DEPARTMENT OF GENETICS AND PLANT BREEDING

1. Course No. : GPBR 111
2. Course Title : **Principles of Genetics**
3. Credit Hours : 3 (2+1)
4. General Objective : To impart knowledge to the students on the ultrastructure of cell and cell organelles, principles of genetics and their applications in plant breeding for improving agricultural productivity
5. Specific Objectives

Theory

By the end of the course, the students will be able to

i. understand the basic concepts of the ultrastructure of cell, cell organelles, chromosomes and nucleic acids
ii. apply the principles of inheritance to plant breeding
iii. acquaint with the fundamentals of chromosomal and cytoplasmic inheritance, sex determination, mutations and chromosomal aberrations

Theory Lecture Outlines

1. Introduction and definitions of cytology, genetics and cytogenetics – interrelationships among cytology, genetics, plant breeding and also with other branches of science – history – historical developments – cell theory and protoplasm theory
2. Cell – differences between plant cell and animal cell – differences between prokaryotic and eukaryotic cell ; Ultrastructure of cell and cell organelles – cell wall – plasma membrane – cytoplasm – endoplasmic reticulum – ribosomes
3. Ultrastructure of cell and cell organelles – golgi complex – lysosomes – cytoplasmic vacuoles – microbodies – microtubules and microfilaments – centrosomes – basal granules – sphaerosomes – microbodies – cilia and flagella
4. Ultrastructure of cell and cell organelles – plastids – classification of plastids – structure of chloroplast – mitochondria – nucleus – nucleolus, nuclear membrane and nucleoplasm
5. Chromosomes – morphology of chromosomes – shape, size and number of chromosomes – structure of chromosome – composition of chromosome – euchromatin and heterochromatin – karyotype and ideogram

6. Chromosomes – special types of chromosomes – lamp brush chromosomes, salivary gland chromosomes, supernumerary chromosomes, iso-chromosomes and sex chromosomes
7. Deoxyribo Nucleic Acid (DNA) and its structure – Watson and Crick model – functions and types of DNA
8. Modes of DNA replication – semi-conservative DNA replication – experimental proof; Ribo Nucleic Acid (RNA) – structure, function and types – messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA)– differences between DNA and RNA
9. Genetic code – properties of genetic code – central dogma – outline of protein synthesis – transcription and translation
10. Gene expression and differential gene activation– Operon concept – Lac Operon
11. Mitosis – definition – process of mitosis – mitotic cycle – significance in plant breeding
12. Meiosis – definition – process – differences between mitosis and meiosis – significance in plant breeding
13. Arrangement of genes on chromosomes – linkage – definition – linkage groups – coupling phase and repulsion phase – types of linkage – distinction between linkage and pleiotropism
14. Theories of linkage – estimation of linkage – Morgan's work in *Drosophila* – importance of test cross in linkage studies – significance in plant breeding
15. Crossing over – mechanism of crossing over – types of crossing over – factors effecting crossing over – crossing over at four strand stage – cytological proof of crossing over in *Drosophila* – significance of crossing over in plant breeding – coincidence – interference
16. Chromosome mapping – two-point and three-point test cross – cytological maps and genetic maps – importance of linkage and chromosome maps in plant breeding
17. Mendelian genetics – terminology – Mendel's experiments – reasons for selection of pea as experimental material – characters studied – reasons for Mendel's success
18. Mendel's Laws – Law of segregation – Law of independent assortment –Principle of dominance– Principle of unit characters – exceptions to Mendel's Laws
19. Monohybrid and dihybrid ratios – modifications of F_2 ratio in monohybrid and dihybrid crosses and lethal factors

20. Gene action – types of gene action – pleiotropism – alleles – characteristic features of alleles – multiple alleles (blood groups in human beings, fur / coat colour in rabbits and self incompatibility alleles in plants) – characteristic features of multiple alleles – pseudo-alleles – penetrance (complete penetrance and incomplete penetrance) and expressivity (uniform expressivity and variable expressivity)

21. Qualitative and quantitative characters – definition – monogenic and polygenic inheritance and their differences – multiple factor hypothesis

22. Sex determination – various mechanisms of sex determination – genic balance theory of sex determination in *Drosophila melanogaster* – sex linked (colour blindness and hemophilia in human beings) sex influenced (horns in some breeds of sheep and baldness in men) and sex limited characters (plumage of male fowls, milk production in female cattle and appearance of beard in men) – pseudo-hermaphrodites – gynandromorphs

23. Cytoplasmic inheritance – definition – chloroplast inheritance (leaf variegation in *Mirabilis jalapa* and iojap in maize) – mitochondrial inheritance (cytoplasmic male sterility in maize and pokyness in neurospora) – characteristic features of cytoplasmic inheritance – differences between chromosomal and extrachromosomal inheritance

24. Gene mutations – introduction – definition – brief history – terminology – classification of mutations – characteristic features of mutations – spontaneous mutations and induced mutations

25. Gene mutations – artificial induction of mutations – physical and chemical mutagens – molecular basis of mutations – detection of sex linked lethals in *Drosophila* by CLB technique – detection of mutations in plants – importance of mutation in plant breeding programmes – chimeras – xenia and metaxenia

26. Structural chromosomal aberrations – breakage-fusion-bridge cycle – deletions (deficiencies), duplications and their significance in plant breeding

27. Structural chromosomal aberrations – inversions – pericentric inversions and paracentric inversions – inversions as cross over suppressors – translocations – simple and reciprocal translocations – meiotic behaviour – their role in plant breeding

28. Numerical chromosomal aberrations – terminology – classification – euploidy and aneuploidy – kinds of polyploids – autopolyploids, allopolyploids and segmental allopolyploids

29. Numerical chromosomal aberrations – euploidy – monoploids – haploids – differences between monoploids and haploids – diploidy – polyploidy – origin of polyploidy – induction of polyploidy – triploids – tetraploids – cytological behaviour and their significance in plant breeding

30. Numerical chromosomal aberrations – polyploidy and evolution of crop species – wheat, cotton, tobacco, *Triticale*, *Brassica* etc.

31. Numerical chromosomal aberrations – aneuploidy – types of aneuploids – monosomics, double monosomics, nullisomics, double nullisomics, trisomics (primary, secondary and tertiary trisomics) and tetrasomics – their cytological behaviour and significance in plant breeding – effects of polyploidy

32. Genomic approaches in agriculture – definitions of genomics, structural genomics and functional genomics – Human Genome Project – genome size – brief outline

References

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Lecture No.: 1

INTRODUCTION

Cytology (Greek words, *Kytos* = hollow vessel or cell; *logous* = to discourse) or **cell biology** is the biological science which deals with the study of structure, function, molecular organization, growth, reproduction and genetics of the cells.

Genetics is the biological science which deals with the mechanism of heredity and causes of variations in living beings. The word genetics was derived from the Greek root *gen* which means to become or to grow into and it was coined by Bateson in 1906.

Cytogenetics is a branch of genetics that correlates the structure, number and behaviour of chromosomes with heredity and variation.

HISTORICAL DEVELOPMENTS IN CYTOLOGY, GENETICS AND CYTOGENETICS

Year	Scientist	Contribution
1485	L. da Vinci	Recommended the use of lenses for viewing small objects
1590	Z. Janssen and H. Janssen	Produced the first operational microscope.
1665	R. Hooke	Introduced the term " cell " and described cork cells.
1668	F. Redi	Disproved the theory of spontaneous generation of maggots.
1672	Malphigi	Classified the tissues.
1674	A.van Leeuwenhoek	Improved lens system of microscope by grinding.
1682	N. Crew	Described bladders and pores in wood and pith.
1694	J.R. Camerarius	Conducted early experiments on pollination and reported the existence of sex in plants.
1700	R. Linnaeus	Classified the biological organisms.
1761	J.C. Kolreuter	Hybridized various species of tobacco and concluded that each parent contributed equally to the characteristics of the progeny.
1779	C.F Wolff	Founder of embryology.
1809	J.B. Lamarck	Coined the word " biology " and stressed the importance of cell in living organisms. He put forth the theory of inheritance of acquired characters.
1824	Dutrochet	Showed that all plants and animals are composed of cells.
1825	F.V. Raspail	Developed the frozen-section technique and used iodine for detection of starch.
1835	H. von Mohli	Emphasized the importance of protoplasm and described cell division.
1837	R. Brown	Discovered the nucleus in cells of flowering plants.

Year	Scientist	Contribution
1838	M.J. Schleiden and T. Schwann	Formulated the cell theory in plants and animals.
1840	J.E. Purkinj	Gave the term “ protoplasm ”.
1845	A. Donne	Used photomicroscopy for the first time.
1846	K. Nageli	Showed that plant cells arise from the division of pre-existing cells.
1846	G.B. Amici	Showed that egg in the ovary is stimulated to develop into an embryo by the entrance of pollen tube.
1858	R. Virchow	Showed that animal cells arise from the division of pre-existing cells.
1859	C. Darwin	Put forth the theory of natural selection.
1862	Kolliker	Used the term “ cytoplasm ”for the living material surrounding the nucleus.
1865	G. Mendel	Developed the fundamental principles of heredity.
1870	W. His	Invented the microtome.
1871	F. Meischer	Isolated nucleic acids from pus cells.
1873	H. Fol	Described spindle and astral rays.
1875	O. Hertwig	Studied reproduction in sea urchins and concluded that fertilization involves the union of sperm and egg nucleus.
1875	E. Strasburger	Discovered cell division in plants and gave the terms “ cytoplasm ” and “ nucleoplasm ”.
1879	W. Flemming	Introduced the term “ chromatin ”.
1879	H. Fol	Showed that only one sperm enters the egg during fertilization.
1881	E.G. Balbiani	Discovered giant chromosomes in salivary glands of Drosophila.
1882	W. Flemming	Coined the term “ mitosis ” .
1883	W. Rouse	Proposed that chromosomes contain genes which are the units of heredity.

Year	Scientist	Contribution
1885	A.F.W. Schimper	Introduced the term “ plastids ”.
1888	Th. Boveri	Coined the term “ centrosomes ”.
1888	W. Waldeyer	Coined the term “ chromosomes ”.
1892	O. Hertwig	Proposed the protoplasm theory of inheritance.
1892	J. Ruckert	Described lamp brush chromosomes in oocytes of shark.
1892	W. Weisman	Stated that chromosomes are the most important part of the nucleus.
1892	Th. Boveri	Described meiosis in Ascaris.
1898	C. Golgi	Described the golgi apparatus in nerve cells.
1898	C. Benda	Discovered mitochondria in spermatozoa and other cells.
1899	S. Altman	Introduced the term “ nucleic acid ”.
1900	C.E. Correns, H. de Vries and E. Tschermak	Re-discovered Mendel’s laws of inheritance.
1901	E. Strasburger	Introduced the term “ plasmodesmata ”.
1902	C.E. McClung	Identified sex chromosomes in bugs.
1902	H. de Vries	Coined the term “ mutation ”.
1902	W.S. Sutton Th. Boveri	Proposed the chromosome theory of heredity and identified chromosomes as carriers of genetic material.
1903	W. Waldeyer	Proved centromeres are the chromosomal regions with which the spindle fibres become associated during mitosis
1905	L.Cuenot	Discovered lethal genes affecting coat colour in mice.
1905	J.B. Farmer and J.E. Moore	Coined the term “ meiosis ”.
1906	W. Bateson	Coined the term “ Genetics ”and proposed the concept of allele.
1906	W. Bateson and R.C. Punnet	Discovered genetic linkage in sweet pea.

Year	Scientist	Contribution
1906	W.L. Johannsen	Coined the terms “gene”, “genotype” and “phenotype”.
1909	W. Bateson	Coined the term “ epitasis ”.
1909	C. Correns	Reported cytoplasmic inheritance in *Mirabilis jalapa.*
1909	F.A. Janssens	Indicated that chiasmata are produced by exchanges between non-sister chromatids of homologous chromosomes.
1910	T.H. Morgan	Studied crossing over and recombination in Drosophila and coined the term “ crossing over ”.
1910	H. Nilsson-Ehle	Proposed the multiple factor hypothesis.
1911	A.H. Sturtevant	Constructed the first linkage map in Drosophila.
1912	Vejdovsky	Coined the term “ chromonema ”.
1915	T.H. Morgan	Correlated genetic studies with cytological studies. He put forth the theory of linkage and studied sex linked inheritance in *Drosophila melanogaster.*
1917	C.E. Allen	Discovered sex determination in plants.
1921	F.G. Banting C.H. Best	Isolated insulin.
1922	C.B. Bridges	Put forth the genic balance theory of sex determination.
1923	C.B. Bridges	Discovered duplications, deletions and translocations in chromosomes.
1923	Crew	Reported complete reversal of sex in hens.
1924	A.F. Blakeslee and J. Belling	Studied trisomics in Jimson weed *(Datura stromonium*).
1924	R. Feulgen	Described a test to confirm the presence of DNA.
1926	A.H. Sturtevant	Discovered inversions in chromosomes.
1927	G.K. Karpechenko	Synthesized Raphano brassica.
1927	H.J. Muller	Induced mutations in Drosophila melanogaster by X-rays
1928	L.J. Stadler	Induced mutations in maize and barley by X-rays.

Year	Scientist	Contribution
1928	F. Griffith	Conducted experiments on transformations in *Diplococcus pneumonia.*
1931	C. Stern	Gave cytological proof for crossing over in Drosophila.
1931	H. Creighton and B. McClintock	Gave cytological proof for crossing over in maize.
1932	M. Knoll and E. Ruska	Developed the electron microscope.
1933	M. Rhodes	Reported cytoplasmic male sterility in corn.
1935	F. Zernicke	Developed the phase contrast microscope.
1935	R.B. Goldschmidt	Coined the term “ phenocopy ”.
1939	R.A. Steinberg	Induced mutations in Aspergillus sp. with chemicals.
1944	O.T. Avery, C.M. MacLeod and M. McCarty	Explained the significance of DNA and proved that it is the genetic material.
1946	C. Auerbach and J.M. Robson	Induced mutations in *Drosophila melanogaster* using chemicals.
1946	E.S. McFadden, E.R. Sears and H. Kihara	Synthesized *Triticum spelta* in the laboratory.
1948	K.R. Porter	Described the endoplasmic reticulum.
1950	B. McClintock	Discovered jumping genes in maize.
1951	A. Muntzing	Synthesized Triticale.
1952	A.D. Hershey and M.J. Chase	Provided experimental proof of DNA as genetic material.
1953	Robinson and Brown	Observed ribosomes in plant cells.
1953	J.D. Watson, F.H.C. Crick and M.H.F. Wilkins	Proposed the double helix model for DNA molecule.
1954	E.R. Sears	Produced monosomic series of “Chinese Spring ” variety of wheat.
1955	S. Benzer	Described the fine structure of gene–Cistron, Recon and Muton.

Year	Scientist	Contribution
1955	C. DeDuve	Coined the term " lysosomes ".
1955	G.E. Palade	Observed ribosomes in animal cells.
1955	L. Pauling	Studied the relationship between the structure of the DNA molecule and protein synthesis.
1958	G.W. Beadle, E.L. Tatum and J. Lederberg	Put forth the one gene – one enzyme hypothesis.
1958	F.H.C. Crick	Explained the central dogma of molecular biology.
1958	M.S. Meselson and F.W. Stahl	Proved experimentally that DNA replicates by semi-conservative mechanism.
1959	A. Kornberg and S. Ochoa	Synthesized the DNA molecule in vitro.
1961	A.E. Jacob and J. Monod	Explained the genetic regulatory mechanism in protein synthesis – Operon concept.
1968	N.W. Nirenberg , H.G. Khorana and H. Holley	Deciphered the genetic code and polynucleotide synthesis.
1968	Woodcock and Fernandez	Isolated DNA from chloroplasts.
1974	Clande, G.E. Palade and C. DeDuve	Re-discovered a number of cell organelles by electron microscope.
1975	R. Dulbecco, H. Temin and D. Baltimore	Discovered the mechanism of reverse transcription – Teminism.
1975	N. Borlaug	Responsible for development of dwarf wheat and green revolution.
1978	D. Nathans , H.O. Smith and W. Arber	Isolated restriction enzymes.
1985	Potrykus	Used electroporation technique for direct gene transfer in plants.
1986	Helentzaris	Developed the RFLP map in maize and tomato.

Year	Scientist	Contribution
1986	Ow	Transferred and studied the expression of gene for enzyme lucifersase (causes fire flies to glow) in tobacco cells.
1987	Fischoff	Developed insect resistant transgenic tomato plants with Bt gene.
1987	K.B. Mullis	Developed polymerase chain reaction technique.
1988	Ouozzo	Developed transgenic tobacco with CMV coat protein.
1991	Oeller	Developed transgenic tomato with an antisense gene.
1992	Vasil	Developed herbicide resistant transgenic wheat.
1993	Sharp Roberts	Proposed the split gene concept.
1993	Smith	Studied site directed mutagenesis.
1994	Gilman and Rodbell	Studied G proteins and their role in turning external signals into action within cells.
1995	Lewis, Volard and Wieschaus	Studied the role of genes in organ differentiation.
1997	I. Wilmut	Cloned sheep – Dolly.
1997	Prusiner	Studied prions – Mad cow disease.
1998	Delta & Pine Co.	Developed the terminator gene technology.
1998	Monsanto Co.	Developed bollguard variety of cotton.
1998	T. Wakayama and R. Yanagimachi	Created the first cloned mice.
2000	Roslin Institute	Created the first cloned pigs.
2001	Advanced Cell Technology	Birth of first cloned Asian ox called “Gaur”.
2002	Natl. Institute of Agronomic Research, France.	Created the first cloned rabbit
2002	Texas A & M Univ. U.S.A.	Created the first cloned cat called “Cc”.

Lecture No.: 2, 3 & 4

CELL

Cell is the basic unit of organization or structure of all living matter. It was first discovered by Robert Hook in 1665 in cork tissue. Loewy and Sickevitz in 1963, defined a cell as “a unit of biological activity de-limited by a semi-permeable membrane and capable of self-reproduction in a medium free of other living system”. The cell has also been defined as “a unit of life that is the smallest unit which can carry on the activities indispensable to life to grow, to synthesize new living material and to produce new cells”.

The cell theory or cell doctrine was formulated by independently by M.J. Schleiden and Theodor Schwann in 1838-39. It states that the animals and plants differ from each other superficially. But they have same pattern of organization and construction. It further states that the bodies of both animals and plants are composed of cells and that each cell can not only act independently but also function as an integral part of complete organism. Thus the cell is considered as morphological and physiological unit of living organisms or in words of Schwann and Schleiden as “functional and biological unit”. Cells reproduce, assimilate, respire, respond to changes in the environment and absorb water and other materials from internal or external courses.

Purkinje in 1840 coined the term protoplasm. (Greek words, protos = first; plasm = organization) for the juicy living substance of animals. The protoplasm theory states that all living matter, out of which animals and plants are formed is protoplasm. Further, the cell is an accumulation of living substance or protoplasm, which is limited in space by another membrane and possesses a nucleus. The protoplasm occurs everywhere in the cell i.e. the plasma membrane, the nucleus and the portion in between the plasma membrane and the nucleus. The portion of the protoplasm which occurs between the plasma membrane and the nucleus is named as cytoplasm and the portion of the protoplasm occurring in the nucleus is named as nucleoplasm.

Classification of cells or cellular organisms

The body of all living organisms (blue green algae, bacteria, plants and animals) except viruses and certain plants such as Rhizopus, Vaucheria, etc. has cellular organization and may contain one or many cells. The organisms with only one cell in their body are called unicellular organisms (Eg : Blue green algae, bacteria, protozoa etc.). The organisms having many cells in their body are called multicullular organisms (Eg : Most plants and animals). The cellular organisms may have only one kind of cell from the following two major types of cells. (a) Prokaryotic cells and (b) Eukaryotic cells. The major differences between prokaryotic and eukaryotic cells are mentioned below:

DIFFERENCES BETWEEN PROKARYOTIC AND EUKARYOTIC CELLS

PROKARYOTIC CELL	EUKARYOTIC CELL
1. Prokaryotes are primitive organisms (Pro = primitive; Karyon = nucleus)	1. Eukaryotes are higher organisms (Eu = good or true; Karyon = nucleus)
2. They are generally uni-cellular	2. They are generally multi-cellular
3. The average diameter of prokaryotic cell ranges from 5 - 10µm	3. The average diameter of eukaryotic cell ranges from 10–100 µm
4. Posses only one envelope system	4. Posses two envelope system
5. Don't posses well defined cytoplasmic organelles	5. Posses well defined cytoplasmic organelles like endosplasmic reticulum., golgi bodies, chloroplast, mitochondria
6. They lack nucleus and chromosomes	6. Posses well developed nucleus and chromosomes
7. DNA is circular and lies free in the cytoplasm	7. DNA is linear and lies within the nucleus
8. Cell division is by amitosis (binary fission)	8. Cell division is by mitosis and meiosis
9. Posses ribosomes of 70 S type	9. Posses ribosomes of 80 S type
10. Nucleolus is absent	10. Nucleolus is present
11. Spindle fibres are absent	11. Spindle fibres are present
12. Cell wall is made up of polysaccharides Eg: Muramic acid	12. Cell wall is made up of cellulose, hemicellulose and pectins
13. Histone proteins are absent	13. Histone proteins are present
14. Pigments are distributed throughout the cytoplasm	14. Pigments are present in plastids
15. Nuclear membrane is absent	15. Nuclear membrane is present
16. Mesosomes support respiration	16. Mitochondria support respiration
17. Eg: Bacteria, blue green algae, *E. coli,* PPLOs (Pleuropheumonia like organisms)	17. Eg: Plant and animal cells

Shape: The plant and animal cells exhibit various forms and shapes. But the shape of the cell may be irregular, triangular, tubular, cuboidal, polygonal, cylindrical, oval, rounded or elongated in different animals and vary from organ to organ. Even the cells of the same organ may display variations in the shape. Generally, the shape of the cell remain correlated with its functions. For example, the epithelial cells have flat shape and the muscle cells are elongated. Moreover, external or internal environment may also cause shape variations in the cell due to internal or mechanical stress or pressure, surface tension etc.

Size: Mostly the eukaryotic cells are microscopic in size, but definitely they are larger in size than the bacterial cells. The size of cells varies from 1µ to 175 mm. The ostrich egg cell is usually considered as largest cell (with 175 mm diameter). But certain longest nurve cells have been found to have a length of 3 to 3.5 feet.

Number: The body of unicellular or acellular organisms (Protozoa and Protophyta) consists of single cell. Most of the animals and plants are multicellular and may have many cells. The number of cells in the multicellular organisms usually remains correlated with the size of the organisms. Small-sized organisms have less number of cells in comparison to large-sized organisms. The major differences between plant cell and animal cell are:

PLANT CELL	ANIMAL CELL
1. Plant cell has a rigid wall on the out side	1. The cell wall is absent
2. Usually larger in size	2. Comparatively smaller in size
3. Can not change its shape	3. Can often change its shape
4. Plastids are found	4. Plastids are usually absent. Chromatophores are present
5. Posses chlorophyll containing plastids called chloroplasts	5. Chloroplasts are absent
6. A mature plant cell contains a large central vacuole	6. Vacuoles are numerous and very small
7. Nucleus lies on one side in the peripheral cytoplasm	7. Nucleus usually lies in the centre
8. Mitochondria are comparatively fewer	8. Mitochondria are generally more numerous
9. Cristae are tubular in plant mitochondria	9. Cristae are plate like in animal mitochondria
10. Plant cells do not burst if placed in hypotonic solution due to presence of cell wall	10. Animal cells burst if placed in hypotonic solution unless and until it posses contractile vacuole
11. Centrioles are usually absent in lower plants	11. Centrioles are found in animal cell
12. Spindle fibres formed during nuclear division are anastral	12. Spindle fibres formed during nuclear division are amphiastral
13. Golgi apparatus consists of a number of distinct / unconnected units called dictyosomes	13. Golgi apparatus is either localized or consists of a well connected single complex
14. Cytoskeleton does not contain intermediate fibres	14. Cytoskeleton contains intermediate fibres
15. Lysosomes are rare and their activity is performed by specialized vacuoles	15. Typical lysosomes occur in animal cell
16. Glyoxysomes may be present	16. Glyoxysomes are absent
17. Crystals of inorganic substances may occur inside the cells	17. Crystals usually do not occur in animal cells
18. Reserve food is generally starch and fat	18. Reserve food is usually glycogen and fat
19. A tissue fluid does not bathe the individual cells	19. A tissue fluid contain in a NaCl bathes the cells
20. Adjacent cells may be connected through plasmadesmata	20. Adjacent cells are connected through a number of junctions

Cell wall

In plants (including bacteria) a cell is always surrounded by a cell wall lined throughout with plasma lemma. The cell wall is found in plants and is absent in animals. In case of animal cells, the outermost layer of cell is plasma lemma, which is also occasionally called 'cell membrane' or 'plasma membrane'.

Cell wall is the outermost part of the cell and is always non-living, though produced and maintained by living protoplasm. It is a rigid structure and protects the inner parts of a cell. It maintains the shape of the cell and provides mechanical support to the tissues. It originates from the phragmoplast (phragma = fence, separation). Endoplasmic reticulum, golgi complex, mitochondria and microtubules play an important role in the formation of the cell wall. It is mainly composed of cellulose. However it may also contain hemicellulose, pectin, chitin, cutin and lignins. The composition of these substances varies from cell to cell.

The cell wall is complex in nature and is differentiated into middle lamella, primary cell wall and secondary cell wall.

1. **Middle lamella:** It is the outmost layer of plant cell wall and connects the two adjacent cells. It is composed of calcium and magnesium pectate and does not contain any cellulose. Some consider middle lamella as intercellular substance or intercellular matrix.
2. **Primary cell wall:** It is thin, elastic and lies between middle lamella and secondary cell wall. It is mainly composed of cellulose. It develops after middle lamella by deposition of hemicellulose, cellulose and pectin substances.
3. **Secondary cell wall:** It is the inner most layer of cell wall and lies between primary cell wall and plasma membrane. It is relatively thick and is primarily composed of microfibrils of cellulose. In some tissues, besides cellulose, lignin and suberin are also found in the secondary cell wall.

The cell wall has minute apertures through which the cells of a tissue are interconnected. These apertures of cell wall are known as plasmadesmata. They are also referred to as canals of the cell wall.

The main functions of cell wall are

1. It determines the shape and size of a cell
2. It provides protection to the inner parts of a cell from the attack by pathogens.
3. It provides mechanical support to the tissues and act as a skeletal framework of plants.
4. It helps in transport of substances between two cells.

Plasma lemma or plasma membrane

The term was coined by J.Q. Plower in 1931. This membrane is present just beneath the cell wall in plant cells, while it is the outer membrane in animal cell. In plants, it lies between the cytoplasm and the cell wall. It is a living, ultra thin, elastic, porous, semi-permeable membrane covering of cell. The plasma membrane is about 75-100 angstroms thick. In most of the cells, it is trilaminar (three layered) and made up of protein and lipids. The outer protein layer is 25 angstroms thick, the middle lipid layer is 25 to 30 angstroms thick and the inner protein layer is 25 to 30 angstroms thick. The three-layered protein-lipid-protein membrane is called a unit membrane. The outer and inner layers are made up of proteins and the middle layer is made up of lipids. Structure can be best explained by fluid mosaic theory. It is found to contain many pores through which exchange of molecules may occur.

The main functions of plasma membrane are

1. Primarily the plasma membrane provides mechanical support and external form to the protoplasm (cytoplasm and nucleus) and it also delimits the protoplasm from the exterior.
2. It checks the entry and exit of undesirable substances.
3. Due to its semipermeability, it transmits necessary materials to and from the cell (selective permeability).
4. Moreover, it permits only one way passage for molecules like minerals into the cell and restricts their outward movement.

Cytoplasm

The plasma membrane is followed by cytoplasm which is distinguished into (a) Cytoplasmic matrix / hyaloplasm and (b) Cytoplasmic structures

a) **Cytoplasmic matrix:** The space between the plasma membrane and the nucleus is filled by amorphous, translucent, homogeneous colloidal liquid known as hyaloplasm or cytoplasmic matrix. The portion of cytoplasm other than cell organelles is known as hyaloplasm. When the cell is active, the cytoplasm is in fluid state. The cytoplasm is in gel condition, when the cell is dormant. The cytoplasmic matrix consists of various inorganic molecules such as water, salts of sodium and other metals and various organic compounds *viz*., carbohybrates, lipids, nucleoproteins, nucleic acids (RNA and DNA) and variety of enzymes. The peripheral layer of cytoplasmic matrix is relatively nongranular, viscous, clear and rigid and is known as ectoplasm. The inner portion of cytoplasmic matrix is granular, less viscous and is known as endoplasm.

b) **Cytoplasmic structures:** In the cytoplasmic matrix certain non-living and living structures remain suspended. The living structures or cytoplasmic organoids are membrane bound and are called organelles or organoids. These living structures include plastids, mitochondria, endoplasmic reticulum, golgi complex, lysosomes, ribosomes, microtubules, microfilaments, centrosome, basal granules, sphaerosomes, microbodies, cilia and flagella etc. The non living structures or cytoplasmic inclusions called paraplasm or deutoplasm include ergastic substances, crystals, fats, oil droplets, starch granules glycogen granules, vacuole etc.

Nucleus

Robert Brown first observed a cell nucleus in flowering plants in 1837. Generally a cell contains single nucleus. However there are a number of exceptions in which more than one nucleus is present. Plant cells with more than one nucleus are called coenocytes. Eg: Certain algae, fungi, Vaucharia, Rhizopus, where as animal cells with this character are called syncytia. Eg: striated muscle cells of higher animals.

The position of the nucleus in the cell varies according to cell type, although it is often in the centre of the cell. The nucleus is surrounded on all sides by cytoplasm from which it is separated by the nuclear envelope or nuclear membrane.

Morphology: The shape of the nucleus varies according to the species or cell type. The range of variation is limited, although in addition to the common spherical nuclei, ellipsoid or flattened nuclei occur. In majority of cells, the margin of the nucleus is quite regular, but some cells like leukocytes contain nuclei with lobes or infoldings of the margins.

Nuclear size is a function of chromosome number. Size of the nucleus varies with ploidy level. The size of the nucleus is also correlated with the DNA content. Variation in the nuclear size is observed at different times during the cycle of cellular activities.

The nucleus includes (a) Nuclear envelop / membrane, (b) Nucleoplasm or karyoplasms, (c) Nucleolus and (d) Chromatin

1. **Nuclear envelop / nuclear membrane :** It is a double membrane, semipermeable structure broken at numerous intervals by pores or openings. Under light microscope, it appears as a thin line between nucleus and cytoplasm. The space between the inner and outer membrane is known as the perinuclear space. In many places the nuclear membrane joins the membrane of endoplasmic reticulum. The main function of nuclear membrane is to provide a pathway for the transport of materials between the nucleus and cytoplasm
2. **Nucleoplasm / Karyolymph:** It is a fluid substance which escapes, if the nucleus is punctured. It fills the nuclear space around the chromosomes and the nucleolus. The karyolymph is composed primarily of protein materials and is rich

in acidic proteins and RNA rich in bases, adenine and uracil. It is the site of certain enzymes in the nucleus.

3. **Nucleolus:** Fontana first described the nucleolus in 1871. it is a relatively large, generally spherical body present within the nucleus. The number of nucleoli present in each nucleus depends upon the species and the number of the chromosomes or sets of chromosomes. In many plant and animal cells there is one nucleolus for each haploid set of chromosomes.

   Heterochromatic portions of specific chromosomes are found to be in contact with the nucleolus during interphase. These are called nucleolar organizing regions of the chromosomes and are responsible for producing much of nucleolar RNA. Generally, the nucleolus disappears during cell division and reappears in daughter cells at the end of cell division in each daughter nucleus. However, a persistent nucleolus is found to present in Spirogyra and Euglena. The important functions of nucleolus are formation of ribosomes and synthesis of RNA.

4. **Chromatin:** The nucleus contain a darkly stained material called chromatin (Greek word, chromatin = colour), which is a combination of DNA, histone and other proteins that make up chromosomes. During interphase, the chromatin material is organized into a number of long, loosely coiled, irregular strands or threads called chromatin reticulum. When the cell begins to divide, the chromatin bodies condense to form shorter and thicker threads, which were termed chromosomes (Greek word, soma = body) by W. Waldeyer.

   The main functions of chromatin are

   - to package DNA into a smaller volume to fit in the cell,
   - to strengthen the DNA to allow mitosis and meiosis and
   - to control gene expression and DNA replication.

Plastids

Plastids are the cytoplasmic organelles of the cells of plants and some protozoans such as Euglena. Where as the cells of the bacteria, fungi and animals contain chromatophores instead of plastids. Plastids perform most important biological activities such as the synthesis of food and storage of carbohydrates, lipids and proteins.

The term plastid is derived from the greek word “plastikas” means formed or moulded and was used by A.P.W. Schimper in 1885. He classified the plastids into the following types based on their structure, pigments and function.

1. Chromoplasts (coloured) 2. Leucoplasts (colourless)

1. **Chromoplasts:** (Greek words, chroma = colour; plast = living) These are the coloured plastids of plant cells. They contain a variety of pigments and synthesize the food

through photosynthesis. Based on the type of pigment present in them, the chromoplasts of microorganisms and plant cells are as follows :

a) Chloroplasts: (Greek words, chlor = green; plast = living) These are most widely occurring chromoplasts of the plants. They occur mostly in the green algae and higher plants. The chloroplasts contain the pigments chlorophyll A and chlorophyll B. They also contain DNA and RNA.

b) Phaeoplasts: (Greek words, phaeo = dark brown; plast = living) These contain the pigment "Fucoxanthin", which absorbs the light. They occur in the diatoms, dinoflagellates and brown algae.

c) Rhodoplast: (Greek words, rhodo = red; plast = living) The rhodoplast contains the pigment phycoerythrin which absorbs light. The rhodoplast occur in red algae.

2. **Leucoplasts:** (Greek words, leuco = white; plast = living) These are the colorless plastids which store the food material such as carbohydrates, lipids and proteins. The leucoplasts are rod like or spheroid in shape and occur in the embryonic cells, sex cells and meristematic cells. The most common leucoplasts of the plants cells are as follows:

   a) Amyloplasts: (Greek word, amyl = starch) These synthesize and store starch and occur in those cells which store starch.

   b) Elaioplasts: These store lipids and occur in seeds of monocotyledons and dicotyledons.

   c) Proteinoplasts or proteoplasts: These are the protein storing plastids which mostly occur in seed and contain few thylakoids.

Chloroplasts: These are the most common plastids of many plant cells and perform the function of photosynthesis.

Distribution: The chloroplasts remain distributed homogeneously by in the cytoplasm of plant cells. But in certain cells, the chloroplasts become concentrated around the nucleus or just beneath the plasma membrane.

Shape: Higher plant chloroplasts are generally biconvex or plano-convex. However in different plant cells, chloroplasts may have various shapes *viz*., filamentous, saucer shape, spheroid, ovoid, discoid or club-shaped. They are vesicular and have a colourless centre.

Size: Generally 2-3 μ in thickness and 5-10 μ in diameter. Polyploid plant cells have larger chloroplasts than diploid plant cells.

Number: The number of chloroplasts varies from cell to cell and from species to species and is related with the physiological state of the cell. But it usually remains constant for a

particular plant cell. The algae usually have a single huge chloroplast. The cells of higher plants have 20 to 40 chloroplasts.

Ultra structure: Chloroplasts are bound by two unit membranes. Each membrane is trilaminar, lipoproteinaceous. Both unit membranes are separated from each other by a distinct space known as periplastidial space. The inner contents of the chloroplasts are heterogeneous and composed of (a) matrix or stroma and (b) grana

(a) Matrix or stroma: The inner periplastidial space of the chloroplasts is filled with a watery, proteinaceous and transparent substance known as the matrix or stroma. The dark reaction of photosynthesis occurs in the matrix or stroma of chloroplasts and the stroma contains the multienzyme complex for the dark reactions. The grana and intergrana connecting membrane remain embedded in the matrix of chloroplasts.

(b) Grana: Chloroplasts consists of many lamellar or membranous, granular and chlorophyll bearing bodies known as the grana, where the light reaction of photosynthesis takes place. The size of the grana may range from 0.3 to 2.7 µ. The chloroplasts may contain 40 to 60 grana in their matrix. Each granum of the chloroplast of a higher plant cell is composed of 10-100 disc like super imposed, membranous compartments known as thylakoids. In a granum, these thylakoids are arranged parallely to form a stack. Each thylakoid is separated from the stroma by its unit membrane. Within the thylakoid membrane, 4 sub units appear to be arranged as a functional entity called quantasomes. Mechanism of energy transfer known as photophosphorylation occurs within quantasomes. Grana are interconnected by network of membranous tubules called stroma lamella or Fret's channels.

The chloroplasts contain the ribosomes which are smaller than the cytoplasmic ribosomes. The ribosomes of the chloroplasts are 70s type and resemble the bacterial ribosomes. Woodcodk and Fernandez (1968) isolated segments of the DNA molecules from the chloroplasts. DNA of chloroplasts is present in stroma and plays an important role in cytoplasmic inheritance. The DNA of chloroplasts differs from the nuclear DNA in many aspects and it resembles closely the bacterial DNA.

The most important and fundamental function of chloroplasts is the photosynthesis.

Mitochondria: (Greek words, mitos = thread; chondrion = granule) These are first observed by Kolliker in 1880 who named them as granules. In 1882, Flemming named them as files, while in 1898, C. Benda gave the name mitochondria. In the cytoplasm of most cells occur many large-sized, round or rod-like structures called mitochondria. The mitochondria occur singly or in groups and their shape and size vary from cell to cell. They are filamentous in shape and bound by two membranes composed of lipids and proteins. Each membrane trilamellar in nature with two protein layers sandwiching a

bimolecular layer of lipid. The outer membrane forms a bag like structure around the inner membrane, which gives out many finger like folds into the lumen of the mitochondria. These folds are known as cristae. The space between the outer and inner mitochondrial membrane as well as the central space is filled up by a viscous mitochondrial matrix. The matrix, outer and inner membranes are found to contain many oxidative enzymes and co-enzymes.

Functions :

- Mitochondria are the sites of cell respiration
- Oxidation of carbohydrates, lipids and proteins occurs in mitochondria.
- Dehydrogenation
- Oxidative phosphorylation
- The mitochondria also contain some amount of DNA within the mitochondrial matrix and are thus associated with cytoplasmic inheritance.
- Mitochondria contain ribosomes and are capable of synthesis of certain proteins
- Oxidative decarboxylation and kreb's cycle takes place in the matrix of mitochondria, while respiratory chain and oxidative phosphorylation occurs in cristae of mitochondria.

Since the major function of mitochondria is energy metabolism, during which ATP is synthesized, the mitochondria are also called power houses of the cell.

Endoplasmic Reticulum (ER): The endoplasmic reticulum was first observed by Porter in 1945 in liver cells of rats. Cytoplasmic matrix is transversed by vast reticulum or network of interconnecting tubules and vesicles known as endoplasmic reticulum. The endoplasmic reticulum is having a single vast interconnecting cavity which remains bound by a single unit membrane. The membrane of endoplasmic reticulum is supposed to have originated by impushing of plasma membrane in the matrix because it has an outer and inner layer of protein molecules sandwiching the middle layer of lipid molecules similar to plasma membrane.

The membrane of endoplasmic reticulum may be either smooth when they do not have attached ribosomes or rough when they have ribosomes attached with it. Rough endoplasmic reticulum is present abundantly in pancreatic cells. One of the important functions of smooth endoplasmic reticulum is the synthesis of lipids and glycogen. Rough endoplasmic reticulum is associated with the synthesis of proteins. The membrane of endoplasmic reticulum is found to be continuous with the nuclear membrane and plasma membrane.

The three principle forms of endoplasmic reticulum are :

1. **Cisternae :** Long, flattened, sac like, unbranched tubules arranged paralelly in bundles 40-50 mμ in diameter.
2. **Vesicles :** Oval, membrane bound, vacular structures, 25-230 mμ in diameter.
3. **Tubules :** Branched structures forming reticulum system along with cisternae and vesicles 50-190 mμ in diameter.

All three forms of endoplasmic reticulum are bound by a 50 A^0 thick single unit membrane of lipoproteinaceous nature.

Functions:

1. The endoplasmic reticulum forms the ultra structural skeletal frame work of cytoplasmic matrix and it provides mechanical support to it.
2. It also acts as an intracellular circulatory system and it circulates various substances into and out of the cell by the membrane flow mechanisms.
3. The endoplasmic reticulum acts as a storage and synthetic organ. For example: It synthesizes lipids, glycogen, cholesterol, glyserides, hormones etc.
4. It acts as a source of nuclear membrane's material during cell division.
5. It protects cell from toxic effects by de-toxification.
6. In certain cases, it transmits impulses intracellularly. In such cases it is known as sarcoplasmic reticulum.

Endoplasmic reticulum which is of specialized nature and present in muscle cells is known as sarcoplasmic reticulum.

Golgi complex: It occurs in all cells except prokaryotic cells. In plant cells, they are called dictyosomes, which secrete necessary material for the formation of new cell wall during cell division. First reported by C. Golgi in 1898. It is a polymorphic structure having cisternae, vesicles and vacuoles. It is disc shaped and consists of central flattened plate-like compartments / cisternae with a peripheral network of interconnecting tubules and peripherally occurring vesicles and golgian vacuoles. The membranes of golgi complex are lipoproteinaceous and originate from membranes of endoplasmic reticulum.

Functions :

1. Storage of proteins and enzymes which are secreted by ribosomes and transported by endoplasmic reticulum.
2. Secretory in function
3. The dictyosomes secretes necessary material for cell wall formation during cell division.
4. It has a role in the formation of plasma membrane.

5. It activates mitochondria to produce ATP, which is later utilized in respiratory cycle.

Lysosomes: The cytoplasm of animal cells contain many speroid or irregular shaped membrane bound vesicles known as lysosomes. The lysosomes originate from golgi complex and contain many digestive enzymes. Their function is the digestion of food material which comes into the cell by pinocytosis and phagocytosis. The lysosomes of plant cells are membrane bound storage organs containing hydrolytic digestive enzymes and are comprised of sphaerosomes, aleuron grains and vacuoles. Lysosomes are useful in the process of fertilization. They are also useful in autodissolution of cells.

Ribosomes: Robinson and Brown in 1953 first observed ribosomes in plant cells, while Palade in 1955 first observed them in animal cells. They are small, dense, round and granular particles occurring either freely in mitochondrial matrix, cytoplasm, chloroplasts or remain attached to membrane of endoplasmic reticulum forming the rough endoplasmic reticulum. They occur in all prokaryotic and eukaryotic cells and are hence called "universal components of all biological organisms". They originate in the nucleus and consist of mainly RNA and proteins. Each ribosome is composed of two structural sub units *viz*., larger sub unit and smaller sub unit. The ribosomes are 70 S type in prokaryotes containing 50 S and 30 S subunits, while in eukryotes, they are 80 S type consisting of 60 S and 40 S subunits. The ribosome remains attached with the membranes of endoplasmic reticulum by larger subunit. The smaller subunit of ribosome is placed onto the larger subunit like a cap on the head. The ribosomes are essential for protein synthesis.

Micro tubules: The cytoplasm of plant and animal cells is transversed by numerous ultrafine tubules composed of tubulin protein and are called microtubules. The main function of microtubules is transportation of water, cytoplasmic streaming, formation of fibres or asters of the mitotic or meotic spindle during cell division. They form the structural units of centrioles, basal granules, cilia and flagella. They determine the shape of the cell.

Microfilaments (or Micro fibrils): The cytoplasm of most animal cells also contain many ultrafine, proteinaceous, solid microfilaments which maintain the structure of cell and form contractile components of muscle cells.

Centrosome : The centrosomes contain dense cytoplasm and is located near the nucleus of animal cells. During the cell division, the centrosome is found to contain two rod shaped granules known as centrioles. Each centriole consists of nine microfibrillar units and each microfibrillary unit is found to contain three microtubules. During cell division, microtubules help in the sepa ration and movement of chromosomes.

Basal granules: The animal or plant cells which are having locomotary organelles such as cilia or flagella contain spherical bodies known as basal granules at the base of the cilia and are composed of nine fibrils. Each fibril consists of three microtubules, out of which two enter into the cilia or flagella. The basal granules may contain both DNA and RNA.

Sphacrosomes: These are organelles having a single membrane and a matrix which contains triglycerides. These are abundant in cell sin which lipids are stored and contain the hydrolytic enzyme, lipase, which probably has a role in mobilization of stored lipids when required in cell metabolism.

Microbodies: The cytoplasmic matrix of many kinds of cells *viz*., yeast, protozoa, higher plant cells, hepatocytes (liver cells) and kidney cells contain certain rough spherical membrane bound particles. They have a central granular crystalloid core containing some enzymes and occur in intimate relation with endoplasmic reticulum, mitochondria and chloroplasts.

The main functions of microbodies are:

1. Utilization of molecular oxygen
2. They contain enzymes for hydrogen peroxide metabolism, purine metabolism, gluconogenesis (conversion of fat into carbohydrates) and photorespiration.

Cilia and flagella: These are the cytoplasmic projections which are hair like and present on the outer surface of the cells. They help in locomotion of the cells. The cilia and flagella consists of nine outer fibrils around two large central fibrils. Each outer fibril consists of two microtubules. The cilia and flagella are originated from the basal granules and chemically consist of tubulin and dynein proteins and ATP (Adenosine Tri-Phosphate).

Cytoplasmic vacuoles: The cytoplasm of many plant cells and some animal cells contain numerous small or large sized, hollow liquid filled structures known as vacuoles. The vacuoles of plant cells are bound by a single semi-permeable membrane known as

tonoplast. These vacuoles contain water, phenols, anthocyanins, alkaloids and storage products such as sugars and proteins.

The cytoplasm without mitochondria and chloroplasts is known as cytosol.

Lecture No.: 5

CHROMOSOMES

E. Strasburger in 1875 first discovered thread-like structures which appeared during cell division. These thread like structures were called chromosomes due to their affinity for basic dyes. The term chromosome is derived from two Greek words; chrom = colour, soma=body. This term was first used by Waldeyer in 1888. Of all components of cell, the chromosomes have been studied most extensively and perhaps more is known about them than any other cell organelle. The chromosome has greater constancy than any other cell component and it maintains it special qualities from one cell generation to another. Chromosomes contributed to the division of cells and they are of prime importance as they carry the genes which are the hereditary material.

Chromosome number: The number of chromosomes in a given species is generally constant. All the members of the species ordinarily have definite and generally a constant somatic and gametic chromosome number. Somatic chromosome number is the number of chromosomes found in somatic cells of a species and is represented by 2n. Generally somatic cells contain two copies of each chromosome except the sex chromosomes. Both the copies are ordinarily identical in morphology, gene content and gene order and hence known as homologous chromosomes. Gametic chromosome number is exactly half of somatic chromosome number and is represented by n. it denotes the number of chromosomes found in gametes of a species. The number of chromosomes varies greatly from 2n = 4 (n = 2) in *Haplopappus gracilis* (Compositae) to 2n = > 1200 in some pteridophytes.

Name of the organism	Chromosome number (2n)
Rice	24
Wheat	42
Maize	20
Upland cotton	52
Human beings	46
Drosophila	8

Name of the organism	Chromosome number (2n)
Tomato	24
Onion	16
Garden pea	14
Evening primrose (*Oenothera*)	14

Size : The size of the chromosome shows a remarkable variation depending upon the stage of cell division. The chromosomes are the longest and thinnest during interphase (resting stage) and hence not visible under light microscope. Chromosomes are the smallest and thickest during mitotic metaphase.

In general, plants have longer chromosomes than animals and species having lower chromosome number have longer chromosomes than those having a higher chromosome number. Among plants, dicots in general have shorter and higher number of chromosomes than monocots.

Among the higher plants, the longest mitotic chromosomes are those of *Trillium* sps., which may reach 32 µ in size. In most fungi all chromosomes are extremely minute. Chromosome size is not proportional to the number of genes present on the chromosome.

Morphology: The outer covering or sheath of a chromosome is known as pellicle, which encloses the matrix. Within the matrix lies the chromatin. Flemming introduced the term chromatin in 1879. The term chromatin refers to the Feulgen positive materials observed in interphase nucleus and later during nuclear division. Chromatin readily stains with basic dyes especially Basic Fuchsin, which is specific for DNA which in turn is a major constituent of chromosomes.

The chromosome morphology changes during cell division and mitotic metaphase is the most suitable stage for studies on chromosome morphology. In mitotic metaphase chromosomes, the following structural features can be seen under the light microscope.

1. **Chromatid:** Each metaphase chromosome appears to be longitudinally divided into two identical parts each of which is called chromatid. Both the chromatids of a chromosome appear to be joined together at a point known as centromere. The two chromatids of chromosome separate from each other during mitotic anaphase (and during anaphase II of meiosis) and move towards opposite poles.

   Since the two chromatids making up a chromosome are produced through replication of a single chromatid during synthesis (S) phase of interphase, they are referred to as sister chromatids. In contrast, the chromatids of homologous chromosomes are known as non-sister chromatids.

2. **Centromere:** Centromere and telomere are the most stable parts of chromosomes. The region where two sister chromatids appear to be joined during mitotic metaphase is known as centromere. It generally appears as constriction and hence called primary constriction. Centromere is a localized and easily detectable morphological region of the chromosomes which helps in the movement of the chromosomes to opposite poles during anaphase of cell division. The centromere divides the chromosomes into two transverse parts called arms. The centromere consists of two disk shaped bodies called kinetochores. The kinetochores do not form part of the chromatid but lie one on each side of the chromosome such that each chromatid is having its own kinetochore. One kinetochore is attached to the spindle fibres towards one pole and the other similarly towards the other pole.

   Depending on position of the centromeres, chromosomes can be grouped as:

   a) **Metacentric:** Centromere is located exactly at the centre of chromosome, i.e. both arms are equal in size. Such chromosomes assume 'V' shape at anaphase.
   b) **Submetacentric:** The centromere is located on one side of the centre point such that one arm is longer than the other. These chromosomes become 'J' or 'L' shaped at anaphase.
   c) **Acrocentric:** Centromere is located close to one end of the chromosome and thus giving a very chort arm and a very long arm. These chromosomes acquire ' J' shape or rod shape during anaphase.

d) **Telocentric:** Centromere is located at one end of the chromosome so that the chromosome has only one arm. These chromosomes are 'I" shaped or rod shaped.

Normally chromosomes are monocentric having one centromere each. Acentric (without centromere) and dicentric (with two centromeres) chromosomes, if produced due to chromosomal aberrations, cannot orient properly on the equatorial plate and lag behind other chromosomes during anaphase movements.

In certain organisms, centromere does not occupy a specific position, but is diffused through out the body of chromosome. Such chromosomes, which do not have a localized centromere, are found in *Luzula* sps. and insects belonging to the order *Hemiptera*.

3. **Telomere:** The two ends of chromosomes are known as telomeres. They are highly stable and do not fuse or unite with telomeres of other chromosomes due to polarity effect. Any broken end of a chromosome is unstable and can join with a piece of any other chromosome. But the telomeres impart stability to the chromosome, which retains its identity and individuality through cell cycle and for many cell generations.
4. **Secondary constriction:** The constricted or narrow region other than that of centromere is called secondary constriction and the chromosomes having secondary constriction are known as satellite chromosomes or sat chromosomes. Chromosome may possess secondary constriction in one or both arms of it. Chromosomal end distal to the secondary constriction is known as satellite. Production of nucleolus is associated with secondary constriction and therefore it is also called nucleolus organizer region and satellite chromosomes are often referred to as nucleolus organizer chromosomes.
5. **Chromomere:** In some species like maize, rye etc. chromosomes in pachytene stage of meiosis show small bead like structures called chromomeres. Chromomeres are visible during meiotic prophase (pachytene) and invisible in mitotic metaphase chromosomes. The distribution of chromomeres in chromosomes is highly characteristic and constant. The pattern of distribution being different for different chromosomes. They are clearly visible as dark staining bands in the giant salivary gland chromosomes. Chromomeres are regions of tightly folded DNA. Chromomeres of single

chromosome show considerable variation in size. They may differ in size as in the case of maize or they may be of uniform size as in the case of rye.

6. **Chromonema:** A chromosome consists of two chromatids and each chromatid consists of thread like coiled structures called chromonema (plural chromonemata). The term chromonema was coined by Vejdovsky in 1912. The chromonemata form the gene bearing portion of chromosomes.
7. **Matrix:** The mass of acromatic material which surrounds the chromonemata is called matrix. The matrix is enclosed in a sheath which is known as pellicle. Both matrix and pellicle are non genetic materials and appear only at metaphase, when the nucleolus disappears.

Composition of chromosomes: The material of which chromosomes are composed is called chromatin. N.Fleming introduced the term chromatin in 1879. Chromatin was classified into two groups by cytologists on the basis of its affinity to basic dyes like acetocarmine or feulgen (basic fuchsin) reagent at prophase. The darkly stained regions were called heterochromatin, while lightly stained regions were called euchromatin. This differential staining capacity of different parts of a chromosomes is known as 'heteropycnosis'. In general heterochromatin is found in centromeric and telomeric regions and these regions of chromosome generally replicate later than the euchromatic regions of chromosomes. The genes within the heterochromatic regions are usually inactive. Most of the genome of an active cell is euchromatic and the genes with in this euchromatic region are expressed.

Heterochromatin is further classified into two groups: a) Constitutive and b) Facultative

a) Constitutive heterochromatin: It is present in all cells at identical positions on both homologous chromosomes of a pair.
b) Facultative heterochromatin: It varies in state in different cell types, at different stages or sometimes, from one homologous chromosome to another. A well known example of facultative heterochromatin is the *Barr body*, an inactivated X chromosome in somatic cells of mammalian female(XX).

Differences between Heterochromatin and euchromatin:

Heterochromatin	Euchromatin
1.Represent darkly stained regions	1.Lightly stained regions
2.Contains few inactive genes	2.Contains lot of active genes
3.Covers small region of chromosome	3.Larger region of chromosome
4.Usually found near centromere and telomere	4.Found in the middle of chromosome between centromere and telomere
5.Two types – Constitutive and facultative	5.Only one type
6.Late replicating	6.Normal replicating
7.Usually no active part in transcription	7.Plays active role in transcription
8.30 nm fibre	8.3-8 nm fibre

Karyotype and Ideogram: The general morphology (size of chromosomes, position of centromere, presence of secondary constriction and size of satellite bodies) of somatic chromosomal complement of an individual constitutes its karyotype. It can be defined as “the characteristic features by which a set of chromosomes of a species is identified”. Generally, karyotype is represented by arranging the chromosomes in descending order of size, keeping their centromeres in the same line. Thus the largest chromosome is placed on extreme left and the shortest on extreme right. The karyotype of a species can be represented diagrammatically showing all the morphological features of chromosomes. Such a diagram is known as ideogram or ideotype.

Lecture No: 6

SPECIAL TYPES OF CHROMOSOMES

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. The following are included under this category:

1. **Giant chromosomes or polytene chromosomes:** These were first discovered by E. G. Balbiani in 1882 in *Dipteran* salivary glands and hence commonly called salivary gland chromosomes. These chromosomes replicate repeatedly but the daughter chromatids do not separate from one another and the cell also does not divide. This phenomenon is known as endomitosis or endoreduplication. It results in the formation of many stranded giant chromosomes known as polytene chromosomes and the condition is known as polyteny. Their size is 200 times or more than the normal somatic chromosomes (autosomes) and very thick. Hence they are known as giant chromosomes. These chromosomes are somatically paired and their number in the salivary gland cells always appear to be half of that in the normal somatic cells. Along the length of chromosomes, a series of dark bands are present alternate with clear bands known as interbands. These bands have greatly helped in mapping of the chromosomes in cytogenetic studies. In the dark band region, the DNA is tightly coiled while in the interband region, DNA is less tightly coiled. The morphological expression of such sites is represented by local enlargements of certain regions called puffs. These puffs are also known as balbiani rings. Puffs are the sites of active RNA synthesis.
2. **Lamp brush chromosomes:** These were first observed by W. Flemming in 1882 and were described in detail in oocytes of sharks by Rukert in 1892. They occur at diplotene stage of meiotic prophase in oocytes of all animal species. Since they are found in meiotic prophase, they are present in the form of bivalents in which the maternal and paternal chromosomes are held together by chiasmata at those sites where crossing over has previously occurred. Each bivalent has four chromatids, two in each homologue. The axis of each homologue consists of a row of granules or chromomeres, each of which have two loop like lateral extensions, one for

each chromatid. Thus each loop represents one chromatid of a chromosome and is composed of one DNA double helix. One end of each loop is thinner than other which is known as thickend. There is extensive RNA synthesis at thin ends of the loop while there is little or no RNA synthesis at the thick ends.

3. **Accessory chromosomes:** In many species some chromosomes are found in addition to normal somatic chromosomes. These extra chromosomes are called accessory chromosomes or B-chromosomes or supernumerary chromos omes. These chromosomes are broadly similar to normal somatic chromosomes in their morphology, but have some peculiar functional aspects. For instance, presence of several such chromosomes often leads to reduction in vigour and fertility in males. These chromosomes are generally smaller in size than the normal somatic complement. They are believed to be generally inactive genetically. However they may not be completely devoid of genes. Origin of these chromosomes in most species is unknown.
4. **Isochromosomes:** An isochromosome is the one in which two arms are identical with each other in gene content and morphology. Such a chromosome is in assense a reverse duplication with entromeres separating the two arms. Every isochromosome is metacentric. The attached 'x' chromosome of *Drosophila* is a classical example of an isochromosome. However its origin is uncertain. There is no evidence that isochromosomes had any evolutionary significane.
5. **Allosomes / sex chromosomes:** Chromosomes differing in morphology and number in male and female are called allosomes. They are responsible for determination of sex. Eg: X and Y chromosomes in human beings and *Drosophila*. Chromosomes which have no relation with determination of sex and contain genes which determine somatic characters of individuals are called autosomes and are represented by letter 'A'.

Lecture No.: 7

DEOXY RIBOSE NUCLEIC ACID (DNA)

In 1869, Friedrich Meischer was the first person who separated cell nuclei from the cytoplasm and extracted an acidic material, nuclein, from the nuclei of pus cells. He found that the acidic material contained unusually large amounts of phosphorous and no sulphur. Later on in 1889, Richard Altmann used the term nucleic acid in place of nuclein. Nucleic acids were found to be associated with various proteins called nucleoproteins. There are two types of nucleic acids *viz.,* Deoxy ribose Nucleic acid (DNA) and Ribose Nucleic acid (RNA). DNA is the genetic material in most of the organisms. RNA acts as genetic material only in some viruses. DNA is mainly found in the chromosomes in the nucleus, while RNA is mostly found in the ribosomes in the cytoplasm.

Levene showed that nucleic acid can be broken into smaller molecules called nucleotides. Each nucleotide consists of a sugar, phosphate group and a nitrogenous base. The combination of nitrogenous base and sugar with out the phosphate group is called nucleoside (riboside and deoxyriboside) where as the combination of nitrogenous base, sugar and the phosphate group is called nucleotide (ribotide and deoxyribotide) (nucleotide = nucleoside + phosphate).

The 5-carbon (pentose) sugar could be either ribose as in case of RNA or deoxyribose in case of DNA. Associated with each sugar is a nitrogenous base with one or two carbon–nitrogen rings. Bases containing one carbon–nitrogen ring are called pyrimidines. The common pyrimidines present in DNA are thymine(T) and cytosine (C), while in case of RNA pyrimidine base thymine is replaced by uracil(U). Bases containing two carbon-nitrogen rings are called purines. The common purines present in nucleic acids are adenine (A) and guanine(G).

Differences between pyrimidines and purines

Pyrimidines	Purines
These are single ring (six member) compounds.	These are double ring (nine member) compounds.
They are of three types, viz., cytosine, thymine and uracil.	They are of two types, viz., adenine and guanine.
They occupy less space in DNA structure.	They occupy more space in DNA structure.
Deoxyribose is linked at position 3 of pyrimidine.	Deoxyribose is linked at position 9 of purine.

Levene proposed that each of the deoxy-ribonucleotides was present in equal amounts and connected together in chains in which each of the four different nucleotides was regularly repeated in a tetranucleotide sequence (AGCT, AGCT etc.). In 1940 Erwin Chargaff and other biochemists showed that all the nucleotide bases were not present in equal amounts and that the ratio of different bases changed between different species. It was also shown by Chargaff that the number of purine bases (A + G) is equal to the number of pyrimidine bases (C + T) i.e. A + G = C + T. It was also shown that the ratios of adenine to thymine and guanine to cytosine are constant and close to one in various eukaryotic species. By the early 1950's X – ray studies of DNA by Wilkins, Franklin and others indicated a well organized multiple stranded fibre of about 22^0A in diameter that was also characterized by the presence of groups or bases spaced, 3.4^0A apart along the fibre and occurrence of a repeating unit at every 34^0A.

Taking into account the facts known at that time Watson and Crick in 1953 proposed a "double helix" structure of DNA which quickly gained wide acceptance.

The salient features of double helix structure of DNA are:

- The DNA molecule consists of two polynucleotide chains wound around each other in a right-handed double helix.
- The two strands of a DNA molecule are oriented anti-parallel to each other i.e. the 5' end of one strand is located with the 3' end of the other strand at the same end of a DNA molecule.
- Each polydeoxyribonucleotide strand is composed of many deoxyribonucleotides joined together by phosphodiester linkage between their sugar and phosphate residues and the sugar phosphate backbones are on the outsides of the double helix with the nitrogen bases oriented toward the central axis.
- The half steps of one strand extend to meet half steps of the other strand and the base pairs are called complementary base pairs. The adenine present in one stand of a DNA molecule is linked by two hydrogen bonds with the thymine located opposite to it in the second strand, and vice-versa. Similarly, guanine located in one strand forms three hydrogen bonds with the cytosine present opposite to it in the second strand, and vice-versa.

The pairing of one purine and one pyrimidine maintains the constant width of the DNA double helix.

- The bases are connected by hydrogen bonds. Although the hydrogen bonds are weaker, the fact that so many of them occur along the length of DNA double helix provides a high degree of stability and rigidity to the molecule.
- The diameter of this helix is 20^0A, while its pitch (the length of helix required to complete one turn) is 34^0A. In each DNA strand, the bases occur at a regular interval of 3.4^0A so that about 10 base pairs are present in one pitch of a DNA double helix.
- The helix has two external grooves, a deep wide one, called major groove and a shallow narrow one, called minor groove. Both these groves are large enough to allow protein molecules to come in contact with the bases.
- This DNA structure offers a ready explanation of how a molecule could form perfect copies of itself. During replication, the two strands of a DNA molecule unwind and the unpaired bases in the single-stranded regions of the two strands by hydrogen bonds with their complementary bases present in the cytoplasm as free nucleotides. These nucleotides become joined by phospho-diester linkages generating complementary strands of the old ones with the help of appropriate enzymes.

The DNA molecule satisfies the requirement of genetic material in the following ways:-

1. It can replicate itself accurately during cell growth and division.
2. Its structure is sufficiently stable so that heritable charges i.e., mutations can occur only very rarely.
3. It has a potential to carry all kinds of necessary biological information.
4. It transmits all the biological information to the daughter cells.

Thus the essential functions of DNA are the storage and transmission of genetic information and the expression of this information in the form of synthesis of cellular proteins.

Types of DNA

The double helix described by Watson and Crick has right handed helical coiling and is called B-DNA. It is a biologically important form of DNA that is commonly and naturally found in most living systems. This double helical structure of DNA exist in other alternate forms such as A-form, C-form etc. which differ in features such as the number of nucleotide base pairs per turn of the helix. The B-form contains ~ 10 (range 10.0 – 10.6) base pairs per turn. The B-DNA is the most stable form and it can change to another form depending upon the humidity and salt concentration of the sample. The A- form is also a right-handed helix, but it has 11 base pairs per turn. The C-form of DNA has 9.3 base pairs per turn, while the D-form of DNA, which is rare form, has 8 base pairs per turn. Another form of DNA, in which the helix is left-handed, called Z-DNA was discovered by Rich. In ZDNA sugar and phosphate linkages follow a zigzag pattern. ZDNA plays a role in the regulation of the gene activity.

Comparison of B-DNA and Z-DNA

Characteristic	**B-DNA**	**Z-DNA**
Coiling	Right handed	Left-handed
Pitch	34^0A	45^0A
Base pairs / pitch	10.4	12
Diameter	~ 20^0A	~ 18^0 A
Rise per base pair	3.4^0 A	3.7^0A
Sugar – phosphate backbone	Regular	Zigzag

(Pitch – The length of the helix required to complete one turn)

Denaturation: The hydrogen bonds between the DNA strands break on heating the DNA to high temperature (nearly 100^{o}C). The process of separation of DNA strands is known as denaturation.

Renaturation: Reunion of the separated or denatured DNA strands on cooling is called renaturation or annealing. The optimum temperature for renaturation is 20 – 25^{o}C.

Lecture No.: 8

DNA REPLICATION

The process by which a DNA molecule makes its identical copies is called DNA replication.

Modes of DNA Replication:

There are three possible modes of DNA replication.

1. **Dispersive:** In dispersive mode of replication, the old DNA molecule would break into several pieces, each fragment would replicate and the old and new segments would recombine randomly to yield the progeny DNA molecule. Each progeny molecule would have both old and new segments along its length.
2. **Conservative:** According to conservative scheme, the two newly synthesized strands following the replication of a DNA molecule would associate to form one double helix, while the other two old strands would remain together as one double helix.
3. **Semi conservative:** In this model of DNA replication, each newly synthesized strand of DNA would remain associated with old strand against which it was synthesized. Thus, each progeny DNA molecule would consist of one old and one newly synthesized strand.

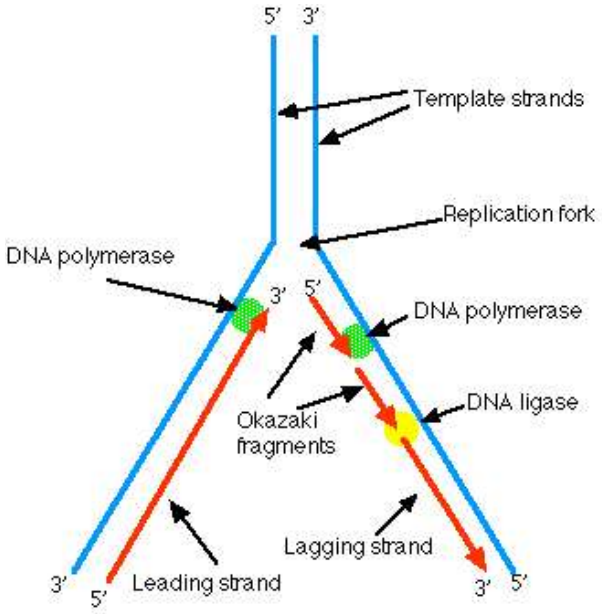
Evidence for Semi conservative replication: The experiment conducted by Matthew Meselson and Franklin Stahl in 1958 on *E. coli* provided a conclusive proof that replication of DNA is by semi conservative model. Meselson and Stahl labeled DNA of *E. coli* bacteria with heavy nitrogen i.e. ^{15}N by growing them on a medium containing ^{15}N for many generations to replace the normal nitrogen (^{14}N). The density of normal and heavy nitrogen differs. The ^{14}N is lighter (1.710 g/cm^3) than ^{15}N (1.724 g/cm^3). It is possible to detect such minute differences in density through density gradient centrifugation. Distinct bands are formed in the centrifuge tube for different density DNA. DNA extracts of *E. coli* with ^{15}N gave a characteristic heavy band at one end of a tube that had been centrifuged at a high speed in an ultra-centrifuge. These labeled cells were then grown on a normal unlabelled media containing ^{14}N for one generation. DNA was again extracted and

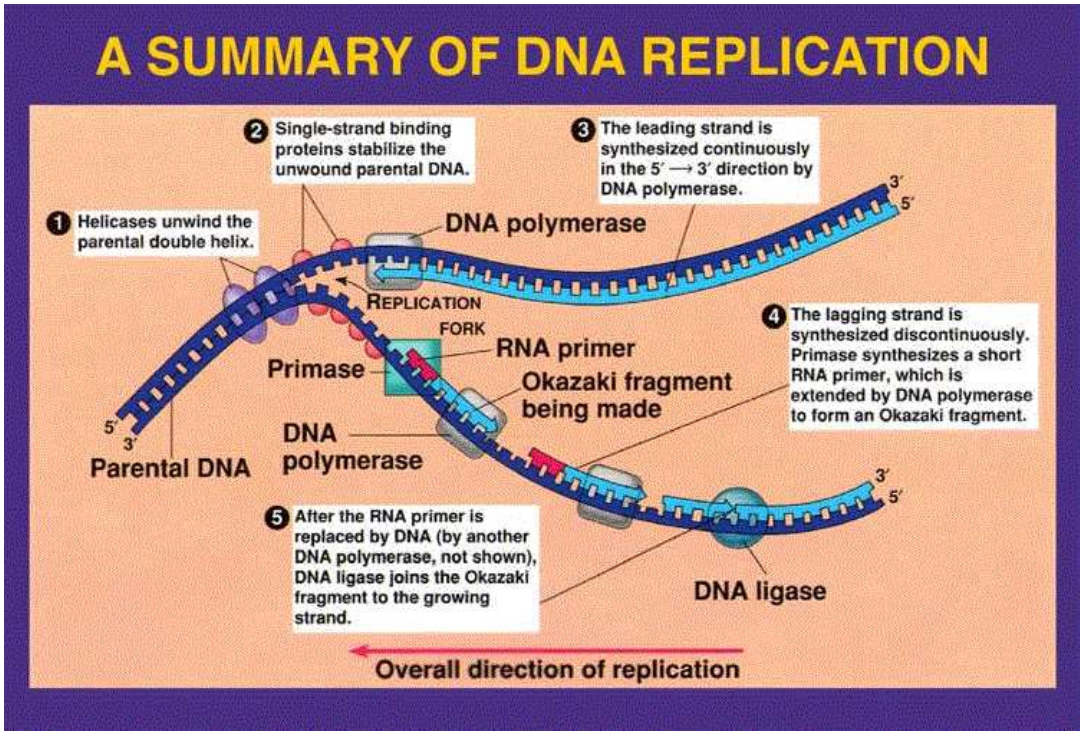
processed and it was found to consist of a hybrid DNA containing both ^{14}N and ^{15}N at the same time. This indicated that the DNA had not replicated in two separate labeled and unlabelled forms. The next generation of growth on unlabelled DNA was found to be in amounts equal to the partially labeled hybrid DNA. Additional generation of growth on unlabelled media gave a relative increase in the amount of unlabelled DNA. After two generations half the DNA was with intermediate density and half with light bands which further confirm semi-conservative mode of DNA replication. After third generation, ¾ DNA was found with ^{14}N and ¼ with hybrid nitrogen ($^{14}N+^{15}N$). When the hybrid DNA was denatured by heating upto 100°C it was found to produce two separate single strands and in the ultracentrifuge density gradient, it was observed to form two separate bands; one band containing ^{15}N and the other ^{14}N. Thus it was concluded that DNA replication was by semi-conservative mode.

The major objection put forth for the semi-conservative replication was that the DNA molecule must unwind a number of times (1/10 of the total number of nucleotides) which cannot be accomplished without breaking with in a short span of time say two minutes. Cairns (1968) provided evidence for this in his experiments with radio-active labeled *E. coli* chromosomes. The *E. coli* chromosome is a double stranded circular chromosome. It was shown that the two circular component strands separate during replication with each strand duplicating individually producing a θ shaped structures during replication. This indicates that unwinding and replication proceed simultaneously.

Method of semi-conservative replication: DNA replication was found to begin at various initiation points, called origin of replication, and proceed bi-directionally. Two enzymes, DNA gyrase and DNA helicase induce unwinding of complementary strands of DNA. Single-strand DNA binding (SSB) proteins bind to the single-stranded DNA, stabilizing it and preventing it from reanneaing. An enzyme, primase, initiates replication by synthesizing the primer. DNA polymerases synthesize the complementary strand by progressively adding deoxyribonucleotides. The DNA replication always proceeds in 5' → 3' direction. During replication one strand of DNA can replicate continuously and the other strand discontinuously or in pieces. The continuously replicating strand is known as leading strand and the discontinuously replicating strand is known as lagging

strand. The replication of lagging strand generates small polynucleotide fragments called ‘Okazaki fragments’ which are later joined together by the enzyme, DNA ligase.





RNA (Ribose nucleic acid)

Structure of RNA: RNA like DNA is a polynucleotide. RNA nucleotides have ribose sugar, which participate in the formation of sugar phosphate backbone of RNA. Thymine is absent and is replaced by Uracil. Usually RNA is a single stranded structure. Single stranded RNA is the genetic material in most plant viruses Eg. TMV. Double stranded RNA is also found to be the genetic material in some organisms. Eg. : Plant wound and tumour viruses. RNA performs non-genetic function. There are three main types or forms of RNA.

1. **messenger RNA (m-RNA):** It constitutes about 5-10% of the total cellular RNA. It is a single stranded base for base complementary copy of one of the DNA strands of a gene. It provides the information for the amino acid sequence of the polypeptide specified by that gene. Generally a single prokaryotic mRNA molecule codes for more than one polypeptide ; such a mRNA is known as polycistronic mRNA. All eukaryotic mRNAs are monocistronic i.e. coding for a single polypeptide specified by a single cistron.

2. **ribosomal RNA (r – RNA):** Occur in association with proteins and is organized into special bodies of about 200 Å A diameter called ribosomes. The size of ribosomes is expressed in terms of 'S' units, based on the rate of sedimentation in an ultracentrifuge. It constitutes about 80% of the total cellular RNA. The function of rRNA is binding of mRNA and tRNA to ribosomes.

3. **transfer RNA (t – RNA):** It is also known as soluble RNA(sRNA). It constitutes about 10-15% of total RNA of the cell. It is a class of RNA which is of small size of 3S type and generally have 70 – 80 nucleotides. The longest t – RNA has 87 nucleotides. It's main function is to carry various types of amino acids and attach them to mRNA template for synthesis of protein. Each t – RNA species has a specific anticodon which base pairs with the appropriate m – RNA codon.

   . The nucleotide sequence of the first tRNA (yeast alanyl tRNA) was determined by Robert Holley (1965), who proposed the clover leaf model of secondary structure of tRNA. Each tRNA is specific for each aminoacid. tRNA

molecule contains the sequence of CCA at the 3'end, which is called amino acid attachment site. At the CCA end, it is joined to the single amino acid molecule for which that tRNA is specific. For example, a tRNA molecule specific for lysine cannot bind to the arginine. tRNA consists of three loops; (a) DHU-loop or D-loop (aminoacyl recognition region), (b) anticodon loop and (c) thymine loop (ribosome attachment region). Anticodon loop contains a short sequence of bases, which permits temporary complementary pairing with the codons of mRNA.

IMPORTANT DIFFERENCES BETWEEN DNA AND RNA

Particulars	DNA	RNA
1. Strands.	Usually two, rarely one.	Usually one, rarely two.
2. Sugar.	Deoxyribose.	Ribose.
3. Bases.	Adenine, guanine, cytosine and thymine.	Adenine, guanine, cytosine and uracil.
4. Pairing.	AT and GC.	AU and GC.
5. Location.	Mostly in chromosomes, some in mitochondria and chloroplasts.	In chromosomes and ribosomes.
6. Replication.	Self replicating.	Formed from DNA. Self replication only in some viruses.
7. Size.	Contains up to 4.3 million nucleotides.	Contains up to 12,000 nucleotides.
8. Function,	Genetic role.	Protein synthesis, genetic in some viruses.
9. Types.	There are several forms of DNA.	Three types, viz., mRNA, tRNA and rRNA.

CENTRAL DOGMA

Dogma = Principle

In 1958 Crick proposed that the information present in DNA (in the form of base sequences) is transferred to RNA and then from RNA it is transferred to proteins in the form of aminoacid sequence and that this information does not flow in the reverse direction i.e. from protein to RNA to DNA. This relationship between DNA, RNA and protein is known as central dogma. DNA molecules however provide the information for their own replication.

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{translation}} \text{Protein}$$

However in cells infected by certain RNA viruses Eg : TMV, the viral RNA produces new copies of itself with the help of RNA replicase. In some viruses, the DNA is synthesized from RNA in the presence of an enzyme, reverse transcriptase. This process is known as reverse transcription. This was first reported by Temin and Baltimore in 1970.

Lecture No.: 9

GENETIC CODE

There are 20 amino acids involved in protein synthesis and there are only 4 bases in the DNA coding for all the amino acids. Nirenberg who has found that three bases code for one amino acid and thus it is possible to code for all the amino acids.

The number and the sequence of bases in mRNA specifying an amino acid is known as a codon. While the set of bases in tRNA which base pair with a codon of mRNA is known as anti-codon. The sequence of bases in an anti-codon is exactly the opposite of that present in the codon. Since the codon and anticodon segments run anti-parallel to each other when they base pair, Codons are written in 5' → 3' direction, where as anticodons are usually written in 3' → 5' direction. The set of all the codons that specify the 20 amino acids is termed as the genetic code.

It is clear from various experiments that a sequence of three nucleotides in mRNA code for an amino acid and hence the genetic code is called the triplet code. Three codons, UAA, UAG and UGA do not code for any amino acid and hence they are called nonsense codons. These codons are also known as stop codons or termination codons as they provide stop signal for termination. The codon i.e. AUG is know as start codon or initiation codon as it starts the synthesis of polypeptide chain. This codon also codes for amino acid methionine. In eukaryotes, the starting amino acid is methionine, while in prokaryotes it is N-formyl methionine.

Nature / Properties of Genetic code; There are several important features or properties of a genetic code.

1. **The code is triplet:** The triplet code was first suggested by Gammow in 1954. In a triplet code, three RNA bases code for one amino acid.
2. **The code is universal:** The same genetic code is applicable to all forms of organisms from microbes to human beings.
3. **The code is comma less:** The genetic code is without a comma or break. The codons are continuous and there are no breaks between the codons. A change or deletion of a single base in the code will alter the entire sequence of aminoacid to be synthesized.

4. **The code is non-overlapping:** Three nucleotides or bases code for one aminoacid and six bases will code for two amino acids. In a non – overlapping code, one base or letter is read only once.
5. **The code is non – ambiguous:** Out of the 64 codons, 61 code for 20 different aminoacid, while 3 are nonsense codons. None of the codons code for more than one aminoacid.
6. **The code is degenerate:** In most cases, several codons code for the same aminoacid. Only two amino acids, *viz*., tryptophan and methionine are coded by one codon each. Nine amino acids are coded by two codons each, one amino acid (isoleucine) by three codons each, five amino acids by four codons each and three amino acids by six codons each. This type of redundancy of genetic code is called degeneracy of genetic code. Such a system provides protection to the organisms against many harmful mutations. If one base of codon is mutated, there are other codons, which will code for the same amino acid and thus there will be no alteration in polypeptide chain.
7. **The code has polarity:** The code has a definite direction for reading of message, which is referred to as polarity. Reading of code in opposite direction will naturally specify for another amino acid example: GUC codes for valine, if reversed, CUG codes for Leucine.

Crick postulated the wobble hypothesis in 1966 to account for the degeneracy of genetic code.

Wobble hypothesis: The first two bases on the codon are usually the important ones, the third base may be in some cases one of the four bases and the triplet would still code for the same amino acid as long as the first two bases are the same and have the same sequence. This concept is known as the Wobble hypothesis.

Exceptions to the Universality of the genetic code: Some exceptions to the universality of the genetic code are known in ciliates (unicellular protozoa) and mitochondria. These exceptions generally involve, initiation or termination codons (and result from either production or the absence of tRNAs representing certain codons). For example, ciliates read UAA and UAG as glutamine instead of termination signals. Similarly in mitochondria, UGA does not specify termination, it means the same as UGG and codes for tryptophan. This change is found in yeast, invertebrates and vertebrates but not in plants.

PROTEIN SYNTHESIS

The genetic information for the synthesis of proteins and other enzymes resides in DNA and the actual synthesis takes place in cytoplasm. Two French scientists of pasture institute, F. Jacob and J. Monod proposed about the existence of a messenger for communication between DNA and the protein synthesizing machinery in the cytoplasm and later this was called the mRNA (messenger RNA).

A particular segment of DNA, that is a gene, which becomes active, in a manner similar to the replication of DNA during cell division, serves as a template for the formation of RNA, which contains a sequence bases of that is complementary to one strand of DNA (antisense strand, which serves as its template) and identical to the other strand of DNA (sense strand). This process of production of mRNA from DNA is called transcription. The RNA polymerase which catalyses the synthesis of RNA from the DNA template is known as transcriptase. Actually in eukaryotic cells, it was found that the RNA molecule immediately after transcription is many times longer than the RNA that enters cytoplasm. Hence, this RNA was named as heterogeneous RNA (hnRNA). This hnRNA is processed in the nucleus itself by deleting intervening sequences called introns and the expressed sequences that are retained are known as exons. This hnRNA is further processed by capping with methyl guanosine at the 5' end and by addition of a number of adenines (poly -A) at 3' end. This capping and addition of poly A tail is supposed to protect the mRNA from degradation by cytoplasmic enzymes.

The process of protein synthesis where amino acids are brought by the tRNA and sequentially arranged based on the codons of the mRNA is known as translation (i. e. the nucleotide sequence is translated into the amino acid sequence). The translation process requires mRNA, rRNA, ribosomes, 20 kinds of aminoacids and their specific tRNAs and many translation factors. The process of translation (protein synthesis) consists of five major steps *viz*., (1) activation of aminoacids (2) transfer of aminoacids to tRNA (3) chain initiation (4) chain elongation and (5) chain termination. Each step is governed by specific enzymes and cofactors.

In the cytoplasm, the amino acids are activated in the presence of ATP and linked to their respective tRNAs by a process called charging of tRNA in the

presence of an enzyme aminoacyl synthetase. Thus a number of tRNA molecules, pick up aminoacids freely floating in the cytoplasm and forms aminoacyl-tRNAs.

The processed mRNA enters the cytoplasm and binds to ribosomes, which serve as work benches for protein synthesis. The ribosome consists of rRNAs and different proteins. Ribosome contains two subunits; the large subunit and the small subunit. The process of translation starts when an initiating aminoacylated tRNA base pairs with an initation codon of an mRNA molecule that has been located by the small subunit of ribosome. Then the larger subunit joins. Two separate and distinct sites are available in the ribosome to which the tRNAs can bind; A (acceptor or aminoacyl attachment) site and P (peptidyl) site. An aminoacyl-tRNA first attaches to site A (acceptor site or aminoacyl attachment site) the kind of aminoacyl t-RNA being determined by the sequence of mRNA (codon) attached to site A. The peptide bonds are formed between the aminoacids which is catalysed by the enzyme peptidyl transferase. The peptidyl tRNA along with the mRNA codon moves to the P (peptidyl) site making the A site available for the attachment of a new aminoacyl-tRNA. Thus the translation proceeds and at the end a releasing factor binds to the stop codon terminating the translation. The ribosome releases the polypeptide and mRNA and subsequently dissociates into two subunits. Further processing of polypeptide chain into proteins and enzymes is done in the cytoplasm itself and depends upon the bonding properties of the amino acids joined in them.

Most of the mRNA molecules are unstable and degraded after the release of polypeptide chain, but some mRNAs such as those coding for hemoglobin may be stable. When the cell needs large quantities of a particular enzyme or protein, more number of mRNA molecules coding for the same protein are produced to meet the demand.

Polyribosomes or polysomes: Many ribosomes read one strand of mRNA simultaneously, helping to synthesize the same protein at different spots on the mRNA.

Cistron : A sub division of gene which acts as a unit of function with a gene.

Muton : A sub division of gene which is the site of mutation.

Recon : The smallest subunit of gene capable of undergoing recombination or a sub unit of gene which is the site of recombination.

Lecture No.: 10

GENE REGULATION

Genes that encode a product required in the maintenance of basic cellular processes or cell architecture are called housekeeping genes or constitutive genes. A constructive gene is an unregulated gene, whose expression is uninterrupted, in contrast to the regulated expression of a gene. The studies of bacterial genetics indicate that all genes not only specify the structure of an enzyme but some of them also regulate the expression of other genes. These genes are called regulator genes. This concept of gene regulation has been studied by F. Jacob and J. Monod in 1961 in *E. coli,* who proposed the operon concept.

According to the operon concept, gene regulation in prokaryotes and bacteriophages involves structural genes, the operator, the promoter, the regulator genes, repressor proteins and an inducer.

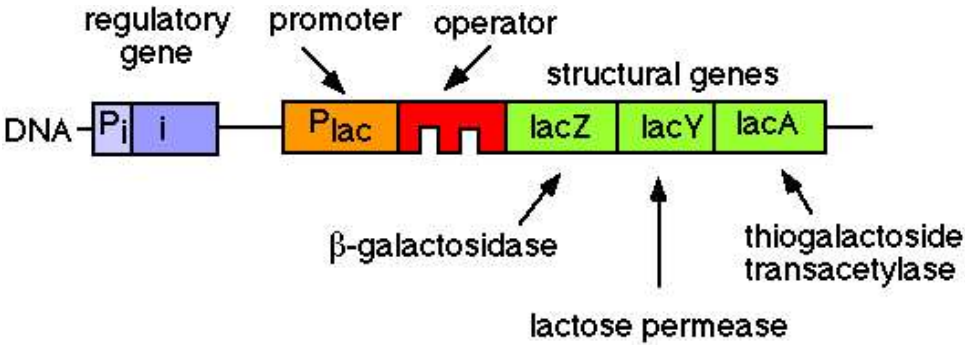
Lac Operon of *E. coli*

A genetic unit that consists of one or more "structural genes" (cistrons that code for polypeptides) and an adjacent "operator – promoter" region that controls the transcriptional activity is called operon. Operator and promoter are up steam to the structural genes. Thus an operon refers to a group of closely linked genes which act together and code for various enzymes required for a particular biochemical pathway. Lac operon consists of several components which are briefly described below:

Structural genes

The lactose operon of *E. coli* is composed of three structural genes *z*, *y* and *a* the '*z*' gene codes for an enzyme ß-galactosidase, which converts lactose into glucose and galactose. The *'y'* gene codes for an enzyme permease, which facilitates the entry of lactose into the cell. The 'a' gene specifies the enzyme thiogalactoside transacetylase, which transfers an acetyl group from acetyl co-A to ß-galactoside. Hence all the three gene products in lac operon are required for the metabolism of lactose. Such genes, which are sequential and transcribed as a single m-RNA from a single promoter are called structural genes. The m-RNA synthesized is the polycistronic mRNA. Only the last cistron has the signals for the termination of transcription.

The Lac operon - showing its genes and its binding sites



The operator region

Operator lies immediately upstream to the structural genes between the promoter and structural genes. Operator is the target site for the attachment of repressor protein produced by the regulator gene. Binding of repressor with operator prevents initiation of transcription by RNA polymerase. When operator is free, the RNA polymerase can bind to the promoter to initiate the mRNA synthesis.

The promoter region

The actual site of transcription initiation is known as promoter region. It also lies upstream to the structural genes next to the operator region. mRNA transcription by the structural gene is catalysed by an enzyme RNA polymerase. This enzyme first binds to the promoter region and then moves along the operator region and structural genes.

Regulator gene

Regulator gene (*i*) specifies a repressor protein, which in the absence of the inducer (lactose), bound to the operator (o), thereby inactivating the operator and preventing transcription of the three structural genes by RNA polymerase. In the presence of an inducer (lactose), the repressor is inactivated by interaction with the inducer. This allows the RNA polymerase to bind to the promoter allowing the transcription of the adjacent structural genes.

Repressor

Repressor is a protein molecule specified by the regulator gene. Repressor may be in active form or inactive form. In the active form, repressor binds to the operator region and prevents transcription. When the repressor is in inactivate form, the transcription takes place.

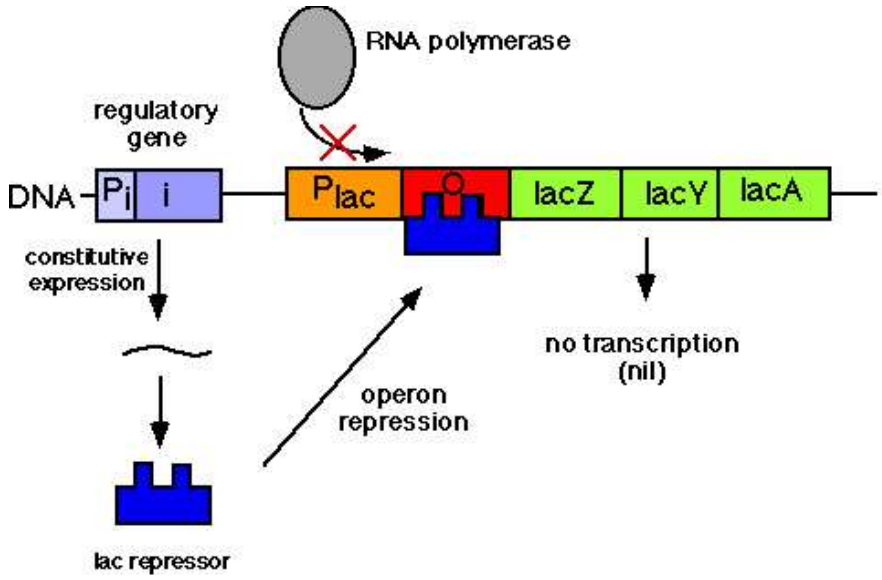
Inducer

The inducer binds to the repressor making it inactive such that it cannot blind to the operator. RNA polymerase path way is cleared allowing the expression of structural genes. A few molecules of lactose present in the cytoplasm of *E. coli* are metabolized into allolacatose, which is an isomer of lactose. Such molecules that induce the expression of any operon by binding to the repressor are called inducers and such operons are inducible operons.

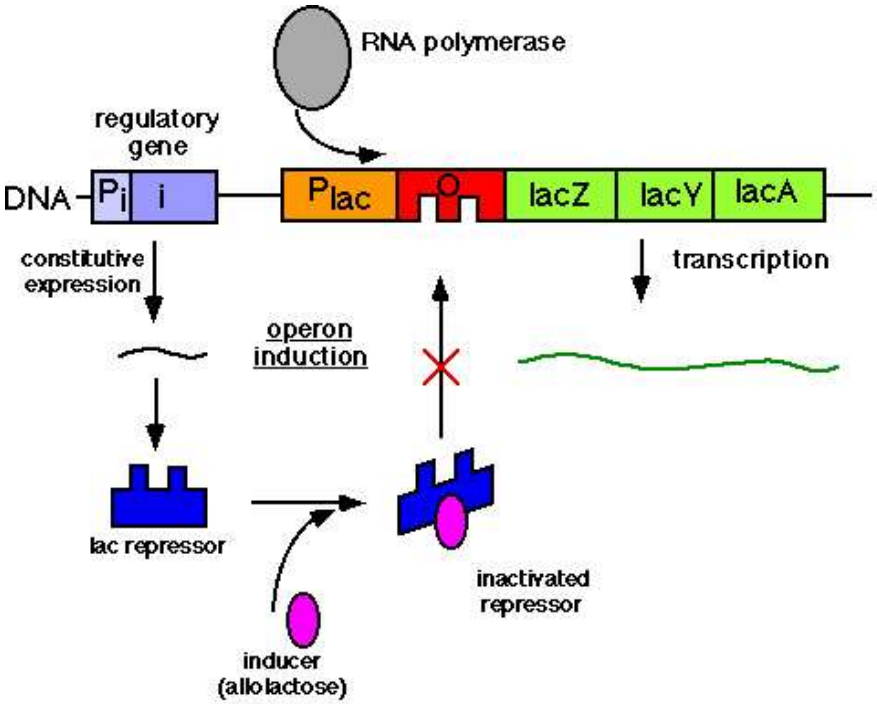
Regulation of Lac Operon

In an uninduced *E. coli,* repressor protein blinds to the operator. Hence, expression of structural genes is not induced. *E. coli* initially contains a few molecules of ß-galactosidase enzyme. A few molecules of lactose slowly diffuse into cytoplasm. ß-galactosidase present in cytoplasm metabolises lactose into allolactose which acts as an inducer. In an induced *E. coli,* allolactose blinds to repressor protein. The repressor protein is detached from the operator. RNA polymerase allows the transcription of structural genes to synthesize a polycistronic mRNA. Permease synthesized from mRNA allows the rapid uptake of lactose. Large number of ß-galactosidase molecules in the cytoplasm metabolise lactose into galactose and glucose.

In the "repressed or uninduced" state, the repressor bound to the operator



In the "induced" state, the lac repressor can not bound to the operator site



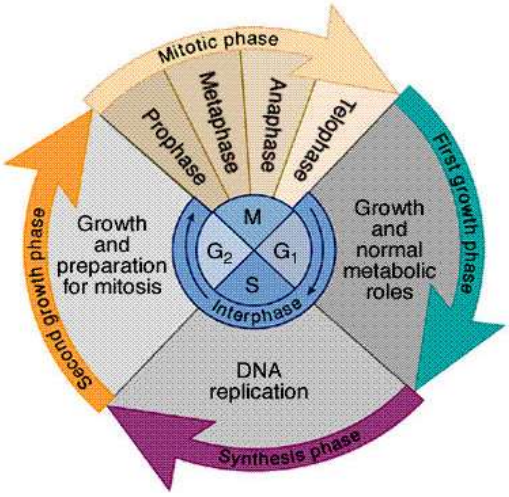
Lecture No.: 11

CELL CYCLE

Cell cycle can be defined as the entire sequence of events happening from the end of one nuclear division to the beginning of the next. A cell cycle consists of two phases, v*iz*., 1) interphase and 2) the cell division proper. The time required for the completion of cell cycle differs from species to species.

Interphase

Interphase is generally known as DNA systhesis phase. Interphase consists of G1, S and G2 sub phases. G1 is the resting phase, S is the period of DNA replication and G2 again is a resting stage after DNA replication.



G1 Phase: It is a pre-DNA replication phase. Thus, this is a phase between telophase and S phase. This is the longest phase which takes 12 hours in *Vicia faba*. It is the most variable period of cell cycle. Synthesis of proteins and RNA take place during this phase.

S (Synthetic) Phase: This phase comes after G1and takes lesser time than G1phase. In *Vicia faba*, it takes six hours. The chromosome and DNA replications take place during this phase.

G2 Phase: This is the post-DNA replication phase and last sub stage of interphase. This phase also takes 12 hours in *Vicia faba*. Synthesis of protein and RNA occur during this stage.

CELL DIVISION

All the cells are produced by division of pre-existing cells. Continuity of life depends on cell division. In the cell division, the division of nucleus is called

karyokinesis and division of cytoplasm is called cytokinesis. The cell division is of two types. 1) Mitosis and 2) Meiosis

MITOSIS

The term mitosis was coined by Flemming in 1882. Mitosis occurs in somatic organs like root tip, stem tip and leaf base etc. Hence it is also known as somatic cell division. The daughter cells are similar to the mother cell in shape, size and chromosome complement. Since the chromosome number is same in the daughter cells as compared to that of mother cell, this is also known as homotypic or equational division.

Mitotic cell cycle includes the following stages:

Interphase : This is the period between two successive divisions. Cells in interphase are characterized by deeply stained nucleus that shows a definite number of nucleoli. The chromosomes are not individually distinguishable but appear as extremely thin coiled threads forming a faintly staining network. The cell is quite active metabolically during interphase.

Mitosis consist of four stage, *viz.,* (a) Prophase, (b) Metaphase, (c) Anaphase and (d) Telophase

a) Prophase: **The nucleus takes a dark colour with nuclear specific stains and also with acetocarmine / orcein. The size of the nucleus is comparatively big and the chromosomes that are thin in the initial stages slowly thicken and shorten by a specific process of coiling. The two chromatids of a chromosome are distinct with matrix coating and relational coiling. The disintegration of nuclear membrane denotes the end of prophase.**

b) Metaphase: **After the disintegration of nuclear membrane, the shorter and thicker chromosomes will spread all over the cytoplasm. Later, the size of the chromosomes is further reduced and thickened. The distinct centromere of each chromosome is connected to the poles through spindle fibres. The chromosomes move towards equator and the centromere of each chromosome is arranged on the equator. This type of orientation of centromeres on the equator is known as auto-orientation. The chromosomes at this stage are shortest and thickest. The chromatids of a chromosome are held together at the point of centromeres and the relational coils are at its minimum.**

c) Anaphase: **The centromere of each chromosome separates first and moves to towards the poles. Depending on the position of the centromeres (metacentric, acrocentric and telocentric), the chromosomes show ` V `, ` L ` and ` I ` shapes respectively as the anaphase progresses. The sister chromatids move to the poles. The chromosome number is constant but the quantity of each chromosome is reduced to half.**

d) Telophase: **Chromosomes loose their identity and become a mass of chromatin. The nucleus will be re-organized from the chromatin. At late telophase stage, the cell plate will divide the cell into two daughter cells.**

Cytokinesis : **The division of cytoplasm usually occurs between late anaphase and end of telophase. In plants, cytokinesis takes place through the formation of cell plate, which begins in the centre of the cell and moves towards the periphery in both sides dividing the cytoplasm into two daughter cells. In animal cells, cytokinesis occurs by a process known as cleavage, forming a cleavage furrow.**

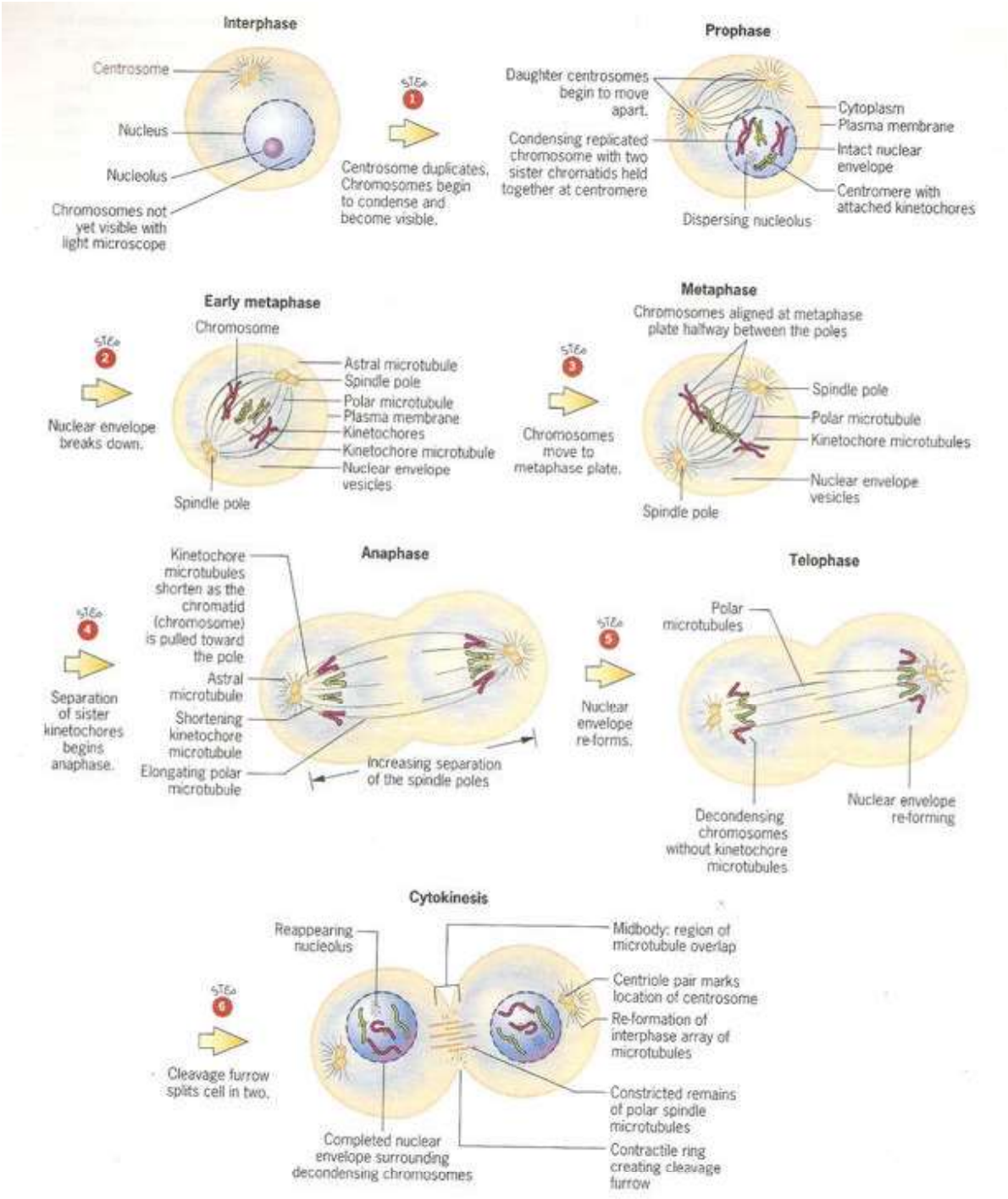
Significance of Mitosis

Mitosis plays an important role in the life of living organisms in various ways as given below:

1. Mitosis is responsible for development of a zygote into adult organism after the fusion of male and female gametes.
2. Mitosis is essential for normal growth and development of living organisms. It gives a definite shape to a specific organism.
3. In plants, mitosis leads to formation of new organs like roots, leaves, stems and branches. It also helps in repairing of damaged parts.
4. It acts as a repair mechanism by replacing the old, decayed and dead cells and thus it helps to overcome ageing of the cells.
5. It helps in asexual propagation of vegetatively propagated crops like sugarcane, banana, sweet potato, potato, etc. mitosis leads to production of identical progeny in such crops.
6. Mitosis is useful in maintaining the purity of types because it leads to production of identical daughter cells and does not allow segregation and recombination to occur.

7. In animals, it helps in continuous replacement of old tissue with new ones, such as gut epithelium and blood cells.

MITOSIS



Lecture No: 12

MEIOSIS

The term meiosis was coined by J.B. Farmer in 1905. This type of division is found in organisms in which there is sexual reproduction. The term has been derived from Greek word; Meioum = diminish or reduce. The cells that undergo meiosis are called meiocytes. Three important processes that occur during meiosis are:

1. Pairing of homologous chromosomes (synapsis)
2. Formation of chiasmata and crossing over
3. Segregation of homologous chromosomes

The first division of meiosis results in reduction of chromosome number to half and is called reduction division. The first meiotic division is also called heterotypic division. Two haploid cells are produced at the end of first meiotic division and in the second meiotic division, the haploid cells divide mitotically and results in the production of four daughter cells (tetrad), each with haploid number of chromosomes. In a tetrad, two daughter cells will be of parental types and the remaining two will be recombinant types. The second meiotic division is also known as homotypic division. Both the meiotic divisions occur continuously and each includes the usual stages *viz.,* prophase, metaphase, anaphase and telophase.

Meiotic cell cycle involves the following stages:

Interphase : Meiosis starts after an interphase which is not very different from that of an intermitotic interphase. During the premeiotic interphase DNA duplication occurs during the S phase

I. Meiosis-I:

(1) Prophase-I: It is of a very long duration and is also very complex. It has been divided into the following sub-stages:

a) **Leptotene or Leptonema:** Chromosomes at this stage appear as long thread like structures that are loosely interwoven. In some species, on these chromosomes, bead-like structures called chromomeres are found all along the length of the chromosomes.

b) **Zygotene or Zygonema:** It is characterized by pairing of homologous chromosomes (synapsis), which form bivalents. The paired homologous

chromosomes are joined by a protein containing frame work known as synaptonemal complex. The bivalents have four strands

c) **Pachytene or Pachynema:** The chromosomes appear as thickened thread-like structures. At this stage, exchange of segments between non-sister chromatids of homologous chromosomes known as crossing over occurs. During crossing over, only one chromatid from each of the two homologous chromosomes takes part. The nucleolus still persists.

d) Diplotene or Diplonema: **At this stage further thickening and shortening of chromosomes takes place. Homologous chromosomes start separating from one another. Separation starts at the centromere and travels towards the ends (terminalization). Homologous chromosomes are held together only at certain points along the length. Such points of contact are known as chiasmata and represent the places of crossing over. The process of terminalization is completed at this stage.**

e) Diakinesis: **Chromosomes continue to undergo further contraction. The bivalents appear as round darkly stained bodies and they are evenly distributed throughout the cell. The nuclear membrane and nucleolus disappear.**

2) Metaphase-I: **The chromosomes are most condensed and have smooth outlines. The centromeres of a bivalent are connected to the poles through the spindle fibres. The bivalents will migrate to the equator before they disperse to the poles. The centromeres of the bivalents are arranged on either side of the equator and this type of orientation is called co-orientation.**

3) Anaphase-I: **The chromosomes in a bivalent move to opposite poles (disjunction). Each chromosome possess two chromatids. The centromere is the first to move to the pole. Each pole has a haploid number of chromosomes**

4) Telophase-I: **Nuclear membranes are formed around the groups of chromosomes at the two poles. The nucleus and nucleolus are re-organized.**

II. Meiosis-II: The second meiotic division is similar to the mitotic division and it includes the following four stages:

1) **Prophase-II:** The chromosomes condense again. The nucleolus and nuclear membrane disappear. The chromosomes with two chromatids each become short and thick
2) **Metaphase -II:** Spindle fibres appear and the chromosomes get arranged on the equatorial plane(auto-orientation). This plane is at right angle to the equatorial plane of the first meiotic division.
3) **Anaphase-II:** Each centromere divides and separates the two chromtids, which move towards the opposite poles.
4) **Telophase-II:** The chromatids move to the opposite poles The nuclear envelope and the nucleolus reappears. Thus at each pole, there is re-organization of haploid nucleus.

Cytokinesis: The division of cytoplasm takes place by cell plate method in plants and by furrow method in animals. The cytokinesis may take place after meiosis I and meiosis Ⅱ separately or sometimes may take place at the end of meiosis II only.

Significance of Meiosis

Meiosis plays a very important role in the biological populations in various ways as given below:

1. It helps in maintaining a definite and constant number of chromosomes in a species.
2. Meiosis results in production of gametes with haploid (half) chromosome number. Union of male and female gametes leads to formation of zygote which receives half chromosome number from male gamete and half from the female gamete and thus the original somatic chromosome number is restored.
3. Meiosis facilitates segregation and independent assortment of chromosomes and genes.
4. It provides an opportunity for the exchange of genes through the process of crossing over. Recombination of genes results in generation of variability in a biological population which is important from evolution points of view.
5. In sexually reproducing species, meiosis is essential for the continuity of generation. Because meiosis results in the formation of male and female

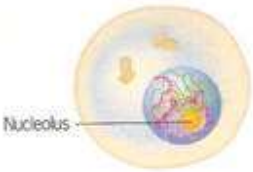
gametes and union of such gametes leads to the development of zygotes and thereby new individual.

MEIOSIS

Prophase I

Leptonema

Replicated chromosomes become visible.



Zygonema

Homologous chromosomes pair.

Pachynema

Homologous chromosomes fully paired. Crossing over occurs.

Diplonema

Homologous chromosomes begin to repel each other. Chromatids become fully visible. Chiasmata become visible.

Diakinesis

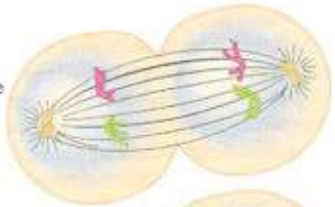
Chromosomes continue to shorten and thicken. Nucleolus and nuclear membrane disappear. Microtubules attach to kinetochores.

Metaphase I

Assembly of spindle is completed. Each chromosome pair aligns across the metaphase plate of the spindle.

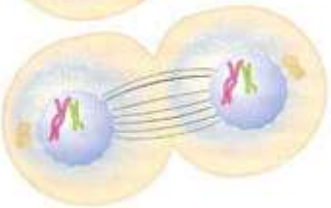
Anaphase I

Homologous chromosome pairs separate and migrate toward opposite poles.



Telophase I

Chromosomes (each with two sister chromatids) complete migration to the poles, and new nuclear membranes may form.



Cytokinesis

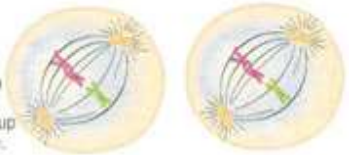
In most species, cytokinesis produces two daughter cells. Chromosomes do not replicate before meiosis II.

Prophase II

Chromosomes condense and move to metaphase plate.

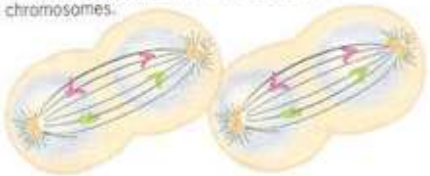
Metaphase II

Kinetochores attach to spindle fibers. Chromosomes line up on metaphase plate.



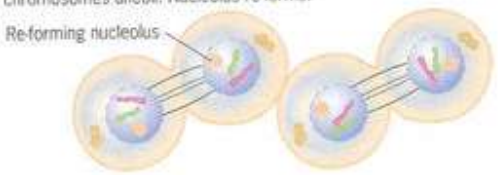
Anaphase II

Sister chromatids separate and move to opposite poles as separate chromosomes.



Telophase II

Nuclear membrane forms around chromosomes and chromosomes uncoil. Nucleolus re-forms.



Four haploid cells form after cytokinesis.

Differences between mitosis and meiosis

Mitosis	Meiosis
1. Consists of one nuclear division	1. Consists of two nuclear divisions
2. One cell cycle results in production of two daughter cells	2. One cell cycle results in production of four daughter cells
3. The chromosome number of daughter cells is the same as that of mother cell (2n)	3. Daughter cells contain half the chromosome number of mother cell (n)
4. Daughter cells are identical with mother cell in structure and chromosome composition	4. Daughter cells are different from mother cell in chromosome number and composition
5. It occurs in somatic cells	5. It occurs in reproductive cells
6. Total DNA of nucleus replicates during S phase	6. About 0.3% of the DNA is not replicated during S phase and it occurs during the zygotene stage.
7. The prophase is not divided into sub stages	7. The prophase I is divided into five sub stages
8. There is no pairing between homologous chromosomes	8. Homologous chromosomes pair during pachytene
9. Segregation and recombination do not occur	9. Crossing over takes place during pachytene
10. Chromosomes are in the form of dyad at metaphase	10. Chromosomes are in the form of tetrad at metaphase
11. The centromeres of all the chromosomes lie on the equatorial plate (auto orientation) during metaphase	11. The centromeres of all the chromosomes lie on either side of the equatorial plate (co-orientation) during metaphase I
12. At metaphase, centromere of each bivalent divides longitudinally	12. The centromere does not divide at metaphase I
13. One member of sister chromatids moves to opposite pole during anaphase	13. One member of homologous chromosomes moves to opposite poles during the anaphase I
14. Maintains purity due to lack of segregation and recombination	14. Generates variability due to segregation and recombination

Lecture No.: 13 & 14

LINKAGE

Sutton and Boveri proposed the chromosome theory of inheritance. According to chromosome theory of inheritance, it is well established that many genes are located in each chromosome in a linear fashion. It may therefore be expected that all genes located in same chromosome would move to same pole during cell division. As a consequence, such genes will fail to show independent segregation and would tend to be inherited together. This tendency of genes to remain together in their original combination during inheritance is called linkage. Mendel's law of independent assortment is applicable only when the genes are located in different chromosomes while linkage refers to the genes located in the same chromosome.

The phenomenon of linkage was first reported by Bateson and Punnet in 1906. They studied flower colour and pollen shape in sweet pea involving two varieties / races.

Phases of linkage: In the two races experimented one parent has purple flowers with long pollen grains. The other parent has red flowers and round pollen grains. The character purple (P) is a simple monogenic dominant to red (p); while long (L) pollen is dominant to round (l) pollen, when these two plants were crossed the F_1 (PL/pl) was purple flowered with long pollen. But in F_2, the ratio of four types of plants deviated from the normal dihybrid ratio of 9:3:3:1 expected on the principle of independent assortment of flower colour and pollen shape.

PARENTS Purple Long x Red Round
PL/PL pl/pl

F_1 Purple Long
PL/pl

F_2	Purple long	Purple round	Red long	Red round	Total
Actual numbers	4831	390	393	1338	6952
Expected numbers	3910.5	1303.5	1303.5	434.5	6952
Expected Ratio	9/16	3/16	3/16	1/16	

The plants with the character combination as in the original parents are known as parental forms or parental combinations, i.e., (i) Plants with purple flowers and long pollen and (ii) Plants with red flowers and round pollen. Plants possessing one character from one parent and another character from the second parent are known as recombined types or recombinations or new combinations or cross-overs, i.e. (i) plants with red flowers and long pollen and (ii) plants with purple flowers and round pollen.

From the above table the following features can be noted that in F_2, there are both parental forms and recombinations. The chief peculiarity of the results in the above table was that parental forms are in far excess of the expected number while the recombinations were fewer. This deviation from the expected ratio is due to linkage of the character pairs, viz., 1. Purple flowers (P) and long pollen (L) are linked because both the genes are located on the same chromosome. Similarly red flowers (p) and round pollen (l) are linked together because the genes are located in the same chromosome (which is the homologue of the previous one). The recombinations are obtained due to crossing over between the two concerned genes in some of the spore mother cells.

Another cross was made in sweet peas with the following combinations:

PARENTS Purple Round x Red Long
PI/PI pL/pL

Purple Long
Pl/pL

F_2	Purple long	Purple round	Red long	Red round	Total
Actual numbers	225	95	97	2	419
Expected numbers	235.7	78.5	78.5	26.2	419
Expected Ratio	9/16	3/16	3/16	1/16	

Here again the parental types are more while the recombinant types are less than expected on the basis of independent assortment, viz., 9:3:3:1. This deviation is also due to linkage.

In the above two examples, it can be seen that in one cross the two dominant factors (PL) are linked in one parent and two recessive factors (pl) are linked in the other. Linkage in such crosses is said to be in coupling phase. In the second cross, dominant allele of one character pair (P) and the recessive allele of another character pair (I) are linked together in one parent, while in the second parent the other recessive (p) and dominant alleles (L) are linked. Linkage in such crosses is said to be in repulsion phase.

Later, T H Morgan put forth the theory of linkage and concluded that coupling and repulsion were two phases of single phenomenon, linkage.

Types of Linkage: Linkage is generally classified on the basis of three criteria viz., (i) Crossing over, (ii) Genes involved and (iii) Chromosomes involved

(i) Based on crossing over: Linkage may be classified into (a) complete and (b) incomplete / partial depending up on absence or presence of recombinant phenotypes in test cross progeny.

(a) Complete linkage: It is known in case of males of *Drosophila* and females of silkworms, where there is complete absence of recombinant types due to absence of crossing over.

(b) Incomplete / partial linkage: If some frequency of crossing over also occurs between the linked genes, it is known as incomplete / partial linkage. Recombinant types are also observed besides parental combinations in the test cross progeny. Incomplete linkage has been observed in maize, pea, *Drosophila* female and several other organisms.

(ii) Based on genes involved : Depending on whether all dominant or some dominant and some recessive alleles are linked together, linkage can be categorized into (a) Coupling phase and (b) Repulsion phase

(a) Coupling phase: All dominant alleles are present on the same chromosome or all recessive alleles are present on same chromosome.

TR tr
----- --- Coupling phase
TR tr

(b) Repulsion phase: Dominant alleles of some genes are linked with recessive alleles of other genes on same chromosome.

```
Tr    tR
-----  ---    Repulsion phase
Tr    tR
```

(iii) Based on chromosomes involved: Based on the location of genes on the chromosomes, linkage can be categorized into (a) autosomal linkage and (b) X-chromosomal linkage / allosomal linkage / sex linkage

(a) Autosomal linkage: It refers to linkage of those genes which are located in autosomes (other than sex chromosomes).

(b) X-chromosomal linkage / allosomal linkage / sex linkage: It refers to linkage of genes which are located in sex chromosomes i.e. either 'X' or 'Y' (generally 'X')

Characteristic features of Linkage:

1. Linkage involves two or more genes which are located in same chromosome in a linear fashion.
2. Linkage reduces variability.
3. Linkage may involve either dominant or recessive alleles (coupling phase) or some dominant and some recessive alleles (repulsion phase).
4. It may involve either all desirable traits or all undesirable traits or some desirable and some undesirable traits.
5. It is observed for oligo-genic traits as well as polygenic traits.
6. Linkage usually involves those genes which are located close to each other.
7. The strength of linkage depends on the distance between the linked genes. Lesser the distance, higher the strength and vice versa.
8. Presence of linkage leads to higher frequency of parental types than recombinants in test cross. When two genes are linked the segregation ratio of dihybrid test cross progeny deviates significantly from 1:1:1:1 ratio.
9. Linkage can be determined from test cross progeny data.

10. If crossing over does not occur, all genes located on one chromosome are expected to be inherited together. Thus the maximum number of linkage groups possible in an organism is equal to the haploid chromosome number.

    Eg. Onion 2n = 16 n = 8 maximum linkage groups possible = 8

    Maize 2n = 20 n = 10 maximum linkage groups possible = 10

11. Linkage can be broken by repeated intermating of randomly selected plants in segregating population for several generations or by mutagenic treatment.

12. Besides pleiotropy, linkage is an important cause of genetic correlation between various plant characters.

Linkage and pleiotropy: A close association between two or more characters may result either due to linkage or pleiotropy or both. Pleiotropy refers to the control of two or more characters by a single gene. A tight linkage between two loci can be often confused with pleiotropy. The only way to distinguish between linkage and pleiotropy is to find out a crossover product between linked characters. Intermating in segregating populations may break a tight linkage, but a huge population has to be raised to find out the crossover product. If a cross over product is not found in spite of repeated intermatings, there seems to be the case of pleiotropy rather than linkage.

Linkage groups : Linkage group refers to a group of genes which are present in one chromosome. In other words, all those genes which are located in one chromosome constitute one linkage group. The number of linkage groups is limited in each individual. The maximum number of linkage groups is equal to the haploid chromosome number of an organism. For example there are ten linkage groups in corn (2n = 20), seven in garden pea (2n = 14), seven in barley (2n = 14), four in *Drosophila melanogaster* (2n = 8) and 23 in man (2n = 46).

Detection of linkage: Test cross is the most common method of detecting the linkage. In this method, the F, heterozygous at two loci (AB/ab) is crossed to a double recessive parent (ab/ab) and the phenotypic ratio of test cross progeny is examined. If the phenotypic ratio of test cross progeny shows 1:1:1:1 ratio of parental and recombinant genotypes, it indicates absence of linkage. If the

frequency of parental types and recombinant types deviate significantly from the normal dihybrid test cross ratio of 1:1:1:1, it reveals presence of linkage between two genes under study.

Another way to detect the presence or absence of linkage is to self pollinate the individual heterozygous at two loci. If there is complete dominance at each locus and no epistasis, the segregation ratio of the progeny will be 9:3:3:1. Presence of linkage either in coupling or repulsion phase will lead to significant deviation from 9:3:3:1 ratio. The deviation of observed values from the expected ratio is tested with the help of x^2 test.

Significance of Linkage in Plant Breeding :

1. Linkage limits the variability among the individuals.
2. Linkage between two or more loci controlling different desirable characters is advantageous for a plant breeder. A linkage between genes controlling two different desirable characters will help in simultaneous improvement of both the characters.
3. Linkage is undesirable when desirable and undesirable genes are linked together.
4. The estimates of genetic variances for quantitative characters are greatly influenced by the presence of linkage

Lecture No.: 15

CROSSING OVER

The term crossing over was first used by Morgan and Cattell in 1912. The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes is called crossing over.

Mechanism of crossing over: It is responsible for recombination between linked genes and takes place during pachytene stage of meiosis i.e. after the homologous chromosomes have undergone pairing and before they begin to separate. It occurs through the process of breakage and reunion of chromatids. During pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids so that each bivalent has four chromatids or strands (four-strand stage). Generally one chromatid from each of the two homologues of a bivalent is

involved in crossing over. In this process, a segment of one of the chromatids becomes attached in place of the homologous segment of the nonsister chromatid and vice-versa. It is assumed that breaks occur at precisely homologous points in the two nonsister chromatids involved in crossing over; this is followed by reunion of the acentric segments. This produces a cross (x) like figure at the point of exchange of the chromatid segments. This figure is called chiasma (which is seen in diplotene stage of meiosis) (plural-chiasmata).

Obviously, each event of crossing over produces two recombinant chromatids (involved in the crossing over) called cross over chromatids and two original chromatids (not involved in crossing over) referred to as noncrossover chromatids. The crossover chromatids will have new combinations of the linked genes, i.e. will be recombinant; gametes carrying them will produce the recombinant phenotypes in test-crosses, which are called crossover types. Similarly, the noncrossover chromatids will contain the parental gene combinations and the gametes carrying them will give rise to the parental phenotypes or noncrossover types. Therefore the frequency of crossing over between two genes can be estimated as the frequency of recombinant progeny from a test-cross for these genes. This frequency is usually expressed as percent. Thus, the frequency of crossing over (%) can be calculated using the formula;

$$\text{Frequency of crossing over(\%)} = \frac{\text{No. of recombinant progeny from a test cross}}{\text{Total number of progeny}} \times 100$$

Types of crossing over: Depending upon the number of chiasmata involved, crossing over is of three types.

1. **Single crossing over**: It refers to the formation of single chiasma between non-sister chromatids of homologous chromosomes. It involves two linked genes (Two point test cross).
2. **Double crossing over**: It refers to the formation of two chiasmata between non-sister chromatids of homologous chromosomes. It involves three linked genes (Three point test cross).
3. **Multiple crossing over**: Occurrence of more than two crossing overs between non-sister chromatids of homologous chromosomes is known as multiple crossing over. However, the frequency of such type of crossing over is extremely low.

Factors affecting crossing over: The frequency of crossing over between the linked genes is affected by several factors.

1. **Distance between the genes:** The frequency of crossing over between the two genes is positively associated with the distance between their location in the chromosome. Crossing over between the two genes would increase with an increase in distance between them.
2. **sex:** The frequency of recombination is markedly influenced by the sex of heterozygotes for linked genes. In general, the heterogametic sex shows relatively lower recombination frequencies than the homogametic sex of the same species. Eg: No crossing over occurs between linked genes in *Drosophila* males and females of silkworm.
3. **Age of female:** The frequency of crossing over shows a progressive decline with the advancing age of *Drosophila* females.
4. **Temperature:** In *Drosophila*, the lowest frequency of crossing over is observed when females are cultured at 22^0C. The frequency of recombination tends to increase both at the lower and higher temperatures than 22^0C.
5. **Nutrition:** The frequency of crossing over in *Drosophila* is affected by the presence of metallic ions Eg: Ca^{+2} and Mg^{+2} in its food. Higher the amount, lower will be the crossing over frequency and vice-versa.
6. **Chemicals:** Treatment of *Drosophila* females with certain antibiotics like mitomycin D and actinomycin D and certain alkylating agents such as ethylmethane sulphonate promotes crossing over.
7. **Radiations:** An increase in frequency of crossing over is observed when *Drosophila* females are irradiated with x-rays and γ-rays.
8. **Plasmagenes:** In some species, plasma genes reduce the frequency of crossing over. Eg: The Tifton male sterile cytoplasm reduces the frequency of crossing over in bajra.
9. **Genotype:** Many genes are known to affect the occurrence as well as the rate of crossing over. For example C_3G gene of *Drosophila* located in chromosome 3 prevents crossing over when present in homozygous state while it promotes crossing over in the heterozygous state.

10. **Chromosomal aberrations:** In *Drosophila*, some chromosomal aberrations Eg: paracentric inversions, reduce recombination between the genes located with in the inverted segment.
11. **Distance from centromere:** Centromere tends to suppress recombination. Therefore genes located in the vicinity of centromeres show a relatively lower frequency of crossing over than those located away from them.

Significance of crossing over in Plant Breeding:

1. It increases variability
2. It helps to break linkages
3. It makes possible to construct chromosome maps

Cytological proof of crossing over: The first cytological evidence in support of genetic crossing over was provided by Curt stern in 1931 on the basis of his experiments with *Drosophila* by using cytological markers. He used a *Drosophila* female fly in which one X-chromosome was broken into two segments and out of these two segments, one behaved as X-chromosome. This chromosome had one recessive mutant allele *car* (carnation) for eye colour and another dominant allele *B* (Bar) for eye shape. The other X-chromosome had small portion of Y-chromosome attached to its one end. This chromosome had the dominant allele + (wild type allele of *car*) producing dull red eye colour and a recessive allele + (wild type allele of *B*) producing normal ovate eye shape. Thus the phenotype of female is barred (since *B* is dominant to +) with normal eye colour (since *car* is recessive to +) and both the X-chromosomes in the female had distinct morphology and could be easily identified under microscope. Such females were crossed with male flies having recessive alleles for both genes (*car* +). As a result of crossing over female flies produce four types of gametes *viz.,* two parental types or non crossover types (*car B* and + +) and two recombinant types or cross over types (*car* + and + *B*). The male flies produce only two types of gametes (car + and Y), because crossing over does not occur in *Drosophila* male. A random union of two types of male gametes with four types of female gametes will produce males and females in equal number of four each.

Stern cytologically examined the chromosomes of recombinant types i. e. carnation with normal eye shape and barred with normal eye colour. It was found that carnation flies did not have any fragmented X-chromosome but rather had normal X-chromosome. On the other hand barred flies had a fragmented

X-chromosome with a segment of Y-chromosome attached to one of the two fragments of X-chromosome. Such chromosome combination in barred is possible only through exchange of segments between non-sister chromatids of homologous chromosomes. This has proved that genetic crossing over was accompanied with an actual exchange of chromosome segments.

Similar proof of cytological crossing over was provided by Creighton and McClintock in maize.

Coincidence: It refers to the occurrence of two or more distinct crossing overs at the same time in the same region of a pair of homologous chromosomes and as a result, a double cross over product is obtained. Coefficient of coincidence is estimated by using the formula:

$$\text{Coefficient of coincidence} = \frac{\text{Observed frequency of double cross over}}{\text{Expected frequency of double cross over}}$$

(The ratio between the observed and the expected frequencies of double crossovers is called coefficient of coincidence)

Interference: The occurrence of crossing over in one region of a chromosome interferes with its occurrence in the neighbouring segment. This is known as interference. The term interference was coined by Muller. It may also be defined as the tendency of one crossing over to prevent another crossing over from occurring in its vicinity. This is called positive interference. Sometimes, one crossing over enhances the chance of another crossing over in the adjacent region. This is termed as negative interference. Eg: *Aspergillus*, bacteriophages. The effect of interference reduces as the distance from the first crossing over increases. The intensity of interference may be estimated as coefficient of interference.

Coefficient of interference = 1 - coefficient of coincidence

Differences between crossing over and linkage

Crossing over	Linkage
1. It leads to separation of linked genes	1. It keeps the genes together
2. It involves exchange of segments between non-sister chromatids of homologous chromosomes	2. It involves individual chromosomes
3. The frequency of crossing over can	3. The number of linkage groups can

never exceed 50 %	never be more than haploid chromosome number
4. It increases variability by forming new gene combinations	4. It reduces variability
5. It provides equal frequency of parental and recombinant types in test cross progeny	5. It produces higher frequency of parental types than recombinant types in test cross progeny

Lecture No.: 16

CHROMOSOME MAPS

Chromosome maps can be prepared by genetical or cytological methods

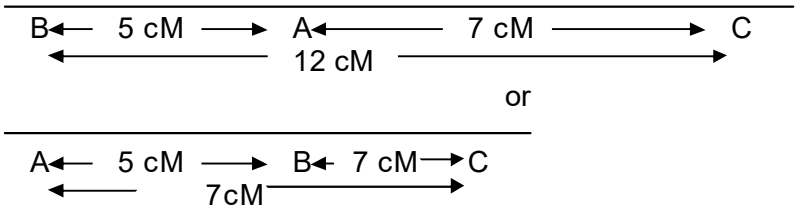
1. Genetical method: This is the general method and is based upon cross over data. The resulting map is the linkage map. Linkage map (cross over map or genetical map) map be defined as a line on which the relative positions of genes proportional to the amount of crossing over between them is represented.

A rule widely followed in plotting genes is that if genes A and B are known to be linked and if a particular gene is found by experiment to be linked with gene A it must also be linked with gene B. This principle follows from the fact that two linked genes are on the same chromosome. The genes, which are linked together on the same chromosomes are called syntenic genes.

Genetic mapping of chromosomes is based on the following assumptions:

a) The genes are arranged in a linear order.
b) Crossing over is due to breaks in the chromatids
c) Crossing over occurs by chance and is at random
d) The percentage of crossing over between the genes is an index of their distance apart.

Map distance: Recombination frequencies between the linked genes are determined from appropriate testcrosses. These percent frequencies are used as map units for preparing linkage maps. A map unit is that distance in a chromosome, which permits one percent recombination (crossing over) between two linked genes. A map unit is also called a centri-Morgan, after the name of the scientist Morgan, who first constructed the linkage map in *Drosophila*. Thus 5 % crossing over between genes A and B is taken to mean that they are situated 5 map units of distance apart on the same chromosome. If a third gene C with 7 % crossing over between A and C is included the relationship of linkage between the three genes A, B and C is indicated as below:



To choose the correct one between these two alternatives, one more information i.e. either the order of arrangement of the three genes or the cross over value between B and C is required. Eg: If the crossover value between B and C is found to be 2 % by actual experiments, the second arrangement is the correct one. Therefore, for preparing a chromosome map of three genes either the map distances (cross over frequencies) between all three gene pairs must be known or the cross over frequencies between any two gene pairs plus the order or sequence of these three genes in the chromosome must be known.

In obtaining cross over value care should be taken about the occurrence of double crossing over between the concerned genes. If two genes A and B are rather far apart in a chromosome and if two crossing overs (i.e. double cross over) occur between A and B, the chromatids involved do not show recombination of marker genes. If double crossing over occurs frequently, the recombination value will be less and gives a false impression that the distance between the concerned two genes is less. To overcome this difficulty, data for chromosome mapping should be taken from linked gene pairs that are quite close together. Usually double crossing over does not occur within distances less than 5 map units or for certain chromosome segments within distances upto 15 or 20 map units.

2. Cytological maps: By cytological studies of chromosomal aberrations and by their behaviour in genetical experiments, it is possible to construct map of chromosomes showing the actual physical location of gene in a chromosome. Such maps are called cytological maps of chromosomes. The work on cytological maps also confirm the theory of linear arrangement of genes in chromosomes.

Comparison between linkage maps and cytological maps: The relative distances between the genes on linkage map and cytological map do not always correspond. The discrepancies are greatest in the vicinity of the centromere where one cross over unit corresponds to a relatively much greater physical distance on the chromosome than in other regions of the same chromosome.

These discrepancies may be explained on the basis that different chromosomes and various regions in the same chromosome may also show variations in frequency of crossing over. Eg: In *Drosophila*, frequency of crossing over seems to be affected by temperature of the mother flies and by environmental factors.

Importance of linkage and chromosome maps in plant breeding:

1. They give an idea whether particular genes are linked or segregate independently.
2. Linkage intensities can be known and the probability of obtaining a given combination of genes can be assessed. If linkage between two genes is close, it is difficult to obtain recombination. In such cases, linkage can be broken artificially by irradiating with x-rays etc. and the desired combinations may be obtained. However, close linkage is useful to preserve desirable gene combinations.
3. Help the geneticist to plan how large the experimental population should be to obtain plants with the desired gene combination.
4. If an easily identifiable qualitative character is found to be linked with the quantitative character, the qualitative character can be used to easily identify the recombinants. This means that when a particular qualitative character is observed in a recombinant plant, it can be understood that the associated linked quantitative character is also present. Eg: Anthocyanin pigment and yield in rice
5. Linkage limits the variability among the individuals.

Lecture No.: 17, 18 & 19

MENDELIAN GENETICS

The term genetics was first used by W. Bateson in 1906. It is often described as a biological science which deals with heredity and variation. Heredity includes those characters which are transmitted from generation to generation and is therefore fixed for a particular individual. The differences among the individual of a species for a character constitutes the variation for that character. Variation on the other hand can be of two types.

1. **Hereditary variation:** refers to the differences in the inherited traits. such variations are found not only in the progeny of different parents but also among the progeny from the same parents. Eg: Differences in pattern of stripes in zebra, differences in length of neck in giraffes.

   Identical twins however are examples where there is no hereditary variation.

2. **Environmental Variation:** It is entirely due to environment.

   Eg: 1. Difference in skin colour. 2. Under inadequate supply of water and N, a tall plant does not grow properly. So it may become dwarf.

The study of genetics, therefore, enables us to differentiate between hereditary and environmental variation. Questions pertaining to nature and the basis of heredity have occupied the thoughts of man for many centuries. But systematic attempts to seek answers to these questions began only in 18^{th} century when several scientists began studies on plant hybridization. One of the notable workers is J. Koelreuter, who conducted extensive study on hybridization in tobacco. He noted uniformity and heterosis in F_1 generation and appearance of variation in F_2 generation. Gaertner used a backcross programme to convert one species into another. Other scientists like Naudin and Darwin also hybridized plants and studied the subsequent generations. However, they could not give an explanation for their results. Gregor Johann Mendel has first offered necessary explanation and hence he is known as “Father of Genetics”. With the help of experiments on garden pea, he was able to formulate laws which explained the manner of inheritance of characters. He presented his findings before the Natural History Society of Brunn in 1865. This paper entitled “Experiments in Plant Hybridization” was presented in German language and published in the annual proceedings of the society in 1866. Although Mendel described his results in 1866, his work was recognized only in 1900 when his laws were re-discovered by Hugo devries, E.V. Tschermark and C.correns, Mendel’s original paper was republished in 1901 in the journal ‘Flora’ Vol. 89. Page 364.

Mendel’s experiments: Mendel chose garden pea (*Pisum sativum*) as the plant material for his experiments, since it has the following advantages.

1. **Convenience of handling:** Peas could be grown easily either in field or in pots and each plant occupies only a small space.
2. **Controlled mating:** The flower structure of pea ensures self pollination, which was experimentally verified by Mendel. Individual pea plants are highly inbred displaying little if any genetic variation from one generation to the next. However since the pea flowers are relatively large, emasculation and pollination is quite easy. Therefore, crossing could be carried out easily.

3. **Short life cycle:** Peas complete their life cycle from seed to seed within 70 days, thus producing many generations in rapid succession.
4. **Large number of fertile off-springs :** Hybrids resulted from crossing two pure strains (true breeding) were perfectly fertile and more in number. Pea seeds are large in size and do not have any problem in germination.
5. **Presence of variation:** Peas have many sharply defined inherited differences like plant height (Tall vs. dwarf), seed shape (round vs wrinkled) etc. In the available varieties, several characters had two contrasting forms, which were easily distinguishable from each other. This permitted an easy classification of F_2 and F_3 progeny from various crosses into clear-cut classes.

Although hybridization experiments were conducted by earlier workers also, they considered the individual as a whole complex of characters. Mendel's success was based on the fact that he considered a single character at one time. Mendel chose seven pairs of contrasting characters for his study. In all the above crosses he obtained a definite ratio of 3:1 in F_2 generation. The determining agent responsible for each trait was called a factor. Since recessive character was not seen in F_1 generation, but reappeared in F_2, Mendel could predict the results to be expected in F_3 generation.

Characters chosen by Mendel for his study

Character	Dominant form	Recessive form
1. Plant height	Tall	Dwarf
2. Seed texture	Round	Wrinkled
3. Seed colour	Yellow	Green
4. Flower colour	Violet	White
5. Pod colour	Green	Yellow
6. Pod shape	Inflated	Constricted
7. Position of flowers	Axial	Terminal

Reasons for success of Mendel:

1. The experiments were very well designed and conducted with great care and skill.

2. The choice of his experimental material, the common garden pea.
3. Mendel studied the inheritance of only one pair of contrasting characters at a time.
4. The characters he chose were well defined and simple; each with only two contrasting forms Eg: Seed coat colour of peas is either green or yellow, with no intermediate types.
5. The seven characters selected by Mendel showed qualitative inheritance.
6. The contrasting forms of each of the seven characters were governed by a single gene and in each case one form was completely dominant over other.
7. Of the seven characters studied by Mendel, the genes for two were located in one chromosome, while three other were present in another chromosome.
8. His greatest innovation was to count the number of progeny in each category to emerge from a given cross for every generation.
9. His knowledge on mathematics was a definite asset for the interpretation of his findings after subjecting the results to more refined mathematical analysis.
10. He maintained particulars of pedigree records, which gave him the exact ancestry of any given plant.

Mendelism / Laws of Mendel: Mendel's cross breeding experiments with garden peas showed certain numerical relations among the progeny. The relationship between the character pairs and the kinds and the ratio of the progeny is known as Mendelism. Mendelism was not a theory of hereditary origin, but it was a theory of the manner in which inheritance had taken place. Mendelism can be summarized in the following laws which are called Mendel's laws of inheritance.

1. **The law of segregation or the law of purity of gametes:** It states that when a pair of alleles / allelomorphs is brought together in a hybrid (F_1) they remain together without contaminating each other and they separate or segregate from each other into a gamete in a complete and pure form during the formation of gametes. The law is universal in its application and it has been found to occur in plants as well as animals.

2. **Law of independent assortment:** The factors in an allelomorphic pair separates independently to the separation of factors in the other allelomorphic pair.
3. **Law of Dominance:** States that in a hybrid one factor of the allelomorphic pair expresses itself completely over the other.
4. **Law of unit characters:** State that each factor (gene) controls the inheritance of single character. These factors occur in pairs in each diploid organism.

Explanation of Laws of Mendel:

Monohybrid ratio: The Mendel's first law i.e. Law of segregation or purity of gametes can be explained by considering the monohybrid ratio i.e. by studying inheritance of only one character. For example: In pea, round seed shape is dominant over wrinkled seed shape.

Generation		Parental	
Parents	Female	X	Male
Phenotype	Round	X	Wrinkled
Genotype	RR	X	rr
Gametes	(R)	↓	(r)
Generation		F_1	Rr (Heterozygous) Round

On selfing:

Parents	Female	X	Male
Phenotype	Round	X	round
Genotype	Rr	X	Rr
Gametes	(R) (r)	↓	(R) (r)
Generation		F_2	

? / ?	R	r
R	RR Round	Rr Round
r	Rr Round	rr Wrinkled

Phenotype ratio: 3 round : 1 wrinkled Genotypic ratio: 1 RR : 2R : 1rr

Two different alleles of the same gene i.e. 'R' and 'r' were brought together in the hybrid (F_1). Even though the hybrid was round seeded in the next generation (F_2) it produced both round and wrinkled seeded progeny. Thus both the alleles for round shape (R) and wrinkled shape (r) remained together in the hybrid without contaminating each other. In F_2 generation (selfing of (F_1) hybrid), the different phenotypes could be recovered because the two alleles in F_1 remained pure and did not contaminate each other thus producing two types of gametes from F_1 i.e. (R) and (r). The separation of homologous chromosomes during anaphase I of meiosis may be regarded as the reason for segregation of the two alleles of a gene. This is because the alleles of a gene are located in an identical position in the two homologous chromosomes.

Dihybrid Ratio:

Mendel's second law i.e. Law of independent assortment can be explained by studying the inheritance of two characters at a time, simultaneously.

Independent segregation for two genes can be explained by assuming that the two genes are located in two different chromosomes. The two alleles of a gene will be located in the two homologues of the concerned chromosome. Independent separation of these two pairs of chromosomes at anaphase I of meiosis will lead to the independent segregation of the genes located in them. Thus any allele of one gene is equally likely to combine with any allele of the other gene and pass into the same gamete. Independent segregation of two genes produces four different types of gametes in equal proportion. A random union among these gametes gives rise to sixteen possible zygotes. These zygotes yield a 9:3:3:1 phenotypic ratio, which is known as the typical dihybrid ratio.

When two pairs of independent alleles enter into F1 combination, both of them have their independent dominant effect. These alleles segregate when gametes are formed But the assortment occurs independently at random and quite freely.

Example: when plants of garden pea with yellow round seeds (Y Y RR) were crossed with plants having green wrinkled seeds (yyrr), yellow round seed plants (YyRr) were obtained in F_1. Thus yellow colour of seed exhibited dominance over green and round seed shape over wrinkled seed shape independently. The F_1 produces four types of gametes YR, Yr, yR and yr. Selfing of F_1 gives rise to yellow round, yellow wrinkled, green round and green wrinkled individuals in

9:3:3:1 ratio. This is possible only when the alleles of two genes controlling the two characters assort independently to one another.

Parents Female X Male

Phenotype Yellow round X Green wrinkled

Genotype YYRR X yyrr

Gametes YR yr

Generation F_1 YyRr (Yellow round)

(Heterozygous)

On selfing/ intermating

Parents Female X Male

Phenotype Yellow round X Yellow round

Genotype YyRr X YyRr

YR Yr yR yr YR Yr yR yr

Generation F_2

Generation F_2

? / ?	**YR**	**Yr**	**yR**	**yr**
YR	YYRR Yellow round	YYRr Yellow round	YyRR Yellow round	YyRr Yellow round
Yr	YYRr Yellow round	YYrr Yellow wrinkled	YyRr Yellow round	Yyrr Yellow wrinkled
yR	YyRR Yellow round	YyRr Yellow round	yyRR green round	yyRr green round
yr	YyRr Yellow round	Yyrr Yellow wrinkled	yyRr green round	yyrr green wrinkled

Phenotypic ratio:

9 Yellow round : 3 Yellow wrinkled : 3 green round : 1 green wrinkled

Genotypic ratio:

1 YYRR : 2 YYRr : 1 YYrr : 2 YyRR : 4 YyRr : 2 Yyrr : 1 yyRR : 2 yyRr : 1 yyrr

Exceptions to Mendel's laws:

1. Paramutations and polyploidy are exceptions to the law of segregation or law of purity of gametes.
2. Linkage is an exception to Mendel's second law i.e. law of independent assortment.
3. Incomplete dominance is an exception to the principle of dominance.
4. Pleiotropism is an exception to the principle of unit characters.
5. Modification of F_2 ratios due to incomplete-dominance, co-dominance, lethal factors, interaction of factors, epistatic factors are all exceptions.

However, Mendel failed to confirm his results when he worked with hawk weed, (*Hieraceum* sp.) because of the formation of embryo from the ovule without fertilization (diploid parthenogenesis) in this plant.

Lecture No.: 20

GENE ACTION

Gene action refers to the manner in which genes control the phenotypic expression of various characters in an organism. Alleles of the gene may interact with one another in a number of ways to produce variability in their phenotypic expression. The dominant and recessive relationship is fundamental and is essentially constant with each pair of alleles.

Gene action can be of the following types:

1. Based on the dominance effect:
    a) Complete dominance
    b) Incomplete dominance
    c) Co-dominance
    d) Over dominance
    e) Pseudo-dominance
2. Based on lethal effects :
    a) Dominant lethals
    b) Recessive lethals
3. Based on epistatic action :
    a) Epistatic factors
    b) Supplementary factors

c) Duplicate factors

d) Complementary factors

e) Additive factors

f) Inhibitory factors

4. Based on number of genes involved:

a) Monogenic

b) Digenic

c) Oligogenic

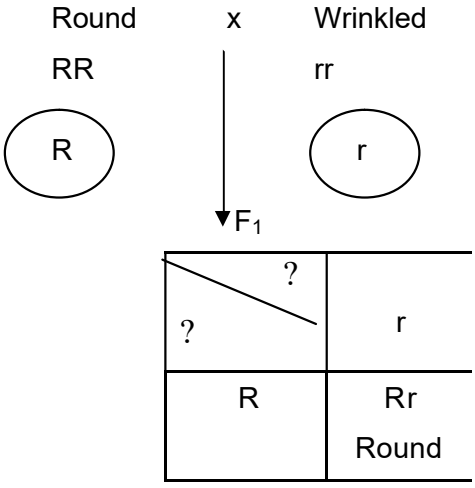
d) Polygenic

5. Based on pleiotropism / pleiotropic gene action

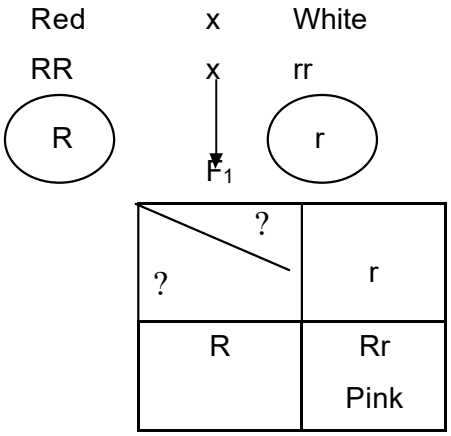
1. Based on dominance effect: It was noted by many workers that many characters of F_1 hybrid were exactly the same as those of one or the other parent of a hybrid. The phenomenon of F_1 hybrid being identical to one of its parents for a character is termed as dominance. The form of a character that is seen in F_1 hybrid is called dominant, while that form of the concerned trait which does not appear in F_1 is known as recessive.

Dominance relationship is of the following types:

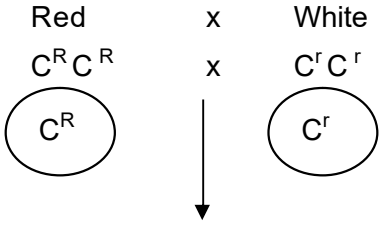
a) Complete dominance: The phenotype produced by a heterozygote is identical to that produced by the homozygotes for the concerned dominant allele. The dominant allele in such a situation is said to be completely or fully dominant. Eg: In garden pea, round seed shape is completely dominant over wrinkled.

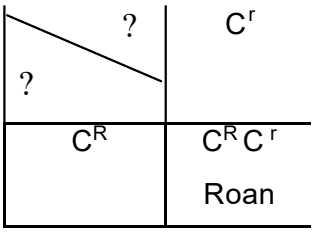


b) **Incomplete dominance**: In many cases, the intensity of phenotype produced by heterozygote is less than that produced by the homozygote for the concerned dominant allele. Therefore the phenotype of heterozygote falls between those of the homozygotes for the two concerned alleles. Such a situation is known as Incomplete or partial dominance and the dominant allele is called incompletely dominant or partially dominant. Eg : In *Mirabilis jalapa* (Four 'O' clock plant) a partially dominant allele 'R' produces red flowers in homozygous state, while its recessive allele 'r' produces white flowers in homozygous state. When a red (RR) flower type plant is crossed with white (rr) flower type plant, the hybrid (Rr) has pink flowers. Thus the intensity of flower colour in F_1 is intermediate between the intensities of flower colour produced by two homozygotes. This phenomenon is also called blending inheritance.



c) **Co-dominance**: Both the alleles of a gene express themselves in heterozygotes. As a result, heterozygotes for such genes possess the phenotypes produced by both the concerned alleles. The coat colour of short horned breed of cattle presents an excellent example of co-dominance.





Roan colour is that which has patches of red and white colours.

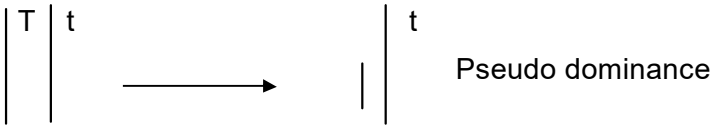
d) Over dominance: In case of some genes, the intensity of charac ter governed by them is greater in heterozygotes than in the two concerned homozygotes. This situation is known as over-dominance. True over dominance is known in case of very few genes. Over-dominance is not the property of an allele but is the consequence of heterozygous state of concerned gene. Eg: white eye gene (W) of *Drosophila* exhibits over-dominance for some of the eye pigments such as sepiapteridine and Himmel blaus. These two eye pigments are present in low concentration in the recessive homozygotes (ww), while the dominant homozygotes (WW) have relatively higher concentrations of these pigments. However, the flies heterozygous for this gene (Ww) have an appreciably higher concentration of these two pigments than the two homozygotes.

Transgressive segregation: The appearance of individuals in F_2 or subsequent generation which exceed the parental types with reference to one or more characters is known as transgressive segregation.

(or)

The segregants which fall outside the range of both the parents are called transgressive segregants and the phenomenon is called transgresive segregation.

e) Pseudo-dominance: Expression of recessive allele of the gene in the hemizygous state / condition either due to sex linkage (Eg: colour blindness in human beings) or chromosomal aberrations (deletion in heterozygotes) is known as pseudo-dominance.



Heterozygous condition	Hemizygous condition

2. Based on lethal effect: One of the most important assumptions for inheritance of any trait is the equal survival of all gametes and zygotes produced as a result of segregation. The assumptions are true for a vast majority of genes. However, some genes affect the survival of those zygotes or individuals in which they are present in the appropriate genotype. Such genes are known as lethal gene. A lethal gene causes death of all the individuals carrying the gene in the appropriate genotype before they reach the adult stage. Most of the lethal genes express their lethal effect only when they are in homozygous state while the survival of heterozygotes remains unaffected. The stage of development at which a lethal gene produces a lethal effect varies considerably from one gene to another. Some genes cause the death of embryo very early in developmental stage. (Eg: 'Y' gene in mice); while others allow survival for a certain period of time and then produce lethal effect (Eg: 'g' gene producing albino seedlings in crop plants like rice, barley etc.

a) **Dominant Lethal gene action:** A lethal gene affecting coat colour in mice was discovered by French geneticist Cuenot in 1905. He found that yellow coat colour in mice was produced by a dominant gene 'Y' while its recessive allele 'y' determines the normal black / grey coat colour. Further, he found that all the mice with yellow coat colour were heterozygous Yy and he was unable to found a mouse homozygous for 'Y' allele (YY). The dominant allele 'Y' is lethal and hence it causes death of homozygous 'YY' embryos at an early stage of development.

Generation	Parental		
Parents	Female	x	Male
Phenotype	Yellow	x	Black / grey
Genotype	Yy		yy
Gametes	Y y		y
Generation		F_1	

?	y
Y	Yy

	Yellow
y	Yy Black / grey

On intermating of yellow progeny

Generation	Parental		
Parents	Female	x	Male
Phenotype	Yellow	x	Yellow
Genotype	Yy		Yy
Gametes	Y y		Y y
Generation		F_2	

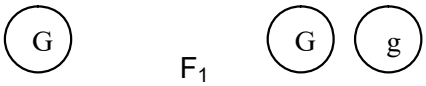
? \ ?	y	y
Y	YY Dies	Yy yellow
y	Yy Yellow	yy Black / grey

Phenotypic ratio : 2 Yellow ; 1 Black / Grey

Genotypic ratio : 2 Yy : 1 yy

b) **Recessive lethals:** Albino seedling character in plants such as rice and barley is governed by recessive alleles. Whenever these alleles are in the homozygous state the seedlings are near white or almost white and totally devoid of chlorophyll. Albino seedlings survive only as long as the food material stored in the seeds is available to them because they are not able to carry out photosynthesis. The heterozygotes, however are normal green and are identical with the dominant homozygotes in their phenotype as well as their survival. Segregation of such genes produces 3 green : 1 albino seedling if they are counted within a week from germination. However, if the plants are counted at maturity, there will be only green plants in the progeny.

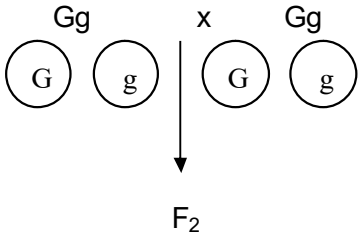
Female	x	male
Green	x	Green
GG		Gg



F_1

? / ?	G	g
G	GG Green	Gg Green

On selfing of Gg individuals



? / ?	G	g
G	GG Green	gg Green
g	Gg Green	Gg albino (dies)

The heterozygous individuals carrying the lethal genes without expression in the heterozygous condition but giving rise to lethals in F_2 generation is called a carrier.

In the above examples Yy, or Gg are carriers

3. Based on epistatic gene action: When expression of one gene depends on presence / absence of another gene in an individual, it is known as gene interaction. Interaction of genes at different loci that affect the same character is called epistasis. The term epistasis was first used by Bateson in 1909 to describe two different genes which control the same character, out of which one masks / suppresses the expression of another gene. Gene that masks the action of another gene is called epistatic gene while the gene whose expression is being masked is called hypostatic gene. Epistatic gene interaction can be of the following types.

(i) Epistatic factors	- 12 : 3 : 1	(ii) Supplementary factors	- 9 : 3 : 4
(iii) Duplicate factors	- 15 : 1	(iv) Complementary factors	- 9 : 7
(v) Additive factors	- 9 : 6 : 1	(vi) Inhibitory factors	- 13 : 3

4. Based on number of genes involved:

a) Monogenic - Each character is controlled by one gene
b) Digenic - Each character is controlled by two genes
c) Oligogenic - Each character is controlled by few genes
d) Polygenic - Each character is controlled by many genes

Polygenic or polymerism: In general, one gene controls or affects a single character. But some characters are known to be controlled by more number of genes. Such genes are called poly genes and the phenomenon is called polymerism.

Eg : Yield in plants.

5. Based on pleiotropism / pleiotropic gene action: In general, one gene affects a single character. But some of the genes are known to affect or control more than one character. Such genes are called pleiotropic genes and the phenomenon is known as pleiotropism. Many fold phenotypic expressions of a single gene is called pleiotropism or pleiotropic gene effects. Eg: White eye gene effects the shape of sperm storage organs and other structures in *Drosophila*. These genes are found in all crop plants. Good example of pleiotropism has been reported in wheat. A gene governing awns in Ona's variety of wheat also increases the yield as well as seed weight.

ALLELES

Alternate forms of a gene is known as allele. Alleles are of two types *viz*., either dominant and recessive or wild type and mutant type.

Characteristic features of alleles:

1. They occupy the same locus on a particular chromosome.
2. They govern the same character of an individual. (T and t – control plant height)
3. A haploid cell has a single copy of an allele for a character. A diploid cell has two copies of an allele for a character, while a polyploidy cell has more than two copies of an allele for a character.
4. An individual may have identical alleles at the corresponding locus of homologous chromosomes in the homozygote or two different alleles in the heterozygote.
5. The alleles may be dominant and recessive or wild and mutant types.

Multiple alleles

Generally a gene has two alternative forms called alleles. Usually one of them is dominant over the other. The two alleles of a gene determine the two contrasting forms of a single character. Ex. Tall (T) and dwarf (t) plant height in garden pea. But in many cases, several alleles of a single gene are known to exist and each one of them governs a distinct form of the concerned character or trait. Such a situation is known as multiple allelism and all the alleles of a single gene are called multiple alleles. Many genes in both animals and plants exhibit multiple alleles. Ex: Blood group in human beings, fur colour / coat colour in rabbits and self-incompatibility alleles in plants.

1. Blood groups in human beings: On the basis of presence / absence of certain antigens, four blood groups in human beings have been established by Karl Landsteiner in 1900. The blood group system in human beings is believed to be controlled by a single gene generally designated as " I." The gene " I" has three alleles. – I^A, I^B and i. Allele I^A controls the production of antigen A, I^B controls the production of antigen B and i does not produce any antigen.

Individuals with the genotype I^AI^A or I^Ai produces antigen 'A' and are classified in blood group A. individuals with genotype I^BI^B or I^Bi are classified in blood group B. Individuals with genotype ii are grouped in 'O' blood group and such individuals produce neither antigen A nor antigen B. individuals with genotype I^AI^B produce both antigens A and B and hence classified as 'AB' blood group.

Human blood groups, their antigen, antibody and compatible blood groups for transfusion:

Blood group	Genotypes	Antigen found	Antibody present	Compatible blood group
A	$I^A I^A$ or $I^A i$	A	B	A and O
B	$I^B I^B$ or $I^B i$	B	A	B and O
AB	$I^A I^B$	AB	None	A, B, AB and O Universal recipient
O	ii	None	AB	O Universal donor

2. Fur or coat colour in rabbits: The fur colour in rabbits is a well known example of multiple alleles. In rabbits, the fur colour is of four types *viz*., agouti, chinchilla, himalayan and albino. It is due to multiple alleles of a single gene 'C'.

Phenotype	Gene symbol	Genotype
1. Agouti	C	CC, Cc^{ch}, Cc^h, Cc
2. Chinchilla	C^{ch}	$c^{ch}c^{ch}$, $c^{ch}c^h$, $c^{ch}c$
3. Himalayan	C^h	c^hc^h, c^hc
4. Albino	c	cc

The order of dominance for fur colour in rabbits can be represented as follows :

C	>	c^{ch}	>	c^h	>	c
Agouti	>	Chinchilla	>	Himalayan	>	Albino
Full colour or wild type		Mixture of coloured and white hairs over the body		Main body is white, while the tips of ears, feet, tail and snout are black		No pigment and with pure white fur colour

3. Self incompatibility alleles in plants: A series of self incompatibility alleles insures cross pollination in many plants. Such alleles were described first in toabacco and later they were found in several other plant species like *Brassica*, radish, tomato, potato etc. In these species, self incompatibility is governed by a single gene 'S' which has multiple alleles *viz*., S_1, S_2, S_3, S_4 and so on.

Characteristic features of multiple alleles:

1. Multiple alleles always belong to the same locus in a chromosome.
2. One allele is present at a locus at a time in a chromosome.
3. Multiple alleles always control the same character of an individual. However, the phenotypic expression of the character will differ depending on the alleles present.
4. There is no crossing over in a multiple allelic series.
5. In a multiple allelic series, wild type is almost always dominant over the mutant type.
6. A cross between two strains homozygous for mutant alleles will always produce a mutant phenotype and never a wild phenotype. In other words, multiple alleles do not show complementation.
7. Further, F_2 generations from such crosses show typical monohybrid ratio for the concerned trait.

Pseudo alleles: Two alleles each of two or more tightly linked genes affecting the same function as a result of which, they appear as multiple alleles. (or) Alleles, which have two separate gene loci, but often inherit together due to close linkage and have very rare chance of crossing over are called pseudo alleles. Eg: Lozenge eye in *Drosophila*.

Iso allele: An allele which is similar in its phenotypic expression to that of other independently occurring allele is called isoallele.

Penetrance; It is the ability of a gene to express itself in all the individuals, which carry it in the appropriate genotype. (or) The frequency with which a gene produces a phenotypic or visible effect in the individuals which carry it in the appropriate genotype is known as penetrance. It refers to the proportion of individuals which exhibit phenotypic effect of a specific gene carried by them.

1. Penetrance of some genes is limited to one sex only. For example : Milk production in cattle or human beings.
2. It may be affected by environmental conditions
3. Penetrance is expressed in percentage

It is of two types

1. **Complete penetrance:** When all the individuals that carry a particular gene exhibit its phenotypic effect, it is known as complete penetrance. In this case, all homozygous dominant individuals will exhibit one phenotype, while all homozygous recessives will exhibit another phenotype. Recessive alleles have no or zero penetrance in heterozygous condition.

   Tall x Dwarf
   TT x tt
   (100%) (100%)
   ↓
   F_1 Tt Tall

   [T exhibits Complete (100%) penetrance while t has no(0%) penetrance]

2. **Incomplete penetrance:** When specific gene does not express their effect in all the individuals which carry them in appropriate genotype, it is known as incomplete penetrance. For example : The recessive gene producing partial chlorophyll deficiency in the cotyledonary leaves of lima bean shows incomplete penetrance as it expresses itself only in 10% of the individuals.

   Almost all the genes showing incomplete penetrance exhibit variable expressivity as well

Expressivity: The degree of phenotypic expression of a gene in different individuals is called expressivity. It is also influenced by environmental conditions in some cases. Expressivity is also two types.

1. **Uniform expressivity:** When the phenotypic expression of a gene is identical or similar in all the individuals, which carry such a gene, it is known as uniform expressivity. Most of the qualitative characters exhibit uniform expressivity. Eg: Seed shape in pea rr genotypes have wrinkled seed shape, while RR or Rr genotypes exhibit round seed shape.

2. **Variable expressivity:** When the phenotypic expression differs in different carriers of a gene, it is known as variable expressivity. Eg: Recessive gene producing partial chlorophyll deficiency in cotyledonary leaves of lima beans.

Lecture No.: 21

QUALITATIVE AND QUANTITATIVE CHARACTERS

The phenotype of any individual can be classified into two types: 1) Qualitative characters and 2) Quantitative characters

1. **Qualitative characters :** The characters that show discontinuous variation and which can not be measured easily are known as qualitative characters. These are also known as classical mendelian traits.

   Eg : Corolla colour – Red → white or pink no continuous variation
   Seed shape – Round → wrinkled variation is not continuous

2. **Quantitative characters** are those showing continuous variation and which can be measured easily. These characters are also known as metric traits. The data obtained from such characters is known as quantitative data. This data can be subjected to statistical analysis and the branch of science which deals with such analysis is known as quantitative genetics or biometrical genetics. Eg : Yield, Plant height

Differences between quantitative and qualitative characters

	Quantitative characters	**Qualitative characters**
Deals with	Traits of degree Eg : Plant height, seed weight, yield etc.	Traits of kind Eg : Corolla colour, seed shape, appearance etc.
Variation	Continuous	Discontinuous
Effect of individual gene	Small and undetectable	Large and detectable
No. of genes involved	Several (polygenic)	one or few (mono / oligogenic)
Grouping into distinct classes	Not possible	Possible
Effect of environment	High	Low
Metric measurement	Possible	Not possible
Statistical analysis	Based on mean, variance, standard deviation etc.	Based on ratios and frequencies
Stability	Low	High

Transgressive segregation of F_2	Yes	No
Dominance effect	No	Yes
Cumulative effect of each gene	Yes	No

MULTIPLE FACTOR HYPOTHESIS

In early days of mendelian genetics it was thought that there was a fundamental difference in inheritance pattern of quantitative and qualitative traits. One of the examples which helped to bridge the gap between these two kinds of traits is "multiple gene model" developed by Swedish Geneticist H. Nilsson Ehle in 1910 to explain inheritance of kernel colour in wheat. In studies on inheritance of kernel colour in wheat and oats, he obtained 3:1, 15:1 and 63:1 ratio between coloured and white seeds from different crosses. It is clear from these ratios that the seed colour was governed by one (3:1 ratio in F_2), two (15:1 ratio in F_2) or three (63:1 ratio in F_2) genes. Nilsson-Ehle found that in crosses showing 15:1 ratio in F_2 kernel colour is governed by two genes. However, on a closer examination of the coloured seeds, he found that there was a marked difference in the intensity of their colour. When he crossed a dark red strain to a white strain (or variety), he observed that all the F_1s were medium red (intermediate between the parental types). But in F_2 generation only one out of the sixteen were of parental types. He interpreted the results in terms of two genes, each with a pair of alleles exhibiting cumulative effects. Each of the dominant alleles R_1 and R_2 adds some red colour to the phenotype, while the recessive alleles r_1 and r_2 add no colour to the phenotype. Thus dark red genotypes contain only R_1 and R_2 alleles while the white genotype contains none of these alleles.

Parents	Female	x	Male
Phenotype	Dark red	x	White
Genotype	$R_1 R_1 R_2 R_2$	x	$r_1 r_1 r_2 r_2$
Gametes	R_1R_2		$r_1 r_2$
Generation		F_1	

? ?	$r_1\ r_2$
$R_1\ R_2$	$R_1\ r_1\ R_2\ r_2$ Medium Red

On selfing

Parents	Female	x	Male
Phenotype	Medium Red	x	Medium Red
Genotype	$R_1\ r_1\ R_2\ r_2$	x	$R_1\ r_1\ R_2\ r_2$
gametes	$R_1 \rightarrow R_2 \rightarrow R_1\ R_2$ $r_2 \rightarrow R_1\ r_2$ $r_1 \rightarrow R_2 \rightarrow r_1\ R_2$ $r_2 \rightarrow r_1\ r_2$		$R_1 \rightarrow R_2 \rightarrow R_1\ R_2$ $r_2 \rightarrow R_1\ r_2$ $r_1 \rightarrow R_2 \rightarrow r_1\ R_2$ $r_2 \rightarrow r_1\ r_2$

Generation F_2

? ?	$R_1\ R_2$	$R_1\ r_2$	$r_1\ R_2$	$r_1\ r_2$
$R_1\ R_2$	$R_1\ R_1\ R_2\ R_2$ Dark Red	$R_1\ R_1\ R_2\ r_2$ Red	$R_1\ r_1\ R_2\ R_2$ Red	$R_1\ r_1\ R_2\ r_2$ Medium Red
$R_1\ r_2$	$R_1\ R_1\ R_2\ r_2$ Red	$R_1\ R_1\ r_2\ r_2$ Medium Red	$R_1\ r_1\ R_2\ r_2$ Medium Red	$R_1\ r_1\ r_2\ r_2$ Light Red
$r_1\ R_2$	$R_1\ r_1\ R_2\ R_2$ Red	$R_1\ r_1\ R_2\ r_2$ Medium Red	$r_1\ r_1\ R_2\ R_2$ Medium Red	$r_1\ r_1\ R_2\ r_2$ Light Red
$r_1\ r_2$	$R_1\ r_1\ R_2\ r_2$ Medium Red	$R_1\ r_1\ r_2\ r_2$ Light Red	$r_1\ r_1\ R_2\ r_2$ Light Red	$r_1\ r_1\ r_2\ r_2$ White

Phenotypic ratio: 1 Dark Red : 4 Red : 6 Medium Red : 4 Light red : 1 white

Genotypic ratio: 1 $R_1\ R_1\ R_2\ R_2$: 2 $R_1\ R_1\ R_2\ r_2$: 1 $R_1\ R_1\ r_2\ r_2$
: 2 $R_1\ r_1\ R_2\ R_2$: 4 $R_1\ r_1\ R_2\ r_2$: 2 $R_1\ r_1\ r_2\ r_2$
: 1 $r_1\ r_1\ R_2\ R_2$: 2 $r_1\ r_1\ R_2\ r_2$: 1 $r_1\ r_1\ r_2\ r_2$

S.No.	No. of alleles for red colour	Phenotype	Frequency	Genotype	Frequency
1.	4	Dark Red	1	$R_1 R_1 R_2 R_2$	1
2.	3	Red	4	$R_1 R_1 R_2 r_2$	2
				$R_1 r_1 R_2 R_2$	2
3.	2	Medium Red	6	$R_1 R_1 r_2 r_2$	1
				$R_1 r_1 R_2 r_2$	4
				$r_1 r_1 R_2 R_2$	1
4.	1	Light Red	4	$R_1 r_1 r_2 r_2$	2
				$r_1 r_1 R_2 r_2$	2
5.	0	white	1	$r_1 r_1 r_2 r_2$	1

Lecture No: 22

SEX DETERMINATION

Sex refers to the contrasting features of male and female individuals of the same species. Thus sex is usually of two types *viz*., male and female. Sex determination is a process of sex differentiation which utilizes various genetical concepts to decide whether a particular individual will develop into male or female. Plants also have sex as there are male and female parts in flowers. All organisms, however do not possess only two sexes. Some of the protozoa may have as many as eight sexes. In most higher organisms, the number of sexes has been reduced to just two. The sexes may reside in different individuals or within the same individual. An animal possessing both male and female reproductive organs is usually referred to as hermaphrodite. In plants where staminate and pistillate flowers occur in the same plant, the term of preference is monoecious Eg. maize, castor, coconut etc. However, most of the flowering plants have both male and female parts within the same flower (perfect flower). Relatively few angiosperms are dioecious i.e. having male and female elements in different individuals Eg: cucumber, papaya, asparagus, date palm, hemp and spinach. The sex cells and

reproductive organs form the primary sexual characters of male and female sexes. Besides these primary sexual characters, the male and female sexes differ from each other in many somatic characters known as secondary sexual characters.

Whether or not there are two or more sexes in the same or different individuals is relatively unimportant. The importance of sex itself is that it is a mechanism, which provides for the great amount of genetic variability characterizing most natural populations.

The various mechanisms of sex determination are:

1. Chromosomal sex determination
2. Genic balance mechanism
3. Male haploidy or Haplodiploidy mechanism
4. Single gene effects (or) monofactorial mechanism of sex determination
5. Metabolically controlled mechanism
6. Hormonally controlled mechanism
7. Sex determination in *Coccinia indica* and *Melandrium album*,
8. Sex determination due to environmental factors

I. Chromosomal sex determination: The chromosomes, which have no relation with sex and contain genes, which determine the somatic characters of an individual are known as autosomes. These chromosomes do not differ in morphology and number in male and female sex. Those chromosomes, which differ in morphology and number in male and female sex and contain genes responsible for the determination of sex are known as allosomes or sex chromosomes.

Differences between Autosomes and Allosomes

Autosomes	Allosomes or Sex chromosomes
1. Refer to other than sex chromosomes.	1. These are sex chromosomes.
2. Morphology is similar in male and female sex.	2. Morphology is different in male and female sex.
3. The number is same in both the sexes.	3. The number is sometimes different in male and female sex.
4. Generally control traits other than	4. Usually determine sex of an

sex.	individual.
5. Number of autosomes differs from species to species.	5. Each diploid organism usually has two allosomes.
6. Do not exhibit sex linkage.	6. Exhibit sex linkage.

The chromosomal influence on sex, in certain insects, has been shown for the first time by McClung in 1902 to be associated with a special sex determining 'X' chromosome. McClung proposed that a male had one 'X' chromosome per cell (XO) and a female has two 'X' chromosomes (XX). Later Stevens and Wilson (1905) found same number of chromosomes in both sexes of milk weed bug. In females all chromosomes were paired and the homologues were equal in size (homomorphic). In the male, all the chromosomes were paired, but the chromosome identified as homologous to the " X" Chromosome was distinctly smaller and was called the "Y" Chromosome (Heteromorphic).

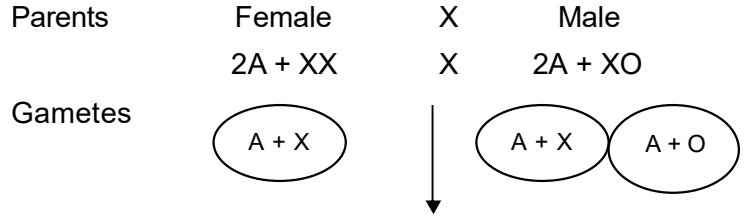
Thus, allosomes are generally of X and Y types, but in birds they are of Z and W types. Sex with similar type of sex chromosomes (XX or ZZ) is known as homogametic sex and with dissimilar type of sex chromosomes (XY or ZW) as heterogametic sex. These are two types: a) Heterogametic male and b) Heterogametic female

a) Heterogametic male: In this mechanism, the female sex has two 'X' chromosomes, while the male sex has only a single 'X' chromosome. As the male lacks a 'X' chromosome during meiosis, 50% of lthe gametes carry 'X' chromosome, while the rest do not have the 'X' chromosome. Such a mechanism, which produces two different types of gametes in terms of sex chromosome is called heterogametic sex. The female sex here is called homogametic sex because it produces similar type of gametes. The heterogametic male may be of the following two types.

i) XX – XO ii) XX – XY

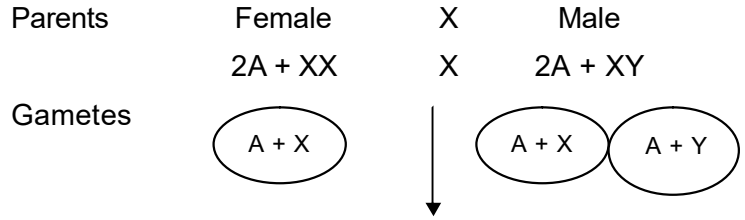
i) XX - XO: In certain insects belonging to orders *Hemiptera* (true bugs), *Orthoptera* (grass hoppers) and *Dictyoptera* (cockroaches), female has two 'X' chromosomes (XX) and are, thus homogametic, while male has only single 'X' chromosome (XO). This mechanism was found by C.E. McClung in 1902. The presence of an unpaired X chromosome determines the masculine sex. The

male being heterogametic sex produces two types of sperms, half with X chromosome and half without X chromosome in equal proportions. The sex of the offspring depends upon the sperm that fertilizes the egg, each of which carries a single X chromosome. Thus fertilization between male and female gametes always produced zygotes with one 'X' Chromosome from the female, but only 50% of the zygotes have an additional X Chromosome from the male. In this way, 'XO' and 'XX' types would be formed in equal proportions, the former being males and the latter being females.



	A + X	**A + O**
A + X	2A + XX Female	2A + XO Male

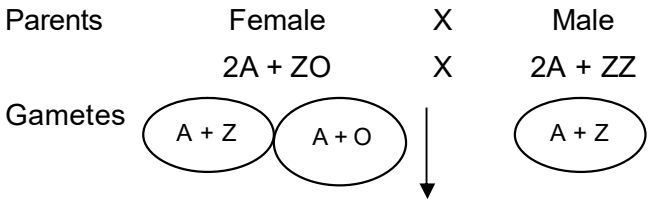
ii) XX – XY: In man, other mammals, certain insects including *Drosophila*, certain angiospermic plants including *Melandrium*, the females possess two X chromosomes (XX) and are thus homogametic and homomorphic, while the males possess one X and one Y chromosome (XY) and are hence heterogametic and heteromorphic. When an egg is fertilized by 'Y' bearing sperm, a male is produced.



	A + X	**A + Y**
A + X	2A + XX Female	2A + XY Male

b) Heterogametic female: In this mechanism the male possess two homomorphic sex chromosomes and are thus homogametic, while the female possesses either a single 'X' chromosome or one 'X' and one 'Y' chromosome and are hence heterogametic. To avoid confusion with earlier types, instead of X and Y, the alphabets Z and W are used. This mechanism of sex determination is also known as "Abraxas mechanism of sex determination" (Kuspira and Walker, 1973) The heterogametic females may be of following two types. i) ZO – ZZ ii) ZW – ZZ

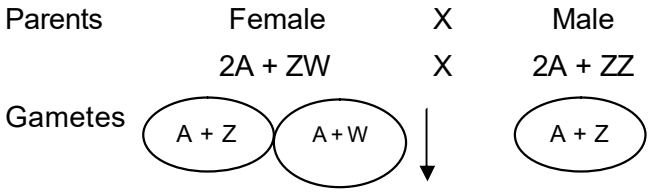
i) ZO – ZZ: This mechanism is found in certain moths and butterflies. In this case, female possess one single 'Z' chromosome and hence is heterogametic. Male possesses two Z chromosomes and thus homogametic.



	A + Z
A + Z	2A + ZZ Male
A + O	2A + ZO Female

The sex of the offspring depends on the kind of egg.

ii) ZW – ZZ: This system is found in certain insects (gypsy moth) and vertebrates such as fishes, reptiles and birds. In this system, the female is heterogametic and produces two types of gametes, one with 'Z' chromosome and the other with 'W' chromosome. On the other hand, male is homogametic and produces all sperms of same type carrying one 'Z' chromosome. The sex of the offspring depends on the kind of egg being fertilized. The 'Z' chromosome bearing eggs produce males, but the 'W' chromosome bearing eggs produce females.



	A + Z

A + Z	2A + ZZ Male
A + W	2A + ZW Female

II) Genic balance mechanism: By studying the sex chromosomal mechanism of sex determination, it may appear at first glance that some genes carried by sex chromosomes i.e. X and Y are entirely responsible for determining sex. But this may not always be true. Extensive experiments on different organisms by different workers have revealed the fact that most organisms generally have inherent potentialities for both sexes and each individual is found to be more or less intermediate between male and female. Hence may be referred to as inter sex. There seems to exist a delicate balance of masculine and feminine tendency in the hereditary compliment of an individual. Such a genic balance mechanism of determination of sex was first observed and studied by C.B. Bridges in 1921 while working with *Drosophila* for the inheritance of vermillion eye colour. According to this mechanism, the sex of an individual in *Drosophila melanogaster* is determined by a balance between the genes for femaleness located in the X-chromosome and those for maleness located in autosomes. Hence, the sex of an individual is determined by the ratio of number of its X chromosomes and that of its autosomal sets, the 'Y' chromosome being unimportant. The ratio is termed as sex index and is expressed as follows.

$$\text{Sex index} = \frac{\text{No. of X chromosomes}}{\text{No. of autosomal sets}} = \frac{X}{A}$$

Different doses of X – Chromosomes and autosome sets and their effect on sex determination

S.No.	Ploidy level	X-Chromo-somes	Sets of autosomes	Sex index (X/A ratio)	Sex Expression
1.	Diploid	3(xxx)	2(AA)	1.50	}Super female
2.	Triploid	4(xxxx)	3(AAA)	1.33	} or meta female }
3.	Haploid	1(x)	1(A)	}	
4.	Diploid	2(xx)	2(AA)	}	
5.	Triploid	3(xxx)	3(AAA)	}1.00	Female
6.	Tetraploid	4(xxxx)	4(AAAA)	}	
7.	Triploid	2(xxy)	3(AAA)	0.67	}

8.	Tetraploid	3(xxxy)	4(AAAA)	0.75	} Inter sex }
9.	Diploid	1(xy)	2(AA)	}	
10.	Tetraploid	2(xxyy)	4(AAAA)	} 0.5	Male
11.	Triploid	1(xyy)	3(AAA)	0.33	Super male or meta male

Individuals with sex index of 0.5 develop into normal males and those with sex index of 1 into normal females. If the sex index is between 0.5 and 1, the resulting individuals will be neither a female nor a male, but have an intermediate sex expression and is called inter sex. Such individuals are sterile. Some flies have sex index of >1, such flies have more pronounced female characteristics than normal fermales and are called super females or metafemales. These are generally weak, sterile and non-viable. Super male flies have a sex index value of <0.5 and are also weak, sterile and non-viable.

Bridges drew the observation by crossing triploid females (3A + XXX) with normal diploid males (2A + XY). From such a cross he obtained normal diploid females, males, triploid females, intersexes, super males and super females. The occurrence of triploid intersexes from such a cross clearly established that autosomes also carry genes for sex determination. Triploid individuals, which had two 'X' Chromosomes as in the case of normal female, here were inter sexes as they had an extra set of autosomes indicating that the autosomes play a definite role in the determination of sex.

Results obtained from a cross of a triploid (3A+XXX) female fly with a diploid (2A+XY) male fly in *Drosophila*

Parents	Female	X	Male
Phenotype	Triploid	X	Diploid
Genotype	3A + XXX	X	2A + XY
Gametes	2A + XX, A + X, 2A + X, A + XX		A + X, A + Y

	A + X	**A + Y**
2A + XX	3A + XXX Triploid female	3A + XXY Triploid inter sex

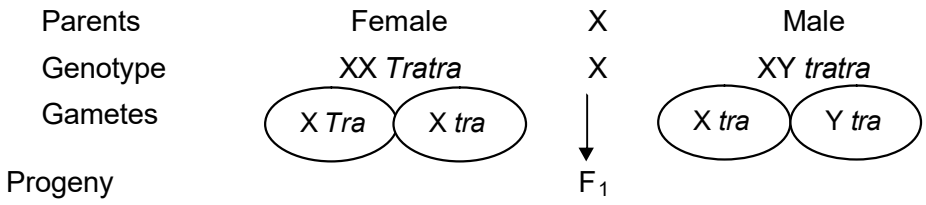
A + X	2A + XX Diploid female	2A + XY Diploid male
2A + X	3A + XX Triploid inter sex	3A + XY Super male
A + XX	2A + XXX Super female	2A + XXY Diploid female

III. Male haploidy or haplodiploid mechanism or arrhenotokous parthenogenesis: It is common in hymenopterous insects (ants, bees, wasps). In honey bees, queens usually mates only once during its life time and the sex ratio of offspring is under the control of queen. Fertilized eggs develop into diploid female and those eggs which the queen chooses not to be fertilized develop parthenogenetically into haploid but fertile males (drones). This phenomenon is known as arrhenotoky and is a form of reproduction as well as a means of sex determination. Meiosis is normal during oogenesis in case of females and produces all haploid eggs. But crossing over and reduction division fails to occur during spermatogenesis in males due to their haploid nature. Thus arrhenotokous parthenogenesis determines the sex in hymenopterans and sex chromosomes have no identity here (unlike *Drosophila*). It seems that heterozygosity for specific genes induces femaleness. The haploid can never be heterozygous. Most of the eggs laid in the hive will be fertilized and developed into worker females. Further during investigation, it has been found that the quantity and quality of food available to the diploid larvae determines whether that female will become a sterile worker or a fertile queen. The diploid larva, which feed on royal jelly, develop into fertile female called queen and the remaining larvae give rise to workers, which are sterile females. Thus, environment here determines sterility or fertility but does not alter the genetically determined sex.

IV. Single gene effect or monofactorial mechanism of sex determination: In *Neurospora*, *Asparagas*, *Drosophila*, maize and *Asparagus*, sex determination is influenced by the differential action of a single autosomal gene, which over rules the effect of sex chromosomes present in the individual.

Autosomal recessive transformer (*tra*) gene of *Drosophila*; when it is present in the homozygous recessive state, it transforms the female (XX) zygote to develop into males which are sterile. The gene *tra* is recessive and hence does

not have any effect in heterozygous condition (*Tratra*) on either sex i.e male or female. In homozygous condition, (*tratra*), this gene has no effect in male. When a heterozygous (XX *Tratra*) female is mated with a homozygous recessive male (XY *tratra*), only 25% of the progeny are females (heterozygous) while remaining 75% are males. Among the males, 1/3 are sterile (XX individuals homozygous recessive for tra gene) as they are transformed to maleness by the *tra*.



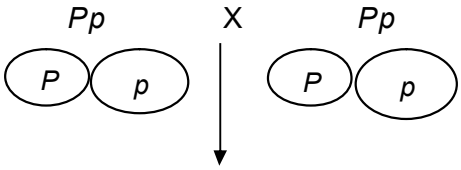
	X *tra*	**Y *tra***
X *Tra*	XX *Tratra* Female fertile	XY *Tratra* Male fertile
X *tra*	XX *tratra* Male sterile (Transformed)	XY *tratra* Male fertile

Ratio: 3 Males : 1 Female

Monogenic control of sex has also been reported in some plants like *Asparagus*, maize, papaya, spinach, etc. *Asparagus* is a dioecious plant. However, rarely female flowers bear rudimentary anthers and male flowers bear rudimentary pistils. Occasionally, male flowers with poorly developed pistils set seed on selfing and segregate in 3 : 1 ratio of male and female.

Segregation of sex in seed obtained from a rare bisexual flower in *Asparagus* showing monogenic control

Rare male plant when selfed



	P	***p***
P	*PP* Male	*Pp* Male
p	*Pp*	*pp*

	Male	Female

Ratio: 3 Males : 1 Female

Maize being a monoecious plant bears both female (silk) and male (tassel) inflorescences on the same plant. A recessive gene *ba* (barren cob) in homozygous condition (*baba*) makes the cobs barren or non-functional. Similarly, a recessive gene *ts* in homozygous condition (*tsts*) converts the male flowers of tassel into female flowers. Thus, homozygous state of gene *ba* (*baba*) converts the monoecious plant into male. Similarly, gene *ts* in homozygous condition (*tsts*) converts the monoecious plant into female. The plants with both dominant genes (*Ba_Ts_*) are monoecious, with *babaTs_* normal male, with *Ba_tsts* female, and with *babatsts* rudimentary females.

In papaya, the sex is postulated to be governed by three alleles, *viz*., *m*, M_1 and M_2 of a single gene. Homozygous recessive (*mm*) produces female plants, heterozygous, *viz*., M_1m and M_2m produce male and hermaphrodite plants, respectively. However, combination of both dominant alleles (M_1 and M_2) produces inviable plants both in homozygous (M_1M_1 and M_2M_2) and heterozygous conditions (M_1M_2). Crosses between female (*mm*) and male (M_1m) produce females (*mm*) and males (M_1m) in 1 : 1 ratio. Similarly, crosses between female (*mm*) and hermaphrodite (M_2m) will produce females (*mm*) and hermaphrodite (M_2m) in 1 : 1 ratio. Selfing of hermaphrodite (M_2m) plants produce hermaphrodite (M_2m) and female (*mm*) progeny in 2 : 1 ratio and about ¼ of the zygotes (M_2M_2) do not survive.

V. Metabolically controlled mechanism: Riddle found that metabolism has some definite role in the determination of sex in pigeon and dove because increased rate of metabolism lead to the development of maleness while decreased rate of metabolism caused femaleness.

VI. Hormonally controlled mechanism: Crew in 1923 reported a complete reversal of sex in hen. Female chicken that have laid eggs been known to undergo not only a reversal of the secondary sexual characteristics such as development of cock feathering, spurs and crewing, but also the development of testis and even the production of sperm cells (primary sexual characteristics). This might have occurred when a disease destroyed the ovarian tissue and in absence

of female sex hormones, rudimentary testicular tissue present in center of ovary began to proliferate or multiply and secrete male hormones, which lead to the development of maleness.

Sexual differentiation in man is influenced by hormones. When the testis of the male is removed before puberty, female characteristics of body form, voice and hair pattern develop in the adult. Tumors of adrenals in women are associated with the development of masculine characters such as low pitched voice and increased growth of hair.

VII. Sex determination in *Coccinia indica* and *Melandrium album*: In these two organisms, studies have revealed that irrespective of number of 'X' chromosomes, and / or autosomal sets, presence of a single 'Y' chromosome is essential to produce male flowers in diploid, triploid and tetraploid species. The pistillate plants are XX and the staminate plants are XY.

Ploidy level	Chromosome Constitution (Autosomes) + (Allosomes)	Sex Expression	X/A ratio
Diploid	AA + XX	Female	1.00
Diploid	AA + XY	Male	0.50 (1/2)
Diploid	AA + XYY	Male	0.50 (1/2)
Triploid	AAA + XXX	Female	1.00
Triploid	AAA + XXY	Male	0.67 (2/3)
Tetraploid	AAAA + XXXX	Female	1.00
Tetraploid	AAAA + XXXY	Male	0.75 (3/4)

VIII. Sex determination due to environmental Factors: In many reptiles, sex is determined by environmental factors like temperature. In most turtle species only females are produced at high temperature (30 – 35ºC) while only males are produced at low temperatures (23 – 28ºC). Sex ratio changes suddenly from all males to all females with just change in temperature of 2ºC during the incubation. In Crocodiles and Lizards, (reverse is the case) the males are produced at high temperature while females are produced at low temperature. In *Bonellia viridis*, a marine worm, all larvae are genetically and cytologically similar. If a particular larva settles near proboscis of adult female, it becomes a male individual. If larva develops freely in water, it becomes a female. In some plants, sex determination depends on day length, temperature and hormones. For example, in cucumber

(*Cucumis sativa*) and muskmelon, treatment with ethylene enhances production of female flowers.

Sex linked inheritance

The characters for which genes are located on sex or 'X' or analogous 'Z' chromosomes are known as sex linked traits. Such genes are called sex linked genes and linkage of such genes is referred to as sex linkage. Inheritance of such genes or characters is known as sex linked inheritance. The sex linkage was first discovered by T.H. Morgan in *Drosophila* and the first sex linked gene found in *Drosophila* was recessive gene 'w' responsible for white eye colour.

Parents	Female	X	Male
Phenotype	White eye	X	Red eye
Genotype	$X^w X^w$	X	X^+Y
Gametes	X^w		X^+ , Y

	$\mathbf{X^+}$	**Y**
$\mathbf{X^w}$	X^+X^w Red eye female	X^wY White eye male

When white eyed females are crossed with wild type (red eye) males, all the male offspring have white eyes like the mother and all the female offspring have red eyes like their father.

In human beings, colour blindness and haemophilia (bleeder's disease) are well known hereditary characters showing a peculiar relationship to sex.

Colour blindness: A marriage between colour blind woman and a normal man gives rise to all normal dauthers and colour blind sons.

Parents	Mother	X	Father
Phenotype	Colour blind	X	Normal
Genotype	$X^c X^c$	X	X^+Y
Gametes	X^c		X^+ , Y

	$\mathbf{X^+}$	**Y**
$\mathbf{X^c}$	X^+X^c Normal daughter	X^cY Colourblind son

The allele for colour blindness (c) is found on both 'X' chromosomes of mother and therefore she is colour blind. The only one 'X' chromosome of father in this marriage carries a wild type (+) allele and hence he has normal vision. The 'Y' chromosome lacks both the alleles (+ and c). The reduction division produces one kind of egg in contrast to two kinds of sperms. Fertilization results in the usual sex ratio of 1 male : 1 female. All the daughters have normal vision since they receive dominant allele '+' from their father. All the sons are colour blind, because their single 'X' chromosome derived from mother carries the allele 'c' for colour blindness. This result is known as crisscross inheritance because daughters are normal like father and sons have colour blindness like mother. However, the daughters are heterozygous carriers. This crisscross method of inheritance is characteristic of sex-linked genes. This peculiar type of inheritance is due to the fact that Y chromosome carries no alleles homologous to those on the X chromosome. Thus males carry only one allele for sex linked traits. This one allelic condition is termed as hemizygous in contrast to homozygous and heterozygous possibilities in female. The expression of recessive gene in hemizygous condition is termed as pseudo-dominance.

The inheritance of colour blindness can be studied in the following three other possible types of marriages:

a) Marriage between normal woman and colour blind man:

Parents	Mother	X	Father
Phenotype	Normal	X	Colour blind
Genotype	$X^+ X^+$	X	$X^c Y$
Gametes	X^+		X^c Y

	X^c	**Y**
X^+	$X^+ X^c$ Normal daughter (Carrier)	X^+Y Normal son

b) Marriage between normal (carrier) woman and normal man:

Parents	Mother	X	Father
Phenotype	Normal	X	Normal
Genotype	$X^+ X^c$	X	$X^+ Y$
Gametes	X^+ X^c		X^+ Y

	X^+	Y
X^+	X^+X^+ Normal daughter	X^+Y Normal son
X^c	X^+X^c Normal daughter	X^cY Colour blind son

c) Marriage between normal (carrier) woman and colour blind man:

Parents	Mother	X	Father
Phenotype	Normal	X	Colour blind
Genotype	X^+X^c	X	X^cY
Gametes	X^+ X^c	↓	X^c Y

	X^c	Y
X^+	X^+X^c Normal daughter	X^+Y Normal son
X^c	X^cX^c Colour blind daughter	X^cY Colour blind son

Results of possible four marriages make it clear why there are more colour blind males than females in the population. In three marriages colour blind sons were produced where as in only one of the marriages, colour blind daughters were observed, where the mother is heterozygous (carrier) and the father is colour blind. Nearly all colour blind women must come from the last type of marriage, since the only other possible source of colour blind females is mating between two colour blind persons – naturally a rare occurrence.

Haemophilia is a recessive sex linked disease and the inheritance pattern of haemophilia is similar to that of colour blindness in human beings.

Genes present in the non-homologous region of the Y chromosome pass directly from male to male. In man, the genes present on Y chromosome (holandric genes) such as the gene causing hypertrichosis (causing excessive development of hairs on the pinna of ear) are transmitted directly from father to son.

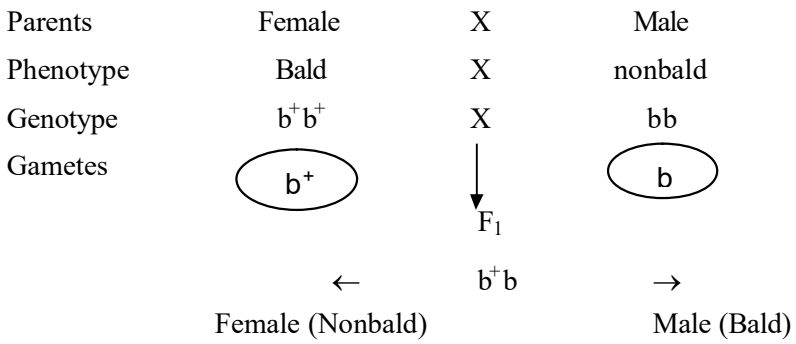
Sex influenced inheritance

Sex influenced genes are the autosomal genes present in both males and females, whose phenotypic expression is different in different sexes in such a way that they act as

dominant in one sex and recessive in the other i.e. in a pair of alleles one seems to be dominant in males while the other in females.

Eg.: Pattern baldness in human beings and horns in sheep. Pattern baldness in human beings is a condition in which a low fringe of hair is present on the head in human beings. It is a genetically inherited condition, where the allele for baldness B is dominant in males and recessive in females. In heterozygous condition, males are bald and females are non-bald. If a woman heterozygous for this gene marries a heterozygous bald man, in the offspring, the ratio of bald to non-bald in males is 3 : 1, while in females it is 1 : 3.

Inheritance of pattern baldness in human being



Phenotypic expression of pattern baldness in man and woman

Genotype	Phenotype	
	Female	**Male**
$b^+ b^+$	Nonbald	Nonbald
b^+b	Nonbald	Bald
bb	Bald	Bald

Sex limited characters or Secondary Sexual characters

Sex limited genes are autosomal genes, whose phenotypic expression is limited to one sex only. Their phenotypic expression is influenced by the sex hormones. The sex limited genes are mainly responsible for secondary sex characters in cattle, human beings and fowl. Eg.: milk production in cattle, beard development in human beings, plumage in male fowls etc.

Milk production in cattle : Just as the cow, the bull carries genes for milk production, but the bull obviously cannot express this trait. Bull may however transmit these genes for

high milk production to the female progeny and the male progeny are unable to express this trait. Some bulls are so well andowed with such genes that they are known to breed calves, which always yield greater milk than their mothers.

However, in plants no secondary sexual characters are known except the absence of one or the other sporangia.

Differences between sex linked and sex limited characters

Sex Linked characters	Sex Limited characters
1. They are located on sex or X chromosome	1. They are located on sex chromosomes or autosomes
2. They can express in both the sexes	2. They can express in one sex only
3. Include characters not related to sex	3. Include primary and secondary sex characters
4. Examples: White eye in *Drosophila*, haemophilia and colour blindness in human beings	4. Examples: milk production in cattle, beard development in human beings, plumage in male fowls etc

Inter sex

In a few rare cases, various mixtures of male and female characteristics may occur in animals, which normally have separate sexes because of various abnormalities of chromosomes or hormonal deficiencies. Such individuals are called inter sex. These can be of two types.

1. **Pseudo-hermaphrodites:** In mammals there are rare cases in which, both sexes are well developed in one body, these are abnormal and hence sterile.
2. **Gynandromorphs or gynanders:** Among animals and insects that do not have sex hormones, there may be sex intergrades with distinct areas of the body showing male and female tissues. For example: *Drosophila gynander*, a bilateral sex mosaic, is male on one side and female on the other.

Lecture No: 23

CYTOPLASMIC INHERITANCE

Inheritance due to genes located in cytoplasm (plasmagenes) is called cytoplasmic inheritance. Since genes govering traits showing cytoplasmic inheritance are located outside the nucleus and in the cytoplasm, they are refered to as plasmagenes. The sum total of genes present in the cytoplasm of a cell or an individual is known as plasmon. The plasmagenes are located in DNA present in mitochondria (mt DNA) and in chloroplasts (cp DNA). Together both the DNAs are called organelle DNA. Therefore, this type of inheritance is often referred to as organellar inheritance, plastid inheritance or mitochondrial inheritance. In this, generally, the character of only one of the two parents (usually female) is transmitted to the progeny. Hence such inheritance is usually referred to as extra-nuclear or extra-chromosomal or maternal or uniparental inheritance.

The cytoplasmic inheritance is of two types: 1) Plastid inheritance and 2) mitochondrial inheritance.

1. **Plastidial or chloroplast inheritance:** Plastids self duplicated and have some amount of DNA and plays an important role in cytoplasmic inheritance. Plastids have green pigments called chloroplasts. chloroplasts contain a unique circular DNA (cp DNA) in the stroma that is completely different from the nuclear genome. Some examples of plastid inheritance are given below:

a) Leaf variegation in *Mirabilis jalapa*: The conclusive evidence for cytoplasmic inheritance was first presented by C. Correns in *Mirabilis jalapa* (Four 'O' clock plant) in 1909. He studied inheritance of leaf variegation in *M. jalapa*. Variegation refers to the presence of white or yellow spots of variable size on the green background of leaves. In *M. jalapa*, leaves may be green, white or variegated. Some branches may have only green, only white or only variegated leaves. Correns made crosses in all possible combinations among the flowers produced on these three types of branches. When flowers from green branch were used as female parent, all the progeny were green irrespective of the phenotype (green, white or variegated) of male parent. Similarly, progeny from crosses involving flowers bloomed on white branches as female parent were all white irrespective of the phenotype of male parent. But in progeny from all crosses involving flowers born on variegated branches as female parent, all the three types i.e. green, white and variegated individuals were recovered in variable proportions.

Female Parent	x	Male Parent	
Green	x	Green	
	x	White	Green
	x	Variegated	
White	x	Green	
	x	White	Pale green
	x	Variegated	
Variegated	x	Green	Green, white and variegated
	x	White	in variable ratio in each of the
	x	Variegated	cases.

The green leaf branches have normal chloroplasts, white branches have mutant chloroplasts and variegated have a mixture of both normal and mutant chloroplasts. The above results indicated that the inheritance is governed by chloroplasts. Since the cytoplasm is contributed to the zygote mainly by female parent, the plastids are transmitted to the zygote from the female parent. Thus the plastids are responsible for variation in the crosses of green, white and variegated leaves.

b) Iojap in maize : In maize, there are three types of leaves i.e. green, iojap (green and white stripes) and white. The green leaves have normal plastids. Iojap leaves have a mixture of normal and mutant plastids and white leaves have only mutant plastids. In a cross between green female and iojap male, only green individuals are produced in F_1 generation. But in the reciprocal cross (iojap female and green male) all the three kinds of progeny are obtained in variable proportions in F_1.

Parents	Female	x	Male
Phenotype	Green	x	Iojap
		↓	
Generation		F_1	
		Green	

Reciprocal cross

Parents	Female	x	Male
Phenotype	Iojap	x	Green
		↓	
Generation		F_1	
		Green, Iojap, White in variable proportion	

These reciprocal differences can be attributed to the type of plastids in the egg cell since only female parent is contributing cytoplasm to the zygote.

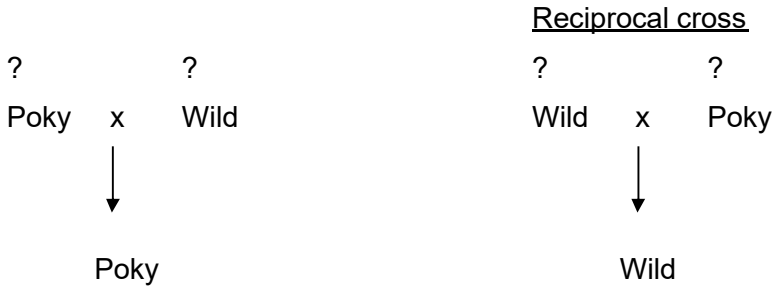
2. Mitochondrial inheritance: The inheritance of some characters, such as cytoplasmic male sterility in plants, pokyness in *Neurospora* etc., is governed by mitochondrial DNA (mtDNA).

a) Cytoplasmic Male Sterility (CMS) in maize : In several crops, cytoplasmic control of male sterility is known. In maize, cytoplasmic male sterility (CMS) is governed by mitochondrial DNA. In such cases, if female parent is male sterile, F_1 progeny also will be male sterile, because cytoplasm is mainly derived from female parent.

?		?
Male sterile	x	Male fertile
	↓	

Male sterile (CMS)

b) Pokyness in *Neurospora*: *Neurospora*, which is a breadmold has two strains i.e. wild and poky. The wild strain has normal growth. While the poky which is a mutant has very slow growth. A cross between a poky female and a wild male produce only poky progeny. In reciprocal cross (a cross between wild female and poky male) all the progeny would be wild. This suggests the presence of cytoplasmic inheritance because only difference between the reciprocal crosses is in the main contributor of cytoplasm.



Characteristic features of cytoplasmic inheritance

1. **Reciprocal difference :** Reciprocal crosses show marked differences for characters governed by plasmagenes. In most cases, plasmagenes from only female parent are transmitted and hence this phenomenon is also called uniparental inheritance.
2. **Lack of segregation :** In general, F_1, F_2, F_3 and subsequent generations do not show segregation for a cytoplasmically inherited trait, as F_1 individuals receive plasmagenes from female parent only.
3. **Somatic segregation :** Plasmagenes generally show the features in somatic tissues such as leaf variegation features which is of rare occurrence in case of nuclear genes.
4. **Association with organelle DNA:** Several plasmagenes have been shown to be associated either with chloroplast or mitochondrial DNA. For example: Cytoplasmic Male Sterility (CMS) in sorghum and maize is associated with mitochondrial DNA.
5. **Nuclear transplantation:** Nuclear transplantation means nucleus of a cell is removed and replaced by nucleus of another genotype from a different cell. If nuclear transplantation reveals a trait to be governed by genotype of cytoplasm and not by that of nucleus, it clearly indicates that the trait or character is governed by cytoplasmic inheritance.

6. **Mutagenesis:** Some mutagens are highly specific mutagens which act only on the plasmagenes and do not affect nuclear genes Eg; ethidium bromide, Induction of mutations by such agents or chemicals in a gene clearly indicates that it is a plasmagene.
7. **Lack of chromosomal location:** In many organisms extensive linkage maps of nuclear genes are available. If a gene is shown to be located in one of these linkage groups, obviously it cannot be a plasmagene.
8. **Transfer of nuclear genome through back crosses:** Nucleus of a variety or species may be transferred into cytoplasm of another variety or species through repeated back crossing with former, which is used as recurrent male parent. Lines produced in this way are called alloplasmic lines, since they have cytoplasm and nucleus from different species.
9. **Lack of association with a parasite or symbiont or virus :** Only those cytoplasmically inherited traits which are not associated with parasites, symbionts or viruses can be regarded to be governed by plasmagenes.

Differences between chromosomal (nuclear) and extra-chromosomal (cytoplasmic or extra-nuclear or maternal) inheritance

S.No.	Character	Chromosomal inheritance	Extra-chromosomal inheritance
1	Location of hereditary factors	Nucleus	Cytoplasm
2.	Associated with	Chromosomes	Chloroplasts and mitochondria
3.	Pattern of Inheritance	Can be explained by mendelism	Cannot be explained by mendelism
4.	Individual hereditary factors are known as	Genes	Plasmagenes
5.	Hereditary factors are collectively known as	Genome	Plasmon
6.	Characters of F_1 progeny	May show dominance or may be intermediate between the parents	Exhibits only the characteristic of the female parent
7.	Reciprocal differences	Not observed	Observed
8.	Segregation of factors and recombination	Present	Absent
9.	Attributes of progeny	Under the control of their own genes	Under the control of cytoplasm of female parent
10.	Action of mutagen	Non-specific	Very specific
11.	Frequency of occurrence	Most common	Rare
12.	Gene mapping	Easy	Difficult

Lecture No.: 24 & 25

MUTATIONS

Mutation in a broad sense include all those heritable changes which alter the phenotype of an individual. Thus mutation can be defined as a sudden heritable change in the character of an organism which is not due to either segregation or recombination.

- The term mutation was first used by Hugo de Vries to describe the sudden phenotypic changes which were heritable, while working with *Oenothera lamarckiana*.
- But the earliest record of mutations dates back to 1791 when Seth Wright noticed a male lamb with unusually short legs in his flock of sheep. This lamb served as a source of short leg trait for the development of Ancon breed of sheep.
- However the systematic studies on mutations were started in 1910 by T.H. Morgan who used *Drosophila melanogaster* for his studies.
- In 1927, H.J. Muller demonstrated for the first time the artificial induction of mutations by using x-rays in *Drosophila*.
- Similarly in 1928, L.J. Stadler demonstrated an increase in the rate of mutations due to x-rays in barley and maize.
- Induction of mutations by chemicals in fungus *Aspergillus* was demonstrated by R.A. Steinberg in 1939.
- C. Auerbach and J.N. Robson in 1946 used chemicals to induce mutations in *Drosophila*.
- The first plant breeding programme using mutations (mutation breeding) was initiated in 1929 in Sweden, Germany and Russia.
- In India it was initiated in early 1930s.

Terminology

Muton: The smallest unit of gene capable of undergoing mutation and it is represented by a nucleotide.

Mutator gene: A gene which causes another gene or genes to undergo spontaneous mutation.

Mutable genes: Genes which show very high rates of mutation as compared to other genes.

Mutant: An organism or cell showing a mutant phenotype due to mutant allele of a gene.

Mutagen: A physical or chemical agent which induces mutation.

Hot spots: Highly mutable sites with in a gene.

Gene mutations or point mutations: The changes which alter the chemical structure of a gene at molecular level.

Classification of mutations: Mutations can be classified in several ways.

1. **Based on direction of mutations :**
   a) **Forward mutation :** Any change from wild type allele to mutant allele
   b) **Backward mutation or reverse mutation:** A change from mutant allele to wild type
2. **Based on source / cause of mutations :**
   a) **Spontaneous mutation:** Mutation that occur naturally
   b) **Induced mutation:** Mutation that originates in response to mutagenic treatment
3. **Based on tissue of origin :**
   a) **Somatic mutation:** A mutation in somatic tissue
   b) **Germinal mutation:** A mutation in germline cells or in reproductive tissues
4. **Based on effect on survival :**
   a) **Lethal mutation:** Mutation which kills the individual that carries it. (survival 0%)
   b) **Sub-lethal mutation:** When mortality is more than 50% of individuals that carry mutation
   c) **Sub-vital mutation:** When mortality is less than 50% of individual that carry mutation.
   d) **Vital mutation:** When all the mutant individuals survive (survival-100%)
5. **Based on trait or character effected :**
   a) **Morphological mutation:** A mutation that alters the morphological features of an individual

b) **Biochemical mutation:** A mutation that alters the biochemical function of an individual.

6. **Based on visibility or quantum of morphological effect produced :**

   a) **Macro-mutations:** Produce a distinct morphological change in phenotype (which can be detected easily with out any confusion due to environmental effects) Generally found in qualitative characters. Eg : colour of flowers, height of plant etc.

   b) **Micro-mutations:** Mutations with invisible phenotypic changes, (which can be easily confused with effects produced due to environment). Generally observed in quantitative characters.

7. **Based on the site of mutation or on cytological basis :**

   a) **Chromosomal mutations:** Mutations associated with detectable changes in either chromosome number or structure.

   b) **Gene or point mutations:** Mutations produced by alterations in base sequences of concerned genes.

   c) **Cytoplasmic mutations:** Mutations associated with the changes in chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA).

Characteristic features of mutations:

1. Mutations are mostly recessive and very rarely dominant.
2. Most mutations have harmful effects and very few (less than 0.1 %) are beneficial.
3. Mutations may be due to a change in a gene, a group of genes or in entire chromosome.
4. If gene mutations are not lethal, the mutant individuals may survive. However, chromosomal mutations are generally lethal and such mutants do not survive.
5. If mutation occur at both loci simultaneously, the mutants can be identified in M_1 generation. However, if it is restricted to one locus only, (dominant to recessive) the effect can be seen only in M_2 generation.

6. Macro-mutations are visible and can be easily identified, while micro-mutations can not be seen with naked eye and need special statistical tests (or statistical analysis).
7. Many of the mutants show sterility.
8. Most mutants are of negative selection value.
9. Mutation for altogether new character generally does not occur.
10. Mutations are random i.e. they can occur in any tissue or cell of an organism. However some genes show higher mutation rate than others.
11. Mutations can be sectorial. The branches arising from mutated sector show mutant characters.
12. Mutations are recurrent i.e. the same mutation may occur again and again.
13. Induced mutations commonly show pleiotropy often due mutation in closely linked genes.

I. Spontaneous mutations: Spontaneous mutations occur naturally without any apparent cause. There are two possible sources of origin of these mutations.

1. Due to error during DNA replication.
2. Due to mutagenic effect of natural environment Eg : UV rays from sunlight

The rate of spontaneous mutations is very low. 1 in 10 lakhs i.e. 10^{-6}. But different genes may show considerably different mutation rates. In crop plants some varieties were developed through spontaneous mutations. They are

	Crop	Variety
1.	Rice	GEB-24, Dee-Geo-Woo-Gen
2.	Wheat	Norin
3.	Groundnut	TMV-10
4.	Sorghum	Co-4 (coimbatore 4)

II. Induced mutations: Mutations can be induced artificially through treatment with either physical or chemical mutagens. The exploitation of induced mutations for crop improvement is called mutation breeding. The rate of induced mutations is very high. The induced mutations did not differ from spontaneous mutations in expression.

Examples of popular induced mutants in crop plants are:

	Crop	Mutant variety	Original variety	Mutagen
1.	Rice	Jagannath	T-141	X-rays
		Mahsuri mutant	Mahsuri,	γ-rays
2.	Wheat	Sharbati sonara	Sonara 64	UV rays
		NP-836	NP-799	x rays
3.	French Beans	Pusa Parvati	Wax podded	x-rays
		Pusa Lal Meeruti	Meeruti	x-rays
4.	Tomato	S-12	Sioux	γ-rays
5.	Castor	Aruna	HC-6	Thermal neutrons
6.	Cotton	MCU 7	1143 EE	x-rays
		MCU 10	MCU 4	γ-rays

Artificial induction of mutations: Mutations can be induced artificially using

1. Physical mutagens or radiations
2. Chemical agents

1. **Physical mutagens:** Include various types of radiations, viz., x-rays, γ-rays, a-rays, ß-rays, fast neutrons, thermal or slow neutrons, UV rays etc. The physical mutagens are classified into

   a) **Ionizing radiations:** They work through the release of ions. They have deep penetrating capacity. Eg : x-rays, γ-rays, a -particles etc.

   b) **Non-ionizing radiations :** They function through excitation and have a very low penetrating capacity. They are used for studies on bacteria and viruses. Eg : UV rays.

   Sources of physical mutagens:

   Gamma garden
   Gamma green house
   Vertical gamma irradiation facility
   Horizontal gamma irradiation facility
   X-ray machine
   Isotopes
   Small portable irradiators, accelerators and cyclotrons
   Nuclear reactors

2. **Chemical mutagens :** These can be divided into four groups.

   a) **Alkylating agents:** This is the most powerful group of mutagens. These are the chemicals which are mainly used to induce mutations in cultivated plants. They induce mutations especially transitions and transversions by adding an alkyl group (either ethyl or methyl) at various positions in DNA. Alkylation produces mutation by changing hydrogen bonding in various ways. Eg: Dimethyl sulphonate (DMS), Ethyl methane sulphonate (EMS), Nitrosomethyl Urea (NMU), Nitrosoethyl Urea (NEU), Methyl methane sulphonate (MMS).

   b) **Base analogues:** These are chemicals which are very similar to DNA bases, such chemicals are sometimes incorporated in DNA in place of normal bases during replication. Thus they can cause mutation by wrong

base pairing. An incorrect base pairing results in transitions or transversions after DNA replication. Eg: 5– bromouracil, 3-bromodeoxy uridine, 2-amino purine.

c) **Antibiotics:** A number of antibiotics like mitomycin and streptomycin have been found to possess chromosome breaking properties. Their usefulness for practical purposes is very limited.

d) **Acridine dyes:** Acridine dyes Eg: proflavin, acriflavin, acridine orange, etc. are very effective mutagens. These are positively charged and they insert themselves between two base pairs of DNA. This is known as intercalation. Replication of intercalated DNA molecules results in addition or deletion of one or few base pairs which produces frame shift mutations.

e) **Miscellaneous:** Hydoxyl amine produce chromosomal aberrations. Nitrous acid (deaminating agent) has strong mutagenic activity in a variety of viruses and micro organisms. But not useful in higher plants.

Materials used for treating with mutagens:

Seeds, pollen, vegetative buds, whole plants, bulbils, tubers, suckers etc.

Molecular basis of mutations:

The term mutation is presently used to cover only those changes which alter the chemical structure of the gene at molecular level. Such changes are commonly referred to as “point mutations”. Point mutations involve a change in the base sequence of a gene which results in the production of a mutant phenotype. Point mutations can be subdivided into the following three classes on the basis of molecular change associated with them.

1. Base substitution
2. Base deletion
3. Base addition

1. **Base substitution:** When a single base in a DNA molecule is replaced by another base it is known as base substitution. This can be of two types.

a) **Transition:** Replacement of a purine by another purine or a pyrimidine by another pyrimidine. (or) The substitution of a purine by another purine or of

a pyrimidine by another pyrimidine base in DNA or RNA is known as transition.

(A ⟷ G or C ⟷ T)

b) **Transversion:** Replacement of a purine by a purimidine and vice versa. (or) The substitution of a purine by a pyrimidine or of a pyrimidine by a purine in DNA or RNA is known as transversion.

(A or G ⟷ C or T or U)

2. **Base deletion :** In base deletion, one or more bases are altogether deleted
3. **Base addition:** There is insertion of one or more bases.

If the number of bases added or deleted is not a multiple of three, a frameshift mutation is obtained, as the reading frame in such case is shifted from the point of addition or deletion onwards. Hence, in a frameshift mutation, all the aminoacids of a polypeptide chain located beyond the site of mutation are substituted / altered.

Frameshift mutations: The mutations which arise due to addition or deletion of nucleotides in mRNA are known as frameshift mutations, because the reading frame of base triplets (codons) beyond the point of addition or deletion is altered as a consequence of such mutations.

Detection of sex linked lethal mutations in *Drosophila* by Muller's ClB technique

Muller developed a system, ClB technique for detecting recessive sex-linked lethal mutations induced by X-ray treatment in *Drosophila*. He used a heterozygous (ClB) stock of Drosophila, which has a special X-chromosome, a large part of which is inverted (paracentric inversion). This acts as a crossover suppressor in the inverted region and is designated by C. A recessive lethal (l) gene and the dominant gene for bar (B) eye are located within the inverted segment, as a result, the l and B are always inherited together in the same chromosome. The other X-chromosome was normal. Consequently all ClB females identified by the bar eye shape, are heterozygous for this chromosome, while the males having the ClB chromosome do not survive [because of the l (lethal) gene].

The male flies were irradiated with X-rays for the induction of sex-linked recessive lethal mutations. Such males are crossed with ClB females. In the F_1, half of the females will have the ClB chromosome, which are easily identified by the bar-shaped eyes. The remaining half of the females will not have the ClB chromosome and are rejected. All the surviving F_1 males will have the normal chromosome from the ClB females, while those receiving the ClB chromosome will die due to the lethal gene 'l'. Each F_1 ClB females is mated to a normal male. Progeny from each such mating is kept in separate culture bottles. Each F_1 ClB female will have one ClB X-chromosome and one X-chromosome from the mutagen treated male parent. So, half of the male progeny receiving ClB X-chromosome will die. The remaining half male progeny will receive their X-chromosome from their mutagen treated grand father which may or may not carry the induced mutation. In case lethal mutation was induced, no males will be observed. On the other hand, if no lethal mutation was induced, half of the males will survive. Thus the ClB method was the simple, rapid and most efficient method for detecting sex-linked lethal mutations.

Detection of mutations in plants :

Generally the seeds of a variety or strain are treated with the mutagens and grown to obtain M_1 plants. The M_1 plants are selfed to avoid out crossing due to partial male sterility in M_1 plants. The seeds thus obtained represent the M_2 generation. The M_2 plants are grown and the plants having mutant features are counted. Then the frequency of a given mutation is estimated as per cent ratio between the number of plants exhibiting a mutant phenotype in M_2 and the total number of plants in M_2.

$$\text{Mutation frequency (\%)} = \frac{\text{Number of plants exhibiting mutant phenotype in } M_2}{\text{Total number of plants in } M_2} \times 100$$

Significance of mutations in Plant Breeding:

1. When a variety is exceptionally good except for one or few characters.
2. When a recessive character is desirable and transfer of that character from wild species is difficult.
3. When a desirable character is linked with an undesirable character.

4. If there is no known source of resistance gene in the available germplasm
5. To create variability
6. To develop male sterile lines
7. To create variations in vegetatively propagated plants

Chimeras

Chimera is defined as a mixture of genetically diverse tissues in the same shoot. These tissues frequently form mosaic pattern. The most common and easily observed chimeras are leaf variegations observed in horticultural plants like Codium and Acalypha. The leaf variegations or chlorophyll variegations are due to plastid mutations, consisting of green and white or green and yellow patterning as also the patterning of anthocyanin distribution. These chimeras can be of three types.

1. **Sectorial:** The tissues possess the characteristics which closely resemble the parents from which these tissues are originally derived.
2. **Periclinal:** The core is of one plant and the epidermal region is of another plant. As a result, the leaves may be of one plant type and the flowers and fruits are of another plant.
3. **Chromosomal chimera:** The most frequent being the ploid-chimera. It has been a very common experience to meet with cells especially in the root tips, with higher chromosomal number in an otherwise diploid tissue. Plants under experimental control have been sometimes observed to give rise suddenly to sectors or whole branches differing in chromosome number.

Xenia

Effect of the genotype of pollen grain on the phenotype of seed tissues (embryo and endosperm) or the genetic effect of pollen parent upon the embryo and endosperm of seeds in some plants.

Lecture No.: 26 & 27

CYTOGENETICS

Cytogenetics is branch of biology devoted to the study of chromosomes and their implications in genetics.

CHROMOSOMAL ABERRATIONS

Occasionally, spontaneous (without any known causal factor) variations in the structure and number of chromosomes have been observed in nature. These variations are called chromosomal aberrations and can be due to either (a) structural changes or (b) numerical changes

Origin of structural aberration

Chromosomes are structures with definite organization. However, through various means they may be broken and their normal structure disrupted. X-rays, atomic radiations and various chemicals are among the agents that can cause breaks in chromosomes. Breaks also sometimes occur under natural conditions, where in most instances the reason for breakage is not known. An initially single deviation from the normal can give rise to a whole series of unusual cytological events.

Breakage-fusion-bridge Cycle : In the gametophyte and endosperm of corn, ends of chromo somes that have recently been broken behave as though they were “sticky,” as is shown by their tendency to adhere to one another. Extensive studies of broken chromosomes in corn have been made by Barbara Mc Clintock. She found that following reduplication of a broken chromosome the two sister chromatids may adhere at the point of previous breakage. The fused sister chromatids would be unable to separate readily. In effect, they constitute a single chromatid with two centromeres, a dicentric chromatid. As the centromeres move to opposite poles at anaphase, the dicentric chromatid stretches out, forming a chromatin bridge from one pole toward the other. This bridge eventually breaks, but the break does not always occur at the point of previous fusion. Therefore, chromosomes may be formed that show duplications or deficiencies if compared with an original type.

Thus, if the original chromosome is

C B A

the type ***C B A A***

is a duplication type, since region *A* is represented twice. The type

C B

is a deficiency, since region *A* is absent.

When a chromosome bridge breaks, perhaps as a the result of tension caused by the movement of two centromeres of the dicentric chromatid two new broken ends are formed. Each of these has the same qualities of adhesiveness that gave rise to the original fusion. This situation permits repetition of more similar events as described above, in cyclic series. Spontaneous production of chromosome aberrations through breakage-fusion-bridge cycles may occur in this manner for some time. But when a broken chromosome is introduced into the sporophytic generation such cycles cease, as the broken ends heal in the zygote.

Structural chromosomal aberrations: Structural chromosomal changes include those chromosomal aberrations which alter the chromosome structure i.e. the number of genes, the sequence or kind of genes present in the chromosome(s) and do not involve a change in chromosome number.

Types of structural chromosomal aberrations: These aberrations may be confined to a single chromosome or more than one chromosome. Hence they are of two types.

1. Intra-chromosomal aberrations
2. Inter-chromosomal aberrations

I. Intra-chromosomal aberrations: When aberrations remain confined to a single chromosome of a homologous pair, they are called intra-chromosomal aberrations.

II. Inter-chromosomal aberrations: When breaks occur in non-homologous chromosomes and the resulting fragments are inter-changed by both the non-homologous chromosomes, they are known as inter-chromosomal aberrations.

I. Intra-chromosomal aberrations: They may be of the following types :

1. Deletions or deficiencies: Deletion is due to the loss of a part of a chromosome. In a deletion, a chromosome lacks either a terminal or an interstitial segment which may include only a single gene or a part of a gene. Hence it is of two types.

i) Terminal deletion: If a break occurs near the end of chromosome and a small piece of terminal chromosome is lost, it is called terminal deletion.

ii) Interstitial or intercalary deletion: Sometimes two breaks may occur at any two points and the broken ends of the original chromosome get fused or reunited and as a result, an interstitial deletion is formed. If the chromosome has a centromere, it will persist. Otherwise it will be lost during cell division.

Both types of deletions can be observed during pachytene stage of meiosis or in the polytene chromosomes. Deficiencies can be artificially induced using radiations. However, in majority of the cases, deficiencies are intercalary because, when a terminal part of the chromosome is lost, it can not be repaired unless it unites with another broken end.

Genetic significance / effects of deletions:

1. Organisms with homozygous deletion do not survive to an adult stage because a complete set of genes is lacking (lethal effect).
2. Small deficiencies, if present in heterozygous condition (deficiency heterozygote) can be tolerated by the organism. In such individuals during pachytene stage of meiosis the unpaired segment of the normal chromosome of an intercalary deletion heterozygote produces a characteristic loop in a bivalent. In case of terminal deletion heterozygotes, the segment towards one end of the normal chromosome remains unpaired. Loops can be observed in salivary gland chromosomes of *Drosophila* or giant chromosomes, which are found in a permanent state of pairing. Therefore even small deficiencies could be detected in these chromosomes.
3. Deficiencies have an effect on the inheritance also. In presence of deficiency a recessive allele will behave like a dominant allele. When an organism heterozygous for a pair of alleles Aa loses a portion of the chromosome bearing the dominant allele 'A', the recessive allele being in the hemizygous condition will be expressed phenotypically. This phenomenon is known as pseudo-dominance.
4. This principle of pseudo-dominance has been utilized for location of genes on specific chromosomes in *Drosophila*, maize and other organisms. Thus, deletions are important cytological tools for mapping genes.
5. Deletions play an important role in species formation and creating variability through chromosomal mutations.

   In *Drosophila*, deletions were recorded on X-chromosome in the regions containing genes-w (for white eye), fa (for facet eye) and v (for vermilion eye colour).

2. Duplications or Repeats: Duplication occurs when a segment of chromosome is represented two or more times in a chromosome of a homologous pair. The extra segment may be a free fragment with a centromere or a chromosomal segment of the normal complement. As a result, in one chromosome of the homologous pair, there will be deletion, while in other there will be a duplication. Duplication was first reported in *Drosophila* by C.B. Bridges in 1919.

Duplications are of four types.

1. Tandem: The extra chromosome segment may be located immediately after the normal segment in precisely the same orientation (i.e. having the same gene sequence)
2. Reverse tandem: The gene sequence in the extra segment of a tandem duplication is in the reverse order i.e. is inverted. (eg. cb in place of bc)

3. Displaced: The extra segment may be located in the same chromosome but away from the normal segment.
4. Reverse displaced: The gene sequence in the extra segment of a displaced duplication is in the reverse order i.e. is inverted (eg. ed in place of de)

If duplication is present in only one of the two homologous chromosomes, at pachytene stage of meiosis, cytological observations characteristic of deficiency will be obtained in duplication also.

Genetic significance / effects of duplications:

1. Duplications are not as harmful as deletions.
2. Some duplications are useful in the evolution of new genetic material.
3. Large duplications can reduce fertility.
4. The phenotype may be altered.

One of the classical examples of duplication in *Drosophila* is that of bar eye. Bar eye is a character where the eyes are narrower as compared to normal eye shape. This phenotypic character is due to the duplication for a part of chromosome. By the study of giant salivary gland chromosomes of *Drosophila melanogaster*, it could be demonstrated that 'Bar' character was due to duplication in the region 16A of x chromosome. Barred individuals (16A 16A) gave rise to ultra bar (16A 16A 16A) and normal wild type (16A) due to unequal crossing over.

3. Inversions: When a segment of a chromosome is oriented in reverse direction, such a segment is said to be inverted and the phenomenon is termed as inversion. Gene sequence in an inverted segment is exactly the opposite of that in its normal homologous pair. Inversions would involve two breaks followed by reunion of interstitial segment in a reverse order i. e . the segment rotates by 180^0.

(Let us imagine that a chromosome 1-2-3-4-5-6-7-8 gives rise to another chromosome having the order 1-2-6-5-4-3-7-8. the segment 3-4-5-6 has rotated here at 180^0 giving an inverted order of genes 6-5-4-3)

Inversions can be of two types depending upon whether centromere is involved or not in inversions.

(a) Paracentric inversions and (b) Pericentric inversions.

(a) Paracentric inversions: Paracentric inversions are those inversions, where the inverted segment does not include centromere.

(b) Pericentric inversions: The inverted segment includes the centromere in pericentric inversions. (pericentric means surrounding the centromere or on the periphery of centromere).

Cytology of inversions: When both the members of a homologous pair have similar type of inversion, it is called inversion homozygote. Meiosis is normal in inversion homozygotes. When only one chromosome of a homologous pair has inversion it is called inversion heterozygote. Due to an inverted segment in one of the two homologous chromosomes, the normal kind of pairing is not possible in an inversion heterozygote. In order to enable pairing of homologous segments, a loop is formed by each of the two chromosomes. This kind of configuration will be observed in paracentric as well as pericentric inversions. However, the products of crossing over and the subsequent stages of meiosis will differ in these two kinds of inversions.

1. **Paracentric inversion:** A single crossing over or an odd number of crossovers in an inverted region will result into the formation of a dicentric chromosome (having two centromeres) and an acentric chromatid (without centromere) when two chromatids are involved in the crossing over. These dicentric chromatid and acentric chromatid will be observed at anaphase I in the form of a bridge and a fragment.
2. **Pericentric inversion:** In a pericentric inversion, although at pachytene, the configuration observed is similar to that described above for paracentric inversion, the products of crossing over and the configurations of subsequent stages of meiosis differ. In this case, two of the four chromatids resulting after meiosis will have deficiencies and duplications. Unlike paracentric inversion, no dicentric bridge or acentric fragment will be observed at anaphase I.

However, in pericentric inversion, if the two breaks are not situated equidistant from the centromere, this will result in the change in shape of the chromosome. For instance, a metacentric chromosome may become sub-metacentric and vice-versa.

Genetic consequences of inversions

1. Simple inversions do not have primary effects other than change in chromosome shape.
2. A peculiar kind of position effect occurs due to suppression of the transcription of gene.
3. Normal linear pairing of homologous chromosomes is not possible.
4. Heterozygosity will be maintained from generation to generation.
5. Among the four chromatids resulting after crossing over, two chromatids would have deficiencies and duplications. The gametes having these chromosomes will not function normally and lead to high sterility. Therefore there should be considerable gametic or zygotic lethality. In plants there will be sufficient pollen sterility.

   However, since the products of single crossing over will not function and the only crossover products recovered will be double cross overs, the observed frequency of recombination between any two genes of interest will be considerably reduced. Due to

this reason, inversions (especially paracentric inversions) are often called crossover suppressors. This reduction in crossing over is not the actual reduction in cytological crossing over, but is the result of lack of recovery of the products of single cross overs. This property of inversion has been utilized in the production of CIB stock used by Muller for the detection of sex-linked lethal mutations in *Drosophila melanogaster*.

4. Shifts: Shifts are altered forms of inversions. In this type of aberrations, the genes are in the right order but a segment is shifted either to the right or to the left.

5. Isochromosome: Isochromosome is the one in which both the arms are identical in both gene content and morphology. It arises when the centromere divides in wrong plane yielding two daughter chromosomes each of which carries the information of one arm only but present twice.

II. Interchromosomal aberrations : These are of the following types.

1. **Translocation :** Integration of a chromosome segment into a non-homologous chromosome is called translocation. It involves shifting of one part of chromosome to another non-homologous chromosome. The phenomenon of translocation was discovered by C.B. Bridges in 1923 in *Drosophila* and by Hugo de Vries in *Oenothera lamarckiana*.

Translocation is of two types

1. **Simple translocation :** In simple translocation, the terminal segment of chromosomes is integrated at one end of a non-homologous chromosome. However, they are rare.
2. **Reciprocal translocation :** If two non-homologous chromosome exchange segments which need not be of same size, it results in reciprocal translocation. Production of reciprocal translocation requires a single break in each of the two non-homologous chromosomes followed by reunion of the chromosome segments thus produced. An individual having reciprocal translocation may be either a translocation homozygote or a translocation heterozygote. When both the chromosomes from each pair are involved, it produces translocation homozygote and when only one chromosome from each pair of two homologues is involved, it gives rise to translocation heterozygote.

In a translocation homozygote, the two homologues of each of the two translocated chromosomes are identical in their gene content. As a result, they form normal bivalent and there is no detectable cytogenetic aberration (peculiarity).

In a translocation heterozygote, one member from each of two homologous pairs is involved in reciprocal translocation, while the remaining chromosomes of the two concerned pairs are normal. Due to the pairing between homologous segments of chromosomes, a cross-shaped (+) figure involving four chromosomes will be observed at pachytene. These four chromosomes at metaphase I will form a quadrivalent, which may exhibit any one of the following three orientations.

1. Alternate 2. Adjacent I 3. Adjacent II

1. **Alternate :** In this case, the centromeres lying alternate to each other in the cross shaped figure move to the same pole. In otherwords, the adjacent chromosomes will orient towards opposite poles. As a result, the two normal ch romosomes move to one pole, while the two translocated chromosomes move to the opposite pole. Such a segregation can take place only when the cross shaped figure of four chromosomes is twisted to form a figure of 'oo'.
2. **Adjacent – I:** In adjacent I orientation, adjacent chromosomes having non-homologous centromeres will orient towards the same pole. In other words, the chromosomes having homologous centromeres will orient towards the opposite poles. Thus a ring of four chromosomes will be observed.
3. **Adjacent – II:** In adjacent II orientation, the adjacent chromosomes having homologous centromeres will orient towards the same pole. In this case also a ring of four chromosomes will be observed.

In both Adjacent-I and adjacent-II disjunctions, one normal and one translocated chromosome move to the opposite poles. Adjacent-I and adjacent-II disjuctions will form gametes which would carry duplications or deficiencies and as a result would be non-functional or sterile. Therefore, in a plant having translocation in heterozygous condition, there will be considerable pollen sterility.

Genetic significance of translocation heterozygotes:

1. They produce semi sterile plants with low seed set.
2. Some genes which earlier assorted independently tend to exhibit linkage relationship.
3. The phenotypic expression of a gene may be modified when it is translocated to a new position in the genome.
4. The presence of translocation heterozygosity can be detected by the occurrence of semi-sterility and low seed set. This can then be confirmed at me iosis by quadrivalent formation. Functional gametes will be formed only from alternate disjunction, which will give rise to three kinds of progeny *viz*., normal, translocation heterozygotes and translocation homozygotes in 1:2:1 ratio.

Role of structural chromosomal aberrations in plant breeding

1. They are useful in the identification of chromosomes
2. Utilization of vigour as in case of duplication.
3. Useful in genome analysis.
4. Useful for the transfer of desirable characters through translocation.
5. They have evolutionary significance.

Lecture No.: 28, 29 & 30

NUMERICAL CHROMOSOMAL ABERRATIONS

Each species of micro-organisms, plants and animals is characterized by particular chromosome complement or set of genome, represented once in gametic (haploid) cell i.e. n and twice in somatic (diploid) cells i.e. 2n. The term genome refers to a complete set of chromosomes of a diploid species. All the members of a genome are distinct from each other in gene content and often in morphology. Members of a genome do not pair. Possession of such sets of chromosomes or genomes, gives a specific chromosome number to each species. But sometimes, some irregularities may occur during mitosis, meiosis or fertilization and may produce cells with variant chromosome number. A deviation from the diploid state represents a numerical chromosomal aberration which is often referred to as heteroploidy. Individuals possessing variant chromosome number are known as heteroploids. Variation in chromosome number (ploidy) may occur through the addition or loss of complete chromosome set or genome (euploidy) or of one or few chromosomes (aneuploidy).

Thus numerical changes in chromosomes (heteroploidy) can be mainly of two types: 1. Euploidy and 2. Aneuploidy.

Euploidy: (Greek word; Eu = true or even; ploidy = unit)

The term euploidy designates a change in chromosome number which involves entire set of chromosomes. Euploids have one or more complete genomes, which may be identical with or distinct from each other. The somatic chromosome number of a euploid individual is exact multiple of basic chromosome number of that species. The basic chromosome number refers to the haploid or gametic chromosome number of a diploid species and in case of

polyploidy species, the haploid chromosome number of parental diploid species; represented by x. Euploidy includes monoploids, diploids and polyploids.

Monoploids: Monoploids contain a single chromosome set and are characteristically sterile. In other words monoploids have the basic chromosome number (x) of a spec ies. Monoploids (x) differ from haploids (n) which carry half or gametic chromosome number. In a true diploid species, both monoploid and haploid chromosome number are same (i. e. x=n).

Haploid: Haploid is a general term used to designate the individuals or tissues with a gametic chromosome number i.e. n.

Differences between monoploids and haploids:

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

Eg :	Maize	2n = 20	x = 10	n = 10
	Wheat	2n = 6x = 42	x = 7	n = 21

Haploids can be of two types

1. Monohaploids: Individuals that arise from a normal diploid species.
   Eg. : Maize 2n = 20 and n = 10
2. Polyhaploids: Individuals that arise from any polyploid species.
   Eg : Wheat 2n = 6x = 42 and n=3x=21

Haploids can arise spontaneously or can be induced. Spontaneous haploids have been obtained in plants like tomato, cotton, barley, coffee, pearl millet and wheat. The first induced haploid was produced by Jorgensen in 1928 by crossing *Solanum nigrum* x *Solanum luteum*

Guha and Maheswari in 1964 obtained haploid plants of *Datura innoxia* directly from the pollen by culturing the anthers. The main reason for plant breeders' interest to obtain the haploids has been to develop a new and rapid method of breeding homozygous diploids or polyploids through diploidization using colchicine. In a haploid, every gene is present only once. Doubling of

chromosomes should theoretically result in complete homozygosity. Repeated inbreeding for homozygosity in plants requires many (8-9) generations. But doubling of the chromosome number of haploids results in immediate homozygosity. Diploids obtained through the chromosome doubling of haploids are known as dihaploids.

Characteristic features of haploid plants:

Haploids are smaller, less vigorous than their diploid phenotypes. Haploids are sterile, as the chromosomes have no regular pairing partner homologous chromosomes during meiosis and they are found as univalents at metaphase I of meiosis. The meiotic products are deficient in one or more chromosomes. For instance, a haploid in maize, (2n=20) will have 10 chromosomes and the number of chromosomes in gametes can range from 0-10. Consequently considerable sterility will be found in a haploid maize.

Haploids can be produced through anther culture, parthenocarpy, delayed pollination etc.

Diploidy: Diploidy is characterized by presence of two genomes in each somatic cell of the diploid organism. Most animals and plants are diploids. The diploidy is related with fertility, balanced growth, vigour, adaptability and survival of diploid organisms.

Polyploidy: The organisms with more than two genomes are called polyploids. Among plants, polyploidy occurs in multiple series of 3, 4, 5, 6, 7, 8 etc. of the basic chromosome number and thus resulting in triploids, tetraploids, pentaploids, hexaploids, heptaploids, octaploids etc., respectively. Generally ploidy levels higher than tetraploid are not commonly encountered in the natural population. However there are some exceptions. Eg: hexaploid (6x) wheat, octaploids (8x) straw berries, many commercial fruits and ornamental plants, liver cells of man etc.

Origin of polyploidy: Different degrees of ploidy are originated by different means. However, two basic irregular processes have been discovered by which polyploids may evolve from diploid plants and become established in nature.

1. **Somatic doubling:** Cells sometimes undergo irregularities in mitosis and give rise to meristematic cells that perpetuate these irregularities in new generations of plants.
2. **Reproductive process:** Reproductive cells may have an irregular reduction division in which the sets of chromosomes fail to separate completely to the poles at anaphase. Both the sets thus become incorporated in the same nucleus resulting in the doubling of chromosome number in the gamete. Thus a triploid originates by irregularities during meiosis (i.e. by union of diploid gamete with haploid gamete) Likewise a tetraploid may originate by the somatic doubling of the chromosome number or by union of unreduced diploid gametes.

Induction of polyploidy: Polyploidy can be induced by two methods. 1. By physical agents and 2. By chemical agents

1. **By physical agents:**
   a) **Temperature shocks:** Extreme changes in temperature results in a higher frequency of polyploid cells.
   b) **Centrifugation:** Centrifugation of seedlings or plants causes polyploidy in their cells.
   c) **X-rays:** X- rays can also induce polyploidy
2. **By chemical agents:** Some chemicals like colchicine, chlorohydrate, mercuric chloride have been found to induce polyploidy in plants. Colchicine treatment is the most effective and most widely used treatment for chromosome doubling. The chromosome doubling effect of colchicine was first described by Blakeslee and Nebel independently. Colchicine interferes or disturbs the formation of spindle fibres during cell division and thus inhibits the movement of sister chromatids to the opposite poles. Colchicine is a poisonous chemical isolated from the seeds and bulbs of autumn crocus (*Colchicum autumnale*). Pure colchicine is $C_{22}H_{25}O_6N$.

Kinds of polyploids: Polyploids are distinguished on the basis of source of chromosomes into three main kinds. 1. Auto polyploids, 2. Allopolyploids and 3. Segmental allopolyploids

1. Autopolyploids: In a plant, when same set of chromosomes of a genome are increased in number, autopolyploids are obtained. The prefix “auto” indicates that the ploidy involves homologous chromosome sets. For example, if a diploid species has two similar sets of chromosomes / genomes designated as AA, an autotriploid will have three similar (AAA) genomes and autotetraploid will have four similar (AAAA) genomes.

Genetical and morphological characters expressed by autopolyploid depend on the genetic constitution of parent plant. In general, expression is exaggerated either in positive / negative direction. For example: vegetative growth may be more vigourous. Leaves may be more broader or dark green in colour. The floral parts, fruits and seeds may be bigger. However, autopolyploids occur rarely in natural populations. Eg: Auto triploids– *Cynodon dactylon* (doob grass).

a) Auto triploids: Auto triploids have three complete sets of genomes of the same species in somatic cell. Triploids can arise in several ways. Generally, in nature they originate by the fusion of a haploid gamete with a diploid gamete (unreduced gamete). Diploid gametes occur sporadically as unreduced germ cell in a diploid organism. They are also produced by meiosis in tetraploid organism or in segments of otherwise diploid organisms where doubling of the somatic chromosome number has taken place. Triploids can be produced artificially by crossing between autotetraploid and diploid species.

Triploids are generally highly sterile. In an autotriploid, there are three sets of homologous chromosomes. If these sets are normally paired, trivalents (as observed in primary trisomics) will be observed. The trivalents can not disjoin normally and will either disjoin 2:1 chromosomes to two poles or will disjoin 1:1 leaving one chromosome as a laggard. The number of chromosomes in the gametes of a triploid organism therefore, will vary from n to 2n. Most of these gametes are unbalanced leading to high degree of sterility. Examples of triploidy in animals are rather rare. Triploids are useful only in those plant species which propagate asexually like banana, sugarcane, apple, sugarbeet, watermelon, tomato, *Cynodon dactylon* (doob grass).

Seedless watermelons are grown commercially in Japan. They are produced by crossing tetraploid (4x, used as female) and diploid (2x, used as male) lines, since the reciprocal cross (diploid female X tetraploid male) is not successful. The triploid plants do not produce true seeds. Almost all the seeds are small, white rudimentary structures like cucumber seeds. For good fruit setting, diploid plants are planted in the ratio of 1 diploid : 5 triploid plants.

b) Auto tetraploids: In autotetraploids, four copies of the genome of same species (AAAA or BBBB) are present. They may arise spontaneously or can be induced artificially by doubling the chromosomes of a diploid species with colchicine treatment. In autotetraploids, since there are four sets of chromosomes, quadrivalents are formed, which disjoin in a normal 2:2 manner giving diploid gametes. Rarely, a quadrivalent may disjoin in 3:1 or may leave one or more chromosomes as laggards at anaphase I. Therefore autotetraploids also have a certain degree of sterility, although it will not be as high as in autotriploids. Autotetraploids are usually larger and more vigorous than the diploid species. Eg: Rye, alfalfa, grapes, groundnut, potato, coffee, *Oenothera lamarckiana*.

In an autotetraploid, four chromosomes are homologous to each other, hence each gene has four copies. A simplex individual has one dominant and three recessive alleles (Aaaa), a duplex has two dominant and two recessive alleles (AAaa), a triplex has three dominant and one recessive alleles (AAAa), a quadruplex has all dominant alleles (AAAA), while a nulliplex has no dominant alleles (aaaa).

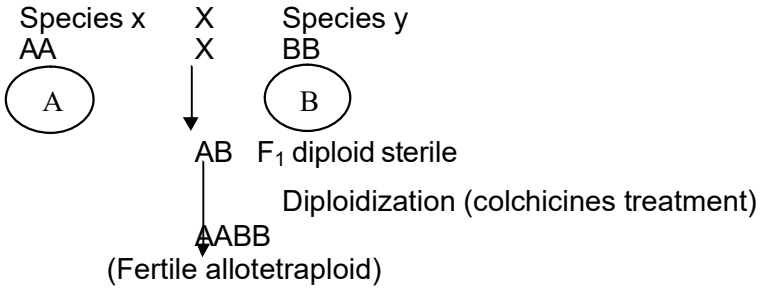
2. Allopolyploids: A polyploid containing genetically different chromosome sets from two or more species is known as allopolyploid. The prefix “allo” indicates the involvement of non-homologous sets of chromosomes.

Origin of allopolyploids: Natural allopolyploids most likely originate through chromosome doubling of F_1 hybrid produced by chance through natural hybridization between two distinct species of the same genus or from different genera. Experimental production of allopolyploids is achieved through chromosome doubling of F_1 hybrid with the help of colchicine. Such allopolyploids are often called synthetic allopolyploids. The synthesis of

allopolyploids involves two steps. 1. Production of F_1 hybrids by crossing two distinct species and 2. Chromosome doubling of such F_1 hybrids.

The man made cereal Triticale is an example of synthetic allopolyploid.

Amphidiploid: It is an allopolyploid (allotetraploid) which arises by combining genomes of two different species.



The amphidiploids are fertile due to the presence of homologous chromosomes and behave as a diploid during meiosis. The term amphidiploid was proposed by Nawashin.

Natural allopolyploids: Inter-specific crossing followed by chromosome doubling in nature have resulted in origin of some natural allopolyploid crops like cotton, tobacco, mustard, wheat, etc. The origin of some natural allopolyploid crops is briefly presented below:

(i) Cotton: The new world cotton (*Gossypium hirsutum*) is an interesting example of allopolyploidy. J.O. Beasley crossed old world cotton (*Gossypium herbaceum*) with American cotton (*Gossypium raimondii*) and doubled the chromosome number in F_1 hybrids. The allopolyploid thus produced resembled the cultivated new world cotton (*Gossypium hirsutum*) and when crossed with it gave fertile F_1 hybrids. These results thus suggested that tetraploid cotton (*Gossypium hirsutum*) originated from two diploid species namely *Gossypium herbaceum* (2n = 26) and *Gossypium raimondii* (2n = 26).

Gossypium herbaceum X *Gossypium raimondii*
Var. *africanum*
(Old world cotton) (American cotton)
2n = 26 (diploid) 2n = 26 (diploid)
13 small bivalents 13 large bivalents
AA DD

F_1 hybrids (highly sterile)
2n = 26
26 (13 small + 13 large) univalents (diploid)
AD

diploidization
(Treated with colchicine)

Gossypium hirsutum,
(New world cotton)
2n = 52 (tetraploid)
26 (13 small + 13 large) bivalents
AADD

(ii)Tobacco: There are two cultivated species of tobacco. i. e. *Nicotiana tabacum* and *Nicotiana rustica*.

a) *Nicotiana tabacum* is an allotetraploid and available evidence suggests that it is derived from a cross between *Nicotiana sylvestris* x *Nicotiana tomentosa*

Nicotiana sylvestris X *Nicotiana tomentosa*
2n = 24 (diploid) 2n = 24(diploid)
AA BB

F_1 Hybrid
2n = 24 (diploid)
AB (sterile)

doubling of chromosomes

Nicotiana tabacum
2n = 48 (Amphidiploid)
AA BB

b) *Nicotiana rustica* is believed to be an amphidiploid obtained from a cross between *Nicotiana paniculata* and *Nicotiana undulata*

Nicotiana paniculata X *Nicotiana undulata*

2n = 24(diploid)
CC

2n = 24(diploid)
DD

↓

F_1 Hybrid

2n = 24 (diploid)
CD (sterile)

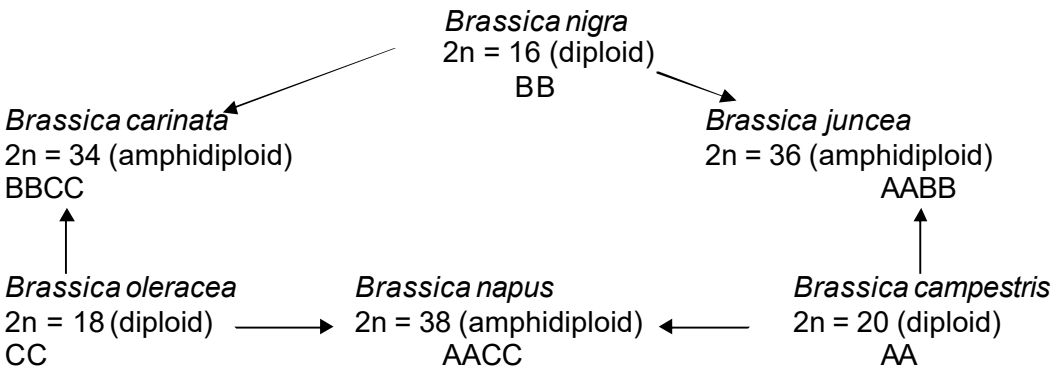
↓ doubling of chromosomes

Nicotiana rustica

2n = 48 (amphidiploid)
CC DD

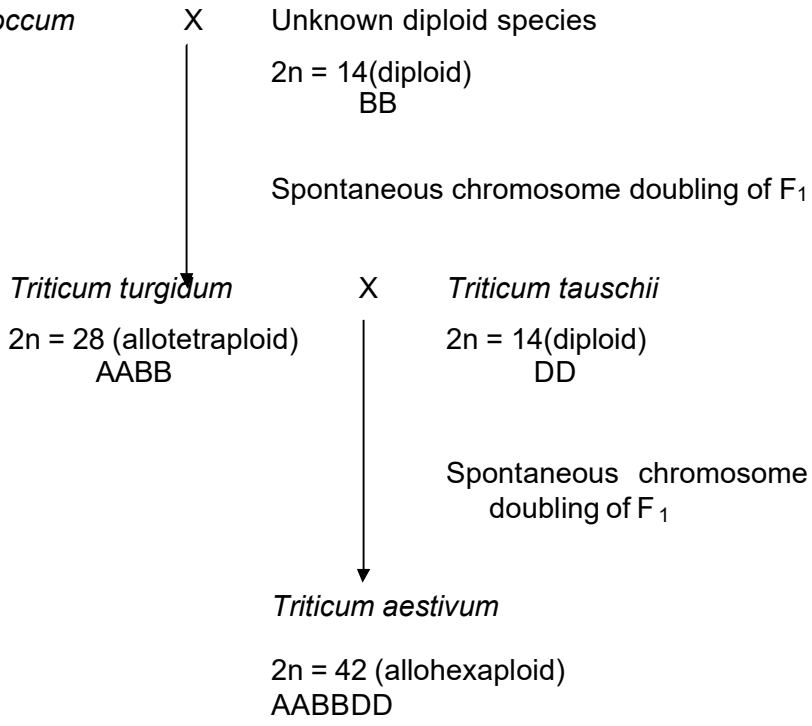
(ii) *Brassica*: Several of *Brassica* species like *Brassica juncea*, *Brassica napus* and *Brassica carinata* are allotetraploids (amphidiploids). It is believed that *Brassica juncea* is an amphidiploid derived from a cross between *Brassica nigra* and *Brassica campestris*; *Brassica napus* is an amphidiploid derived from a cross between *Brassica oleracea* and *Brassica campestris* and *Brassica carinata* is an amphidiploid derived from a cross between *Brassica nigra* and *Brassica oleracea*.

Brassica Triangle



(iv) Wheat: The common or bread wheat, *Triticum aestivum* (formerly *Triticum spelta*) is an allohexaploid. It was artificially synthesized in 1946 by Mc Fadden and Sears. It has two copies each of the genomes A, B and D and its somatic complement is represented as AA BB DD. The sources of A and D genomes are more or less unanimously accepted as *Triticum monococcum* (AA) and

Triticum tauschii (DD) (formerly *Aegilops squarrosa* –goat grass), respectively. There is considerable doubt about the source of B genome. According to one hypothesis, *Aegilops speltoides* may be the source of this genome. But recent evidences do not support this idea. Most likely, the donor of B genome is now extinct and its identity is still not clear. Most likely, the amphidiploid AABB was produced initially. This gave rise to a tetraploid wheat, *Triticum turgidum* (formerly, *Triticum dicoccum* – emmer wheat). This amphidiploid (AABB) was subsequently outcrossed with *Triticum tauschii* (formerly *Aegilops squarrosa* – goat grass) to ultimately yield the hexaploid wheat, *Triticum aestivum* (AABBDD)



Artificial allopolyploids: Artificial allopolyploids have been synthesized in some crops either to study the origin of naturally available allopolyploids or to explore the possibilities of creating new species. Some examples of artificial allopolyploids are given below:

i) Triticale: Triticale, a man made cereal, is first produced by Muntzing. Triticale is a new crop species synthesized by crossing wheat and rye (*Secale cereale)*.

a) Some triticales are hexaploids and are developed from a cross between tetraploid wheat (*Triticum turgidum)* and rye.

Wheat X Rye

Triticum turgidum X *Secale cereale*

2n = 28 (Tetraploid) 2n = 14 (diploid)
AABB RR

↓

F_1 Hybrid

2n = 21 (allotriploid)
ABR (sterile)

↓ doubling of chromosomes

Triticale hexaploide

2n = 42 (allohexaploid)
AA BB RR

b) Octaploid triticales are produced from a cross between hexaploid wheat (*Triticum aestivum*) and rye.

Wheat X Rye

Triticum aestivum X *Secale cereale*

2n = 42 (hexaploid) 2n = 14 (diploid)
AA BB DD RR

↓

F_1 Hybrid

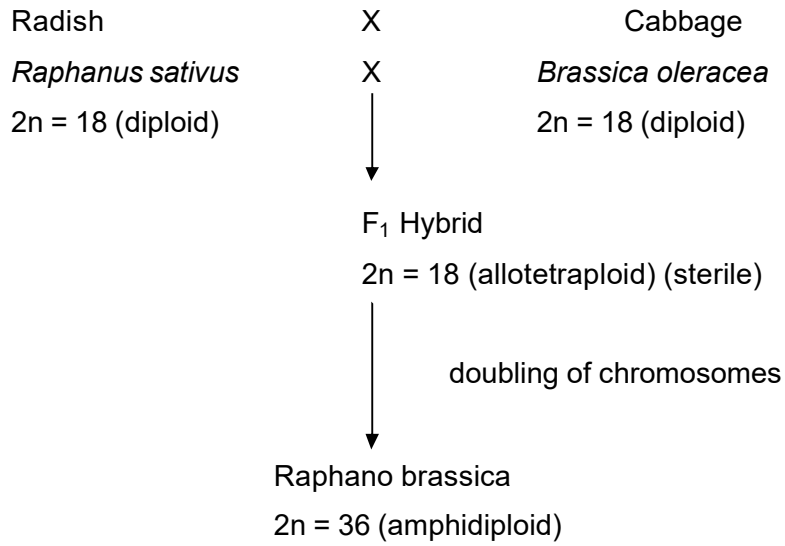
2n = 28 (allotetraploid)
ABDR (sterile)

↓ doubling of chromosomes

Triticale octaploide
2n = 56 (allooctaploid)
AA BB DD RR

(iii) Raphanobrassica: Russian geneticist G.D. Karpechenko in 1927 synthesized Raphanobrassica, which is an allopolyploid resulting from a cross between Radish and cabbage. He wanted to develop a fertile hybrid between these two species with roots of radish and leaves of cabbage. However, the F_1 hybrids, he got, were diploid having roots of cabbage and shoots of radish. They were highly sterile because of failure of each set of chromosomes to provide sufficient genetic homology to effect pairing. Among these sterile F_1 hybrids, he found certain fertile allotetraploids which contained 36

chromosomes due to spontaneous doubling and were named as Raphanobrassica.



3. **Segmental allopolyploids:** In some allopolyploids, different genomes present are not quite different from one another. Consequently, in these polyploids, chromosomes from different genomes do pair together to some extent and mulitvalents are formed. This means that certain segments of chromosomes and not the entire chromosomes are homologous (Homeologous chromosomes). Therefore such allopolyploids are called segmental allopolyploids according to Stebbins (1943 – 1950). These are intermediate between autopolyploids and allopolyploids and can be identified by their meiotic behaviour. The common hexaploid bread wheat is also regarded as a segmental allopolyploid, because the three diploid genomes i.e. A, B and D are related (homoeologous) to each other.

Effects of polyploidy:

1. **Genetical effects:** The polyploidy often results in sterility. For example, an extra set of chromosomes in case of triploids is distributed in various combinations resulting in genetically imbalanced gametes.
2. **Phenotypic effects:** Most usual phenotypic effect of polyploidy is gigantism in morphology of plants. Eg: The tetraploid plants may have large sized pollen grains, cells of leaves, stomata, xylem etc. than a normal

diploid plant. They are also more vigorous. As a result, large sized fruits, seeds and flowers are obtained from economically important plants.

3. **Physiological effects:** The ascorbic acid content has been reported to be higher in tetraploid cabbage and tomato than in corresponding normal diploid species. Corn flour of tetraploid maize has been found to contain 40% more vitamin A than that of normal diploid species.

Lecture No.: 31

Aneuploidy

It is any deviation from a euploid condition. This condition can be expressed either as an addition of one or more entire chromosome or as a loss of such chromosomes to a genomic number.

Aneuploidy can be due to

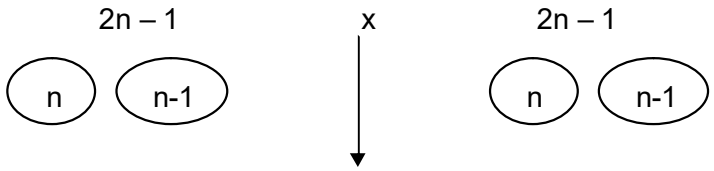
1. Loss of chromosomes in mitotic or meiotic cells due to laggards (lagging chromosomes), which are characterized by retarded movement during anaphase.
2. Irregularities of chromosome distribution during meiosis of polyploids with uneven number of basic genomes like triploids and pentaploids.
3. The occurrence of multipolar mitosis resulting in irregular chromosome distribution during anaphase.

Aneuploids can be of the following types:

1. **Monosomy:** The diploid organism which lacks one chromosome of a single homologous pair is called monosomic with genomic formula 2n-1. A monosomic produces two types of gametes n and n-1 because single chromosome without a pairing partner may go to either of poles during meiosis. The monosomics are usually weaker than normal diploids. Monosomics are normally found in polyploids and the diploids cannot tolerate them. The polyploids have several chromosomes of same type and therefore, this loss can be easily balanced by homologous or partially homologous chromosomes from other genomes. The number of possible monosomics in an organism will be equal to the haploid chromosome number. In common wheat, since 21 pairs of chromosomes are present, 21 possible monosomics are known. These 21 monosomics in wheat were produced by Sears in 1954 in the variety Chinese spring and are being used for genetic studies all over the world. Monosomics have been used extensively in wheat breeding for the purpose of chromosome substitution.

Double monosomics (2n-1-1) or triple monosomics (2n-1-1-1) could also be produced in polyploids like wheat. In double monosomics the missing chromosomes are non-homologous.

2. **Nullisomy:** Diploid organisms which have lost a pair of homologous chromosomes are called nullisomics with genomic formula 2n-2. In double monosomy and nullisomy, the chromosome number is same but the genomic formula differs. In nullisomy (2n-2), a complete homologous chromosome pair is missing. In double monosomy, (2n-1-1), two chromosomes of different chromosome pairs (one each from two different chromosome pairs) are missing. Nullisomics are not usually found in natural populations, but have to be obtained by intercrossing or selfing of monosomics (2n-1). These can occur by fusion of two gametes that are lacking in the same chromosome.



? / ?	n	n-1
n	2n Diploid	2n-1 Monosomic
n-1	2n-1 Monosomic	2n-2 Nullisomic

Nullisomic series are not of great agronomic importance, but used for genetic studies. They exhibit reduced vigour, fertility and survival.

Double nullisomy (2n-2-2) involves loss of two pairs of homologous chromosomes.

Monosomics and nullisomics are together known as hypoploids, which refers to loss of one or two chromosomes from the normal diploid.

3. **Trisomy**: Trisomics are those organisms which have one extra chromosome (2n+1). Since the extra chromosome may belong to any one of the different chromosome pairs, the number of possible trisomics in an organism will be equal to the haploid chromosome number. For instance, in

barley (2n = 14) the haploid chromosome number is n = 7. Consequently seven trisomics are possible, in a trisomic, one of the pairs of chromosomes has an extra member and forms a trivalent during anaphase I of meiosis. Two chromosomes will go to one pole and one chromosome will go to other pole. As a result, two types of gametes are formed i.e. n and n+1. This is very common in plants and has variable effects on phenotype. In plants, the first case of trisomy was investigated in Jimson weed i.e. *Datura stramonium* by A.F. Blakeslee and J.Belling in 1924. *Datura* (2n = 24) normally has 12 pairs of chromosomes in somatic cells. But in an individual, they discovered 25 chromosomes. The size, shape and spine characteristics of seed capsule of this trisomic plant were different from seed capsule of wild type species. Through experimental breeding, Blakeslee and his associates succeeded in producing all 12 possible trisomics. When these were grown, each was found to have a distinguishable phenotype that was attributed to extra set of genes present on the extra chromosome contained in each of the 12 pairs of chromosomes.

An individual having two extra chromosomes each belonging to a different chromosome pair is called double trisomic (2n + 1 + 1).

Depending on the nature of extra chromosome, simple trisomics are of three types.

a) **Primary trisomics:** The additional chromosome is normal one in primary trisomics.

b) **Secondary trisomics:** Trisomics having isochromosome as additional chromosome.

c) **Tertiary trisomics:** When additional chromosome in a trisomic is translocated one, it is known as tertiary trisomic.

The first human trisomic syndrome discovered was the one involving 'G' group of chromosomes called Mongolism or Down's syndrome.

4. **Tetrasomy:** Tetrasomics have a particular chromosome represented four times. Therefore the general chromosome formula for tetrasomics is 2n+2. All the 21 possible tetrasomics in wheat are viable.

Tetrasomics often behave more regularly than the aneuploids with odd number of chromosomes. The four homologues tend to form a quadrivalent at meiosis and disjunction often proceeds fairly regularly, two by two.

Trisomics and tetrasomics are together known as hyperploids or polysomics, which refers to addition of one or two chromosomes to a single or two different homologous pairs.

Applications of aneuploids: Aneuploids are useful in crop improvement and genetic studies as detailed below:

1) Aneuploids have been used to determine the phenotypic effects of loss or gain of different chromosomes.
2) They are used to produce chromosome substitution lines. Such lines provide information on the effect of different chromosomes of a variety in the same genetic background.
3) They are also used to produce alien addition and alien substitution lines.
4) Monosomics are also used in transferring chromosomes with desirable genes from one species to another.
5) Aneuploid analysis permits the location of a gene as well as of a linkage group on to a specific chromosome. Monosomics and nullisomics are used for this purpose.
6) Studies on nullisomic and tetrasomic combinations made it possible to establish homoeology among the chromosomes of A, B and D genomes of wheat.
7) Aneuploids are also useful in identifying the chromosomes involved in translocations (tertiary trisomics).
8) Aneuploids are also useful in the preparation of molecular maps.
9) They may be used for obtaining chromosome specific probes.

   (Probe is a DNA sequence that is used to detect the presence of the same DNA sequence in a test DNA sample).

A summary of terms used o describe heteroploidy (variation in chromosome number):

	Term	Type of change	Symbol*

	Heteroploid	A change from diploid	
A.	Euploid	Number of genomes or copies of a genome is more or less than two	
a)	Monoploid	One copy of a single genome	x
b)	Haploid	Gametic chromosome complement	n
	i) Monohaploid	Haploid individuals that arise from a normal diploid	
	ii) Polyhaploid	Haploid individuals that arise from a polyploid	
	iii) Dihaploid	Diploids obtained through the chromosome doubling of haploids	
d)	Diploid Polyploidy	Two copies of genome More than two copies of one genome or two copies each of two or more genomes**	2x
1.	Autoployploid	Genomes are identical with each other	
i.	Autotriploid	Three copies of one genome	3x
ii.	Autotetraploid	Four copies of one genome	4x
iii	Autopentaploid	Five copies of one genome	5x
iv	Autohexaploid	Six copies of one genome	6x
	Autoheptaploid	Seven copies of one gnome	7x
v	Autooctaploid	Eight copies of one genome	8x
2.	Allopolyploid	Two or more distinct genomes (Generally each genome has two copies)	
i	Allotetraploid	Two copies each of two distinct genomes	$(2x_1 + 2x_2)$** or (AA BB)
ii	Allohexaploid	Two copies each of three distinct genomes	$(2x_1 + 2_2 + 2x_3)$*** or (AA BB CC)
iii	Allooctaploid	Two copies each of four distinct genomes	$(2x_1 + 2_2 + 2x_3 + 2x_4)$*** or (AA BB CC DD)

	Term	Type of change	Symbol*
B.	Aneuploid	One or few chromosomes extra or missing from 2n	2n± few
a)	Monosomic	One chromosome missing	2n-1
b)	Double monosomic	One chromosome from each of two different chromosome pairs missing	2n-1-1
c)	Nullisomic	One chromosome pair missing	2n-2
d)	Trisomic	One chromosome extra	2n+1
e)	Double trisomic	One chromosome from each of two different chromosome pairs extra	2n+1+1
f)	Tetrasomic	One chromosome pair extra	2n+2

*2n = somatic chromosome number } of the species, whether diploid
n = gametic chromosome number } or polyploid

x = The basic chromosome number or genomic number
x_1, x_2, x_3, x_4 = Distinct genomes from different species.

** In general this condition occurs other situation may also occur.

Lecture No: 32

GENOMICS

Genomics is the sub-discipline of genetics that focus on the structure and function of entire genomes, mapping, sequencing and analyzing the functions of entire genomes. The term "Genomics" was coined by Thomas Roderick in 1986. Genomics includes sequencing of genomes, determination of the complete set of proteins encoded by an organism, and the functioning of genes and metabolic pathways in an organism.

Genomics is often divided into two domains:

1. **Structural genomics:** It deals with the determination of the complete sequence of genomes or the complete set of proteins produced by an organism. This has progressed in steps as follows: (i) construction of high resolution genetic and physical maps, (ii) sequencing of the genome, and (iii) determination of complete set of proteins in an organism
2. **Functional genomics:** It refers to the study of functioning of genes and metabolic pathways, *i.e.,* the gene expression patterns in organism. It includes the analyses of transcriptome (a complete set of RNAs transcribed

from a genome) and proteome (the complete set of proteins encoded by a genome).

Human Genome Project:

The human genome project was launched in 1990, with the goal of sequencing the entire human genome by 2005 at an estimated cost of $3 billion. Some of the important goals of HGP were as follows.

i) Identify all the genes in human DNA
ii) Determine the sequences of the 3 billion chemical base pairs that make up human DNA
iii) Store this information in public databases
iv) Develop tools for data analysis
v) Transfer related technologies to the private sectors and
vi) Address the ethical, legal, and social issues (ELSIs) that may arise from the project

Sequencing of human genome was carried out by the public-funded Human Genome Sequencing Consortium and the private company Celera genomics established by Craig Venter. A nearly complete sequence of the human genome comprising 99 percent of the euchromatic DNA was released in October, 2004. The first draft of human genome sequence, which is over 3 billion nucleotide long, consists of 24 chapters, *viz.,* 22 autosomes and X and Y sex chromosomes.

Salient Features of Human Genome:

i) The human genome contains over 3 billion nucleotide pairs
ii) On an average there is one gene per 145 kb in the human genome.
iii) Human genome is estimated to have about 20,000 – 25,000 genes that encode protein products.
iv) Average gene consists of 3000 bases. But sizes of genes vary greatly, with the largest known human gene encoding dystrophin containing 2.5 million base pairs.
v) Only about 1.1%of the genome encodes amino acid sequences of polypeptides
vi) The functions are unknown for over 50% of the discovered genes

vii) The repetitive sequences makeup very large portion of human genome. Repetitive sequences have no direct coding function but they shed light on the chromosome structure, dynamics and evolution.

viii) Chromosome 1 has most genes (2968) and Y chromosome has the lowest (231)

ix) Almost all nucleotide bases are exactly the same in all people. Genome sequences of different individuals differ for less than 0.2% of base pairs. Most of these differences occur in the form of single base differences in the sequence. These single base differences are called single nucleotide polymorphisms (SNPs). One SNP occurs at every ~ 1,000 bp of human genome. About 85% of all differences in human DNAs are due to SNPs.