkled seed will segregate for colour in the proportion of $3/4^{th}$ yellow and $1/4^{th}$ green.

iv) The expected $F_2$ phenotypic ratio is 9: 3: 3: 1, of which 9/16 round yellow, 3/16 round green, 3/16 wrinkled yellow and 1/16 wrinkled green.

v) The cross indicates a simple dihybrid inheritance.

**Proof**

**i) Punnet square method:**

Parent Round, Yellow x Wrinkled, green

RRYY rryy

Gamete R Y r y

$F_1$ Round yellow

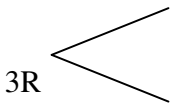
RrYy

| **Parents** | **RY** | **Ry** | **rY** | **ry** |
|---|---|---|---|---|
| **RY** | Round Yellow RRYY | Round Yellow RRYy | Round Yellow RrYY | Round Yellow RrYy |
| **Ry** | Round Yellow RRYy | Round green Rryy | Round Yellow RrYy | Round green Rryy |
| **rY** | Round yellow RrYY | Round Yellow RrYy | Wrinkled Yellow rrYY | Wrinkled yellow rrYy |
| **Ry** | Round Yellow RrYy | Round green Rryy | Wrinkled Yellow rrYy | Wrinkled green rryy |

**ii) Branching / Forked system/ Schematic representation:**

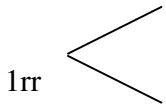
| $F_2$ | | Genotype | Frequency | Phenotype |
|---|---|---|---|---|
| | 1YY | **RRYY** | 1 | Round Yellow |
| 1 RR | 2 YY | RRYy | 2 | Round yellow |
| | 1 yy | RRyy | 1 | Round green |
| | | | | |
| | 1YY | RrYY | 2 | Round yellow |
| 2Rr | 2Yy | RrYy | 4 | Round yellow |
| | 1yy | Rryy | 2 | Round green |
| | | | | |
| | 1YY | rrYY | 1 | Wrinkled yellow |
| 1 rr | 1Yy | rrYy | 2 | Wrinkled yellow |
| | 1yy | Rryy | 1 | Wrinkled green |

To arrive at the phenotypic frequencies, this can be drawn in the branching system as follows:




3Y 9 R-Y- Round yellow

1yy 3 R-yy Round green




3Y 3 rrY – Wrinkled yellow

1yy 1 rryy Wrinkled green

**Chi square test**

| Sl. No. | Class | Expected ratio | Observed value O | Expected value E | Deviation d = (O-E) | $d^2/E$ |
|---|---|---|---|---|---|---|
| 1. | Round yellow | 9 | 304 | 31.5 | 2.5 | 0.020 |
| 2. | Round green | 3 | 102 | 100.5 | 1.5 | 0.022 |
| 3. | Wrinkled yellow | 3 | 95 | 100.5 | -5.5 | 0.300 |
| 4. | Wrinkled green | 1 | 35 | 33.5 | 1.5 | 0.607 |
| Total | | 16 | 536 | 536 | 0 | 0.409 |

The calculated $X^2$ value is (0.409, for d.f. 3) less than the tabular $X^2$ value (7.816), so the deviation of observed data is due to chance only. The observed data agree well with the expected ratio of 9: 3: 3: 1.

**2. Dominance in a- and incomplete dominance in B- ($F_1$ AaBb x $F_1$ Aabb)**

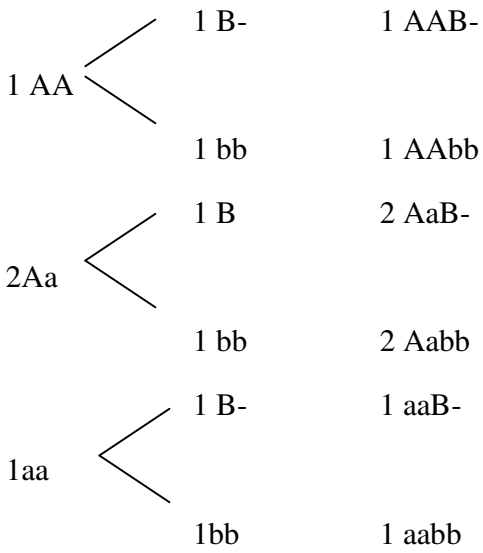
| $F_2$ | | 1 BB | 3 A-BB |
|---|---|---|---|
| | 3 A - | 2 Bb | 6 A-Bb |
| | | 1 bb | 3 A-bb |
| | | 1BB | 1 aaBB |
| | 1 aa | 2 Bb | 2 aaBb |
| | | 1 bb | 1 aabb |

3. **Incomplete Dominance in A and dominance in B ($F_1$ AaBb x $F_1$ AABb)**

| $F_2$ | | 3 B | 3 AAB- |
|---|---|---|---|
| | 1 AA | | |
| | | 1 bb | 1 AAbb |
| | | 3 B- | 6 AaB- |
| | 2Aa | | |
| | | 1 bb | 2 Aabb |

| | | | |
|---|---|---|---|
| | | 3B- | 3 aaB- |
| | 1aa | | |
| | | 1bb | 1 aabb |

4. **Incomplete dominance in a- and test cross bb ($F_1$ AaBb x Aabb)**




5. **Dominance, in A- and B-, test crossed ($F_1$ AaBb x aabb)**

1 B 3 AaB-

3 A

1 bb 3 Aabb

1 B- 1 aaB-

1 aa

1 bb 1 aabb

6. **Incomplete dominance in both the alleles ($F_1$ AaBb x $F_1$ AaBb)**

| | | | |
|---|---|---|---|
| | | 1 BB | 1 AABB |
| $F_2$ | 1 AA | 2 Bb | 2 AABb |
| | | 1 bb | 1 AAbb |
| | | 1 BB | 2 AaBB |
| | 2 Aa | 1 Bb | 4 AaBb |
| | | 2 bb | 2 Aabb |
| | | 1 BB | 1 aaBB |
| | 1aa | 2 Bb | 2 aaBb |
| | | 1 bb | 1 aabb |

The ratios are further modified depending upon whether the first or second locus is recessive lethal.

**Exercise:**

1. In the garden pea, Mendel found that yellow seed colour was dominant to green and round seed shape was dominant to shrunken.
   a) What phenotypic ratio would be expected in the $F_2$ from a cross of a pure yellow round x green shrunken?
   b) What is the $F_2$ ratio of yellow: green and of round: shrunken
   c) Give the test cross ratio.
2. In Drosophila, ebony body colour is produced by a recessive gene e and wild type (gray) body colour by its dominant allele e+. Vestigial wings are governed by a recessive gene vg and normal wing size (wild type) by its dominant allele Vg+. If wild type dihybrid flies are crossed and produce 256 progeny, how many of these progeny flies are expected in each phenotypic class?
3. The normal cloven-footed condition in swine is produced by homozygous recessive genotype mm. A mule footed condition is produced by the dominant genotype M-. white coat colour is governed by a dominant allele of another locus B and black by its recessive allele b. a white mule footed boar is crossed to a sow of the same phenotype. Among the $F_1$ offspring, all black mule footed types were to

be crossed. What phenotypic ratio would be expected among the test cross progeny? If the sow was to be test crossed what phenotypic ratio is to be expected?

4. A genetic condition on chromosome 2 in the fruit fly is lethal when homozygous (pm/pm), but when heterozygous (pm/pm+) produces a purplish eye colour called plum. The other homozygous condition (pm+/pm+) produces wild type colour. On chromosome 3, a gene called stubble produces short, thick bristles when heterozygous (sb/sb+), but is lethal when homozygous (sb/sb). The homozygous condition of its alternative allele (sb+/sb+) produces bristles of normal size (wild type).

    a) What phenotypic ratio would be expected among progeny from crossed between plum, stubble parents?
    b) If the $F_1$ progeny are allowed to mate at random to produce an $F_2$, what is the phenotypic ratio that is expected?

5) Tall tomato plants are produced by the action of a dominant allele D and dwarf plants by its recessive allele d. hairy stem are produced by dominant gene H and hairless stem by recessive allele h. A dihybrid tall, hairy plant is test crossed. The progeny were observed to be 118 tall hairy, 121 dwarf hairless, 112 tall hairless, 109 dwarf hairy.

    a) Represent the cross diagrammatically?
    b) What is the ratio of tall: dwarf or hairy: hairless?
    c) Are these loci assorting independently? Give proof.

6) In man, assume that brown eyes (B) are dominant over blue eyes (b) and right-handedness (L) is dominant over left-handedness (l). A brown eyed, right handed man marries a blue-eyed right-handed woman and their first child is blue eyed and left handed. What are the genotypes of the two parents?

7) In soybean, broad leaf is incompletely dominant over narrow. The heterozygote is intermediate. Purple is dominant over white.

    a) What will be the phenotypic ratio of $F_2$ of a broad leaved plant with a homozygous purple flower crossed with a narrow leaf white flowered plant?
    b) What will be the offspring of the cross between $F_1$ and narrow leaf white flowered plant?

## Ex.No:11 Dominant epistasis, recessive epistasis and duplicate dominant epistasis

**Epistasis**

The interaction of alleles is explained by the internal factors such as hormones and enzymes. Genes specify the structure of proteins. All known enzymes are proteins. Chemical reactions occur as stepwise conversion of one substance into another, each step being mediated by a specific enzyme. Genetic interaction occurs whenever two or more genes specify enzyme(s) which catalyze steps in a biosynthetic pathway.

Assume that the metabolites A and B modify the resulting metabolite. In these situations one gene interferes and determines the phenotypic expression of another non allelic gene (genes), so that the phenotype is determined effectively by the former and not by the later, when both the genes occur together in the genotype. In other words, expression of one gene masks the expression of the other. A gene that prevents the expression of another gene is termed as epistatic, and the gene that is suppressed is hypostatic. The phenomenon of non allelic inter allelic gene interaction is called Epistais, while dominance involves intra allelic gene interaction.

The classical phenotypic ratio of 9: 3: 3: 1 observed in $F_2$ of dihybrids becomes modified by epistasis into ratios which are various combinations of 9; 3: 3: 1, reducing the occurrence of phenotypes to less than four. Depending upon the allelic action they are referred to by different names.

**Epistatic interaction**

| Sl. No. | Epistasis | Ratio | Nature of alleles at A locus | Nature of alleles at B locus |
|---|---|---|---|---|
| 1. | Dominant epistasis | 12 : 3 : 1 | Dominant alleles (A) expresses itself even in presence of B or b | Alleles B or b expressed only when A locus is homozygous recessive (aa).<br>NB: A – B } Same phenotype<br>A – bb |

| | | | | |
|---|---|---|---|---|
| | | | | aaB- } additional phenotypes<br>aabb |
| 2. | Recessive epistasis (supplementary gene action) | 9 : 3 : 4 | Recessive aa suppresses alleles at B locus. | B or b expressed only when A locus has dominant allele (A)<br>A – B – additional phenotype<br>a-bb<br>aaB- } same phenotype<br>aabb |
| 3. | Duplicate gene epistasis with cumulative effect (Additive gene action) | 9 : 6 : 1 | Dominant A- produces 1 phenotype (with B- dominant alleles produces another) | Dominant B-produces same phenotype as A (with dominant alleles A – Produces another)<br>A – bb }<br>aaB- same phenotype<br>aabb }<br>A-B- additional phenotype |
| 4. | Duplicate dominant genes | 15 : 1 | Dominant A- produces one phenotype, with or without dominant B - | Dominant B-produces same phenotype as A, with or without dominant A-<br>A – bb }<br>aaB- } same phenotype<br>A-B-<br>Aabb- additional phonotype |
| 5. | Duplicate Recessive genes (complementary gene action) | 9 : 7 | Single dominant gene A- produces same phenotype as B – or aabb, with dominant B-, produces another | Single dominant gene B-produces same phenotype as A- or aabb, with dominant A-produces another.<br>A – bb }<br>aaB- } same phenotype<br>aabb<br>A-B- additional |

| | | | | |
|---|---|---|---|---|
| 6. | Dominant and recessive gene action (inhibitory gene action) | 13 : 3 | Dominant A – and recessive bb produces same phenotype | Dominant B-alone produces different phenotype<br>A – bb } same phenotype<br>A-B- }<br>Aabb<br>aaB -different/additional phenotype |

**i. DOMINANT EPISTASIS (12: 3: 1)**

Two pairs of factors in which one factor (A-) under dominant condition expresses itself irrespective of the condition in another locus (B-) (*i.e*., dominant/ recessive). Locus B-expresses only when locus A is in recessive condition. The recessive of both expresses a different phenotype.

**Model**

An inhibition of pigment production in onion bulbs (I-) exhibits dominant epistasis over another locus, the genotype iiR-producing red bulbs and iirr producing yellow bulbs. (a) A pure white strains crossed to a pure red strain and produce all white $F_1$s and $F_2$ white 12/16 white: 3/16 red and 1/16 yellow. What are the genotypes of the parents? (b) In $F_2$, 32 were found to be of genotype iirr. Workout the proportion of others.

**Data**

White onion bulbs : I-R- or I-rr

Red onion bulbs : iiR-

Yellow onion bulbs : iirr

ii) Pure white x Pure Red

$F_1$ White

$F_2$ 12/16 white, 3/16 Red and 1/16 yellow

iii) In $F_2$, 32 are of genotype iirr found.

**Inference**

1. Since there is no segregation observed in $F_1$ both the parents are homozygous.
2. Since Red can express only in the absence of I, the genotype of the red parent should be iiRR.
3. Since in $F_2$, 16 zygotic combinations are observed, should be double heterozygote; white parent should be of genotype IIrr and the $F_1$ is I-R-(White)
4. In the 16 combinations since the dominant gene produces white, 12/16 will be white and double recessive yellow and genotype iiR- 3/16 will be red.
5. In $F_2$ iirr is 32, it is 1/16. The others can be calculated.

**Proof**

| | | | | | |
|---|---|---|---|---|---|
| Parents | Pure white | | x | Pure red | |
| | IIrr | | ↓ | iiRR | |
| | $F_1$ | White | | | |
| | | IiRr | | | |
| | | | | Frequency (Since 1/16 = 32) | |
| | $F_2$ | | 3 R- | 9 I-R-White | 288 |
| | | 3R- | | | |
| | | | 1 rr | 3 I-rr White | 96 |
| | | | 3 R- | 3 iiR- Red | 96 |
| | | lii | | | |
| | | | 1 rr | 1 iirr-yellow | 32 |

**Conclusion**

i) It is a case of dominant epistasis

ii) Frequency of the phenotypes:

| | | |
|---|---|---|
| White | : | 384 |
| Red | : | 96 |

Yellow : 32

**RECESSIVE EPISTASIS (9: 3: 4)**

The dominant condition at one locus (A) expresses a phenotype whose expression is intensified by another dominant gene in another locus (B). The recessive genotype of locus A suppresses the expression of the dominant allele at B locus which is similar to the double recessive.

**Model:** In flax, two blue flowered parents were crossed and the offsprings segregated into 65 Blue, 20 Lilac and 27 White.

What are the genotypes of the parents? Give proof.

Data: Parents phenotype : Blue x Blue

Progenies : Blue 65; Lilac 20; White 27

What are the genotypes of the parents?

**Inference**

1. When two phenotypically similar blue flowered parents were crossed, the progeny segregated into blue, lilac and white showing that both the parents are heterozygous.
2. Since lilac is an intermediate form between blue and white and few in number, incomplete dominance is rule out.
3. Lilac is the basic colour in which another allele interacts and intensifies it to blue, i.e. two dominant genes are necessary to produce blue.
4. Since the progeny segregated both the parents should be heterozygous.
5. In the absence of basic colour viz., lilac, the intensifying gene has no action and therefore it is white and the double recessive that lacks both the dominant alleles also is white.
6. In other words, a gene that has no action of its own intensifies or supplements the action of another dominant gene. Here the expression of lilac is intensified by a supplementary gene into blue.
7. Since the recessive allele of one locus suppressed the expression of another locus (here the intensifying factor). It is called recessive epistasis.

8. Of the zygotic combinations expected in double heterozygote progenies, 9/16 where two dominant genes are parents will be blue, 3/16 where the basic colour allele in present will be lilac, and 3/16 where the recessive epistatic gene is present and 1/16 recessive will be white.
9. Hence the ratio expected is 9 : 3 : 4 of blue : lilac : white

**Proof**

Parents Phenotype Blue x Blue

LlQq LlQq

Gamete: LQ, Lq, lQ, lq : LQ, Lq, lQ, lq

| Progenies | | Genotypes | Frequency | Phenotype |
|---|---|---|---|---|
| 3L - | 3Q- | L-Q | 9 | Blue |
| | lqq | L-qq | 3 | Lilac |
| lll | 3Q- | 11Q- | 3 | White |
| | 1qq | 11 qq | 1 | White |

**Chi square test**

| **Sl. No.** | **Class** | **Expected ratio** | **Observed value (O)** | **Expected value (E)** | **Deviation (O-E) = d** | **$d^2/E$** |
|---|---|---|---|---|---|---|
| 1. | Blue | 9 | 65 | 63 | 2 | 0.063 |
| 2. | Lilac | 3 | 20 | 21 | -1 | 0.047 |
| 3. | White | 4 | 27 | 28 | -1 | 0.035 |
| **Total** | | 16 | 112 | 112 | 0 | 0.145 |

**Conclusion**

i) It is a case of recessive epistasis involving two non allelic genes

ii) The gene Q that has no action of its own, intensifies the action of another dominant gene 'L' changing lilac into blue.

iii) The genotype of the parent is LlQq.

'll' is epistatic gene, Q is hypostatic.

## DUPLICATE DOMINANT EPISTASIS (15 : 1)

The dominant alleles of both loci each produce the same phenotype without cumulative effect.

### Model

In Shepherd's purse, triangular capsule is dominant over round and it is due to duplicate genes C and D. What are the genotypes of the parents that would produce the following results.

a) 15 Triangular and one round

b) 3 Triangular and one round

### Data

Triangular capsule is dominant over round

Triangular capsule is governed by duplicate genes C and D

### Inferences

a) i) Since there is segregation in the immediate progeny either one or both the parents should be heterozygous.

ii) Since there are 16 zygotic combinations, interaction between the two pairs of non allelic genes is involved, of which the double recessive produces the round capsule.

ii) The genotype of the parents should be Cc Dd.

b) i) Since the segregation is in a 3 : 1 ratio of triangular and round, and triangular character is governed by two duplicate genes, the possibilities are,

1) One of the parents should be a double heterozygote. When crossed with a recessive round, 3 will have two or one of the genes to produce triangular, while the recessive one will be round (or)
2) Both the parents may be heterozygous for one gene, say Cc or Dd, the other gene being recessive

or

3) One of the parents is heterozygous for one gene, say Cc and the other recessive dd. The other parent may be heterozygous for Dd and recessive for cc.

In all the cases, the segregation will be 3 triangular and one round.

**Proof**

a) Parents phenotype : Triangular x Triangular

Genotype : CcDd CcDd

Gamete : CD, Cd, cD, cd x CD, Cd, cD, cd

| Offspring : | Genotype | Frequency | Phenotype |
|---|---|---|---|
| 3D - | C-D- | 9 | Triangular |
| 3C - | | | |
| 1dd | C-dd | 3 | " |
| 3D- | ccD- | 3 | " |
| 1cc | | | |
| 1dd | ccdd | 1 | Round |

b) i) Parental Phenotype Triangular x Round

(Genotype) Cc Dd ccdd

Gamete CD, Cd, cD, cd cd

| Offspring | Genotype | frequency | Phenotype |
|---|---|---|---|
| | CcDd | 1 | Triangular |
| | Ccdd | 1 | " |
| | ccDd | 1 | " |
| | ccdd | 1 | Round |

ii) Parents Phenotype Triangular x Triangular

Ccdd ccdd

Gamete Cd, cd cd

Offspring 3C-dd Triangular

1ccdd round

(or)

iii) Parents Phenotype Triangular x Triangular

Ccdd ccDd

Gamete Cd, cd cD, cd

| Offspring | **Genotype** | **Frequency** | **Phenotype** |
|---|---|---|---|
| | CcDd | 1 | Triangular |
| | Ccdd | 1 | " |
| | ccDd | 1 | " |
| | ccdd | 1 | round |

**Conclusion**

i) It is a case of duplicate dominant epistasis in which two genes have similar effect either alone or in combination i.e. Triangular capsule

ii) The recessive of both the genes forms the round capsule.

**EXERCISE**

1. Matings between black rats of identical genotype produced offspring as 14 cream coloured, 47 black and 19 albinos.
   A) What epistatic ratio is approximated by these offspring?
   B) What type of epistasis is operative?
   C) What are the genotypes of parents and offsprings?
2. The black leghorn breed of chickens had feathered shanks. When Langshans are crossed to the Buff Rock breed with unfeathered shanks, all $F_1$ have feathered shanks. Out of the 360 $F_2$ progenies, 24 were found to have non-feathered shanks and 336 had feathered shanks.
   a) What is the mode of interaction of this trait?
      Give proof using chi-square test.
3. An inhibitor of pigment production in onion bulbs (I-) exhibits dominant epistasis over another locus. The genotype ii R- producing red bulbs and iirr producing yellow bulbs. A pure white strain is crossed to a pure red strain and produces all white $F_1$ and $F_2$ with 12/16 white, 3/16 red and 1/16 yellow. What were the genotypes of the parents? Prove with statistical analysis.

## Ex.No:12 Duplicate recessive epistasis, additive epistasis, duplicate and recessive epistasis

**Duplicate recessive epistasis (9: 7)**

**Model:** When pure breeding two white flowered sweet pea plants were crossed, the $F_1$ was purple. In $F_2$, the progenies segregated into 94 purple and 75 white. (Statistically would this be considered as 1: 1 ratio. If not, how do you interpret i.e., and) what would be the genotype of the parents?

**Data;** Pure breeding white x white

| | | |
|---|---|---|
| $F_1$ | Purple | |
| $F_2$ | 94 purple | 75 white |

What is the interaction?

Is it a test cross ratio?

**Inference**

1. Since parents are pure breeding white and $F_1$ is purple, the factor for purple colour is available under homozygous condition in both the parents.
2. $F_1$ should be a double heterozygote as alleles responsible for colour production are available in both the parents and their corresponding alternative forms are available in either parent in a recessive condition. Thus the genotype of the parents could be parent 1 = $P_1P_1$ $P_2P_2$ : Parent 2 = $p_1p_2$ $P_2P_2$ and the $F_1$ = $P_1p_1P_2p_2$
3. When two pairs of factors are involved, $F_2$ of pure breeding lines will give a 9 : 3 : 3 : 1 ratio, in which 9/16 will have both the dominant genes. In this case it produces colour. Since the same condition does not exist in all other classes they are white.
4. Here the recessive alleles interfere with the expression of the dominant alleles at other locus. Since two recessive alleles are involved the interactions referred to as Duplicate **recessive epistasis**.

5. In other words the two dominant genes ($P_1$ and $P_2$) having no action of their own, complement each other in producing the purple phenotype.
6. Since 1 : 1 is a monohybrid test cross ratio and the $F_1$ is double heterozygote, the segregation is more probable to be a 9 : 7, rather tan 1 : 1 ratio.

**Proof**

Schematic representation

Parents : White x white

Genotype : $P_1P_1p_2p_2$ $p_1p_1P_2P_2$

$F_1$ : Purple

$P_1p_1P_2p_2$

| | | | **Genotype** | **Frequency** | **Phenotype** |
|---|---|---|---|---|---|
| $F_2$ | $3P_1$ – | $3P_2$ - | $P_1 – P_2 –$ | 9 | Purple |
| | | | | | |
| | | $1p_2p_2$ | $P_1 – p_2p_2$ | 3 | White |
| | $1\ p_1p_1$ | 3P2 – | $p_1p_1P_2 –$ | 3 | White |
| | | | | | |
| | | $1\ p_2p_2$ | $p_1p_1p_2p_2$ | 1 | White |

Purple : 9 White : 7

**Chi square test**

| **S. No.** | **Class** | **Expected ratio** | **Observed value (O)** | **Expected value (E)** | **Deviation = d (O-E)** | **$d^2/E$** |
|---|---|---|---|---|---|---|
| 1. | Purple | 9 | 94 | 95.06 | -1.06 | 0.0118 |
| 2. | White | 7 | 75 | 73.94 | 1.06 | 0.0118 |
| Total | | 16 | 169 | 169 | 0 | 0.0236 |

**Chi square test based on 1: 1 ratio**

| S. No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation = d (O-E) | $d^2/E$ |
|---|---|---|---|---|---|---|
| 1. | Purple | 1 | 94 | 84.5 | 9.5 | 1.06 |
| 2. | White | 1 | 75 | 84.5 | -9.5 | 1.06 |
| Total | | 2 | 169 | 169 | 0 | 2.12 |

**Conclusion**

1. It is a case of duplicate recessive epistasis in which the recessive alleles of the two factors mask the expression of the other dominant non allelic gene.
2. Though statistically the 1 : 1 ratio fits well, genetically the segregation observed cannot be 1 : 1

**DOMINANT AND RECESSIVE EPISTASIS (13:3)**

Dominant genotype at one locus (A-) and the recessive genotype at the other locus (bb) produce the same phenotypic effect. The locus B-expresses only when locus A is in recessive condition (aa).

**Model**

In paddy, purple sheath is dominant over green. In a cross with purple sheath with green sheath, the $F_1$ is found to be green. In $F_2$ the progenies segregated as 1291 green and 307 purple. Interpret the segregation and give proof.

**Data**

Parents : Purple x Green

F1 Green

Purple Green

**Inference**

1. Since purple is dominant to green, and the $F_1$ appeared as green, the expression of purple colour could be inhibited by the gene present in green parent.
2. Since $F_1$ is not segregating, both the parents should be homozygous.
3. The genotype of purple parent should be PPii and green parent is ppII. If green parent is ppII, there won't be any segregate for 'P' and the progeny will segregate in 3 : 1 rather 13 : 3.
4. It is a case of dominant and recessive epistasis in which dominant gene in one locus and the recessive allele at another locus express a similar phenotype viz., green, while purple expresses only when the dominant inhibiting gene is recessive condition.

**Proof**

| | | | | | |
|---|---|---|---|---|---|
| Parent | : | Phenotype | Purple | x | Green |
| | | | PPii | | ppII |
| | | $F_1$ | Green | | |
| | | | PpIi | | |
| | | | Genotype | Frequency | Phenotype |
| | 3I- | | P-I- | 9 | Green |
| | 3p- | | | | |
| | | Iii | P-ii | 3 | Purple |
| | 3I- | | ppI- | 3 | Green |
| | 1pp | | | | |
| | | lii | ppii | 1 | Green |

**Chi Square test**

| Sl. No. | Class | Expected ratio | Observed value (O) | Expected value (E) | Deviation d (O-E) | D2/E |
|---|---|---|---|---|---|---|
| 1. | Green | 13 | 1291 | 1298.38 | 7.38 | 0.04 |
| 2. | Purple | 3 | 307 | 299.62 | -7.38 | 0.01 |
| | Total | 16 | 1598 | 1598 | 0 | 0.05 |

**Conclusion**

i) It is a case of dominant and recessive epistasis in which purple colour can be expressed only in the absence of dominant inhibitory gene.

ii) It segregated for 13: 3 ratio.

**DUPLICATE EPISTASIS / CUMULATIVE EFFECT (9 : 6 : 1)**

Considering that at each locus, the dominant allele produces one unit of a substance such as pigment independently. When two loci exist in dominant condition, the expression is cumulative. The recessive genotype produces no pigment.

**Model**

Three fruit shape are recognized in summer squash (*Cucurbita pepo*.): Disk, elongated and sphere shape. Pure disk was crossed with elongated and 45 disks were observed.

1. interpret the interaction
2. Give proof

Data: Parents Phenotype disk x elongated

$F_1$ disk

$F_2$ Disk; 45; Sphere: 30; elongated : 5

**Inference**

1. When pure breeding disk and elongated are crossed, the $F_1$ is disk showing disk is dominant over elongated.
2. In $F_2$ sphere appears showing that the characters for sphere is available in both the parents.
3. Reducing the proportion of genotypes in $F_2$, it is observed that elongated appears as 1/16, - probably the double recessive.
4. Since in $F_2$, 16 zygotic combinations are observed $F_1$ should be a double heterozygote, and disk parent should be double dominant.
5. The genotype of the parents could be disk DDSS and elongated ddss, and sphere may be either ddS- or D-ss
6. Disk is due to the cumulative expression of factors responsible for sphere and the double recessive is elongated.

**Proof**

| | | | | |
|---|---|---|---|---|
| Parents | | Disk | x | elongated |
| | | DDSS | ↓ | ddss |
| $F_1$ | | | Disk | |
| | | | Dd Ss | |
| $F_2$ | | 3S- : | 9D-S- | Disk |
| | 3D- | | | |
| | | 1ss : | 3D-ss | Sphere |
| | | 3S- : | 3dds- | Sphere |
| | 1dd | | | |
| | | 1ss : | 1ddss | elongated |

**Excersise**

1. A dominant gene S in Drosophila produces a peculiar eye condition called star. Its recessive allele S+ produces normal eye of wild type. The expression of S can be suppressed by the dominant allele of another locus su-s. The recessive allele of the locus su-s+ has no effect on s+. What is the type of interaction involved?
2. Two white flowered strains of sweet pea (*Lathyrus odoratus*) were produced an $F_1$ with purple flowers. Random crossing among $F_1$ produced 96 progeny plants, 53 exhibiting purple flowers and 43 with white flowers.
    b) What phenotypic ratio is approximated by the $F_2$?
    c) What is the type of interaction involved?
    d) What are the probable genotypes of parents and offsprings?
3. Three fruit types are recognized in summer squash (*Curcurbita pepo*): disc shaped, elongated and spear shaped. A pure disc shaped variety was crossed to a pure elongated variety. The $F_1$ were all disc shaped. Among 80 $F_2$, there were 30 spear shaped, 5 elongated and 45 disc shaped. Reduce the $F_2$ numbers to their lowest ratio and comment on their inheritance.
4. A homozygous yellow rat when mated with a homozygous black rat produces $F_1$ all grey in colour. Brother-sister mating of $F_1$ produced $F_2$ progeny in the phenotypic ratio of 27 grey: 9 yellow: 8 black:3cream coloured.

    (a) Explain the type of inheritance.
    (b) Give the genotypes of $F_2$ progeny.

(c) What proportion of $F_2$ rats are expected to be homozygous among the black coloured progeny?

## Ex.No: 13 Multiple allele and polygenic inheritance

The majority of heritable traits governed by a pair of genes exhibiting minimum two contrasting phenotypic classes. Such characters are known as quantitative or discontinuous. For example, cattle may have horns or no horns, flowers may be red, or white, man may have blood group A or B or AB or O. Drosophila many have red eyes or white eyes.

For example, if a group of one hundred persons is classified according to their heights, a gradual gradation in the height can easily be recognized. Such a character exhibits continuous phenotypic variation. F.C. Galton (1883) noted that many of these continuous variations are heritable. Characters like height, weight, intelligence or colour exhibit continuous variations in human population. Plant height and the size, shape and number of seeds and fruits have also been found to exhibit quantitative differences. These gradations in characters are determined by a number of gene pairs and also the gene pairs have additive effect or are cumulative. Each gene has a certain amount of effect, and the more is the number of dominant genes, the more is the degree of expression of the character. This is known as quantitative inheritance, or polygenic inheritance. The genes associated in a cumulative manner are known as cumulative factors or polygenes.

**Polygenes**

The polygenes can be defined as two or more different pairs of alleles which have cumulative effect and govern quantitative characters.

**Characters of polygenes**

- The effects of each contributing gene are cumulative or additive.
- Each contributing allele in a series produces on equal effect.
- There is no dominance involved.
- Epistasis does not exist among genes at different loci.
- No linkage is involved in the process.
- Effects of environment are absent or may be ignored.

The above values for frequency of genotypes is calculated by the expansion of binomial equation, $(a = b)^{n.}$ Where you have is the number of contributing dominant alleles.

| **Binomial $(a + b)^n$** | **Expansion of binomials** |
|---|---|
| $(½ + ½)^1$ | 1 : 1 |
| $(½ + ½)^2$ | 1 : 2 : 1 |
| $(½ + ½)^3$ | 1 : 3 : 3 : 1 |
| $(½ + ½)^4$ | 1 : 4 : 6 : 4 : 1 |
| $(½ + ½)^5$ | 1 : 5 : 10 : 10 : 5 : 1 |
| $(½ + ½)^6$ | 1 : 6 : 15 : 20 : 15 : 6 : 1 |
| $(½ + ½)^7$ | 1 : 7 : 21 : 35 : 35 : 21 : 7 : 1 |

We obtain the binomial expansion as:

$(a + b)^1 = a + b$

$(a + b)^2 = a^2 + 2ab + b^2$

$(a + b)^3 = a^3 + 3a^2b + 3ab^2 + b^3$

$(a + b)^4 = a^4 + 4a^3b + 6a^2b^2 + 4\ ab^3 + b^4$

**Example**

In a study on the inheritance of skin colour in man, the following results were observed comment on the inheritance.

Negros x White

Mullatoes ($F_1$)

The intermating among $F_1$ progenies produces the following $F_2$ children.

1/16 – Negroes

4/16 – Dark

6/16 – Intermediate

4/16 – Light

1/16 – White

**Data** Negroes x White

Mullatoes ($F_1$)

$F_2$: Negroe : Dark: Intermediate: Light: White

1 : 4 : 6 : 4 : 1

**Inference**

1. Since the parents are pure breeding, the $F_1$ must have been a heterozygote.
2. Since this gives segregates. Upon intermating and the segregants show differential shades, this might be due to polygenic inheritance.
3. Since 1/16 of the $F_2$ were extreme in colour as either of the parental plant, 2 pairs of genes are involved in controlling the production of melanin pigment, responsible for the production of skin where in man.

**Proof**

**P1** **Negroes** **x** **white**

AABB aabb

$F_1$ Mullatoes

AaBb

$F_2$

| **Gametes** | **AB** | **Ab** | **aB** | **Ab** |
|---|---|---|---|---|
| **AB** | AABB (Negroe) | AABb (Dark) | AaBB (Dark) | AaBb (Intermediate) |
| **Ab** | AABb (Dark) | AABB (Intermediate) | AaBb (Intermediate) | Aabb (Light) |
| **aB** | AaBB (Dark) | AaBb (Intermediate) | AaBB (Intermediate) | aaBb (Light) |
| **ab** | AaBb (Intermediate) | Aabb (Light) | aaBb (Light) | aabb (White) |

The reclassification of the squares, bases on genotypes and phenotypes of $F_2$ genotypes and phenotypes of $F_2$ progeny of Negro & white parents is as follows:

| **Genotypes** | **No. of dominant genes** | **Frequency** | **Phenotype** | **Phenotypic ratio.** |
|---|---|---|---|---|
| AABB | 4 | 1 | Black (negro) | 1/16 |
| AaBb<br>AABb | 3 | 2<br>2 } 4 | Dark | 1/16 |
| AaBb<br>aaBb<br>AAbb | 2 | 4<br>1 } 6<br>1 | Intermediate | 6/16 |
| Aabb<br>aaBb | 1 | 2<br>2 } 4 | Light | 4/16 |
| Aabb | | 1 | White | 1/16 |

**Conclusion**

Inheritance of skin colour in man is governed by polygenes, determining quantitative inheritance. The amount of melanin determines the skin colour, governed by two pairs of genes. These genes are present at two different loci and each dominant gene is responsible for the synthesis of fixed amount of melanin. The effect of all the genes is additive and the amount of melanin produced is always proportional to the number of dominant genes.

**Do it yourself**

1. In tomato, genotype aab bcc. produces 100 g tomatoes and AABBCC produces 160 g tomatoes, each gene (capital letters) causing an increase of 10g. Give the weight of tomatoes in the parents and progenies in the following crosses:
    a) AAbbcc x aaBBcc
    b) AAbbcc x AaBbCc
    c) aaBbCc x aaBbCc
    d) AaBbCc x aaBBCc
2. Height in man is caused by polygenes.
    i) Can matings between individuals with intermediate height produce children taller than either of the parent?
    ii) Can mating between two short parents produce children taller than either of the parents?

3. If the wheat kernel colour is determined by three pairs of polygenes, in a cross AABBCC x aabbcc. What fraction of $F_2$ would be expected to be like other parent? How may $F_2$ phenotypic classes result?

**MULTIPLE ALLELES**

Bertstein (1925) discovered that the inheritance of different blood groups in man is determined by a number of multiple allelic series. There are alleles concerned with the determination of blood group of any person. For convenience, group O is regarded as normal and alleles A and B are said to represent two dominant mutations which have occurred on the same locus. These represent codominant alleles. If normal gene is represented by +, the three gene will be +, A and B. Since + is recessive, individuals of group O will have a genotype +/+; gene A is dominant individuals of blood groups A might have A genotype., +/A or A/A and similarly individuals of group B might have +/B or B/B and individuals of group AB will have a genetic composition A/B.

**Multiple alleles (Morgan, 1914)**

Individual alleles of a group consisting of more than two are called a series of multiple alleles. The dominant relationship varies with series. The alleles are arranged in a descending series in which every allele is dominant over all alleles below it.

Example: Coat colour in rabbits.

CC ; Wild, CchCch – Chinchilla ; ChCh – Himalayan;

CaCa = Albino. The dominant relationship is

C > Cch > Ch > Ca

The number of genotypes in a series of multiple alleles is

= ½ [n (n + 1)] where in n = number of alleles of the group.

When n= 4;

½ [4 (4 + 1)] = 10 genotypes are produced

In the example, apart from the above four homozygotes, the following are possible.

Wild = $Cc^{ch}$, $C^{ch}$, $C^{ca}$

Grey = $C^{ch}C^{h}$, $C^{ch}C^{a}$

Himalayan : $C^{h}C^{a}$, 10 Genotypes

**Model:**

A yellow mouse crossed with another yellow mouse gave 8 yellow, 2 black and tan offspring. Two of these black and tan offspring gave at least one black progeny of their own. What is the probability of obtaining yellow, black and tan and black offspring from

a) a cross of $F_1$ black and tan to $F_1$ black and tan
b) a cross of $F_2$ black to $F_2$ black
c) a cross of $F_1$ yellow to $F_1$ yellow

The coat colour in mouse is controlled by a series of alleles consisting of

AY = yellow; AL = Yellow with black belly; A = Agouti

at = black and tan ; a = black

**Data**

| | | |
|---|---|---|
| Yellow | x | Yellow |
| $F_1$ 8 yellow | | 2 black and tan |
| | | Black |
| 1. $F_1$ black and tan | x | $F_1$ black and tan |
| 2. $F_2$ black | x | $F_2$ black |
| 3. $F_1$ yellow | x | $F_1$ yellow |

**Inference**

i. In mouse homozygous yellow is lethal. Since there is segregation in the immediate progeny, both the parents should be heterozygous. Gene responsible for black and tan should be present in both the parents.

ii. Since black and tan progeny segregate on inter breeding to give at least one black progeny, it should be also heterozygous.

iii. To obtain a heterozygous progeny in $F_1$ the alleles responsible for the production of black and tan and black should be present in the two different parents.

iv. Since yellow colour is due to '$A^Y$' allele, it should be present in both the parents. To obtain a heterozygous black and tan which on further interbreeding gives atleast one black progeny, the alleles '$a^t$' and 'a' should be present in the two different parents. Hence the possible genotypes of the parent can be $A^Y$ $a^t$ and $A^Y$a. Since $A^Y$ is dominant, the parent will be yellow only.

v. Since $F_1$ is black and tan is heterozygous, interbreeding is expected to give black and tan and black offsprings on 1 : 1 ratio, in order words, at least one black offspring.

vi. Interbreeding of $F_2$ black will give only black offspring.

vii. The Possibilities of $F_1$ yellow x $F_1$ yellow are,

a) $A^Ya^t$ x $A^Ya$ Progeny : 2/3 yellow 1/3 black and tan

b) $A^Ya$ x $A^Ya$ Progeny : 2/3 yellow 1/3 black

c) $A^Ya^t$ x $A^Ya^t$ Progeny : 2/3 yellow 1/3 black and tan

In all the cases $A^YA^Y$ is lethal.

**Proof**

| Yellow | x | yellow |
|---|---|---|
| $A^Ya^t$ | | $A^Ya$ |

| Offspring : | Genotype | Phenotype | Frequency |
|---|---|---|---|
| | $A^YA^Y$ | lethal | |

$A^Ya$ Yellow

$A^Ya^t$ Yellow 2/3

$a^ta$ Black and tan

Black and tan x Black and tan

Offspring: $a^ta$ : black and tan 3 = 3/4

a a : Black = 1/3

2) $F_2$ black x $F_2$ black

Progeny all black

3) $F_1$ yellow x $F_1$ yellow

$F^1$ yellow can be $A^Ya^t$ or $A^Ya$

Possibilities (a) $A^YA$ : aa : 2/3 : 1/3 : Yellow: Black

(b) $A^Ya^t$ : aa : 2/3 : 1/3 : Yellow: Black and tan

(c) $A^Ya^t$ : $A^Ya$ : 2/3 : 1/3 : Yellow

**Conclusion:**

It is a multiple inheritance in which the coat colour of mouse is controlled by a series of alleles in which dominance of one over the other is as follows:

$A^Y$ – yellow $a^t$ Black and tan a black

Additional Problems

1. Plumage colour in mallard ducks is dependent upon a set of three alleles; MR Restricted mallard pattern: M mallard and 'm' dusky mallard. The dominance hierarchy is MR M m. Determine the genotypic and phenotypic ratios expected in the $F_2$ from the following crosses.

   i) $M^RM^R$ x $M^RM$

   ii) $M^RM$ x M m

iii) $M^R m$ x mm

2. Write the phenotype and genotype segregation in the following multiple allelic series crosses where the dominance relationship is

   C > $C^{ch}$ > $c^h$ > c

   C: Coloured, $C^{ch}$ ; Chinchilla, $c^h$ : Himalayan ;

   c: colourless

   1) C $c^h$ x $c^{ch}$ $c^h$ 2) C c x $c^{ch}$ $c^h$ 3) $c^{ch}$ c x $c^h$c

   4) C c x C c 5) cc x C $c^{ch}$

3. A couple believed that they have brought the wrong baby home from the hospital. The wife is group O, her husband is group B and the child is group O. Could the baby be theirs?

4. A couple preparing for marriage have their blood typed along with other required blood tests. Both are AB. They ask you what type of blood group their children may have? What would you tell them and how would you explain your conclusions?

5. A man has type A blood and his wife has type B blood. A physician types the blood of their four children and is amazed to find one each of the four blood types among them. He is not familiar with genetics and calls upon you to explain how such a thing could happen. What would you tell?

6. A wealthy, elderly couple dried together in an accident soon, a man shows up to claim their fortune, contending that he is their only son who ran away from home when a boy. Other relatives dispute this claim. Hospital records show that the deceased couple were blood type AB and O. The claimant to the fortune fortunately has the type O. Do you think that the claimant was an impostor? Explain.

7. Two sets of parents. Mr. X and Mrs. X and Mr. Y and Mrs. Y are claiming the same baby. Blood tests give the information that Mr. X and Mrs. X belongs to A group of blood and Mr. Y. belong to O group and Mrs. Y is of AB groups. The child belongs to O. Explain with reasons to which parents the child could be given.

## Ex.No: 14 Estimation of linkage with $f_2$ and test cross data, coupling and repulsion

Exceptions to Mendal's law of independent assortment were discovered in 1906 by Bateson and Punnet. They found that two pairs of alleles in sweet peas did not assort independently. When the two alleles A and b came from the same parent (AABB x aabb), they tended to enter the same gamete and transmitted together, and when the same allele came from different parents (AAbb x aaBB) they tended to enter different gametes and remain apart. The first situation was referred to as coupling and the second repulsion.

**Coupling**: The two alleles of two linked genes are said to be in coupling phase if the two dominant alleles are located in one chromosome and recessive alleles in its homologous chromosome (AB/ab).

**Repulsion**: A double heterozygote is said to be in repulsion phase when the dominant alleles are located one in each of the homologous chromosomes (Ab/aB).

**Linkage**: i) It is the tendency of two linked genes, to remain in their original combination due to their residence in the same chromosome.

ii) The degree of strength of linkage depends upon the distance between the linked genes.

**Model**

In maize, coloured pericarp and full endosperm are dominant, Two experimental results are given below:

a) Pure breeding parents: Coloured, full endosperm x Colourless shrunken

$F_1$ coloured, full x test cross

b) Pure breed parents: Coloured, shrunken x Colourless, full

$F_1$ coloured, full x test cross

| **Progenies**<br>**Characters** | **Experiment (a)** | **Experiment (b)** |
|---|---|---|
| Coloured, full | 4032 | 639 |
| Coloured, shrunken | 149 | 21379 |
| Colourless, full | 152 | 21906 |
| Colourless, shrunken | 4035 | 672 |

Interpret the segregation

**Inference**

i) The data are reclassified for individual characters as follows

**Experiment (a)**

Coloured: Colourless : 4032+149=4181; 152+4035=4187

Full : Shrunken : 4032+152=4184; 149+4035=4184

**Experiment (b)**

Coloured: Colourless : 21379+639=22018; 21906+672=22578

Full : Shrunken : 639+21906=22445; 21379+672=22051

Individual characters segregate into a 1:1 ratio. However, when both the characters are considered together, the expected 1 : 1 : 1 : 1 test cross ratio is not observed. Since there is a deviation from the normal test cross ratio and the parental combination appears more, linkage is suspected between the two factors.

ii) In experiment (a) the two dominant characters enter from the same parent, and parental combination appear more, explaining the coupling phase, while in experiment (b) the two dominant characters enter from different parents, tend to enter different gametes, and parental combination appear more, explaining the repulsion phase.

**Experiment (a)**

Parents: Phenotype: Coloured, full x colourless, shrunken

CCSS ccss

$F_1$ x test cross Coloured, full colourless, shrunken

CS cs

--- x --

Cs cs

**Test cross progenies**: Coloured full: Coloured shrunken: Colourless full: Colourless shrunken

| CS | Cs | cS | cs |
|---|---|---|---|
| ---- | --- | --- | --- |
| cs | cs | cs | cs |
| 4032 | 149 | 152 | 4035 |

Parental combination: $\frac{4032 + 4035}{8366} \times 100 = 96.4\%$

Recombination % : $\frac{149 + 152}{8366} \times 100 = 3.6\%$

**Experiment (b)**

Parents : Phenotype: Coloured shrunken x Colourless full

Cs cS

--- x ---

Cs cs

$F_1$ x test cross : Coloured full x Colourless, shrunken

CS cs

--- x ---

cS cs

| Test cross progenies: | Coloured: shrunken | Coloured: full | Colourless: Shrunken | Colourless full |
|---|---|---|---|---|
| | Cs | CS | cs | cS |
| | ---- | --- | --- | --- |

| cs | cs | cs | cs |
|---|---|---|---|
| 21379 | 639 | 672 | 21906 |

$$\text{Parental combination: } \frac{21379 + 21906}{44595} \times 100 = 97.06\%$$

$$\text{Recombination \% : } \frac{639 + 672}{44595} \times 100 = 2.94\%$$

(Note: Note the method of writing symbols where linkage is involved – eg: AB/ab and not AaBb)

**Conclusion**

1. Experiment (a) is the result of coupling phase and experiment (b) is the result of repulsion phase.
2. The recombination % or crosses over value in coupling and repulsion phase are 3.66 and 2.94 respectively. The variation is due to the population size handled.
3. Precision of the experiment depends upon the population size. Higher the population, higher the precision.

**Note:**

For a given $F_2$ data, proportion of gametes formed based on cross over percentage and parental combination can be worked out and product of proportion of gametes will be zygotic combination and all 16 combinations will add to 1.

**Exercise**

1. In a cross between a dihybrid parent (Aa/Bb) and a test cross parent (ab/ab) whose linkage relationships were unknown, the following progenies were obtained.

   42% AaBb

   Parental combination

   42% aabb

8% Aabb

Recombinant genotypes

8% aaBb

Could you comment on the type of inheritance and linkage relationship?

2. In the same experiment with the above parental genotypes, another cross between them yielded the following $F_1$ progenies.

42% Aabb

Parental types

42%aaBB

8% AaBb

Recombinant types

8% aabb

How would you explain the above inheritance? Do you find any difference between the previous experiment and the outcome of this cone? Give reasons.

**LINKAGE ESTIMATION FROM $F_2$ DATA**

The degree or intensity with which two independent genes are linked together is called the linkage value and is always expressed in percentage. The intensity of linkage is measured in inverse sense as the fraction of the total number of chromosome pairs in which the change over takes place at gametogenesis. This is known as recombination fraction. The smaller the fraction, more intense is the linkage.

**Model**

In linseed, the following data were gathered

| | | | | |
|---|---|---|---|---|
| Parents: | Petals | deep lilac | x | lilac |
| | Stigma | deep purple | | White |
| $F_1$ | | lilac petal, white stigma | | |

| $F_2$ petal | Lilac petal | | *Deep lilac petal* | | Total |
|---|---|---|---|---|---|
| | **White AB (a)** | **Deep purple Ab (b)** | **White aB (c)** | **Deep purple ab (d)** | |
| Observed | 357 | 37 | 33 | 94 | 521 |
| Expected on 9 : 3 : 3 : 1 | 293.04 | 97.68 | 97.68 | 32.56 | 501 |
| Deviation | 63.96 | -60.68 | -64.68 | 61.44 | |

**I. Additive method (Emmerson)**

$$p^2 : \frac{E-M}{n}$$

Where P= Linkage value

E = Sum of end classes

M = Sum of middle classes

n = Total population.

1-p = cross over value expressed in %

$$P^2 = \frac{(357+94) - (33+37)}{521} = \frac{381}{521}$$

$P^2 =$ 0.73; P=0.854, 1-p = 1-0.854 = .146 or 14.6%

Cross over value = 14.6. Linkage value 85.4%

**II. Product ratio method (Emmer)**

$$P^2 = \frac{p + 1 - \sqrt{3P + 1}}{P - 1}$$

$$P : \frac{ad}{bc}$$

Substituting the figures,

$$P: \quad \frac{357 \times 94}{37 \times 33} : 27.48$$

$$P^2: \quad \frac{27.48 + 1 - \sqrt{(3 \times 27.48 + 1)}}{27.48 - 1} = 0.728;$$

P = 0.85 and 1-P = 0.15

Cross over value: 15% Linkage value = 85%

**III. Square root method**

In a double heterozygote AB/ab, ½ of the frequency of all non cross over gametes will be equal to the frequency of ab gametes. If cross over % is 20, then there are 80% non crossover gametes (40% AB and 40%ab). The probability of two ab gametes uniting to form the double recessive ab/ab = $(0.4)^2 = 0.16$ or 16%. If cross over % is not known, 16% of double recessive in $F_2$ can e used to calculate the percentage of non cross over gametes =

$$2\sqrt{\frac{\text{frequency of double recessive}}{\text{Total}}} = 2\sqrt{\frac{\text{Double recessive}}{\text{Total progeny}}}$$

$\sqrt{2\ 0.16}$ = 2(0.4) = 0.8 or 80%. If 80% non crossovers, 20% must be cross over types.

**Problems** (Practice with all the three methods)

1. In groundnut, spreading habit is dominant over bunch and branching is dominant over non branching. When spreading branched variety was crossed with a bunch non branching variety. The $F_1$s were spreading and branching. In $F_2$, the following observations were made. Spreading branched: 876, bunch branched; 174, spreading non branched; 192, bunch non branching 160. Calculate the strength of linkage.
2. Two recessive genes in the third linkage group of corn produce crinkly leaves and dwarf plants respectively. When a pure normal plant was pollinated by a pure crinkly dwarf plant,

the $F_2$ progeny consisted of 104 normally; 43 dwarf: 51 crinkly: 2 dwarf crinkly. Calculate the strength of linkage.

3. Coloured kernel is dominant to colourless in corn. Full kernel is dominant to shrunken. A pure coloured, full variety was crossed to a colourless shrunken variety. In the $F_2$, there was 73% coloured full; 2% coloured shrunken: 2% colourless full and 23% colourless shrunken. Calculate the strength of linkage.

## Ex.No:15 Problems on two point test cross, three point test cross –three point test cross and gene mapping

**Three point test cross**

**Linkage relationships from a three point testcross:**

In a test cross involving three linked genes, the parental types are expected to be most frequent and double crossovers to be least frequent. The gene order is determined by manipulating parental combinations in proper order for the production of double cross over types.

Based on recombination frequency, gene map is drawn which is referred as genetic map. Morgan (1911) suggested that the strength of linkage of a pair of genes, (that is, the amount of crossing over *i.e*., recombinations observed) is a function of distance on the chromosome between the genes. Greater the distance, more frequently the cross over occurs. Genes located close to each other are strongly linked.

Sturtevant, a student of Morgan suggested that the double crossovers between the loci are likely to affect the estimates of the distance and proposed a three point test cross.

In a three point test cross, the actual recombination frequency between any two loci is the sum of the single recombination and double recombination frequency.

Note: A single recombinant event results in exchange of alleles at one or the other end of the loci, whereas double recombinant event results in an exchange of alleles at the middle loci.

**Interference (Muller, 1916)**

The effect that increases or decreases the frequency of subsequent recombinations on the same chromosome after one cross over event has occurred is called interference. Interference is expressed in terms or coefficient of coincidence (CC).

$$CC = \frac{\text{No. of double recombinant observed}}{\text{No. of double recombinants expected}} \times 100$$

No. of double recombinations  Product of two single

Expected  =  recombinant frequency

In general CC is low and interference is high with closely linked loci in comparison to more distantly linked loci.

**Model**

In maize $F_1$ plants from the cross between coloured, shrunken, starchy and colourless, full, waxy were back crossed to colourless, shrunken, waxy plants. The following progenies were observed.

| **Progeny** | **Frequency** |
|---|---|
| Coloured, shrunken, starchy | 2538 |
| Colourless, full, waxy | 2708 |
| Coloured, full, waxy | 116 |
| Colourless, shrunken, starchy | 113 |
| Coloured, shrunken, waxy | 601 |
| Colourless, full, starchy | 626 |
| Coloured, full, starchy | 4 |
| Colourless, shrunken, waxy | 2 |
| **Total** | 6708 |

Map the distance between, C, S, W and determine the coefficient of coincidence

**Data**

Parents: Coloured, shrunken, starchy x Colourless, full, waxy

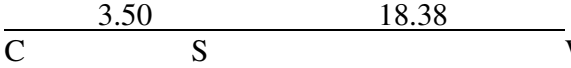
$F_1$ x Colourelss, Shrunken, waxy

Progenies (as in problem)

**Inference**

1. When a triple heterozygote is crossed with a triple recessive parent, the expected segregation ratio of the offspring is 1: 1: 1: 1: 1: 1: 1: 1. The deviation from the expected ratio and occurrence of parental combination *viz*., coloured, shrunken, starchy and colourless, full, waxy appearing more leads to show that linkage is involved between these genes.
2. The data on reclassification based on individual characters for colour, shape and nature, show a 1: 1 ratio which a test cross ratio. Hence, the colour, full, starchy characters are dominant over colourless, shrunken and waxy respectively.
3. The low frequency of recombinants for full and starchy *viz*., 4 and 2 shows that it is a double cross over progeny, the gene for shape being located in between the loci for colour and nature of endosperm.
4. To find the distance between two genes, the recombinants characters are calculated taking two characters at a time.
5. The genetic map will be,

Scale 1 mm: 2 map units




6. The coefficient of coincidence is the ratio of the frequency of observed double cross over to the expected double cross over frequency expressed in % = 13.9%.

**Proof**

**Data reclassified for single characters**

1. Coloured : 2538+116+60+4 = 3257
   Colourless : 2708+113+626+2 = 3449

2. Full : 2708+116+626+4 = 3444
   Shrunken : 2538+113+601+2 = 3254

3. Starchy : 2538+113+626+4 = 3281
   Waxy : 2708+116+601+2 = 3427

**Data classified for two characters**

**i) Colour and Shape**

| | | | | | |
|---|---|---|---|---|---|
| **Parental** | Coloured, | Shrunken | : | 2538+601 | : 3139 |
| **Combinations** | Colourless | Full | : | 2708+626 | : 3334 |

**Recombinations**

| | | | |
|---|---|---|---|
| Coloured, Full | : | 116+4 | : 120 |
| Colourless, Shrunken | : | 113+2 | : 115 |

$$\text{Percentage of recombination} = \frac{115 + 120}{6708} \times 100 = 3.53\%$$

**ii) Shape and nature of endosperm**

**Parental**

| | | | |
|---|---|---|---|
| **Combinations** | Shrunken, Starchy | : 2538+113 | : 2651 |
| | Full, waxy | : 2708+116 | : 2824 |
| **Recombinations** | : Shrunken, Waxy | : 601 + 2 | : 602 |
| | Full, Starchy | : 626 + 4 | : 620 |

$$\text{Percentage of recombination: } \frac{603 + 630}{6708} \times 100 = 18.38\%$$

**iii) Colour and nature of endosperm**

**Parental**

| | | | |
|---|---|---|---|
| **Combinations** | : Coloured, Starchy | : 2538+4 | : 2542 |
| | Colourless, Waxy | : 2708+2 | : 2710 |
| **Recombinations** | : Coloured, Waxy | : 116+ 601 | : 717 |
| | Colourless, Starchy | : 113+626 | : 739 |

$$\text{Percentage of recombination: } \frac{717 + 739}{6708} \times 100 = 21.7\%$$

**From the reclassified data the following linkage relationship are drawn**

**A. Non crossover**

Coloured, Shrunken, Starchy : 2538

Colourless, Full, Waxy : 2708

**B. Single cross over between colour and shape**

Coloured, full : 116

Colourless, Shrunken : 113

**C. Single cross over between shape and nature**

Shrunken, Waxy : 601

Full, Starchy : 626

**D. Double cross over one between colour and shape; one between shape and nature**

Coloured, full, Starchy : 4

Colourless, Sarunken, Waxy : 2

**Corrected cross over percentage**

1. Cross between colour and shape

$$\frac{116 + 113 + 4 + 2}{6708} = 0.0353 \text{ or } 3.53\%$$

2. Cross over between shape and nature

$$\frac{601 + 626 + 4 + 2}{6708} = 0.1838 \text{ or } 18.38\%$$

3. Double cross over between colour and nature

$$\frac{4 + 2}{6708} = 0.00089 = 0.089\%$$

**Coefficient of coincidence**

$$= \frac{\text{Observed double crossover frequency}}{\text{Expected double crossover frequency}} \times 100$$

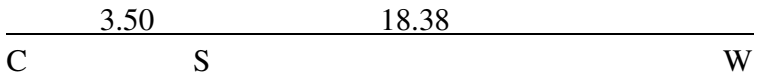
$$= \frac{0.00089}{0.0353 \times 0.1838} = 0.1390 = 13.9\%$$

Distance between C – S: 3.53; Strength of linkage : 3.53%
S – W: 18.38; Strength of linkage : 28.38%

Interference = 1-CC = 1-0.139 = 0.861

**Conclusion**

i) **Genetic map** 1 mm: 2 map unit




ii) Coefficient of coincidence: 13.90%

**Exercise**

1. In a test cross of a trihybrid parent, the following observations were made.

| ***Parents*** | ***trihybrid parent*** | ***x*** | ***test cross parent*** |
|---|---|---|---|
| | Aa, Bb, Cc | | abc/abc |
| | (Linkage unknown) | | |

| $F_1$ | | |
|---|---|---|
| | 36% Aabbcc | 9%aabbCc |
| | 36% aaBbCc | 9% AaBbcc |
| | 72% | 18% |
| | 4% AabbCc | 1%AaBbCc |
| | 4%aaBbcc | 1%aabbcc |
| | 8% | 2% |

With the above data, determine which of the sequence is of the parental genes and which one of them would be in the middle locus?

For the same problem, designating the distance B-A as region I and A-C distance as region II give the single cross ever products in region I and region II.

## Ex.No:16 Gene mapping – combining map segments; genetic variation in human

**Combining map segments**

Segments of map determined form three- point linkage experiments may be combined whenever two of the three genes are held in common.

**Worked example**

Consider three map segments:

1. a 8 b 10 c
   ---

2. c 10 b 22 d
   ---

3. c 30 e 2 d
   ---

Now, to combine these segments into a single map, first superimpose each of these segments by aligning the genes shared in common.

1. a 8 b 10 c
   ---

2. d 22 b 10 c
   ---

3. d 2 e 30 c
   ---

combine the three segments into one map now:

The a to d distance = (d to b) – (a to b)

= 22 - 8 =14

The a to e distance = (a to d) – (d to e)

= 14 - 2 = 12

So the final map would be,

d 2 e 12 a 8 b 10 c

---

Additional segments of map added in this manner can produce a total linkage map over100 map units long. However, the maximum recombination between any two linked genes is 50%. That means, genes very far apart on the same chromosome may behave as though they were on different chromosomes (*ie*., assorting independently).

**Interference and coincidence**

When interference is complete (100%), no double cross overs will be observed and coincidence becomes zero. When we observe all the double cross overs expected, coincidence is unity and interference becomes zero. When interference is 30% operative, coincidence becomes 70% and so on.

**Example:**

Given the map distances of A-B = 10 and B –C = 20 then 0.1 x 0.2 = 0.02 or 2 % double cross overs are expected if there is no interference. Suppose we observe 1.6% double cross overs in a test cross experiment,

Coincidence = 1.6 / 2.0 = 0.8.

This simply means that we observed only 80% of the double cross overs that were expected on the basis of combining independent probabilities (map distances)

Interference = 1.0 – 0.8 = 0.2.

Thus 20% of the expected double cross overs did not form due to interference.

The percentage double cross overs that will be probably be observed can be predicted by multiplying the expected double cross overs by the coefficient of coincidence.

**Example**

Given a segment of a map

a 10 b 20 c

---

With 40% interference we expect 0.1 x 0.2 = 0.02 or 2% double cross overs on the basis of combining independent probabilities. However, we will only observe 60% of those expected because of interference. Therefore, we should observe 0.02 x 0.6 = 0.012 or 1.2% double cross over types.

**Exercise**

1. Two dominant mutants in the first linkage group of the pig govern the traits pollex (px) which is the atavistic return of the thumb and little toe and rough fur. When dihybrid pollex, rough pigs (with identical linkage relationships) were crossed to normal pigs, their progeny fell into four phenotypes as 79 rough,103 normal, 95 rough, pollex and 75 pollex.
   a) Determine the genotype of the parents.
   b) Calculate the amount of recombination between px and R.

## Ex.No:17 Final practical examinations

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