Principles of Genetics 2+1

Theory

Unit I: Cytology

Definition of genetics, heredity, inheritance, cytology, cytogenetics; Brief history of developments in genetics and cytogenetics; Physical basis of heredity: Structure and function of cell and cell organelles – Differences between Prokaryotes and Eukaryotes.Cell division – mitosis, meiosis and their significance, cell cycle - zygote formation and embryo development - identical and fraternal twins. Chromosome structure, chemical composition, nucleosome, centromere, telomere, euchromatin, heterochromatin, NOR, satellite chromosome, karyotype, ideogram – chromosome banding; Types of chromosomes based on position of centromere, based on structure and function: normal and special chromosomes - polytene, lampbrush, based on the role in sex determination: autosomes and allosomes, Other types of chromosomes - B, ring and isochromosomes; Chromosomal aberration: Variation in chromosome structure – deletion, duplication, inversion and translocation – genetic and cytological implications; Chromosomal aberration: Variation in chromosome number – euploid, aneuploid, types of aneuploids and their origin; Nondisjunction - Klinefelter syndrome and Turner syndrome; Definition of eugenics and euthenics; Polyploid - auto and allopolyploids, their characters; meaning of genome; evolution of wheat, Triticale, cotton, tobacco, Brassicas.

Unit II: Mendelian laws and modifications of Mendelian laws

Pre-Mendelian ideas about heredity – Vapour and fluid theory, Magnetic power theory, Preformation theory, Lamarck's theory, Darwin's theory, Germplasm theory and Mutation theory; Work of Mendel – Characters studied reasons for Mendel's success, Law of dominance, Law of segregation and Law of independent assortment. Rediscovery of Mendel's work; Chromosomal theory of inheritance. Allelic interactions – Dominance vs. recessive, complete dominance, codominance, incomplete dominance, over dominance; Terminologies: gene, allele, locus, homozygous, heterozygous, hemizygous, genotype, phenotype, monohybrid, dihybrid, trihybrid, polyhybrid. Deviation from Mendelian inheritance – Non allelic interaction without modification in Mendelian ratio – Batson and Punnet's experiment on fowl comb shape. Non allelic interaction with modification in Mendelian ratio – i.) Dominant epistasis (12:3:1) ii.) Recessive epistasis(9:3:4) iii.) Duplicate and additive epistasis((9:6:1). iv.) Duplicate dominant epistasis(15:1) v) Duplicate recessive epistasis (9:7) vi.) Dominant and recessive epistasis(13:3); Summary of epistatic ratios (i)to (vi); Lethal genes, Pleiotrophy, penetrance and expressivity, phenocopy: Multiple alleles, blood group in humans, coat colour in rabbits, self incompatibility in plants; pseudo alleles, isoalleles;

Unit III: Quantitative inheritance, Linkage and Crossing over

Quantitative inheritance – Multiple factor hypothesis – Nilsson Ehle, his experiment on wheat kernel colour; Polygenes – transgressive segregation, comparison of quantitatively and qualitatively inherited characters; modifiers; Types of gene action controlling quantitative traits; Linkage - coupling and repulsion; Experiment on Bateson and Punnet – Chromosomal theory of

linkage of Morgan – Complete and incomplete linkage, Linkage group; Crossing over – significance of crossing over; cytological proof for crossing over - Stern’s experiment; Factors

controlling crossing over; Strength of linkage and recombination; Two point and three point test cross; Double cross over, interference and coincidence; genetic map, physical map.

Unit IV: Sex determination, sex linkage and cytoplasmic inheritance

Sex determination: Autosomes and sex chromosomes - chromosomal theory of sex determination - different types – sex determination in human, fowl, butterfly, grasshopper, honey bee, fumea; Genic balance theory of Bridges, quantitative theory, hormonal theory, barr bodies, metabolic differentiation theory; Gynandromorphs – sex reversal in chicken; Sex linked inheritance – cris cross inheritance – reciprocal difference; holandric genes; sex influenced and sex limited inheritance; Sex determination in plants – Melandrium, papaya, maize. Cytoplasmic inheritance and maternal effects – features of cytoplasmic inheritance, chloroplast, mitochondrial - plastid colour in *Mirabilis jalapa* - iojap gene of maize, cytoplasmic male sterility in rice, kappa particles of paramecium - plasmid and episomic inheritance.

Unit V: Modern concept of genetics and mutation

DNA, the genetic material – Griffith's experiment, experiment of Avery, McCleod and McCarthy – confirmation by Hershey and Chase; RNA as genetic material – Frankel, Conrat and Singer experiment; Structure of DNA – Watson and Crick model – Central dogma of life; Proof for semi conservative method of DNA replication; Models of DNA replication; steps involved in DNA replication; RNA types - mRNA, tRNA, rRNA; genetic code, transcription; Translation – protein synthesis; Regulation of gene expression – operon model of Jacob and Monad; Structural genes and regulator genes; Cistron, muton and recon; Complementation test; exons, introns – split genes – plant genome structure; Mobile genetic elements; Meaning of Developmental genetics, DNA methylation, siRNA, RNA_i, Functional genomics, Metagenomics, Transcriptomics, Proteomics, Metabolomics and Phenomics. Mutation – characteristics of mutation – micro and macro mutation – ClB technique - molecular basis of mutation; major physical and chemical mutagens.

Practical

Study of cell and cell organelles – Preparation of fixatives and stains – pre treatment of materials for mitosis and meiosis – study of mitosis and meiosis. Study of genetic ratios of – monohybrid, dihybrid – incomplete dominance. Gene interaction - multiple alleles and multiple factors. Study of linkage, estimation of strength of linkage and recombination frequency in two point and three point test cross data and F_2 data – Drawing of genetic map – interference and coincidence.

Theory schedule

1. Definition of genetics, heredity, inheritance, cytology, cytogenetics; Brief history of developments in genetics and cytogenetics.
2. Physical basis of heredity: Structure and function of cell and cell organelles – Differences between Prokaryotes and Eukaryotes.

3. Cell division – mitosis, meiosis and their significance, cell cycle; zygote formation and embryo development - identical and fraternal twins.
4. Chromosome structure, chemical composition, nucleosome, centromere, telomere, euchromatin, heterochromatin, NOR, satellite chromosome, karyotype, ideogram – chromosome banding.
5. Types of chromosomes based on position of centromere, based on structure and function: normal and special chromosomes - polytene, lampbrush, based on the role in sex determination: autosomes and allosomes, Other types of chromosomes - B, ring and isochromosomes.
6. Chromosomal aberration: Variation in chromosome structure – deletion, duplication, inversion and translocation – genetic and cytological implications.
7. Chromosomal aberration: Variation in chromosome number – euploid, aneuploid, types of aneuploids and their origin; Nondisjunction - Klinefelter syndrome and Turner syndrome; Definition of eugenics and euthenics.
8. Polyploid - auto and allopolyploids, their characters; meaning of genome; evolution of wheat, Triticale, cotton, tobacco, Brassicas,
9. Pre-Mendelian ideas about heredity – Vapour and fluid theory, Magnetic power theory, Preformation theory, Lamarck's theory, Darwin's theory, Germplasm theory and Mutation theory.
10. Work of Mendel – Characters studied reasons for Mendel's success, Law of dominance, Law of segregation and Law of independent assortment. Rediscovery of Mendel's work
11. Chromosomal theory of inheritance. Allelic interactions – Dominance vs. recessive, complete dominance, codominance, incomplete dominance, over dominance.
12. Terminologies: gene, allele, locus, homozygous, heterozygous, hemizygous, genotype, phenotype, monohybrid, dihybrid, trihybrid, polyhybrid.
13. Deviation from Mendelian inheritance – Non allelic interaction without modification in Mendelian ratio – Batson and Punnet's experiment on fowl comb shape. Non allelic interaction with modification in Mendelian ratio – i.) Dominant epistasis (12:3:1)
14. ii.) Recessive epistasis(9:3:4) iii.) Duplicate and additive epistasis((9:6:1). iv.) Duplicate dominant epistasis(15:1)
15. v) Duplicate recessive epistasis (9:7) vi.) Dominant and recessive epistasis(13:3); Summary of epistatic ratios (i)to (vi).
16. Lethal genes, Pleiotrophy, penetrance and expressivity, phenocopy: Multiple alleles, blood group in humans, coat colour in rabbits, self incompatibility in plants; pseudo alleles, isoalleles.
17. **Mid Semester Examination**
18. Quantitative inheritance – Multiple factor hypothesis – Nilsson Ehle, his experiment on wheat kernel colour.
19. Polygenes – transgressive segregation, comparison of quantitatively and qualitatively inherited characters; modifiers; Types of gene action controlling quantitative traits.
20. Linkage - coupling and repulsion; Experiment on Bateson and Punnet – Chromosomal theory of linkage of Morgan – Complete and incomplete linkage, Linkage group.
21. Crossing over – significance of crossing over; cytological proof for crossing over - Stern's experiment; Factors controlling crossing over.
22. Strength of linkage and recombination; Two point and three point test cross.
23. Double cross over, interference and coincidence; genetic map, physical map.

24. Sex determination: Autosomes and sex chromosomes - chromosomal theory of sex determination - different types – sex determination in human, fowl, butterfly, grasshopper, honey bee, fumea; Genic balance theory of Bridges, quantitative theory, hormonal theory, barr bodies, metabolic differentiation theory; Gynandromorphs – sex reversal in chicken
25. Sex linked inheritance – cris cross inheritance – reciprocal difference; holandric genes; sex influenced and sex limited inheritance.
26. Sex determination in plants – Melandrium, papaya, maize.
27. Cytoplasmic inheritance and maternal effects – features of cytoplasmic inheritance, chloroplast, mitochondrial - plastid colour in *Mirabilis jalapa* - iojap gene of maize, cytoplasmic male sterility in rice, kappa particles of paramecium - plasmid and episomic inheritance.
28. DNA, the genetic material – Griffith's experiment, experiment of Avery, McCleod and McCarthy – confirmation by Hershey and Chase; RNA as genetic material – Frankel, Conrat and Singer experiment.
29. Structure of DNA – Watson and Crick model – Central dogma of life
30. Proof for semi conservative method of DNA replication; Models of DNA replication; steps involved in DNA replication.
31. RNA types - mRNA, tRNA, rRNA; genetic code, transcription.
32. Translation – protein synthesis; Regulation of gene expression – operon model of Jacob and Monad; Structural genes and regulator genes;
33. Cistron, muton and recon; Complementation test; exons, introns – split genes – plant genome structure; Mobile genetic elements; Meaning of Developmental genetics, , DNA methylation, siRNA, RNA_i, Functional genomics, Metagenomics, Transcriptomics, Proteomics, Metabolomics and Phenomics.
34. Mutation – characteristics of mutation – micro and macro mutation – ClB technique - molecular basis of mutation; major physical and chemical mutagens.

Practical schedule

1. Use of microscopes and study of cell shapes and cell organelles of active mitotic and meiotic tissues.
2. Principles of killing and fixing; preparation of stains and preservatives.
3. Study of the mitotic phases in root tips of onion / *Aloe sp*.
4. Study of behaviour of chromosomes in mitosis.
5. Procedure for fixing and observing different meiotic phases in the inflorescence of maize.
6. Procedure for fixing and observing different meiotic phases in the inflorescence in pearl millet/ sorghum/ forest tree.
7. Observation of bivalents, trivalents, quadrivalents and chromosome banding
8. Repetition of meiotic studies in maize/ sorghum/ pearl millet/ forest tree and making temporary and permanent slides.
9. Principles of dominance, recessive, back cross, test cross, incomplete dominance, codominance and lethal factor; Chi square test; Monohybrid genetic ratio with dominance, with incomplete dominance and test cross.
10. Dihybrid ratio with dominance, with incomplete dominance and test cross
11. Simple interaction of genes-comb character in fowls; Dominant epistasis.
12. Recessive epistasis, Duplicate and additive epistasis.
13. Duplicate dominant epistasis, Duplicate recessive epistasis, Dominant and recessive epistasis.

14. Multiple alleles and polygenic inheritance
15. Estimation of linkage with F_2 and test cross data; Coupling and repulsion.
16. Problems on two point test cross and three point test cross; Working out interference, coincidence and drawing genetic maps.
17. **Final Practical examination.**

References

1. Gupta P.K., 1997. Cytogenetics. Rastogi Publications, Meerut
2. Strickberger. M.W. 1996. Genetics. Prentice-Hall of India Pvt. Ltd. New Delhi.
3. Singh, B.D. 2004. Fundamentals of genetics, Kalyani Publishers, Chennai.
4. Verma,P.S. and V.K.Agarwal. 2007. Genetics. S.Chand and Company Ltd./ New Delhi.
5. Stansfield, W.D.1990. Theory and problems of genetics. Mc-Graw Hill Book Co.,New York

Further reading

1. Daniel Sundararaj, G. Thulasidas and M.Stephen Dorairaj, 1997. Introduction to Cytogenetics and Plant Breeding. Popular Book Depot, Chennai –15.
2. Benjamin Lewin 2005 Genes IX Oxford University Press, Oxford.
3. Gupta P.K., 1993. Genetics, Rastogi publications, Meerut.
4. Reddi, O.S., 1992. Understanding Genetics. Sunil Sachdev Publishers, New Delhi – 64.
5. Russel, P.J. 2000. Fundamentals of genetics. Addition Wesley Longman Publishers, USA.
6. singh, R.J.2002. Plant cytogenetics. CRC Press, USA

Web resources

1. www.nmsu.edu
2. www.biology200.gsu.edu

Lecture 1. Definition of genetics, heredity, inheritance, cytology, cytogenetics; Brief history of developments in genetics and cytogenetics.

GENETICS

Genetics is the science of **heredity and variation**. The science of genetics deals with the principles that explain the similarities between parents and their progeny and the differences among individuals of a single species.

Heredity - Process which brings about the biological similarity between parents and progeny. Deals with **inheritance** of characters from parents to off springs.

Inheritance is the transmission of genetic information from parents and ancestors to offspring.

Variation: The differences among individuals of a single species for a particular character.

Genes: are the functional units that govern the development of characters of an individual.

Gene - unit of inheritance

Cytology: Study of cell structure and functions of cell organelles

Cytogenetics -The study of various aspects of chromosomes and their effects on the development of characters of organism.

Chief Events in Cytology

Year	Scientist	Discovery/ Event
1665	Robert Hooke	discovered Cell
1831	Robert brown	presence of nucleus
1838	Schleiden and Schwann	Cell theory
1861	Schultzee	Protoplasm theory
1870	Fredrick Meischer	Isolated Nucleoprotein
1879	Flemming	described chromatin in nucleus
1882	Flemming	described cell division (Mitosis)
1888	Waldeyer	Described chromosomes
1902	Mc Clung	Sex Chromosomes

1903	Sutton	Chromosome theory
1905	Farmer and Moore	Coined the term Meiosis
1913	Sturtvent	Built first chromosome map
1937	Blakeslee	Induced polyploidy with Colchicine

Robert Hook (1665)

He described the cell as empty vessel. He introduced term cell.

Cameraious:

He proved pollen is important for fertilizations. He is the first man to produce first artificial hybrid plant.

Koelreutes

He showed that F_1 might resemble either male (or) female parents (or) combination of both. Hereditary contribution of the two parents to their offspring was equal.

Knight

He obtained the dominant forms in F_1 and segregation of various characters in F_2.

Gaertner

F_1 are uniform and their F_2 produced considerable variation.

Naudin

Hybrids races and species of plants are often luxurient than either of the parents.

Robert Brown

He described the cell nucleus in the flowering plants.

He observed random thermal motion of small particles known as Brownian movement.

Schleiden and Schwann:

They discovered the formation of nucleus in the cell and formulated cell theory, which says

1. The cell is the smallest building element of a multicellar organism.
2. Each cell has a specific work to complete.
3. The cell can only the produced from another by cell division.

Strasberger:

He described fertilization in Angiosperms.

Van Beneden - Meiosis:

He showed number of chromosomes in the gametes, is half of the number of body cells. In fertilization the chromosome contribution of eggs and sperms to the zygote are numerically equal.

Flemming - Mitosis:

1. He proposed mitosis in cell.
2. He showed the chromosome spliited during nuclear division and the formation of daughter nuclei.
3. He also applied the name chromatin which is to the stainable position of the nucleus.

History of Genetics

Gregor John Mendel

- An Austrian botanist who laid foundation for the science of genetics.

- Born in the year **1822** near Brunn in Austria
- He worked with *Pisum sativum* - Garden pea
- Presented a paper **in 1865 – "Experiments in Plant Hybridization"** before the Natural History Society of Brunn
- **Mendel's Paper was published in 1866**
- Formulated two important laws of inheritance **in 1866**
    1. Law of segregation
    2. Law of independent assortment
- Died in the year **1884**
- Importance of his work was **realized only in 1900**
- For this pioneer work he was called as the **"Father of Genetics"**

Rediscovery of Mendels work in 1900 by Correns, Hugo devries, Tschermak

Carl Erich Correns

- A german botanist who rediscovered Mendel's work in 1900
- He conducted research with garden pea and came to the same conclusion as drawn by Mendel in 1865.
- He worked with *Mirabilis jalapa* (4'O' clock plant) and established the first conclusive example for **Extrachromosomal inheritance**

Hugo devries

- Rediscovered the mendel's law of inheritance independentantly but simultaneously with Correns and Tschermak in 1900,
- He coined the term **Mutation** - sudden heritable changes in the characters

Tschermak

- One of the co discoverers of Mendel's classic papers on garden pea
- He applied mendel's law of heredity in **barley, wheat rye hybrids and oat hybrids** for the development of new plants

Morgan, T.H.

- Established the **chromosome theory of heredity in 1910**.
- He showed that genes are linked in a series on chromosomes and are responsible for observable genetic traits.
- Received Nobel Prize for Physiology and Medicine in 1933.
- Discovered hereditary transmission mechanisms and **sex linkage in Drosophila.**

Bridges, C.B.

- Established the chromosomal basis of heredity and sex.
- Constructed detailed **gene map of the giant chromosomes** found in the salivary gland cells of fruit fly larva.
- Discovered **genic balance theory of sex determination** and gene duplication in Drosophila.

Muller, H.J.

- **X- rays speed up the natural process of mutation**.
- For experimental induction of mutation he was awarded Nobel Prize

Beadle and Tatum

- Proposed the concept of **one gene one enzyme hypothesis** for which they received Nobel prize in 1958

Avery, Mac Leod and Mc carty

- Discovered the **phenomenon of transformation**
- They (Avery, Mac Leod and Mc carty) reported that the substance which caused the transformation was DNA and demonstrated the **DNA was the genetic material**

Watson and Crick (1953) and Wilkinson

- The **X ray diffraction studies of DNA by Wilkinson** resulted in the Discovery of the molecular structure of DNA, in 1953 by Watson and Crick

Watson and Crick - proposed the double helix model of DNA

- The three scientists received Nobel prize in 1962

Barbara Mc Clintock

- Discovered **transposons/ jumping genes** in Maize in 1950- Genes that are capable of changing their position on a chromosome and from one chromosome to another
- Awarded Nobel Prize in 1983

Benzer

- Detail structure of viral genes and **coined the term cistron** to denote functional sub unit of genes
- Gave sub divisions of genes viz., **Cistron, Recon and Muton**. These are the units of function, recombination and mutation within a gene.

Meselson and Stahl

- Experimentally confirmed the **semi-conservative replication of DNA**

F.H.C. Crick

- Proposed the **Central Dogma of Molecular Biology in 1958**
- Gave evidence for the triplet nature of genetic code in 1961

Nirenberg - Responsible for deciphering the **genetic code- 1961**

Jacob and Monad

- Explained gene regulation in cell metabolism by directing the biosynthesis of enzymes - **Operon concept in 1962**
- Awarded Nobel prize in 1965

Khorana

- Discovered how the **genetic components of the cell nucleus control the synthesis of protein**
- Awarded Nobel prize in 1968 with Nirenberg and Holley
- He prepared the first **artificial copy of a yeast gene** in 1970

Stanley Cohen and Herberd Boyer

- Genetic Engineering in 1973

A.M. Maxam and W.Frederick Gilbert; F. Sanger and Coulson

- Sequencing of DNA in 1977

Craig Venter (2001) – human Genome Project – Complete sequencing of human genome

Goff *et al* and Yu *et al* (2002) – Rice Genome Sequencing

Lecture 2. Physical basis of heredity: Structure and function of cell and cell organelles – Differences between Prokaryotes and Eukaryotes.

CELL

A Cell may be defined as the structural and functional unit of a living being. It is the minimal biological unit capable of maintaining and propagating itself. A study of the structural and functional organization of different structures within a cell is known as '**Cytology**'. Cytogenesis concerns with the study of various aspects of chromosomes and their effects on the development of characters of organisms. It is universally accepted that genes are located in chromosome. Cytogenetics originated as a result of bringing two different branches of biology namely cytology and genetics together.

HISTORY- the word '**Cell**' has been derived from the Latin word Cellula meaning a small compartment. The term was first used by Robert Hook (1665). Robert Hook who constructed the first compound microscope observed the sections of Cork and opined that they contain honeycomb like compartments. German biologists M.J. Schleiden and T.S. Schewann (1838) established the 'Cell theory' that all organisms are made up of cells. One of the significant discoveries of the cell came from 'Robert Brown' (1830). He discovered the presence of a spherical body in the centre of every cell, which he named 'Nucleus'. In 1835-37, Purkinje and Mohi independently discovered that protoplasm is an important constituent of every cell and it

plays an important role in every cell activity including division. Golgi (1838) discovered the golgi apparatus, Balbiam (181) discovered chromosomes in the salivary glands of chironomus. At amount the same time, Flemming (1882) studied cell division in detail and gave the name ' Mitosis'. Endoplasmic reticulum was discovered by proter in 1945, while Benda gave the name mitochondria to organells originally discovered by Hemming. Lyzosomes were discovered in 1955 by de Duve. The shape of cell may be variable like spherical, rectangular, flattened, oval, polygonal triangular come like column etc., There is a great rang eof variation among cells in size also. This small cell size can be encountered in coccus bacteria (0.2 to 0.5 m) while the largest size of the cell is seen in Ostrich egg (Marly 15 cm).

Gross morphology of the cell

A generalized plant cell has an outer most envelope called the 'Cell wall'. This is absent in animal cells. Internal to this in the plasma membrane. This encloses the nucleus and other cytoplasmic inclusions suspended in cytoplasm. The inclusions are Ribosomes, Lysosomes, Mitochondria, Plastids, Golgi complex, Endoplasmic reticulum, Vacuole and non-living inclusions like crystals, raphids etc., The primitive organisms like certain bacteria blue green algae, the nucleus is not properly organized hence such cells are called Prokaryotic, while in evolved organisms, the nucleus is organized. Such cells are called Eukaryotic. The following are some of the fundamental differences between eukaryotic and prokaryotic cells.

CELL WALL - Plant cells are surrounded by a non-living and rigid coat called a 'cell wall'. The main functions of a cell wall are to provide plant cells a definite shape and mechanical support and strength to tissue and organs. Cell wall has 3 distinct parts ;

1. Middle lamella
2. Primary cell wall
3. Secondary cell wall

Middle lamella - In plants, the wall of contiguous (immediate neighbour) cells are joined by middle lamella, which is composed mainly of pectin. Secondary wall Middle lamella Primary wall Plasmodesmata The pectin of middle lamella is most likely in the form of calcium (Ca++) and Magnesium (Mg ++) salts. Adhesian of the walls of cotinguos cells in primarily dependant on the presence of Ca ++ and Mg++ ions in the middle lamella a removal of these ions results in

the separation of cells from each other. Pectin is readily hydrolysed by the enzyme pectinase as well as by strong acids.

PRIMARY CELL WALL - is deposited after the formation of middle lamella and lies between middlle lamella and plasma lemma. It is main constituent are hemi cellulose (53%) and cellulose (30%). In addition it contains pectin (5%). Protein (5%) and lipid (7%).

SECONDARY CELL WALL - Is the last to be deposited and lies between cell wall and plasma lemma in a cell, it is the inner most laayer of wall. It is composed of mainly of cellulose. The cellulose microfibrils are relatively more closely packed and they are arranged more or less parallel to each other. Several microfibrils associate to form a macrofibril, which is the structural unit of secondary cell wall.

PLASMA LEMMA (PLASMA MEMBRANE)

The membrane enclosing cytoplasm of a cell is known as plasma lemma or plasma membrane. It is composed of lipids and preoteins, the ratio between the two being quite variable among different cell types. Three distinct layers are seen under electron microscope, two or three are relatively dense and osmiphilic in nature; each of them is about 20oA thich. The two osmaphilic layers are separated by a relatively light osmiphobic layer of about 35oA thickness. The three layers together are known as 'Unit membrane"' this term coined by Robertson. The chief function of plasma lemma in to regulate the movements of various molecules into and out of the cytoplasm. In addition to the passive movement of molecules, some ions are transported across plasma lemma by means of active transport.

CYTOPLASM

The substance, except nuclear, surrounded by the plasmalemma is known as 'Cytoplasm". Electron microscope reveals a member of membraneous and other structures in the cytoplasm; the portion of cytoplasm other than therse structure is known as 'hyaloplasm. Of the various structures present in the cytoplasm, mitochondria and plastids contain DNA; as a result they are autonomous to a limited degree. However, the remaining cytoplasmic structures do not contain DNA and they are specified exclusively by nuclear genes.

The cytoplasm may contain the following structures -endoplasmic reticulum (ER), ribosomes, Golgi bodies, Lysosomes, Sphersomes, Vacuoles, certioles (in animals only), microtubrils, Mitochondira and plastids (in green plants only).

ENDOPLASMIC RETICULUM (E.R)

The cytoplasm contains an extensive network of membrane-enclosed space; these space along with the membranes enclosing them are known as E.R. It consists of 3 types of membrane-enclosed elements.

1. Vesicles of 25-500 in m diameter
2. Tubules of 50-100 m m diameter
3. 40-50 m m thick cisterns of variable length and width.

The tubulus may or may not be extensivley branched, and the cisterns may ormmay not be connected with each other. The ultastructure of E.R membrane in the same as that of a unit membrane, that is, it has two osmiophilic layers separated by an osmiopholic layers. E.R is grouped into two categories,

1. Smooth E.R.
2. Rough E.R.

In smooth E.R elements, both outer and inner surfaces are regular and smooth. In those cells where little or no protein synthesis takes place, only smooth ER is found. The rough ER elements, ther outer surfaces of membranes have a rough appearance due to the attachment of ribosome's on the outer surface. Rough ER is mainly composed of cisterns (membrane-enclosed plate like elements) and is found in cells actively involved in protein synthesis. Smooth and rough E.R change into each other as per the needs of cells.

Functions of ER

i. it provides the structural base for protein (rough ER), lipid, phopholipid synthesis.
ii. it proivides channel for the transport of materials synthesized in association with ER to the various parts of cells and even outside the cells.
iii. it provides a controlled passage for the export of m.RNA moelecules from nucleus to rought ER.
iv. Several enzyme molecules are embedded in the membranes of E.R.

RIBOSOMES

These are dense granular nucleoprotein structures occurring in cytoplasm, matrix of mitochondria and chloroplasts. In many instances ribosomes are attached to the ER. Observed

first in plants cells in 1953 by Robinson and Brown, while studying bean roots. Ranging in diameter from 150 to 200Ao, they have RNA and protein in equal quantities. Ribisomes are isolated by differential centrifugation depending on sedimentation co-efficient. The sedimentation coefficient is expressed in terms of Sved berg units. The 'S' units are related with the size and weight of the ribosome molecules.

TYPES

Two types of ribosome's have been identified based on the sedimentation coefficient. If the organelle is heavier, its sedimentation co-efficient is more. The two types are 70s ribosome's and 80s ribosome's. Ribosome's may occur singly as isolated units when they are called 'monosomes'. When they occur in clusters or groups, they are called 'polyribosomes'. The poly ribosomes may have a sedimentation coefficient of 100s-600s. The number of ribosomes per cell varies, it may be 10,000 (bacterial cell) or up to 10 million (eukaryotic cell). Ribosomes of chloroplasts and mitochondria have their own protein synthesis.

They have sedimentation co-efficient of 55s with two sub units 40s and 30s.

ULTRA STRUCTURE

Ribosomes are oblate or spheroidal structures having two sub units (a large and a small). The larger sub unit in dome likes and the smaller subunit is placed above like a cap. The 70 s ribosome has two units 50 s and 30s.

FUNCTIONS

Ribosomes are the sites of protein synthesis. The polyribosomes serve as a plat form in the assembly of amino acids brought together by specific to RNA from cytoplasm.

GOLGI COMPLEX

Described first by Camilio Golgi in 1890. Golgi complex found in plant cell is often referred to as 'Dictyosomes". Each golgi body consists of following parts;

1. Cisternae
2. Tubulus
3. Vesicles
4. Golgian vacuoler.

Functions

i. Absorption of compounds

ii. Sites of enzyme production

iii. Sites of hormonal production

iv. Sits of protein storage

v. Formation of plant cell wall-by synthezing pectin, hemicellulose and cellulose microfibrils. They also help in the formation of cell plate during mitosis.

PLASTIDS

These are living cytoplasmic inclusions found inmost of the plants. The plastids are of three categories viz., chromoplasts, leucoplasts and chloroplasts.

Chromoplasts

They are pigmented plastids. The pigments are non-chlorophyllus like carotenes, yellow xanthophylls, phycoerythrin etc.,

Leucoplasts

They are colorless plastids. They lack pigments and are usually present in cells which do not receive direct light. Leucoplast may be seen in the storage leaves of onion. Leucoplasts that store starch are called amyloplast, those that store oil are called elaioplasts and the ones storing proteins are called protenoplasts.

Chloroplast

These are by far, the commonest and the most plastids. As the primary sties for trapping and converting solar energy they are very vital for the existence of not only the green plants, bu for the whole living world.

Chloroplasts have varied shape and varied size. Chloroplast of polyploid cells are generally larger than in the diploid cells. They are uniformly distributed all over the cytoplasm, but in some instances they cluster towards the nucleus. The concentration of chloroplasts will also depend on light intensity.

Structure

It has a covering of two membranes with an inner membrane space. These membranes are smooth and there are no perforations or particles. The membranes are differentially permeable.

A section of chloroplasts reveals an intricate system of membranes enclosed in a granular matrix. These membranes are called lamellae and the surrounding matrix-the stroma. In a sectional view, the lamellae can be seen packed and there stacks are called thylakoids. In higher plants, the thylakoids themselves form highly compact bundles called grana. Some thylakoids of granum extend into the stroma and maintain contact with other grana. These are called stroma thylakoid or stroma lamellae or inter grana. Ribosomes and RNA have also been isolated from the chloroplasts indicating a machinery for protein synthesis. Some of the important pigments present in chloroplast are chlorophylls, carotenoids, cytochromes etc.,

NUCLEUS

It is the most important organelle of the cell which regulates all its activities. It was discovered by Robert Brown (1831). Most of the cells are uninucleate. It has the following parts;

1. Nuclear membrane
2. Karyolymph (Nuclear sap)
3. Chromonemata
4. Nucleolus
5. Endosperms

NUCLEAR MEMBRANE

It helps in effective communication between nucleus and cytoplasm. The elements of E.R. contribute to the nuclear envelope during cell division. The nuclear membrane is a double membrane with a number of pores called ‘Nucleopores'. The space between these two membranes is called 'perinucelar space' or cisterna.

KARYOLYMPH (NUCLEAR SAP)

It is proteinaceous, but also has nucleic acids, enzymes and minerals. It is quite probable that in plants the nuclear sap contributes to the spindle.

CHROMONEMATA

Enclosed in the karylymph and visible in the interphase nucleus are found a number of fibrillar structures constituting a network called chromonemata or chromatrin fibrils. Some coarsegranules are deposited on the chromatin net work. These are called chromocentres and

constitute the points of condensation of chromosomes. During cell division the chromatin network brakes up into specific number of chromosomes. Two regions can be identified in the chromatin material. These are heterochromatic region and euchromatic region. The heterochromatic region stains darkly and shows numerous bead like structure called 'Chromomeres'. The heterochromatic region has less DNA. This region is believed to the genetically and metabolically inert. The light staining region of the chromatin is called the 'euchromatin region" This region contains more of DNA and is supposed to be genetically active. **NUCLEOLOUS**

Nucleolus was first discovered by Fortana (1874). A sphearoidal body, situated either in the central or peripheral position, the necleolous is supported to regulate the synthetic activity of the nucleus. Usually 2 or more chromosomes are associated with the nucleus (this can be seed during late prophase) and these are called nucleolar organisms as they play a role in re-appearance of the nucleolus after cell division. The number of nucleoli per nucleus varies from one to 2 or 3. Chemically the nucleoli are rich in RNA.

Functions

i. It is the active site of RNA synthesis

ii. It is the source of ribosomal RNA

iii. It produces precursors of ribosome's

Lecture3. Cell division – mitosis, meiosis and their significance, cell cycle; zygote formation and embryo development - identical and fraternal twins.

CELL DIVISION

MITOSIS

All cells originate through division of pre-existing cells. Bodies of all multicellular organisms are derived from unicellular zygote through repeated divisions of zygote and the cells derived through its division. The division of chromosomes and cytoplasm of a cell into daughter

cells is known as 'Cell division'. The cell that undergoes division is termed as 'parent cell', while the cells derived from the division of a parent cell are known as daughter cells.

Functions of cell division

To produce two daughter cells, which are involved in the following;

i. Growth and development of somatic tissue of organism

ii. Regeneration of damaged tissues

iii. Production of new tissues

iv. Reproduction

v. Keeping the size of cells within a limited range.

Two types of cell division i. Mitosis ii. Meiosis

In addition, bacterial cells divide by fission (similar to mitosis). The various events occurring in division may be grouped into

i. Karyokinesis - Division of chromosomes

ii. Cytokinesis - Division of cytoplasm

MITOSIS

It was first used by Fleming in 1882. In plants, mitosis is confined to the meristamatic tissues of root and shoots tips, young leaves flower buds. On the basis for changes in the morphology of nucleus and the chromosomes, the events in a mitotic cell division are grouped into five stages;

i. Interphase

ii. Prophase

iii. Metaphase

iv. Anaphase

v. Telophase.

Interphase

In this stage of cell after the telophase of previous division and before onset of prophase of the next one. During interphase, chromosomes are fully extended and uncoiled so that they do not take-up sufficient stain. Interphase in the longest stage. In a cell undergoing mitosis every 24 hours i.e. having a cell cycle of 24 hr., interphase may occupy upto 23 hours, while the division

or mitotic phase may take up onluy 1 hour. DNA replication occurs during the middle part of interphase. This provides the basis for classifying interphase into three sub stages.

1. G1 (first gap)
2. S (Synthesis of DNA)
3. G2 (Second gap)

G1, G2 - Protein + RNA synthesis

S - DNA synthesis

M - Chromosome movement, division

PROPHASE

i. The appearance of define thread like structures in nuclear is the most important event of prophase. In the beginning, chromosomes appear as a loose ball of thin wool. As prophase proceeds, chromosomes become increasingly shorter and thicker due to increased condensation by mid prophase; the two chromatids of each chromosome become visible. By the end of prophase all the chromosomes become considerably shorter and thicker.

ii. During prophase nucleolus and nuclear membrane remain present.

Chromosome condensation that is decrease in length, with increased in thickness is mainly due to the coiling of chromosomes.

The two sister chromatids of each chromosomes are coiled in relation to each other this is referred to as relational coiling. This relational coiling is of two types.

i. **Plectominic coiling** - The two sister chromatids cannot separate from each other without the chromosomes being rotated. It is happening during prophase of mitosis.

ii. **Paranimic coiling** - Sister chromatids are note twisted round each other, they are simply slipped into those of other they can easily separated without rotating the chromosomes. This type of coiling is found during meiotic prophase. The relational coiling between sister chromatids goes on decreasing as the chromosomes become smaller, it disappears by late prophase.

METAPHASE

At the end of prophase four important events take place;

The main features of metaphase are

i. Absence of nucleolus

ii. Disappearance of nuclear membrane.

iii. Arrangement of chromosomes on the equatorial plate

iv. Shortest and thickest chromosomes (Condensation)

v. Coils are less in number and largest in diameter

vi. Presence of spindle apparatus

vii. Absence of relation coiling between sister chromatids.

ANAPHASE

The two sister chromatids of each chromosomes separate and migrate towards the opposite poles of the cell. Anaphase begin when the centromeres of chromosomes appear to divide longitudinally so that the sister chromatids separate from each other and ends with the reaching of the chromosomes to opposite poles centromete in the first portion of each of the chromosomes to begin to move towards the poles. Spindle fibres originate at two points located near the periphery of a cell and opposite to each other. These points are known as 'poles' Chromosomes become somewhat more condensed as compared to those at metaphase, so that they appear relatively smaller in size.

TELOPHASE

Anaphase ends and Telophase begins when sister chromatids of all the chromosomes of a cell reach the opposite poles. During telophase, the following events occur in the two groups of chromosomes collected at the opposite poles.

i. The chromosomes uncoil so that they become very long and thin and appeared to be coiled into a loose ball of fine thread.

ii. Nucleus reappears

iii. Nuclear membrane is reorganized around each group of chromosomes.

iv. At the end of telophase, middle lamella appears at the equatorial plate of the cell.

The nuclear envelope dissociates into small elements which become part of E.R. of the cell. During telophase, there elements re-originate around the two groups of chromosomes and fuse to produce nuclear envelope around them. In terms of duration, prophase in the longest stage of the

division phase of cell cycle. In comparison anaphase is the shortest stage, while metaphase and telophase are considerably longer than anaphase.

CYTOKINESIS

It is complete by the end of Telophase. At the equatorial plate, elements of E.R. and products of Golgi bodies organize and give rise to cell plate and subsequently of cytoplasm begin in the centre of the cell and gradually extend outwards on each side in a plane, perpendicular to the axis of the spindle. The two daughter cells produced by mitosis contain one nuclear sac; each nuclear has the same number of chromosomes as the parent cell. Each daughter cell enlarges in size till it becomes comparable to the parent cell.

Significance of Mitosis

- After fusion of male and female gametes the zygotes are formed. So, Mitosis is responsible for development of zygote into adult organism.
- it is essential for normal growth and development of living organism. It gives shape to a specific organism.
- Mitosis, in plants leads to formation of new parts – roots, leaves, stem branches. It helps in repairing of damaged parts.
- Mitosis, leads to production of identical progenies in vegetatively propagated crops.
- It is useful in maintaining purity of types because it leads to production of identical daughter cells and does not allow segregation and recombination to occur.
- In animals, it helps in continuous replacement of old tissues. Eg. Blood cells.

MEIOSIS

Meiosis takes place during gamete formation and hence it is confined to reproductive cells only. As a consequence of meiosis, gametes contain only half (h) of the somatic chromosome number (2n). Therefore union between one male and one female gamete during fertilization restorers the chromosome number to the diploid (2n) state. Thus the chromosome number of a species remains constant from one generation to the next generation produced by sexual reproduction. In the absence of meiotic cell division, the chromosome number of a species would be doubled in

every generation, due to the fusion of male and female gametes, an impossible biological situation. The nucleus of each cell undergoes two successive divisions referred to as the first and second meiotic division.

Pre-Meiotic Interphase

During 'S' phase of pre-meiotic interphase chromosomes replication takes place. But approximately 0.3% of the total DNA present in the nucleus does not replicate during the 'S' phase this DNA replicates during the zygotine sub stage of prophase I. A special type of histone specific to cells preparing for meiosis is synthesized during S phase. This histone is not found in cells undergoing mitosis, and it may be related to the entry of cells into meiosis.

FIRST MEIOTIC DIVISION

Significant events;

i. Pairing between homologous chromosomes.

ii. Crossing over between them during pachytene stage of prophase I

iii. Separation of homologus chromosomes and their migration to the opposite poles of a cell during Anaphase I. As a result, the two daughter nuclei produced by this division receive only half of the chromosomes present in somatic cells. For this reason, the first division is often referred as 'Reduction division'.

Prophase I - is divided into 5 sub stage viz.,

i. Leptotene
ii. Zygotene
iii. Pachytene
iv. Diplotene
v. Diakinesis

LEPTOTENE

i. There is a marked increased in the nuclear volume

ii. There is chromosome condensation so that they become visible as fine threads like a loose ball of knitting wool. Each chromosome consists of two chromatids.

ZYGOTENE

It begins with the initiation of pairing between homologous chromosomes. The main events are as follows:

i. Paring between homologous chromosomes.

ii. Completion of replication of the remaining 0.3% DNA of each nucleus, this DNA synthesis is referred to as Z-DNA synthesis or Zygote DNA synthesis.

iii. Synthesis of a specific nuclear protein

iv. Development of the synaptenemal complex and

v. Progressive condensation of chromosomes.

Pairing of homologous chromosomes is often referred as 'Synapsis'.

PACHYTENE

It begins when synapsis comes to an end and it ends when the homologous chromosomes begin to move away from each other. The main events are ; i. Thre is a further condensation of chromosomes, so that chromosomes pairs become shorter and thicker. ii. Chromosomes are easily recognizable during this stage and each bivalent has four chromatids. iii. The nucleolus is distinct and quite large. iv. **Crossing over between homologous chromosomes takes place during this stage**.

DIPLOTENE

i. Homologous chromosomes of each bivalent begin to move away from each other.

ii. The two homologous of each bivalent appear to be attached with each other at one or more points, these attachments are known as chiasma. It is believed that initially chiasma are located at the points of actual crossing over between homologous chromosomes.

iii. As diplotene progress, chiasmata, slowly move towards the ends of the homologous chromosomes; this movement is referred to as **chiasma terminalization** i.e. movement of chaisma towards terminal positions in the chrommosomes. Chiasma terminalization occurs mainly due to the movement of homologous chromosome away from each other.

iv. There is further condensation of chromosomes so that they become progressively shorter and thicker.

DIAKINESIS

i. Bivalents move away from each other and spread towards the periphery cells.

ii. Nucleolus, nuclear envelope disappears.

iii. The spindle apparatus is organized. The bivalents now migrate to the equatorial plate of cells; this marks the ends of diakinesis. Bivalents may be in the form of (1) a closed ring, (2) an open ring or (3) rod shaped.

METAPHASE -I

i. Bivalents are arranged at the metaphase plate

ii. Centomeres of the two homologues of each bivalent lie on the either side of the equatorial plate.

iii. Metaphase terminates as soon as homologous chromosomes begin to separate from each other and to migrate to opposite poles of the cell.

ANAPHASE -I

i. Separation of the two homologous chromosomes of each bivalent marks the beginning of anaphase stage.

ii. One chromosome from each bivalent begins to migrate to one pole, while the other migrates to the opposite pole.

As a result the number of chromosomes at each pole is exactly half (h) and each pole receives one homologue from each of the bivalents present in a cell. Thus the reduction in chromosomes number is not only a quantitative one but a qualitative one as well. Thus at the end of AI, the chromosome present is somatic cells are effectively and precisely separated into two identical groups.

TELOPHASE -I

i. The chromosomes uncoil only partially

ii. Nuclear envelope becomes organized around the two groups of chromosomes.

iii. Nucleolus also reappears.

CYTOKINENSIS

The cytoplasm of each cell divides into two halves, with a single haploid nuclear in each half. The two halves of each cell do not separate, but they stay together, and this two celled structure is known as dyad.

SECOND MEIOTIC DIVISION / MEIOSIS II

During Meiosis II, two sister chromatids of each chromosome separate and migrate to the opposite pole. As a result, the number of chromosomes in each of the two haploid nuclei remains the same (i.e haploid)., at the end of this division. The second division of meiosis is often referred to as equational division. Sometimes, it is called as 'Meiotic Mitosis'. The second meiotic division is also divided into four stages.

i. Prophase II

ii. Metaphase II

iii. Anaphase II and

iv. Telophase II

PROPHASE - II

There is no relational coiling between sister chromatids. At the end, nucleus, nuclear envelope disappears and spindle apparatus is organized.

Cytokinesis

Dyad divides into two parts. One parent cell produces from haploid daughter cells after meiosis. The four daughter cells present together and are known as tetrad.

Significance of Meiosis

- Meiosis play an important role in all living organisms
- It helps in maintaining the chromosome number constant in a species.
- Meiosis results in production of gametes with haploid chromosome number.
- Union of female and male leads to formation of Zygote which receives ½ chromosome number from female and ½ chromosome number from male, thus the original somatic chromosome number is restored.
- Meiosis facilitates segregation of independent assortment of chromosome and genes.

- Recombination of genes results in Creation of Variability which is essential for evolution of new crop plants.
- In sexually reproduction crops Meiosis helps for continuity of generation.

zygote formation and embryo development

- The development of the embryo is called embryogenesis.
- In organisms that reproduce sexually, once a sperm fertilizes an egg cell, the resultant cell is called as zygote that has half of the DNA of each of two parents.
- In plants, animals, and some protists, the zygote will begin to divide by mitosis to produce a multicellular organism. The result of this process is an embryo.
- **Plant embryogenesis** is the process that produces a plant embryo from a fertilized ovule by asymmetric cell division and the differentiation of undifferentiated cells into tissues and organs.
- It occurs during seed development, when the single-celled zygote undergoes a programmed pattern of cell division resulting in a mature embryo.

Identical and fraternal twins

- A **twin** is one of two offspring produced in the same pregnancy
- Identical twins (*monozygotic)* : they develop from one zygote that splits and forms two embryos
- Fraternal twins (*dizygotic*) - they develop from two separate eggs that are fertilized by two separate sperm.

Lecture 4. Chromosome structure, chemical composition, nucleosome, centromere, telomere, euchromatin, heterochromatin, NOR, satellite chromosome, karyotype, ideogram – chromosome banding.

CHROMOSOME

Chromosomes are rod shaped, dark stained bodies seen during metaphase. The term 'chromsome' was first used by Waldeyer in 1888. (Chrom- coloured soma =body), deeply stained, while cytoplasm remained unstained. Each speceies has a definite chromosome number. Each species has a definite chromosome number, represented by 2n. Somatic cells contain two copies of each chromosome, which are identical in morphology, gene content and gene order and they are known as homologous chromosomes. Gametic chromosome number is precisely one half of the somatic number, is represented by 'n' zygote is produced by fusion of one male and one female gamete (n+n=2n).

MOROPHOLOGY

Cell division, the following structural features can be seen under light microscope by staining.

1. Chromatid
2. Centromere
3. Telomere
4. Secondary constriction and satelite
5. Chromosome

CHROMATID

It is the structural and functional unit of chromosomes. At Metaphase, each chromosome appears to the longitudinally divided into two identical parts, each of which is known a 'Chromotid'. The chromatids of a chromosome appear to joined the giller at a point called 'centromere'. The two chromatid making up a chromosome are produced through replication of a single chromatid, they are referred as 'Sister chromatids'. In contrast the chromatrids of homologous chromosomes are known as non-sister chromatids.

CENTROMERE

The region where thre two sister chromatids of a chromosome appeared to held together is known as 'centomere' under light microscope, centomere generally appears as a constriction in the chromosome, hre it is also termed as 'primary constriction'. Centromeres are the first part moving towards the opposite poles during anaphase; the remaining regions lag behind and appear as if they wree being pulled by the centomere. Therefore, chromosome movement is due to the centromeres of chromosomes hence they are also known as 'Kinetochoes'. In most speceis each chromosome has a single centromere in a fixed position which does not change except due to structural chromosome aberrations. Therefore, the position of centromere serves as an important land mark in the identification of different chromosomes of a species. Each chromosomes is divided into two transverse parts by its centromere; these parts are called 'Arms'. On the basis of the position of centromere, the chromosome may be divided into your classes.

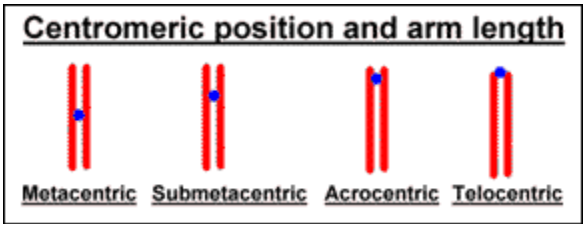
i. **Metacentric** - Cntremere is at the centre of chromosome having equal arms and appeared as 'V' shaped during anaphase.

ii. **Submeta centric chromosoem** - Centromere is on one side called 'Submedian. 'L' ' shaped during anaphase.

iii. **Acrocentric** - When centromere is located close to one end, they are called as "Sub terminal 'j' or rod shaped.

iv. **Telo centric** - Occasionally, the centromere appeared to be at one end of the chromosome, called as 'Terminal' Rod shaped during anaphase. They are unstable. In most species each chromosomes has a single centromere such chromosomes are termed as 'Monocentric'. But in some speceis each chromosomes as 'Poly centric'- Polycentric chromosomes often break into smaller chromosomal units each of which is stable and functions normally. Centromeres,

ccontain highly repetitive DNA called "Satellite -DNA" or "Sat- DNA", distinct from the rest of the Chromosomal DNA. It constitutes about10% of total DNA present in the genome. In many species Sat-DNA consists of only one sequence, while in others more thanone distinct sequences are found.



TELOMERE

The two ends of chromosomes are known as 'Telomeres'. They are highly stable and do not fuse with other chromosomes. It is generally accepted that, the structural integrity and individuality of chromosomes is maintained due to the telomeres and that all stable chromosome ends are composed of telomeres.

SECONDARY CONSTRICTION AND SATELITE

In some chromosomes a secondary constriction, in addition to that due to centromere (primary constriction) is also present. It is known as "Secondary constriction. It is present in short arm near one end, or in many chromosomes they are located in the long arm nearer to the centromere. The region between the secondary constriction and the nearest telomere is known as satellite. Therefore, chromosomes having secondary constriction are called "Satellite Chromosome" or "Sat -Chromosomes. The position of secondary constriction in Sat-Chromsome is fixed and remains constant. The number of Sat-Chromosomes in the genome varies from one species to the other. The number of Sat. Chromosomes may ranged from 2,4,6 or 10, 13,14,15,21 and 22. Human somatic cells have 10 Sat Chromosomes. Nucleolus is always associated with the secondary constriction of Sat. Chromosomes. Therefore secondary

constrictions are also called as "Nucleolus organizer Region" (NOR) and Sat-Chromosomes are often referred as Nucleolus organism chromosome (NOC) NOR contains several hundred copies of the gene coding for ribosomal RNA (r RNA).

CHROMOSOME

In some species (Maize, amphibian etc.,) chromosomes during Prophase I of meiosis, particularly during pachytene stage, show small bead like structures called 'Chromomeres'. The distribution of chromomeres in a chromosome is highly characteristic and constant, the patterns of distribution being different for different chromosomes; homologous chromosomes show an identical pattern.

- Flemming (1882) discovered two structures at cytological level
- Heitz (1928) coined as
- **HETEROCHROMATIN -** Found proximal to centromere, indistinguishable from Euchromatin at metaphase- genetically inactive region
- **EUCHROMATIN** is genetically active and less contracted regions. Principal functional regions
- Pachytene stage of meiosis is ideal for studying chromosome morphology

Two types of heterochromatin

- Constitutive heterochromatin or inherited
- Facultative heterochromatin or appearing during development

S.N	Euchromatin	Heterochromatin
1.	Less condensed region	More condensed region
2.	Loosely coiled chromatin fibres	More tightly folded chromatin fibres
3.	Deeply stains- cell division; less stain -interphase	Less stain - cell division; Deeply stain -interphase
4.	Genetically active - has unique DNA	Genetically less active – has repetitive DNA
5.	Can synthesise mRNA in vitro	Unable to synthesise mRNA in vitro

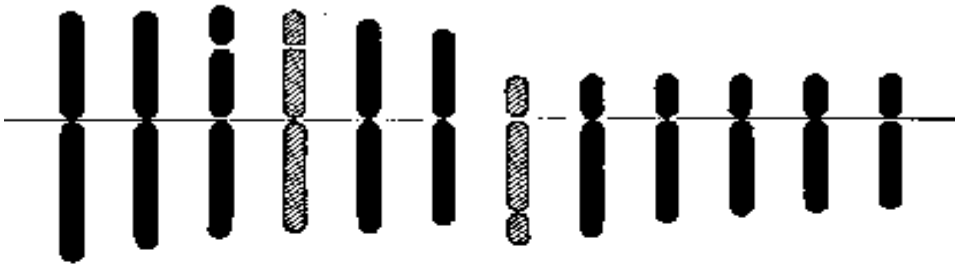
6.	DNA replication is earlier – in the S phase	DNA replication is at later stage –at the start of cell division
7.	This region is not sticky	This region is sticky
8.	Cross over frequency is more	Cross over frequency is less
9.	Does not show heteropycnosis	show heteropycnosis

KARYOTYPE

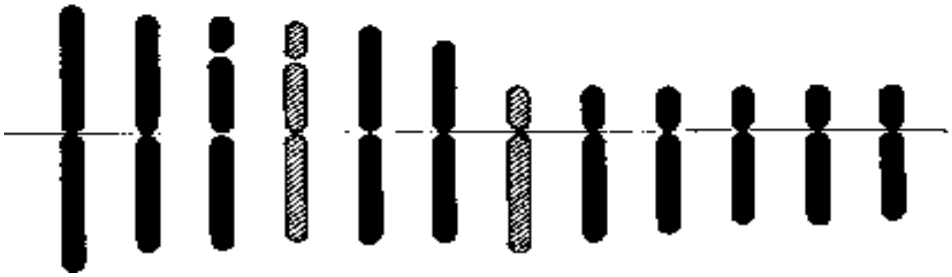
The general morphology, i.e. the size of chromosomes, the position of centromeres, the presence of secondary constriction and the size of satellite bodies of the somatic chromosomes complement of an individual constitutes its "Karyotype". It is represented by arranging the chromosomes in a descending order of size keeping their centromeres in a straight line. Each chromosome in the karyotype is designated by a serial number according to its position. A perfectly symmetrical karyotype has all metacentric chromosomes of the same size. Karyotypes showing a deviation from this state are called asymmertical. It is believed that, perfectly symmetrical karyotypes represent a primitive state from which more advanced asymmetrical Karyotypes have evolved through structural changes in chromosomes.

Idiograms: Diagrammatic representation of chromosome arrangement based on size and length.
Idiograms of larch species: related species often have different karyotypes (idiograms) as is shown by these idiograms of Larix decidua and L. sibirica.

LARIX DECIDUA



LARIX SIBIRICA



Chromosome banding techniques

A **chromosome** **banding** pattern is comprised of alternating light and dark stripes, or bands, that appear along its length after being stained with a dye. A unique **banding** pattern is used to identify each **chromosome** and to diagnose chromosomal aberrations, including **chromosome** breakage, loss, duplication or inverted segments.

Chromosome banding has since become a standard and indispensible tool for cytogenetic analysis., and several banding techniques have been developed::

Q banding: chromosomes are stained with a fluorescent dye such as quinacrine . Chromosomes are treated with quinicrine mustard solution, a fluorescent stain, to identify specific chromosomes and structural rearrangements. It is especially useful for distinguishing the Y chromosome (also Y bodies in interphase nuclei) and various polymorphisms involving satellites and centromeres of

specific chromosomes.

G banding: produced by staining with Giemsa after digesting the chromosomes with trypsin. Chromosomes are G-banded to facilitate the identification of structural abnormalities. Slides are dehydrated, treated with the enzyme trypsin, and then stained.

C banding: chromosomes are treated with acid and base, then stained with Giesma stain. C-**banding** stains areas of heterochromatin, which is tightly packed and repetitive DNA. NOR-staining, where NOR is an abbreviation for "nucleolar organizing region," refers to a silver staining method that identifies genes for ribosomal RNA that were active in a previous cell cycle.

R-banding methods are useful for analyzing deletions or translocations that involve the telomeres of chromosomes. R-**banding** is the reverse pattern of G bands so that G-positive bands are light with R-**banding** methods, and vice versa. R-**banding** involves pretreating cells with a hot salt solution that **denatures** DNA that is rich in adenine and thymine. The chromosomes are then stained with Giemsa. R-**banding** is helpful for analyzing the structure of **chromosome ends,** since these areas usually stain light with G-**banding**.

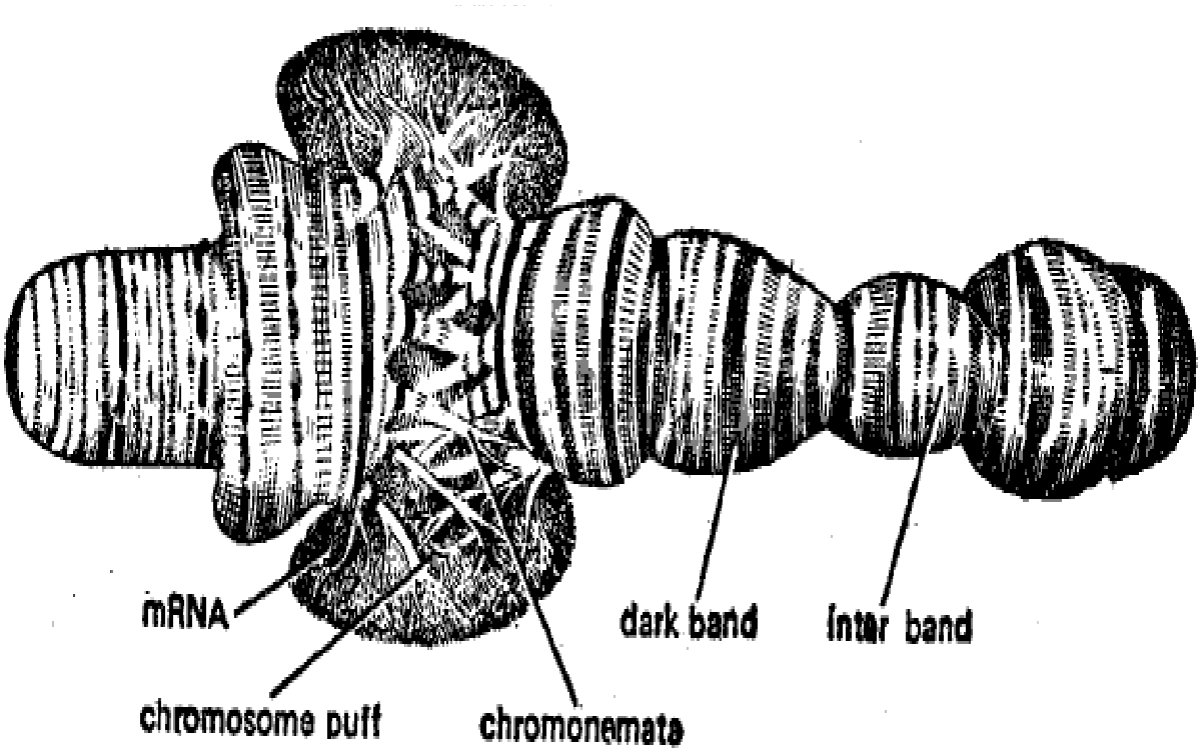
T-banding is used to stain the telomeric regions of chromosomes for cytogenetic analysis. Telomeric (or terminal) banding was first reported by Dutrillaux, who used two types of controlled thermal denaturation followed by staining with either Giemsa or acridine orange. The T bands apparently represent a subset of the R bands because they are smaller that the corresponding R bands and are more strictly telomeric..

Lecture 5. Types of chromosomes based on position of centromere, based on structure and function: normal and special chromosomes - polytene, lampbrush, based on the role in sex determination: autosomes and allosomes, Other types of chromosomes - B, ring and isochromosomes.

Special chromosomes

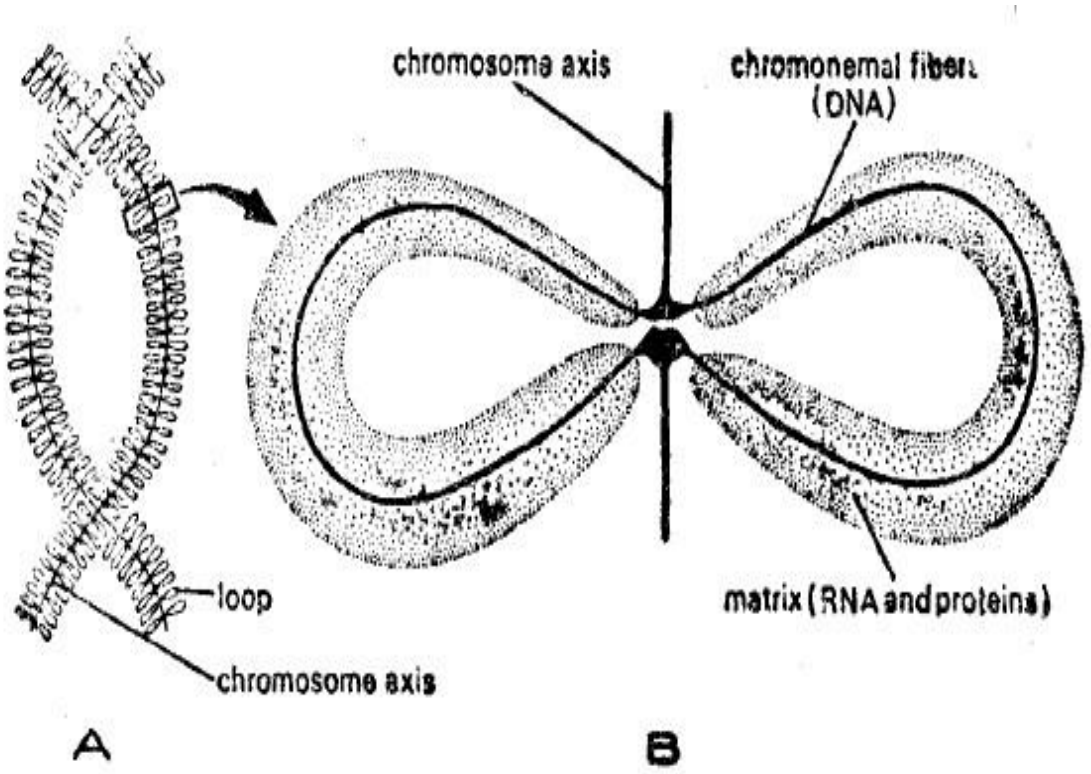
1. **Polytene chromosomes**

- Also called as Giant chromosomes / Salivary gland chromosomes. First reported by Balbiani in 1881
- The nuclei of the salivary gland cells of the larvae of *Drosophila* have unusually **long and wide chromosomes, 100 or 200 times in size of the normal chromosomes**.
- The **salivary gland cells do not divide** after the glands are formed. But their chromosomes replicate several times **(a process called endomitosis)** and become exceptionally giant – sized to be called polytene chromosomes
- The polytene chromosomes of the salivary gland cells of *D. melanogaster* contain **1000 to 2000 chromosomes, which are formed by nine or ten consecutive multiplication cycles and remain associated parallel to each other.**
- polytene chromosomes have **alternating dark and light bands along their length**.
- The dark bands are comparable with the chromomeres of simple chromosomes and are disc-shaped structures occupying the whole diameter of chromosome. They contain **euchromatin**.
- The light bands or inter bands are fibrillar and composed of heterochromatin.
- The swollen regions are known as chromosome **"puffs" or Balbiani rings**. They are the regions of genetic activity .
- **Chromosome puffs** are diffuse uncoiled regions of the **polytene** chromosome that are sites of RNA transcription. A **Balbiani ring** is a large chromosome puff.
- Such puffs **change location** as development proceeds, at specific locations.
- The presence of a specific puff is related with the appearance of a specific protein



Polytene chromosome

2. **Lampbrush chromosome** One of the large **chromosomes** found in the eggs (primary oocytes) of amphibians, with paired loops which extend from most of their chromomeres giving a furry, brush-like appearance under the microscope. They are particularly obvious at the diplotene stage of meiosis.**Lampbrush** chromosomes occur during the diplotene stage of meiosis I. **Lampbrush** chromosomes are meiotic bivalents, each consisting of 2 sister chromatids. Each halve-bivalent is represented by two long strands that form many **brushlike loops** along the main axis of the **chromosome**. The outgrowths make DNA available for transcription during the maturation of the egg. Usually there is a little gene expression at meiosis, so it is not so easy to identify the activities of individual genes. Giant chromosomes in the **lampbrush** form can solve this problem, since they allow the individual transcription units to be examined. **Lampbrush** and chromosomal puffs in a cell indicate that the transcription of tRNA is taking place.

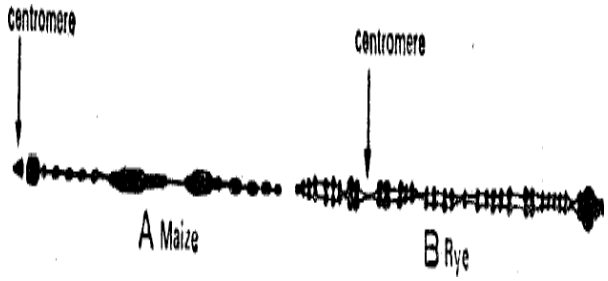


A- At low magnification, B- A loop magnified (after Robertis, *et al.*, 1970).

Lampbrush chromosomes

3 B-Chromosomes

– Many plant (maize) and animal (insects and small mammals) species, besides having autosomes (A-chromosomes) and sex-chromosomes posses a special category of chromosomes called B-chromosomes without obvious genetic function. These B-chromosomes usually have a normal structure, are somewhat smaller than the autosomes.

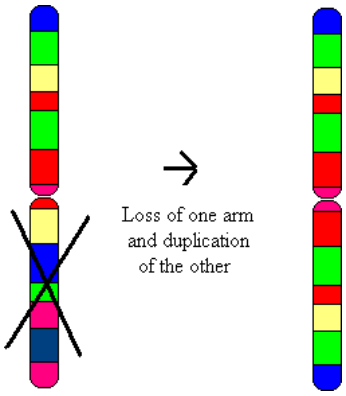


4.ISO Chromosomes- An isochromosome is a chromosome in which both arms are identical. It is thought to arise when a centromere divides in the wrong plane, yielding two daughter chromosomes, each of which carries the informations of one arm only but present twice. The isochromosomes are formed during mitosis and meiosis.

If a gamete having a isochromosome is fertilized by a normal gamete, the zygote will possess an unbalanced karyotype.

In *Drosophila,* the misdivision of centromere of telocentric X chromosome changes that into an "attached-X" isochromosome, In man X-isochromosome causes the disease called gonadal dysgenesis.

An isochromosome is a chromosome that has lost one of its arms and replaced it with an exact copy of the other arm



5.Ring chromosome

- A ring chromosome is a chromosome whose arms have fused together to form a ring.
- A ring chromosome is denoted by the symbol r.
- Ring chromosomes may form in cells following genetic damage by mutagens like radiation, they may also arise spontaneously during development.
- Ring chromosomes found in prokaryotes
- Ring chromosomes otherwise called as genophores
- Ring chromosomes were Also reported in humans and drosophila

- Ring chromosomes thoroughly studied in maize by Mc Clintock
- Ring chromosomes are meiotically unstable

Lecture 6. Chromosomal aberration: Variation in chromosome structure – deletion, duplication, inversion and translocation – genetic and cytological implications.

Types of structural chromosomal aberrations

The chromosomal aberrations may remain confined to a single chromosome or may extend to both of the member of the homologues pair and, therefore, may be of following types:

A. Intrachromosomal aberrations,

B. Interchromosomal aberrations.

A. Intrachromosomal Aberrations

When aberrations remain confined to a single chromosome of a homologous pair, they are called intrachromosomal or homosomal aberrations.

1. Deficiencies (Deletions): Loss of a portion of segment from a chromosome is called Deletion

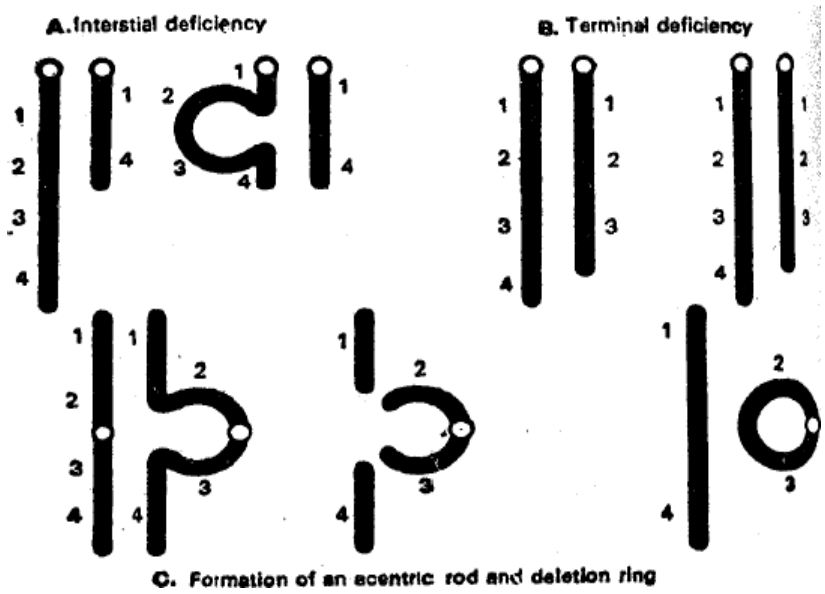
Two types - Terminal deficiency
Intercalary or interstitial deficiency**.**

Terminal deficiency

In deletion or deficiency type intrachormosomal aberration a chromosomal lacks either in an interstitial or terminal chromosomal segment which may include only a single gene or part of a gene.

Intercalary or interstitial deficiency: Loss of a portion of segment from a chromosome from the intermediate portion or between telomere and centromere.

- Intercalary deletions are more common than terminal deficiency
- The deleted portion may have one / two / several genes



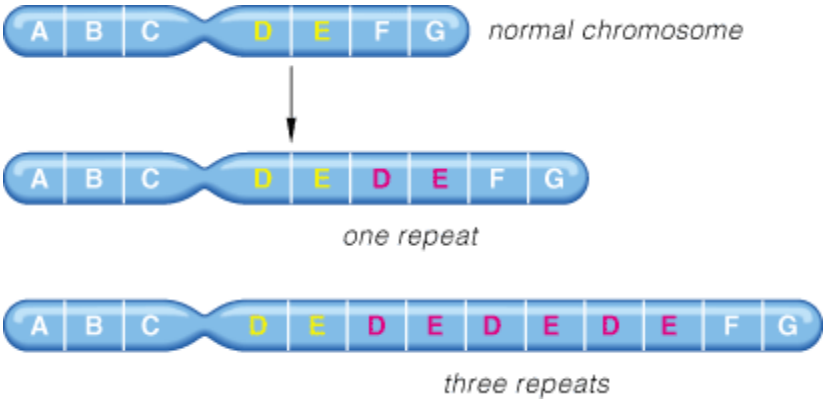
Genetic Significance of Deficiencies

Lethal effect: Organisms with homozygous deficiency usually do not survive to an adult stage because a complete set of genes is lacking.

2. Duplications (Additions)

Duplication occurs when a segment of the chromosome is represented two or more times in a chromosome of a homologous pair. This extra-chromosomal segment may be a free fragment with a centromere or a chromosomal segment of the normal complement

- Reported by Bridges (1919) in Drosophila
- Recent reports is on several crops – rice , wheat, maize, Tobacco, Tradescantia, Barley

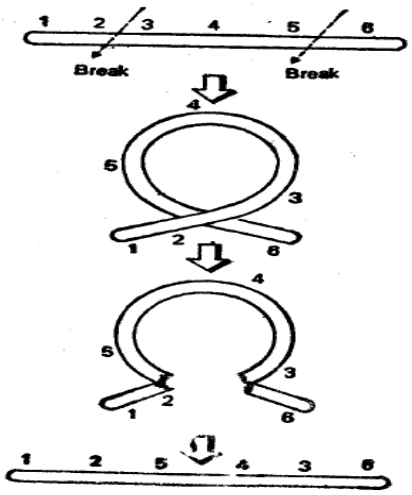


Genetic significance of Duplications

- The duplications of chromosomes are not deleterious to the organism like the deficiency, but, they usually protect the organism from the effect of a deleterious recessive gene or from an otherwise lethal deletion.
- some duplications are useful in the evolution of new genetic material. In an organism with duplications, because the old genes can continue to provide for the present requirements of the organism, the superfluous genes may be free to mutate to new forms without a loss in immediate adaptability.
- Large duplications can reduce the fertility as a result of meiotic complication, and in this way reduce their own probability of survival (Sybenga, 1972).
- Relocation of chromosomal material without altering its quantity may result in an altered phenotype, this is called position effect.

INVERSIONS

An inversion is an intra-chromosomal aberration in which a segment is inverted 180 degrees. For example if a chromosome has segments in the order of 1-2-3-4-5-6 and breaks occur in regions 2-3 and 5-6 and the broken piece (3-4-5-) is reinserted in reverse order, then the inverted chromosome will have segments in order of 1-2-5-4-3-6



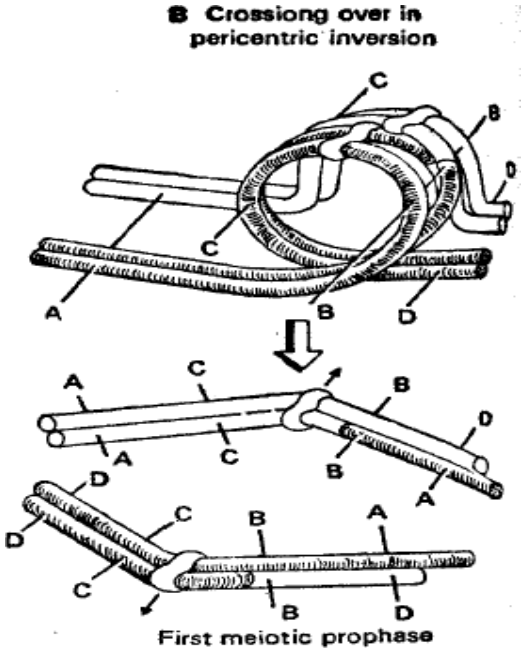
The origin of an inversion (after Stansfield, 1969).

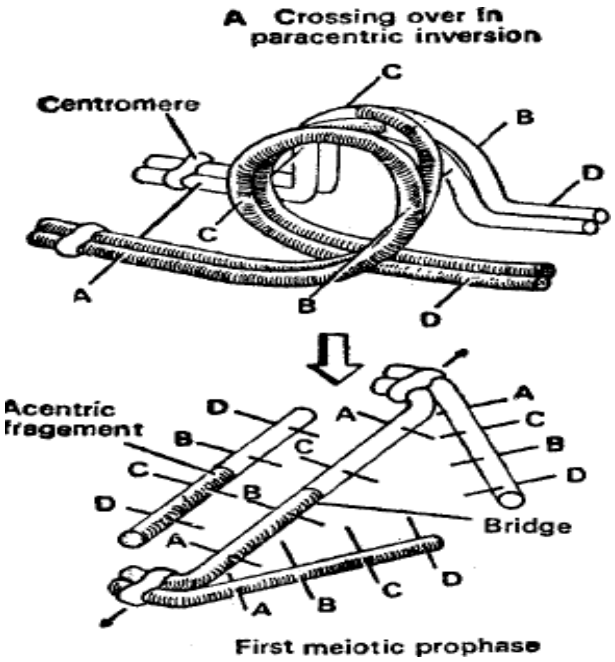
Types of inversions

The inversions are of following types:

i) Pericentric inversions – When the inverted segment of chromosome includes or contains centromere, then such inversions are called heterobranchial or pericentric inversions.

ii) Paracentric inversions – When the inverted segment includes no centromere and the centromere remains located outside the segment, then such type of inversion is called homobranchial or paracentric inversion.





Genetic significance of inversions

i) Simple inversions do not have primary phenotypic effects other then on chromosome shape. Frequently, however, some DNA at a break point has been damaged and this may result in an observable mutation, often recessive (e.g., clB lethal mutation in Drosophila).

ii) Due to inversion a peculiar kind of position effect occurs. The position effect is caused by the transfer of a gene from a euchromatic segment to the vicinity of heterochromatic segment. Heterochromatinization may then extend into a displaced, originally euchromatic region and suppress the transcription of the gene in it.

iii) Normal linear pairing is not possible in inversion heterozygotes. The difficulties encountered with pairing cause a reduction of exchange (crossing over) in and around the inversion.

iv) They maintain heterozygosity from generations to generations.

B. Interchromosomal aberrations

When breaks occur in non-homologous chromosomes and resulting fragments are interchanged by both of the non-homologous chromosomes, the inter-chromosomal or heterosomal aberrations occur. The inter-chromosomal aberration is of following type:

TRANSLOCATION It is an inter-chromosomal aberration where in exchange of chromosomal segments occurs between non-homologous chromosomes

RECIPROCAL TRANSLOCATION: Translocation involves the shifting of a part of one chromosome to another non-homologous chromosome. If two non-homologous chromosomes exchange parts, which need not be of the same size, the result is a reciprocal translocation. The reciprocal translocation may be of following types:

Homozygotic translocation - In homozygotic translocation normal meiosis occur and cannot be detected cytologically. Genetically they are marked by altered linkage group by the fact that a gene with new neighbors may produce a somewhat different effect in its new location (position effect).

Heterozygotic translocation – In heterozygotic translocation a considerable degree of meiotic irregularity occur. During meiosis, an individual which is heterozygous for a reciprocal translocation must form a cross-shaped configuration in order to affect pairing of all homologous segments. This cross-shaped configuration often opens out into a ring as chiasmata terminalize. The meiotic products (gametes) are of three types –normal balanced and unbalanced gametes

Genetic significance of Heterozygotic Translocation:

1. The heterozygous translocation produce semi-sterile organisms because between half and two third gametes fail to receive the full complements of genes required for normal development of sex.
2. Some genes which formerly assorted independently, exhibit linkage relationships after translocation has occurred; a single reciprocal translocation will reduce the number of linkage groups by one.
3. The phenotypic expression of a gene may be modified when it is translocated to a new position in the genome (position effect).

Lecture7. Chromosomal aberration: Variation in chromosome number – euploid, aneuploid, types of aneuploids and their origin; Nondisjunction - Klinefelter syndrome and Turner syndrome; Definition of eugenics and euthenics

The term **genome** refers to a complete set of chromosomes of a diploid species. 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**.**

Numerical changes in chromosomes

- **A) Alterations in whole chromosome sets (Euploidy)**
- **B)Additions or subtractions of individual chromosomes (Aneuploidy)**

Euploidy individuals having the chromosome number which is an exact multiples of the basic or genomic number

Aneuploidy is the term for cells, tissues, and individuals with excess or lacking one or few individual chromosomes; It is a change in the number of chromosomes that can lead to a chromosomal disorder

- **hyperploidy** is a type of aneuploidy when there is an excess number of chromsomes (trisomics, tetrasomics)
- **hypoploidy** is another type of aneuploidy when one or more number of chromosomes are lacking(monosomics, nullisomics).

Types of Aneuploid

Nullisomics(2n – 2): individuals from which one chromosome pair is missing

Monosomics(2n – 1): those lacking a single chromosome

Double monosomic(2n – 1 – 1) : individual has two chromosomes missing, but the two chromosomes belong to two different chromosome pairs.

Trisomic(2n + 1): An individual having one extra chromosome

Double trisomic (2n + 1 +1): that having two extra chromosomes each belonging to a different chromosome pair

Tetrasomic (2n + 2): When an individual has an extra pair of chromosomes

Origin of Aneuploids:

- **Non-disjunction – failure of separation of paired chromosomes**
- occurs when paired chromosomes do not separate either during meiosis I or meiosis II.

Sources of Aneuploids

1. Spontaneous occurrence from normal 2n plants, usually from random segregation of a univalent.
2. From asynaptic disomics and aneuploids – meiotic mutants.
3. Haploids and other polyploids – Unequal segregation from autopolyploids (2x X 4x).
4. Numerical nondisjunction from multivalent configurations may generate trisomics and monosomics – Interchange heterozygotes.

Aneuploids in human disease

Monosomy (2n-1) condition

Turner's Syndrome females have lost one of the X chromosomes (XO), sterile

Trisomics (2n+1) condition

Klinefelter's Syndrome: XXY feminized males; sterile

Down Syndrome: Result of a trisomy of chromosome #21 (although a few cases due to a translocation)

Trisomy 13 (Patau syndrome) and trisomy 18 (Edwards syndrome) can also survive to birth.

Eugenics" The study of the agencies under social control that may improve or impair the racial qualities of future generations either physically or mentally.

Euthenics: the study of methods of improving human well-being and efficient functioning by improving environmental conditions. (Or) Measures to improve the environment in order to improve health, appearance, behavior, or well-being of society.

Euphenics: Measures to improve the individual or phenotype (the body) by biological or medical means.

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.

Euploids

In euploids, the chromosome number is an exact multiple of the basic or genomic number. Euploidy is more commonly known as polyploidy. When all the genomes present in a polyploidy species are identical, it is known as autopolyploid and the situation in termed as autopolyploidy. In the case of allopolyploids, two or more distinct genomes are present. Euploids may have 3 (tripoid), 4 (tetraploid), 5 (pentaploid), 6 (hexaploid), 7 (heptaploid), 8 (octaploid) or more genomes making up their somatic chromosome number.

In case of autopolyploidy, they are known as autotriploid autotertaploid, autopentaploid, autohexaploid, autoheptaploid, autooctaploid and so on, while in the case of allopolyploidy they are termed as allotriploid, allotetraploid, allopentaploid, allohexaploid, alloheptaploid, allooctaploid etc.

Amphidiploid is an allopolyploid that has two copies of each genome present in it and, as a consequence, behaves as a diploid during meiosis. A segmental allopolyploid contains two or more genomes, which are identical with each other, except for some minor differences.

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

Lecture 8. Polyploid - auto and allopolyploids, their characters; meaning of genome; evolution of wheat, Triticale, cotton, tobacco, Brassicas,

POLY PLOIDS

Individuals with more than two sets of chromosomes are called ‘ polyploid’. Poly ploid in otherwise called as ‘Euploidy’.

Individual with one set of chromosomes – haploid or Monoploids.

Individual with two sets of chromosomes – True diploid

Most plants and animals posses two sets of chromosomes.

GENOME

The complete set of chromosome found in the gamete of a true diploid is called a genome. e.g. p. glaucm, 14 chromosome 2 sets n=7, 2n=14.

POLYPLOIDS

Individuals with more than two sets of chromosomes are called ‘ polyploid’..

Somatic No. (2n)	Multiples of Basic No.(x-12)	Level of ploidy
24	2x	Diploid
36	3x	Triploid
48	4x	Tetraploid
60	5x	Pentaploid
72	6x	Hexaploid

CLASSIFICATION

POLYPLOIDS

Based on origin Based on genome

Natural Induced Autopolyploids Allopolyploids

Autopolyploid: when same set of chromosomes of a genome are increased in number, it is autopolyploid. 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

Origin of autopolyploids

- The effective method to obtain autopolyploids is using **colchicine.**
- Colchicine is a spindle fiber poison or suppressant.
- It inhibits the spindle mechanism at mitosis, resulting multiples of normal chromosome number.

Types of Autopolyploids

Auto triploids (3x): Auto triploids have three complete sets of genomes of the same species in somatic cell. AAA

- Autotriploids can be produced by crossing diploids with their corresponding autotetraploids (2x x 4x).
- The high sterility of autotriploids has been explored in plant breeding.
- Triploid bananas (2n = 33) are vigorous but seedless and therefore preferred for food consumption.
- Triploid watermelons have only undeveloped seeds.
- Triploid is also applied in seedless Citrus cultivars

Important triploid plants include, some potatoes, bananas, watermelons and Winesap apples

Autotetraploids: four copies of the genome of same species (AAAA or BBBB) are present.

- Examples of autotetraploids are alfalfa, coffee, peanuts and McIntosh apples.
- These also are larger and grow more vigorously
- All of these crops must be propagated asexually

Use of Autopolyploids in Plant breeding

- i) Autopolyploid manifests greater vegetative growth but reduced seed production.
- This implies that autopolyploid induction would be more useful for vegetative parts of the plants, such as forage, or root, but not the seed.
- ii)Autopolyploid produced from diploids with lower chromosome number have been relatively more successful.

Allopolyploids A polyploid containing genetically different chromosome sets from two or more species is known as allopolyploid. (AABB)

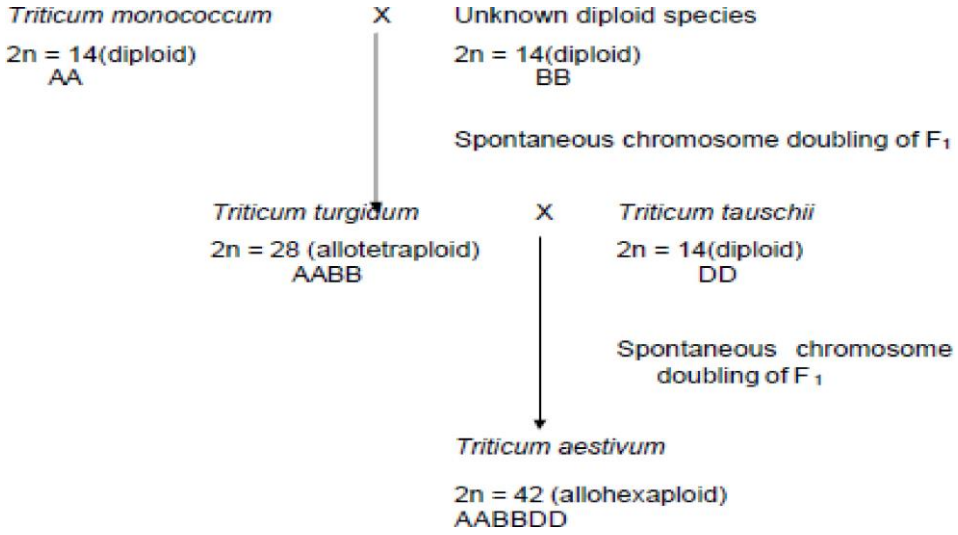
- Allopolyploid are polyploids with chromosomes derived from different species.

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**.

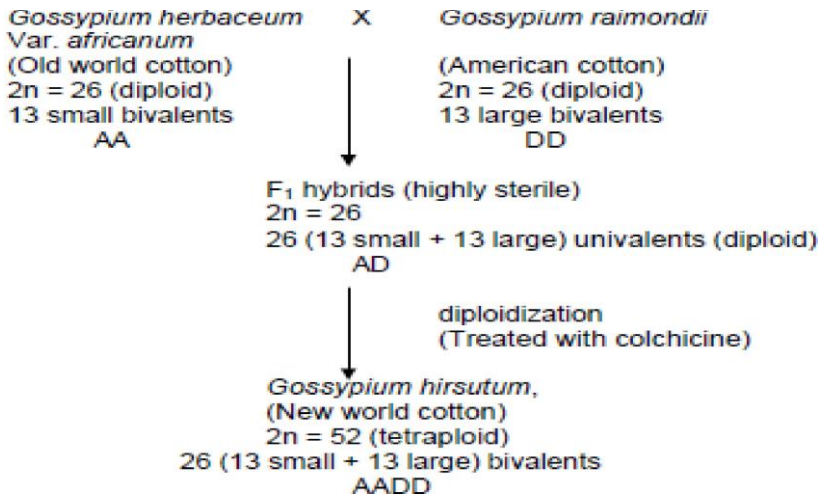
Role of polyploidy in evolution of crops - wheat, cotton, Tobacco and Brassica.

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)Wheat: The common or bread wheat, ***Triticum aestivum is an allohexaploid***. 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.

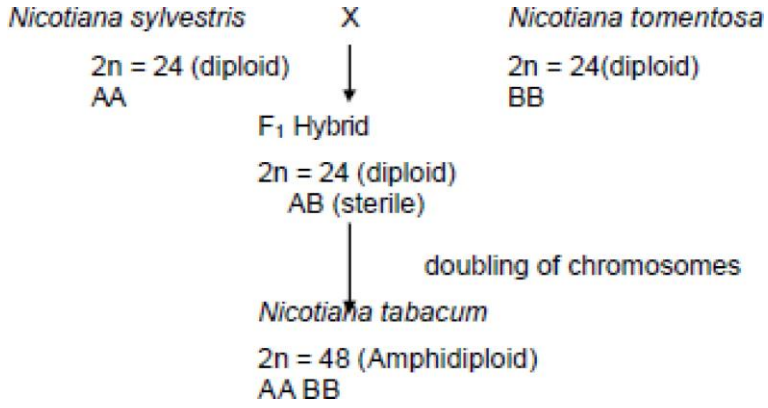


(i) Cotton: The new world cotton (Gossypium hirsutum) is an interesting example of allopolyploidy.

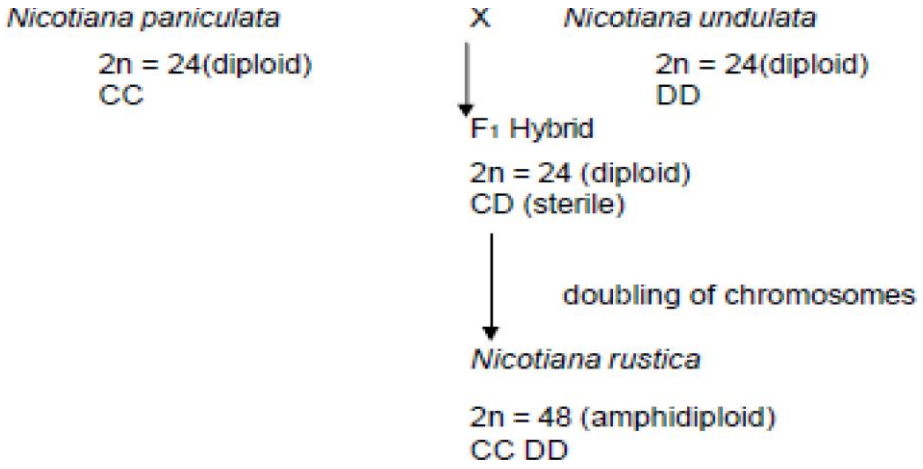


(ii)Tobacco: There are two cultivated species of tobacco. i. e. *Nicotiana tabacum* and *Nicotiana rustica.*

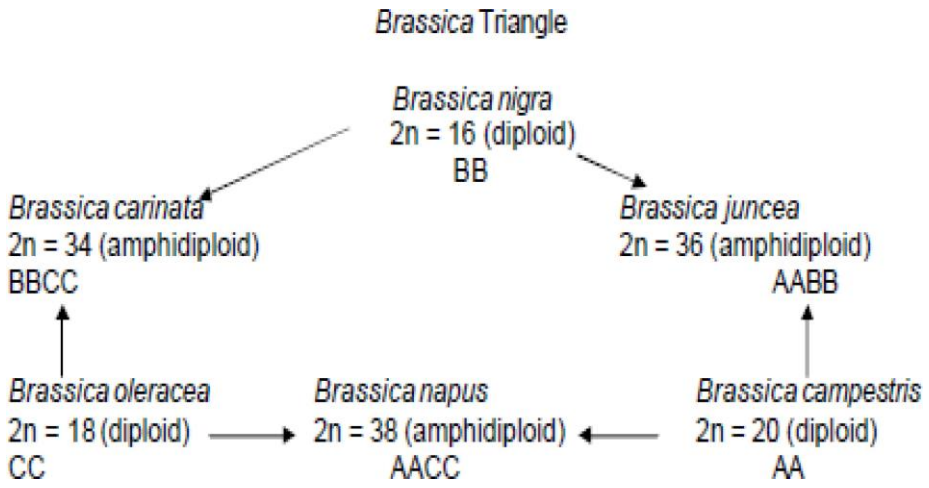
a) Nicotiana tabacum is an allotetraploid and available evidence suggests that it is derived from a cross between *Nicotiana sylvestris x Nicotiana tomentosa*



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


Nicotiana paniculata
2n = 24(diploid)
CC
X
Nicotiana undulata
2n = 24(diploid)
DD
F1 Hybrid
2n = 24 (diploid)
CD (sterile)
doubling of chromosomes
Nicotiana rustica
2n = 48 (amphidiploid)
CC DD

(ii)Brassica:


Brassica Triangle
Brassica nigra
2n = 16 (diploid)
BB
Brassica carinata
2n = 34 (amphidiploid)
BBCC
Brassica juncea
2n = 36 (amphidiploid)
AABB
Brassica oleracea
2n = 18 (diploid)
CC
Brassica napus
2n = 38 (amphidiploid)
AACC
Brassica campestris
2n = 20 (diploid)
AA

Lecture 9. Pre-Mendelian ideas about heredity – Vapour and fluid theory, Magnetic power theory, Preformation theory, Lamarck's theory, Darwin's theory, Germplasm theory and Mutation theory.

Early concept:

Among the biological sciences the science of genetics originated 1900 with the rediscovery of Mendelian Principles. Though pre-historic plants and animal breeders employed hybridizations and selection they were not aware of principles of genetics.

Early works forwarded various speculation and theories explain the phenomenon of heredity. The ideas of early workers can be grouped into the following headings.

1. Vapour and fluid theory:

Early Greek Philosophers thought hereditary information of the parents exist in the form of vapour and fluid. Pythagorus 500 B.C. proposed that the moist vapour from dry nurves and other parts of the body organs from the nerves form an embryo in the uterus of the female.

2. Magnetic power (Harvey (1578 - 1657)

He proposed the uterus had some magnetic power to consume an embryo.

3. Pre-formation theory

With discovery of Sperms and egg by 17th century biologist pre-formation theory was proposed. According to this new individuals of completely pre-formed in miniature size in gametes.

4. Epigenetic theory (Wolf) 1738

He proposed that each egg had a granule which gradually develops into various organs of the embryo.

5. Particulate - Maupertius (1698 - 1759)

Maupertius proposed that both the parents produce the gametes. Egg and sperm united to produce embryo. Each organ of embryo is made of two parts of embryo one is come from male (Γ) another one female (E).

6. Theory of Lamarck (1744 - 1829)

Lamarck proposed that environmental changes cause modification in organism and that such modification transmitted to subsequent generation. That is the character acquired in one generation to another generation. Acquire characters are inheritable.

e.g. Giraffie live in the interior part of the Africa. So it feed on the leaves of tall tree and to strain itself continuously to reach them. Such exercise caused the legs and necks to grow in length. The increased length was inherited by the progeny touched the legs and neck and over generation it will continue. This has evolved the present day 6 metre giraffie.

7. Theory of pangenesis - Charles Darwin (1809 - 1882)

Darwin proposed theory of natural selection. Many individuals of each species and there is always existence. If hereditary differences occurs with in a species of plants natural selection allowed only the fittest individuals of the species to survive and eliminating others. This is known as survival of fittest due to natural selection.

In 1868 Darwin published origin of species. He also proposed theory of Pangenesis . he called it hereditary particles. Germules that are produced every part of the body during the life

time. The germules from all the organs are transported to the gonads. The gonads distributed gametes if male (Γ) gametes unit during fertilisation, germmules from the migrate in respective organ and determine the development of organ. Organ is modified some way. The gemmules of the organ also modified accordingly which is transmitted to the offspring through gametes.

Germplasm theory(Weismann) (1814 - 1914)

Weismanns suggested the reduction in chromosome number takes places during formation of eggs / sperm and the original number resorted when eggs sperm fused. According to him acquired character not inherited. To prove this in mice to cut off tails 22 generation at last 1592. All of them having normal fail end. If the acquired characters are inherited the tails condition of mice produced by cuttings of their tails should be transmitted to offsprings. If the theory of pangenesis was correct the germmules for tail would be absent in the gamets of the tail less mice and their progency would be tails less.

Weismann proposed germplasm theory of heredity particles called IDS (Genes) situated on idants (chromosome) constitute the germplasm. The germplasm transferred to parents to offsprings and give rise body or some. The germplasm is independent of the body. Whatever happens to the body has not effect on the germplasm. This was prooved by ovary transplantation in guinea pigs. When albino guinea pigs are mated with alblono only **albino** progenies are produced.

Castle & Phillips (1909) removed the ovary of the albino guinea pigs and grafted in their place the ovary of guinea pig. The albino animal with the ovary of the black one when mated with the albino on the offsprings is black only. This proved the germplasm is not affected by body.

The history of genetics can therefore be reviewed under three periods (1) Pre-Mendelian, (2) Mendelian, and (3) Post-Mendelian.

PRE-MENDELIAN PERIOD

Some of the scientists prior to Mendel tried to account for the differences existing between individuals and suggested theories to explain their inheritance. The most important theories are the following:

Theory of Lamarck

The French biologist Jean Baptiste de Lamarck (1744-1829) proposed the theory that environmental changes cause modifications in organisms and that such modifications are transmitted to subsequent generations. He believed that environment acts directly on plants and indirectly on higher animals.

Lamarck said that changes in environmental conditions create new needs in animals. Conscious efforts of the animals to adapt to the environment involves the use of certain organs, thereby causing them to become large, strong and well-developed. Other organs are not used and so become smaller, weaker and less well-developed. Such bodily changes are called acquired characters since an animal achieves them by its own exertions to adapt to the environment. Acquired characters, according to Lamarck, are then passed on to the offspring of the organism that acquired them, and new species originate by accumulation of these modifications.

The giraffe dwells in the interior arid parts of Africa where there is not much herbage. According to Lamarck, the giraffe was obliged to feed on the leaves of tall trees and to strain itself continuously to reach them. Such exercise caused the necks and legs to grow in length. The increased length was inherited by the progeny, which, in turn, stretched their necks and legs and transmitted their increased length to their own offspring. Thus has evolved the present day six-metre high giraffe.

Detailed studies have failed to show that acquired characters are inherited. Most biologists have therefore abandoned the theory of inheritance of acquired characters, otherwise known as Lamarckism.

Darwin's Theory

In 1858, Charles Darwin (1809-1881) and Wallace independently proposed the 'Theory of Natural Selection'. According to this theory, many more individuals of each species are born than can possibly survive and consequently there is always a struggle for existence. If hereditary differences occur within the wild species of plants, nature will eliminate some and select others.

Over-production, struggle for existence, hereditary variations and survival of the fittest are thus the important principles of the theory of natural selection.

Ten years after the publication of the Origin of Species (1859), Darwin adopted the doctrine of the inheritance of acquired characters but he proposed a new theory of how it happened. He modified the views of Spencer and proposed the 'Hypothesis of Pangenesis'(1868).

Darwin assumed that hereditary particles termed pangenes of gemmules, are produced by every part of the body during the life time of an organism and that, these assume the characters of the various parts of the body from which they were derived, together with whatever modifications the latter may have acquired. Eventually all the pangenes accumulate to form the germ cells which give rise to the new individual, thus ensuring the development of the parental characters and inheritance of acquired characters.

Weismann's Germplasm Theory

Weismann(1834-1914), a German zoologist, suggested in 1887 that a reduction in chromosome number took place during the formation of the egg and the sperm, and that the original number was restored when the egg and the sperm fused. In 1892, he suggested that the maternal and paternal chromosomes separated during the reduction division and that they recombined when the gametes united.

According to Weismann's Germplasm Theroy of Heredity', the hereditary particles called ids (what we now call as genes) situated on idants (what we now call chromosomes) constituted the germplasm. The germplasm is handed down from parent to offspring and it gives rise to the body or soma (somatoplasm) whose character it determines. The germplasm is independent of the body and whatever happens to this body has no effect on the germplasm which is contained within it.

According to Weismann, acquired characters cannot therefore be inherited. To prove this he cut off the tails of mice for twenty-two generations and found that the progeny consisting of 1,592 individuals had tails of normal length.

The independence of the germplam from the somatoplasm was shown by the ovary transplantation experiment in guinea pig. Ordinarily, when albino guinea pigs are mated with albinos, only albinos are produced. Castle and Phillips removed the ovaries of an albino guinea pig and grafted in their place the ovaries of a black guinea pig. The albino animal with the ovary of the black one was then mated with an albino. All the offspring were found to be black, thereby

proving that the germplasm (i.e., the ovary from the black guinea pig) is not affected by the somatoplasm (i.e., the body of the albino).

De Vries' Mutation Theory

Charles Darwin believed that evolution is due to natural selection of small hereditary variations occurring among individuals of any species. Bateson did not agree with Charles Darwin. He believed that evolution is due to large discontinuous variations. De Vries (1848-1935) introduced the term `mutation' for these large, discontinous changes in the genotype and proposed the `Mutation Theory', according to which sudden hereditary changes lead to evolution.

De Vries (1901) observed that the evening primrose Oenothera lamarckiana, a native of America, was growing wild in Holland. In a population of this weed, he observed some plants which differed in some characters from the typical Oenothera lamarckiana. Since it is a self -fertilised species, he felt that these variants have arisen suddenly rather than as hybrids. He transplanted them to his garden and studied them for several years. He observed that variations continued to arise spontaneously and that these variations were inherited. He called these drastic changed as mutations and maintained that mutations play an important role in the evolution of new species.

Lecture 10. Work of Mendel – Characters studied reasons for Mendel's success, Law of dominance, Law of segregation and Law of independent assortment. Rediscovery of Mendel's work

Work of Mendel

Gregor John Mendel was born in 1822 near Brunn in Austria. In 1843, Mendel entered Augustinian monastery at Brunn. He completed his theological studies in 1848 and was appointed as a substitute teacher in high school. In 1851 – 53, the monastery sent him for studies at university of Vienna. He continued as a substitute teacher for 12 more years. In addition he worked as a priest in the local church. He lived in a house located within the premises of the church. He began to collect pea seeds for his experiments in 1857 from commercial seed growers all over the Europe. He conducted all his experiments within the kitchen garden of his house with the help of his own resources.

Mendel carried out his experiment in the monastery gardens from 1856 – 63. His experimental material is **garden pea (*Pisum sativum*).** He studied **seven contrasting characters**. He presented the result of his experiment before **"The National History society at Brunn"**. His paper entitled **"Experiments in plant hybridization"** was presented in German language. No. one appreciated the importance of his work until 1900. Gregor Johan Mendel died in 1884, at the age of 62 years.

Seven Characters studied by Mendel

	Character	**Dominant**	**Recessive**
1.	Seed shape	Round	Wrinkled
2.	Petal colour	Purple	White
3.	Cotyledon colour	Yellow	Green
4.	Pod colour	Green	Yellow
5.	Pod shape	Full	Constricted
6.	Position of flower	Arial	Terminal
7.	Length of stem	Tall	Dwarf

PEA as an experimental material

Pea offered several advantages as an experiment material.

i. In the pea varieties available commercially, several characters had two contrasting form which were easily distinguishable from each other.

ii. The flower structure of pear ensured self pollination this was experimentally verified by Mendel. This greatly facilitated the production of F2 and F3 progeny as well as avoided contamination by foreign pollen.

iii. Pea flowers are relatively large. Therefore emasculation and pollination is quite easy, which allows easy artificial hybridization in pea.

iv. The duration of pea crop is of a single season. As a result, every year one generation of pea can be grown.

v. Pea seeds are large and present no problem in germination. Pea plants are relatively easy to grow and each plant occupies only a small space. This persists a large number of plants to be grown in a relatively small area. (In addition, Mendel worked in Raj mash, *P. vulgaris*)

Mendel's findings:

1. Both male and female make equal contribution to the development of character in progeny, since the results from reciprocal crosses are identical.
2. In F_1 generation, character of only one of the parent is expressed. This is known as dominant character. The character of the other parent which is not expressed is referred as recessive.
3. In F_2 characters of both the parents ie., dominant and recessive appeared in a definite proportion of 3:1
4. The recessive character is not modified in F_1 generation and its expression is prevented.
5. The dominant character may be of 2 types.
    i) It may be pure like that in the parent producing progeny with the dominant trait.
    ii) It may be a hybrid similar to F_1 hybrid (heterozygote) producing ¾ progeny with dominant character and remaining ¼ with recessive character.

Reasons for Mendel success:

1. He accurately diagnosed the weakness of earlier experimental materials, techniques and approaches. He carefully avoided mistakes in his experiment.

2. Mendel studied the inheritance of only one pair of contrasting character at a time.
3. He selected pea varieties, which have clearly different forms of one or more character.
4. He classified all the plants of a population on the basis of contrasting character under studying and kept accurate record of the no. of plants in each category for every generation.
5. He carried out his experiments with great care, i.e he grow the parents in two seasons to avoid mechanical mixture.
6. His knowledge on mathematics was a definite asset for the interpretation of his result.

The Laws of Mendel:

1. Law of dominance:

On crossing homozygous organisms for a single pair of contrasting characters, only one character make its appearance in F_1 generation and is named as dominant character.

2. Law of segregation (law of purity of gametes): (Mendel's First Law)

If two alleles are brought into a hybrid, they do not contaminate or blend with each other but segregate and pass into different gametes.

3. Law of independent Assortment: (Mendel's Second Law)

When two or more pairs of alleles are brought into a hybrid, the segregation of any one pair of allele is independent to that of segregation of any other pair of allele.

Rediscovery of Mendel's work:

In the year 1900, Mendel's paper was rediscovered. Three scientists working independently of each other, de Vries in Holland, Correns in Germany and Tschermak in Austria, arrived at the same conditions as those of Mendel. After this rediscovery, there was a spurt of interest in the Mendel's findings and the science of Genetics was truly borne.

Mono hybrid

The progeny derived by crossing two individuals (or) strains which differ for one gene.

Di hybrid:

The progeny from a cross between two homozygous parent different for two gene.

Tri hybrid

The progeny from a cross between parents differing in three genes.

No. of gene	Phenotypic ratio	No. of phenotypes	No. of genotypes	Genotypic ratio
1	(3:1)	2	3	(1:2:1)
2	(9:3:3:1)	4	9	(1:2:1:2:4:2:1:2:1)
3	27:9:9:9:3:3:3:1	8	27	
	Different kinds of gametes produced	2^n	3^n	

Where' n' is no. of genes for a character

Mendel's monohybrid cross

As mentioned earlier, Mendel studied the inheritance of each of the seven pairs of contrasting characters selected by him in peas in separate crosses. For example, he crossed a variety of peas having round seeds with a variety having wrinkled seeds. The hybrid seeds from this cross were all round. He planted these hybrid round seeds and obtained an F_2 generation consisting of round and wrinkled seeds in the proportion of 3:1. Similarly, he crossed a variety of peas having yellow cotyledons (the colour of which could be seen through the `transparent' or thin seed coats) with a variety having green cotyledons. The hybrid seeds resulting from this cross were all yellow. He planted these hybrid yellow seeds and obtained an F_2 generation consisting of yellow and green seeds in the proportion of 3 yellow : 1 green.

Mendel's dihybrid cross

Mendel then crossed a variety with round and yellow seeds with a variety with wrinkled and green seeds. The F_1 hybrid seeds were all round and yellow. The plants raised there from yielded seeds of four sorts which frequently presented themselves in one pod. In all, 556 seeds were yielded by 15 plants and of these, there were:

315	Round and yellow
108	Round and green
101	Wrinkled and yellow
32	Wrinkled and green

Mendel observed that the segregation for form of seed and colour of cotyledons separately was in the ratio of 3 dominants: 1 recessive.

Round seeds	(315 + 108) / 556	=	76.08%
Wrinkled seeds	(101 + 32) / 556	=	23.92%
Yellow seeds	(315 + 101) / 556	=	74.82%
Green seeds	(108 + 32) / 556	=	25.18%

F_2 ratio of hybrid round	**F_2 ratio of hybrid yellow**		**F_2 ratio of hybrid round and yellow**
	3 **Y**	=	9 **RY (**round yellow)
3 **R**	1 **y**	=	3 **Ry** (round green)
	3 **Y**	=	3 **rY (**wrinkled yellow)
1 r	1 **y**	=	1 **ry** (wrinkled green)

Mendel concluded that the F_2 of a cross involving two pairs of contrasting characters (i.e., a dihybrid cross) shows four kinds of individuals in the ratio of 9 : 3 : 3 : 1.

Mendel's Second Law

Mendel arrived at the following conclusion:

When an individual forms gametes, the members of a pair of alleles always segregate (i.e., separate) from each other but the members of different pairs of alleles assort independent of each other.

Mendel's Second Law of Inheritance or the Law of Independent Assortment can, therefore, be expressed as follows:

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

Test cross- Crossing of F1 with recessive parent

That the dihybrid forms four kinds of female gametes and four kinds of male gametes in equal numbers can be shown by crossing it with the double recessive.

From a cross between the dihybrid as female and the wrinkled green seeded plant as male, Mendel obtained 31 round yellow, 26 round green, 27 wrinkled yellow and 26 wrinkled green seeds. As the recessive plant produces only one kind of male gamete, **ry**, a 1 : 1 : 1 : 1 ratio is possible only if the dihybrid produces four kinds of female gametes, **RY, Ry, rY** and **ry,** in equal numbers.

Back cross

Crossing of F1 with any one of the parent

From a back cross between the double recessive as the female and the dihybrid as the male, he obtained 24 round yellow, 25 round green, 22 wrinkled yellow and 27 wrinkled green seeds. The progeny is in the ratio of 1 round yellow : 1 round green : 1 wrinkled yellow : 1 wrinkled green, thereby showing that the dihybrid produces four types of gametes, **RY, Ry, rY** and **ry** in equal numbers.

Trihybrid ratio

Trihybrid is a hybrid resulting from a cross between parents differing in three genes.

As an example, we can consider Mendel's cross between pea plant with round seeds, yellow cotyledons and grey-brown seed coats and one with wrinkled seeds, green cotylendons and white seed coats. All the hybrid seeds resulting from this cross are round, yellow and grey-brown.

An individual heterozygous for three independently assorting pairs of alleles produces eight types of gametes in equal numbers as follows:

RYB	**RYB**
Ryb	**Ryb**
RyB	**RyB**
Ryb	**Ryb**

Eight kinds of male gametes fertilising at random eight corrresponding kinds of female gametes produce an F_2 consisting of 64 possible combinations composed of 27 different genotypes. Since **R** is dominant over **r, Y** over **y**, and **B** over **b**, the 27 different genotypes fall into eight visibly different types (i.e., phenotypes) as follows:

		3 B = 27 RYB	(round, yellow, brown)
	3Y	**1 b = 9 Ryb**	(round, yellow, white)
3 **R**		**3 B = 9 RyB**	(round, green, brown)
	1 y	**1 b = 3 Ryb**	(round, green, white)
		3 B = 9 rYB	(wrinkled, yellow, brown)
	3Y	**1 b = 3 rYb**	(wrinkled, yellow, white)
1 **r**		**3 B = 3 ryB**	(wrinkled, green, brown)
	1 y	**1 b = 1 ryb**	(wrinkled, green, white)

Lecture 11. Chromosomal theory of inheritance. Allelic interactions – Dominance vs. recessive, complete dominance, codominance, incomplete dominance, over dominance.

Chromosomal theory of inheritance: **(Sutton and Boveri)**

The idea of chromosomal basis of segregation and independent assortment was put forth by Sutton in 1902. He explained the law of segregation and independent assortment on the behavior of chromosomes during meiosis. The hypothesis that genes are located in chromosome was designated by Sutton and Bovery. Sutton and Bovery hypothesis was known as chromosome theory of inheritance. Evidence for chromosome theory of inheritance

1. Each somatic cell contains 2 copies of each gene i.e., A, a similarly each somatic cell has 2 copies of homologous of each chromosome. Each somatic cell produced during embryonic and subsequent development receives 2 copies of each gene present in the zygote.
2. A gamete contains only one copy of a gene or one allele of a gene. This phenomenon is termed as segregation. It is assumed that 2 alleles of a gene are located in the 2 chromosomes of a homologous pair. Separation of 2 homologous chromosomes during anaphase I of meiosis will account for segregation of 2 alleles.
3. Several subsequent studies and Mendel's studies found that 2 or more genes assorted independently to yield typical dihybrid, trihybrid ratios. Members of homologous chromosomes assort independently to that of the other.

4. In 1901 Meclung discovered the accessory or x chromosome of grass hopper and stated that, this chromosome was involved in sex determinanation since a specific chromosome is involved in sex determination, the genes governing this trait may be located on x chromosomes.

5. In 1910, T.H. Morgan presented a more direct evidence supporting the chromosome theory of heredity. He found that the pattern of transmission of white eye gene was identical with that of the x chromosome of Drosophila. This prompted Morgan to postulate that the gene for white eye was located in the x chromosome.

6. In 1910, Morgan described the phenomenon of linkage and crossing over between 2 sex linked genes in drosophila. He proved that the linkage between any 2 genes depends on the distance between them in the chromosome. Morgan explained that the recombination of linked gene is due to exchange of genetic material between homologous chromosomes.

7. When a segment of chromosome is inverted, the inverted chromosome shows as inverted gene sequence corresponding to the inverted segment. Hence genes must be located in chromosome.

8. In addition to structural changes in chromosomes, numerical chromosome changes also furnish in favour of this theory. In monosomics and trisomics there will be significant deviation from the normal inheritance pathern ie from 3:1 ration in F_2 for a single gene.

Parallelism of the chromosome and genes.

i. Chromosomes occur in pairs one received from male (Γ) parent other received from female (E) parent. Gene also exist in pairs (allele) one from Γ another E.

ii. Each pair of chromosome differ from other pairs. Likewise each gene has individuality and produce specific effect.

iii. At meiosis the member of one pair of chromosomes separate and go into different gametes. According to law of segregation each gene separate from its allele and each one enter into different gamete.

iv. The segregation of one pair of chromosome has been observed is independent of the segregation in another pair chromosome. According to law of independent assortment, segregation of one pair of allele to another pair of alleles is independent.

Allelic interaction –interaction within the allele of same gene is called as intra-allelic interaction or **dominance**

Types of dominance

1. Complete dominance:

In case of complete dominance, the phenotype produced by heterozygotes is identical with that produced by homozygotes for the concerned dominant allele. The dominant allele in such a situation is known is completely or fully dominant.

Eg: In peas, round seed shape is produced by the dominant allele W, while wrinkled shape is determined by its recessive allele w. Seeds having the genotype Ww are round and indistinguishable from those having the genotype WW. As a result, characters showing complete dominance yield the typical 3:1 monohybrid ratio in F_2.

2. Incomplete Dominance:

The phenotypic expression of heterozygote for a gene being intermediate between those of the two concerned homozygote. Such a situation is known as incomplete or partial dominance.

Eg:- Flower colour in 4^0 O Clock plant

(Mirabilis jalapa)

RR x rr

(Red) (White)

F_1 : Rr (pink)

F_2 : 1 RR : 2 rr : 1 rr

Red : pink : white

Here the phenotypic ratio in F_2 is 1: 2 :1 instead of 3:1 in Complete dominance

3. Co – Dominance: Both the alleles of a gene express themselves in the heterozygotes. As a result, heterozysotes for the such genes posses the phenotype produced by both the alleles of such genes.

Eg. Blood group antigens of man.

$I^A I^B$ x $I^A I^B$

(AB) (AB)

$I^A I^B$: $2\ I^A I^B : 1\ I^B I^B$

1 A: 2 AB : 1B

coat colour in cattle

$C^R C^R$ x $C^W C^W$

(Red) (White)

F_1 $C^R C^W$ (Roan)

F_2 1 $C^R C^R$: 2 $C^R C^W$: 1 $C^W C^W$

Also called as mosaic dominance

4) **Over dominance:**

In case of some genes, the intensity of character governed by them is greater in heterozygotes than in the two concerned homozygotes. This situation is known as over dominance.

Eg: - AA x aa

(120 cm) (100mm)

F_1 Aa (140 cm)

F_1 is superior to the dominant parent. It is called as **Hetero**, **Super** (or) **over dominance.**

Lecture 13. Deviation from Mendelian inheritance – Non allelic interaction without modification in Mendelian ratio – Batson and Punnet's experiment on fowl comb shape. Non allelic interaction with modification in Mendelian ratio – i.) Dominant epistasis (12:3:1)

Gene interaction: For the determination of single phenotypic character, two alleles of a single gene interacted in various way.

Eg: complete dominance, incomplete dominance or codominance. These kind of genetic interactions occur in between the two alleles of a single gene is referred as **Allelic interaction or intra geneic interaction.**

Non – allelic interaction or intergenetic interactism (or) Epistasis

Deviation from Mendelian inheritance – Non allelic interaction without modification in Mendelian ratio – Batson and Punnet's experiment on fowl comb shape

A classical case of two pairs of alleles affecting the same characteristic and producing in the F_2 four different phenotypes in the ratio of 9 : 3 : 3 : 1 was discovered in fowls by Bateson and Punnett.

Each breed of pultry possesses a characteristic type of comb. The Wyandotte breed has a comb known as the 'rose' comb, the Brahma has a 'pea' comb, the Leghorn has a 'single' comb and the Malay breed has a comb known as the 'walnut' comb. Each of these breeds true.

Crosses between rose-combed and single-combed types show that rose is dominant to single comb and that there is a segregation of 3 rose : 1 single comb in the F_2. In matings between pea-combed and single-combed birds, pea comb is found to be dominant over single comb and a 3 : 1 ratio appears in the F_2.

When, however, a rose-combed fowl is crossed with a pea combed one, all the F_1 birds show the walnut comb. When the F_1 walnut combed birds are bred together, there appears in the F_2 9 walnut : 3 rose : 3 pea : 1 single comb.

These results can be interpreted as follows: The rose comb is due to a gene R and the pea is due to another gene P. The walnut comb is due to the presence of both the dominant genes, R and P and the single comb is due to their recessive alleles, r and p.

The breeding behaviour of the different genotypes of the F_2 is summarised.

F_2			Breeding behaviour
Phenotype	Genotype	Ratio	
Walnut	RRPP	1	All the progeny walnut-combed
	RRPp	2	3 walnut (RP) : 1 rose (Rp)
	RrPP	2	3 walnut (RP) : 1 pea (rP)
	RrPp	4	9 walnut : 3 rose : 3 pea : 1 single
Rose	RRpp	1	All the progeny rose-combed
	Rrpp	2	3 rose (Rp) : 1 single (rp)
Pea	rrPP	1	All the progeny pea-combed
	rrPp	2	3 pea (rP) : 1 single (rp)
Single	rrpp	1	All the progeny single-combed

The above example depicts a case of non-epistatic intergenic interaction in which two genes that determine the same character produce a new phenotype by mutual non-epistatic interaction.

Epistasis: (Non-allelic interaction) Interaction between two genes.

E**xpression of one gene masks the expression of the other** is **called epistasis**

A gene which suppressing the other character is known as epistatic gene

Gene expression which was suppressed by a epistatic gene is known as hypostatic gene

The term epistasis is used for any kind of gene interaction.

Types of Epistasis

The various types of epistatic gene interaction include

1. Dominant epistasis or Simple epistasis (12:3:1)

In Dominant Epistasis, the dominant allele at one locus mask the expression of both dominant and recessive alleles at another locus resulting in 12 : 3 :1 ratio

2. Recessive epistasis or Supplementary gene action (9:3:4)

In Recessive Epistasis, the recessive alleles at one locus mask the expression of both dominant and recessive alleles at another locus resulting in 9 :3 : 4 ratio

3. Dominant and recessive epistasis or Inhibitory gene action (13:3)

In this type of Epistasis, the dominant and recessive alleles at one locus mask the expression of both dominant and recessive alleles at another locus resulting in 13 : 3 ratio

4. Duplicate recessive epistasis or Complementary gene action (9:7)

In this epistasis, the recessive alleles at either of the two loci mask the expression of dominant alleles at the two loci, resulting in 9 : 7 ratio

5. Duplicate dominant epistasis or Duplicate gene action (15:1)

In this epistasis, the dominant alleles at either of the two loci mask the expression of recessive alleles at the two loci, resulting in 15 : 1 ratio

6. Duplicate genes with cumulative effect or polymeric gene action (9: 6 : 1)

In this type of epistasis, two dominant alleles have similar effect when they are separate but produced enhanced effect when they are together, resulting in 9 : 6 : 1

Difference between Dominance and Epistasis

Sl.No	Dominance	Epistasis
1.	Interaction of two alleles of the same gene, thus involving single locus	Interaction of two or more genes, thus involving two or more loci
2.	Always refers to Heterozygotes therefore, it is not fixable	Refers to homozygotes and heterozygotes therefore, it is fixable in homozygotes
3	Dominance is of three types viz., complete, incomplete and overdominance	Epistasis is of several types viz., dominance, duplicate and recessive
4.	Partial dominance alters the normal segregation ratio of 3:1 into 1:2:1	It modifies the normal dihybrid ratio in F_2
5.	It is known as intragenic or intralocus gene interaction	It is known as intergenic or interallelic or interlocus gene interaction
6.	Recessive genes can express only in	Recessive genes can also exhibit masking

	homozygous condition	effect

Dominant epistasis: (12:3:1)

Two genes affecting the same character produce distinct phenotypes when they are alone. But when both the genes are present together, the expression of one gene masks the expression of the other.

In Barley seed coat color is governed by two dominant B and Y

B - Black b- white

Y - Yellow Y - white

When both B_ Y_ present both the gene produce their effect but the B- Black colour is so intense and it masks the expression of Y gene.

9 - B – Y - Black

3 - B-yy Black

3 - bb Y- Yellow

1 - bb yy White

When two non allelic genes affect the same trait of an organism, expression of one dominant gene mask the expression of another gene. A gene thus mask the expression of another gene is called epistatic gene and the gene hidden is called hypostatic gene.

Lecture 14. ii.) Recessive epistasis(9:3:4) iii.) Duplicate and additive epistasis((9:6:1). iv.) Duplicate dominant epistasis(15:1)

Recessive epistasis: (9:3:4) (supplementary factor)

In this gene interaction, the dominant allele of one of the two genes governing a character produces a phenotypic effect. However, the dominant allele of the other gene does not produce a phenotypic effect of its own, but when it is present with the dominant allele of the first gene it modifies the phenotypic effect produced by that gene.

Eg:- Maize grain colour / Aleurone colour.

	RRPP x	rrpp	RR – Red
	(purple)	(white)	
	RrPp purple		(RR – red PP – no. colour when it (pp) combine with another Dominant gene (RR) it modify its phenotype)
	3P – 9 R-P - purple		
3R-	1pp – 3R –pp – Red		
	3 P – 3 rr P_		
1 rr		White	

1 p - 1 rr pp

Phenotypic ratio 9:3:4

Duplicate and additive epistasis: (9:6:1)

In this gene action, the two genes controlling a character produce identical phenotypes when they are alone. But when the genes are present together, their phenotypic effect is enhanced as if the effect of the two genes were cumulative (or) additive.

Eg:- In barley, two completely dominant genes A and B affect the length of awns. Gene A or B alone gives rise to awns of medium length. But when both the genes A and B are present together, they produce long awns indicating that the effects of A and B are added together.

Parents AA BB x aa bb

Long awn awnless

A a B b long awn.

F_2

3B_ 9A- B - Long awn

3 A - 1 bb 3A - bb

3 B - 3 aa B - Medium

1 aa

1 bb aa bb awnless

Duplicate dominant interactions : (15: 1)

Characters showing duplicate gene action are determined by two completely dominant gene these dominant genes produce the same phenotype, whether they are alone (or) together.

The contrasting phenotype is produced only both the genes are in the homozygous recessive state.

Eg: Non floating habit in rice is controlled by two dominant genes DW_1, and Dw_2. genes DW_1 and DW 2 alone, as well as together produce the same phenotype viz., non floating. The floating habit is obtained only when both these genes are in recessive state.

Parents

DW_1 DW 2 DW_2 x dw_1 dw_1 dw_2 dw_2

(Non floating) floating

3Dw_2 - 9 DW 1 _ Dw2_

N. floating

3 DW_1-- 1dw_2 - 3 DW 1 _ dw_2 dw

2

3Dw_2 - 3 dw1dw1 Dw2-

1 dw1dw1

1dw2dw2- 1 dw_1 dw 1 dw_2 dw_2 - floating

Phenotypic ratio 15:1

Lecture 15. v) Duplicate recessive epistasis (9:7) vi.) Dominant and recessive epistasis(13:3); Summary of epistatic ratios (i)to (vi).

Duplicate recessive epistasis: ((9:7) complementary factor.

In this type of gene interaction, the production of one of the two phenotypes of a trait requires the presence of dominant alleles of both the genes controlling the concerned traits when any are of the two or both the genes are present in the homozygous recessive state, the contrasting phenotype is produced.

Eg: In sweet pea, the development of purple flowers requires the presence of two dominant genes C and R when either C or R or both the genes are present in recessive condition. Purple colour flower cannot be produced as a result of which white flowers are obtained.

Parents CC RR x cc rr

(Purple) (white)

Cc Rr (purple)

F_2 3 R _ 9 C _ R _ Purple

3 C _

r r 3 C _ rr

1 cc 3 R _ 3 cc R _ White

1 rr 1 cc rr

Phenotypic ratio (9:7)

Dominant and recessive epistasis (13:3)- Inhibitory epistasis

In this gene action, one of the two completely dominant genes produces concerned phenotype or the character while its recessive allele in homozygous state produces contrasting phenotypes. The second dominant gene has no effect of its own. However it has the ability to stop the expression of the first gene. As a result when the two dominant genes are present together, they produce same phenotype as that produced by the recessive homozygous of first gene.

Eg: Development of aleurone colour in maize. A dominant gene 'R' produces red colour, while its recessive allele 'r' reduces no colour. Another dominant gene 'I' does not produce any colour by itself; it only prevents the color production by 'R' when both 'I' and 'R' are present together.

(i)	Dominant Epitasis	12:3:1	Simple epistasis
(ii)	Recessive Epitasis	9:3:4	(Supplementary factor)
(iii)	Dominant & Recessive Epitasis	13:3	Inhibitory
(iv)	Duplicate Recessive interaction	9:7	(Complementary)
v)	Duplicate Dominant interaction	15:1	(Duplicate factor)

vi)	Duplicate gene with cumulative effect	9:6:1	(Additive factor)

Lecture 16. Lethal genes, Pleiotrophy, penetrance and expressivity, phenocopy: Multiple alleles, blood group in humans, coat colour in rabbits, self incompatibility in plants; pseudo alleles, isoalleles.

Lethal Genes: Lethal genes are genes which in the homozygous state have such a marked deleterious effect that such homozygous organisms are inviable. A lethal gene causes the death of all the individuals carrying this gene in the appropriate genotype before these individuals reach adulthood.

When seeds from self-pollinated maize plants are sown, sometimes green seedlings and white seedlings (i.e., albinos) emerge in a ratio of approximately 3 green : 1 white. The albinos die within a few says after germination and only green plants remain. **The 3:1 ratio will be modified into 2:1 .**

Maize being a highly cross-pollinated crop is likely to be heterozygous. If the gene for chlorophyll is represented as **W** and its recessive allele for albinism as **w**, the plant is likely to have the genotype **Ww**. When this is selfed, homozygous recessives appear and these die.

Green plants **Ww**			
Selfed			
WW	**Ww**	**Ww**	**Ww**
Green	Green	Green	White (die)
Selfed	Selfed	Selfed	
Green only	Green and white	Green and white	

Seedlings of genotype **ww** always die after a short time in the field because they have no chlorophyll which is absolutely essential for plants.

Lethal genes may be grouped into the following five categories.

1. Recessive Lethals

Most of the lethal genes are recessive lethal since their lethal effect is expressed only when they are in the homozygous state and the survival of the heterozygote is unaffected.

Eg. In mouse hydrocephaly is due to a recessive lethal gene in homozygotes (recessive) the gene causes abnormal growth of cartilage during embryonic development. This leads to irregularly formed skull and brain and accumulation of cerebrospinal fluid. Such homozygotes do not survive while their heterozygotes are apparently normal.

2. Dominant lethal:

Dominant lethal genes are lethal in homozygous conditions and produce some defective or abnormal phenotype in heterozygous condition. Their most serious effect in heterozygotes may also cause death.

Eg: Yellow lethal in mice

When ever the yellow males crossed with yellow females always yellow and brown were obtained in the ratio of 2:1

Yy x Yy

(Yellow) (Yellow)

1 YY : 2Yy: 1yy

Die as : 2 yellow: 1 brown

Embryo

3. Balanced lethal:

Lethal genes can be used in establishing balance lethal system, where both homozygous dominant and homozygous recessive die leaving only heterozygotes. Such balanced lethal systems are known in Oenothera , Drosophila etc.,

4. Conditional lethal:

The genes which may be normal to the individual in a particular environment may prove to be lethal when environment is changed.

Eg: - In Drosophila pseudoobscura. Flies live normally at a temperature at 16.5^0 C when it raised to 25^0C, the flies begin to die.

5. Gametic lethal:

Some genes leads to the in-viability of a class of gametes or make them incapable of fertilization such genes are termed as gametic lethal.

Eg: A dominant Segregation Distarter(SD) gene is known in the chromosome II of Drosophila melanogaster

Complete lethal genes cause death of the zygote, developing embryo, fully developed embryo or the fully developed organism before reproductive stage as in the case of coat colour in mice, seedling colour (albino) in maize, snapdragon and many other plants.

Pleiotrophy:

A single gene controlling more than one character of the organism ie. To say that a gene produces a major phenotypic trait. But in addition to that influences some other phenotypic traits.

The phenomenon of multiple or manifold phenotypic expression of a single gene is called Pleiotropism and such genes are known as pleiotropic genes.

Eg: (i) In cotton, the Punjab hairy lintless gene “lic produces seeds which are without lint. This gene also causes incomplete lancination of leaf, reduction in number and length of internodes, reduction in boll size and fertility.

(ii) In Drosophila, the recessive gene for vestigial wings also affects structure of reproductive organs, reduction in egg production, reduction in longevity and the bristles on the wings.

(iii) In man, gene producing disease called Phenyl ketoneuria also produces number of abnormal traits which are collectively known as syndrome other effects are short stature, mental retardation, widely spaced incisors, pigmented patches on the skin and excessive sweating.

Penetrance

The ability of a gene or gene combination to be expressed phenotypically to any degree is called penetrance.

The proportion of individuals of a specified genotype that show the expected phenotype under a defined set of environmental conditions called penetrance. It is the ability of the gene to express its character.

Complete penetrance

Most dominant and recessive genes in homozygous condition and many completely dominant gene even in heterozygous condition give their phenotypic expression. Heterozygous condition also produces the normal phenotype.

Incomplete penetrance:

Heterozygous condition unable to express fully the normal phenotype.

Eg: In the case of polydactyly in man where one extra finger is present in the palm or foot, the heterozygous condition (**Pp**) brings forth polydactyly in some and normal condition in others. Some heterozygotes individuals were not polydactyles some had an incomplete penetrance.

Expressivity:

The degree of phenotypic expression of a gene in the different individuals is known as expressivity, ie; the degree of effect produced by the Penetrance genotype:

Change of temperature, nutrition, hormone deficiency etc., influence the expressivity of the curly wing in *Drosophila*.

Eg:- In man polydactyl condition may be penetrance in left hand (6fingers) not in right hand (5 fingers) or may be penetrance with the feet but not in the hand.

Phenocopy:

The phenotype becomes altered by the environment in such a way that the new phenotype resembles another phenotype produced by known genes. The induced phenotype is not inherited and is called as phenocopy. Eg:- Generally the body colour of the fruit fly Drosophila melanogaster is light brown. When the larvae of the normal brown bodied fruit flies were reared on food with silver salts, the emerging adults had yellow body

Multiple alleles

More than two alleles at the same locus give rise to a multiple allelic series. Therefore, the existence of more than two alleles at the same locus of a homologous chromosome is referred to as multiple alleles

Presence of multiple alleles adds variability for a character

The number of possible genotypes in a series of multiple alleles can be calculated by using the formula ½ {n x 1 (n + 1)} where, n is the number of identified alleles in that group. For example, if there is 4 alleles in the multiple allelic series, then, ½ (4 x 5)=10 genotypes are possible

Main features of multiple alleles

1. Multiple alleles always belong to the same locus and one allele is present at a locus at a time in a chromosome
2. Multiple alleles always control the same character of an individual. However, the expression of the character will differ depending on the allele present.
3. There is no crossing over in a Multiple allelic series. When two alleles are involved in a cross, the same two alleles are recovered in the F_2 or test cross progeny. This is based on the classical concept of the gene, according to which crossing over takes place between gene and not within a gene.
4. In a series of Multiple alleles, wild type is always dominant. Rest of the alleles in the series may exhibit dominance or intermediate phenotypic expression when two alleles are involved in a cross.
5. The cross between two mutant alleles will always produce mutant phenotype (intermediate). Such cross will never produce wild phenotype. In other words, Multiple alleles do not show complementation (Complementation refers to appearance of wild phenotype when two mutants are crossed)

Examples of Multiple Alleles

Several cases of multiple alleles are known to occur in both plants and animals. Some well known examples for expression of multiple alleles include,

1. Fur color in Rabbits
2. Wing type in Drosophila
3. Eye colour in Drosophila
4. Self incompatability alleles in Plants
5. ABO Blood group in man

1. Fur colour in Rabbits

It is a well known example for multiple alleles

In rabbits, the fur colour is of four types, agouti, chinchilla, Himalayan and albino

1. **Agouti:**
   - ♦ This has full colour, known as wild type
   - ♦ This colour is dominant over all the remaining colours
   - ♦ Produces agouti colour in F1 and 3:1 in F2 when crossed with any of the other three colours in rabbits.
   - ♦ This colour is represented by **C**
2. **Chinchilla:**
   - ♦ This is lighter than Agouti.
   - ♦ This colour is dominant over Himalayan and albino
   - ♦ Produces chinchilla in F1 and 3:1 ratio in F2 when crossed with either Himalayan or albino.
   - ♦ This colour is represented by $\mathbf{c^{ch}}$
3. **Himalayan:**
   - ♦ The main body is white while the tips of ear, feet and tail and snout are coloured
   - ♦ This colour is dominant over albino
   - ♦ Produces 3:1 ratio in f2 when crossed with albino.
   - ♦ This is represented by $\mathbf{c^{h}}$
4. **Albino:**
   - ♦ This has pure white fur colour and is recessive to all other types.
   - ♦ This is represented by **c**

Thus the fur colour in rabbits can be represented in the order of dominance as,

Agouti	**Chinchilla**	**Himalayan**	**Albino**
(C)	**(c^{ch})**	**(c^{h})**	**(c)**

Cross between	**Expression in F_1**	**Segregation in F_2**
Agouti x chinchilla	Agouti	3 Agouti : 1chinchilla
Agouti x Himalayan	Agouti	3 Agouti : 1himalayan
Agouti x albino	Agouti	3 Agouti : 1albino

Chinchilla x Himalayan	Chinchilla	3 Chinchilla : 1Himalayan
Chinchilla x albino	Chinchilla	3 Chinchilla : 1 albino
Himalayan x albino	Himalayan	3 Himalayan : 1albino

Blood Groups in Human

The A-B-O blood group in human being controlled by three alleles I^A, I^B and I^O to form a multiple allelic services.

Blood Group	Possible genotype
O	$I^O I^O$
A	$I^A I^A$, $I^A I^O$
B	$I^B I^B$, $I^B I^O$
AB	$I^A I^B$

I^A and I^B are codominant of each other and both are completely dominant over I^O.

Pseudoalleles

Pseudoalleles are non alleles so closely linked as often inherited as one gene, but shown to be separate by cross over studies.

One of the first demonstrations of pseudoallelic condition was that of star-asteroid analysed by Lewis in 1951. He found a recessive mutation in Drosophila producing a small rough eye when homozygous. It was at locus 1.3 in the second chromosomes. This was also the identical location of gene star, a dominant mutation also affecting the morphology of the eye. The eye was rough and had a slight gleam, hence the name star, with gene symbol **S**. In crosses among these flies, recombination between star and asteriod occurred at a low frequency of one in five thousand.

Pseudoallelic effects are found in Drosophila, corn, cotton, Aspergillus, Neurospora, bacteria and in viruses.

Isoalleles

Usually wild type allele (represented as +) is dominant over its recessive allele. In some natural populations, different wild type alleles affecting the same character were found and these wild type alleles had similar allelic dominance or they may differ in their degree of expression that could be detected in special combinations. Such alleles are called isoalleles.

Timofeev - Ressovsky and Muller found that the wild type Drosophila from different natural populations had different dominant (red eye) alleles as judged by their stability or by their different effects in combinations. Stern found three different wild type alleles of another Drosophila mutant, cubitus interruptus, which showed different degrees of dominance over the same mutant allele. He called such alleles as isoalleles because they were alike in their homozygous effect and their differences appeared only in special combinations.

Modifying genes

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

In the Guernsey breed of dairy cattle, the `solid' colour (fawn i.e., light yellowish brown) of the coat is due to dominant gene **S** and the `spotted' coat (white spotting) is due to its recessive allele **s**. A number of these modifying genes influence the intensity of spotting. If a large number of these modifying genes is present in animals with **ss**, the animals are highly `spotted'. If only a small number of these modifying genes is present in animals with **ss,** they are medium `spotted'. If the modifying genes are absent, animals with **ss** have only few `spots'. These modifying genes have no effect in the presence of the gene for `solid' colour and animals with **SS** or **Ss** have solid-coloured coats irrespective of the number of modifyig genes present.

In Gossypium barbadense the presence of petal spot is due to a gene **S** and the absence of petal spot is due to its recessive allele **s**. A number of modifying genes increases the intensity of colour in the presence of the gene **S**.

Lecture 18. Quantitative inheritance – Multiple factor hypothesis – Nilsson Ehle, his e x p e r i m e n t

It is quite natural that small differences exist among individuals of similar genotype due to the effect of environment on genotype.

- On the other hand, there are some heritable differences also exist with continuous variation.
- Most of the economical traits show continuous variation and they are measurable or quantifiable.

Quantitative characters

- Quantitative characters are traits which show
- continuous variation and
- governed by a large number of genes called multiple genes or
- multiple factors or polymeric genes or polygenes.
- Their inheritance follows same mendelian principles.

Qualitative characters

Qualitative characters show

- discontinuous variation and
- are governed by one or two major genes or oligognes.

Multiple factor Hypothesis (Nilson – Ehle -1908) experiment on wheat kernel color

Multiple factor hypothesis (Nilson - Ehle)

i) for a given quantitative trait there could be several genes, which were independent in their segregation, but had cumulative effect on phenotype

ii) Dominance is usually incomplete

iii) **Each gene contributes something** to the strength of expression of character whereas its recessive allele does not of genes present dominance gene.

- Nilson-Ehle studied Kernel colour in wheat
- concluded that is a quantitative character

- He crossed true breeding red kernel whet (RR) with true breeding white (rr) and the F1 was red (Rr) and the F2 segregated for red and white in 3:1 ratio indicating the dominance of red over white.
- However, careful examination indicated the variation in red color among the red color progenies
- F1 red was not as intense as one of the parents
- In F2 he could observe two grades of red ie., one was red as that of one of its parent, two were higher red as that of F1 individuals.
- In some crosses, a ratio of 15 red : 1 white was found in F2 indicating that there are **two pairs of genes** for red colour that either or both of these can produce red kernels.
    - Finally he observed different shades of red in F2 for red kernel types. The F2 showed **red shades** and white as follows;

Dark red	:	1	
• Medium dark red	:	4	
• Medium red	:	6	15
• Light red	:	4	
• White	:	1	
Total	:	16	

- It was concluded two duplicate dominant alleles R1 and R2 **cumulatively decide** the intensity of red colour
- and both R1 and R2 are in completely dominant over white.
- **The high intensity of red colour depends on the number**.

The F2 ratio in wheat

Genotype	Genotypic ratio	Phenotype
R_1R_1 R_2R_2	1	Dark red
R_1R_1 R_2r_2	2	Medium dark red
R_1r_1 R_2R_2	2	Medium dark red
R_1r_1 R_2r_2	4	Medium red
R_1R_1 r_2r_2	1	Medium red
r_1r_1 R_2R_2	1	Medium red
R_1r_1 r_2r_2	2	light red
r_1r_1 R_2r_2	2	light red

r_1r_2 r_2r_2	1	white

- Hence, if two parents differ for the two genes the segregation was 1:4:6:4:1 provided both R1 and R2 contribute equally to the colour.
    - If three genes are involved in F2 segregation showed 1:6:15:20:15:6:1 for red shades and 1 for white.

Multiple factor –Quantitative inheritance

The alleles that contribute for the trait are called "contributing alleles",

If four genes are determining the kernel color then,

R1R1 R2 R2 x r1r1 r2r2

dark red white

F1 R1r1 R2r2

Medium red

♂ ♀	R_1R_2	R_1r_2	r_1R_2	r_1r_2
R_1R_2	R1R2 R_2R_2 (dark red)	R_1R_1 R_2r_2 (Medium dark red)	R_1r_1 R_2R_2 medium dark red	R_1r_1 R_2r_2 light red
R_1r_2	R1R1 R_2r_2 medium dark red	R_1R_1 r_2r_2 light red	R_1r_1 R_2r_2 light red	R_1r_1 r_2r_2 very light red
r_1R_2	R1r1 R_2R_2 medium dark red	R_1r_1 R_2r_2 light red	r_1r_1 R_2R_2 light red	r_1r_1 R_2r_2 very light red
r_1r_2	R1r1 R_2r_2 light red	R_1r_1 r_2r_2 very light red	r_1r_1 R_2r_2 very light red	r_1r_1 r_2r_2 white

Dark red is due to the presence of **four contributing genes** medium dark is due to three genes and medium red is due to two contributing genes and light red is due to one contributing gene

Quantitative inheritance in ear length of Maize (Emerson & East)

- In maize, the ear (cob) length is governed by multiple factors.
- Two varieties of maize *viz.,* long eared sweet corn and short eared popcorn.
- The earlength of sweet corn ranges from 13-21 cm with an average of 16.8 cm.
- While that of pop corn was ranging from 5 to 8 cm with an average of 6.6 cm when these varieties were crossed,
- the F1 progeny was found to have intermediate size ranging from 9-15 cm with an average of 12.1cms.

- In F2 there were relatively few of these **extreme and relatively large number of these extreme** and
- relatively large number of these with intermediate ear size which implies that ear size differences between two parents may be due to one, two or several gene differences.

Similarly in tobacco (*Nicotiana longiflora*) the corolla length shows polygenic inheritance.

Lecture 19. Polygenes – transgressive segregation, comparison of quantitatively and qualitatively inherited characters; modifiers; Types of gene action controlling quantitative traits

Polygenes: Any of a group of nonallelic genes, each having a small quantitative effect, that together produce a wide range of phenotypic variation. Also called *multiple factor, quantitative gene*.

Transgressive segregation

When the range in the F2 progeny goes beyond the original parents, it is known as transgressive segregation ". Transgressive segregation implies that the parents donate contributing alleles from different genes to the hybrid .

The appearance of individuals in F2 with very higher or lower intensity of expression than their both parents is known as transgressive segregation and such individuals are called transgressive segregants.

Transgressive segregants are produced when

i) the two parents involved in a cross which have positive alleles of different genes affecting a quantitative characters.

Segregation for these genes produces the two extreme homozygotes in F2 which transgress the parental limits for the character

.Quantitative inheritance for skin color in human beings

$$\text{Heritability} = \frac{\text{Genotypic variance}}{\text{Total or phenotypic variation}}$$

The fraction of the total variance that is due to genotype is called in the broad sense.

The differences between qualitative and quantitative traits

Qualitative traits	Quantitative traits
1. Segregation of alleles cause differences in phenotypic character	Segregation of contributing genes causes differences in phenotyeic characters
2. Qualitative traits are governed by Oligo genes or mendelian genes	Quantitative traits are governed by polygenes or multiple factors
3. Effect of each allele is easily distinguishable	effect of individual contributing genes is not distinguishable
4. discrete variation	continuous variation
5. Traits are classified by more counting	quantative traits are measured on a continuous scale (mean, variance) with statistical method)
6. Influence of environment is very less	More influence of environment
7. Dominance is complete in mendelian ratio	each dominant gene has a quantitative effect
8. Lesser role of modifying genes	more role of modifying genes

Features of quantitative inheritance or polygenic inheritance

1. The trait is governed by polygenes
2. segregation occurs at an indefinitely large number of loci

3. Polygenes are always under the influence of environmental factors and hence the quantitative trait is altered.
4. Multiple genes are usually incompletely dominant duplicate genes with cumulative effect
5. The phenotypic differences (variation) within a parental line or F1 population are purely due to environment
6. The F1 individuals have the same genotype and they are intermediate between parents
7. The F2 exhibits considerable variability. The variation within individuals is mainly due to genotype and the variation is continuous.
8. The F2 mean is equal to the F1 mean and the parental mean
9. The individual effect of individual contributing genes can not be distinguished early when compared to the environmental influence
10. The estimates of quantitative inheritance are calculated with mean, variance, heritability and genetic advance
11. They are measurable characters height of 100 cm. The recessive alleles t1t1t2t2 do not influence the height of plant. T1 and T2 due incompletely dominant over t1 and t2 respectively.

Lecture 20. Linkage - coupling and repulsion; Experiment on Bateson and Punnet – Chromosomal theory of linkage of Morgan – Complete and incomplete linkage, Linkage group

LINKAGE AND CROSSING OVER

Bateson and Punnett discovered in 1906 that the principle of independent assortment of members of different pairs of alleles at the timeof formation of gametes is not universsal but has same exception. Thomas Hunt Mongan (1910) found similar situations in Droshphila to give a satisfactory explanation for such deviation.

Linkage

- The tendency of two or more genes to stay together during inheritance is known as linkage
- Linked genes do not show independent segregation
- Group of genes situated on the same chromosome is known as **linkage group**
- The number of linkage group = the number of haploid chromosome number
- Any two genes on a linkage group is known **syntenic**

According to 'Chromosome Theory of Inheritance', the genes are carried in the chromosomes. But the number of genes per individual far exceeds the number of chromosome pairs. It means each chromosome bears many genes. The genes located on the same chromosome cannot assort independently, rather these tend to be inherited together. *This phenomenon of inheritance of genes together and to retain their parental combination even in the offsprings is known as* linkage. The genes located in the same chromosome and being inherited together are known as linked genes, and the characters controlled by these are linked characters.

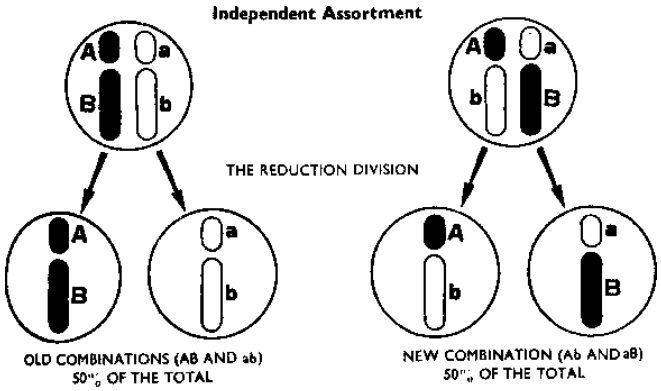


Fig. 8.1. Diagram showing results of independent assortment.

The difference between linkage and independent assortment can be illustrated by the following figure

i

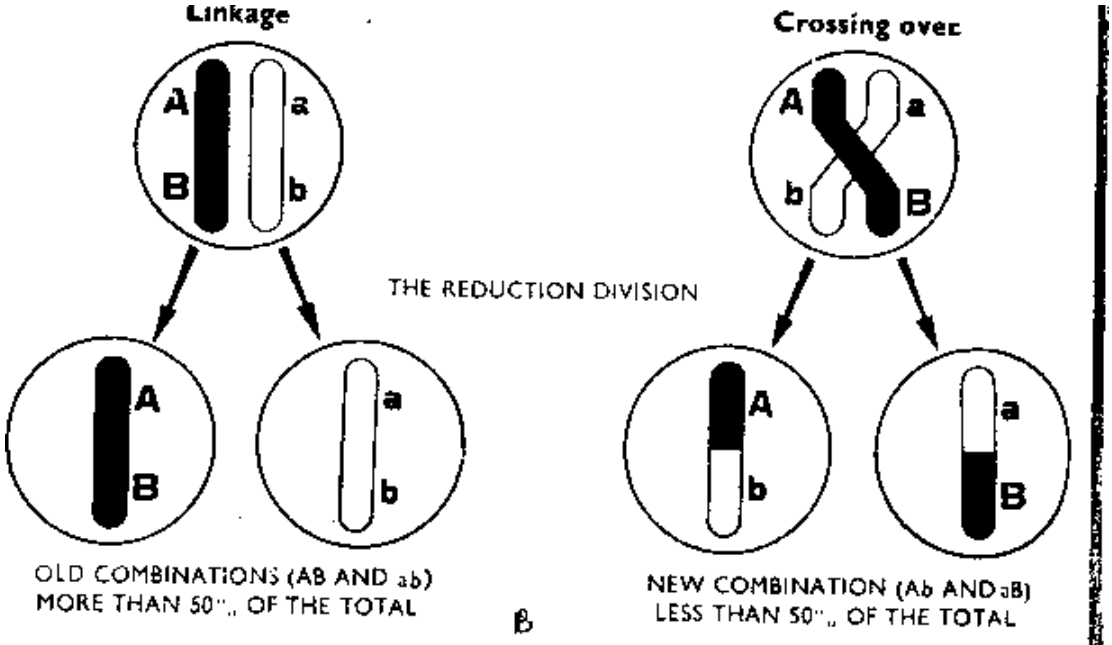


Fig. 8.2. Diagram showing results of linkage and crossing over.

All those genes which are located in the single chromosome! constitute a linkage group. The total number of linkage groups in *mu* organism is equal to the number of chromosome pairs. For example, there are 4 linkage groups in *Drosophila melanogaster,* 23 in man and 7 in sweet pea.

HISTORY OF LINKAGE

The theory of linkage was propounded by T. H. MORGAN in 1911. But its existence was predicted even before that and was described under!- different names.

1. Sutton's Hypothesis

Sutton (1903) suggested that each chromosome bears more than one genes and all the genes, situated in one chromosome are inherited together in the offsprings, but he was unable to support his hypothesis experimentally.

2. Coupling and Repulsion Hypothesis

The event of linkage was first observed by BATESON and PUNNET in 1906 in pea plant, but it was described as coupling**.** They found that the results of dihybrid cross in sweet pea, *Lathynis, odoratus,* involving colour and shape of pollen grains, do not agree with the law of independent! assortment. The results obtained are shown in the table below

P1 Purple flower; long pollen x Red flower, round pollen

F_1 Purple flower; long pollen

Generation	Phenotype	Number	Ratio
F_2	Purple long	296	11
	Purple round	29	1
	Red long	27	1
	Red round	85	3

When these F_1 purple, long (heterozygous) hybrids were crossed with the double recessive red and round)homozygous) individuals (test cross) failed to produce expected 1: 1: 1: 1 ratio in F_2 generation. These actually produced following four combinations in the ratio of 7:1:1:7.

P1 Purple long x Red round

RRLL rrll

F_1	Purple long	x	Red round (double recessive)
	RrLl		rrll

Test cross

Progeny

Purple long	Purple round	Red long	Red round
7	1	1	7

7 purple long : 1 purple round:1 red long : 7 red round

The above results of the test cross indicate that the parental combinations are seven times more numerous than the non-parental combinations. Bateson and Punnet suggested that the alleles coming from the same parent tend to enter the same gamete and to be inherited together (genetic coupling). Similarly, the same genes coming from two different parents tend to enter different gametes and to be inherited separately and dependently (repulsion).

Bateson and Punnet could not explain the exact reasons for coupling and repulsion. Although, the theory is obsolete now, the terms ‘coupling phase’ and 'repulsion phase' have been retained on account of heir descriptive significance.

3. Morgan's Concept of Linkage

Morgan (1910) while working on Drosophila stated that coupling and repulsion are two aspects of the same phenomenon, which he described as 'linkage'. He defined linkage 'the tendency of the genes, as present in the same chromosome, to remain in their original combination and to enter together in the same gamete.

4. Chromosome Theory of Linkage

Morgan and Castle formulated The chromosome Theory of linkage. It has following characteristics: —

1. Genes that show linkage are situated in the *m* chromosome.
2. Genes are arranged in a linear fashion in the chromosome. Linkage of genes is linear.

3. The distance between the linked genes is inversely proportional to the strength of linkage. The genes which are closely located show strong linkage, whereas those, which are with separated, have more chances to get separated by cross over.
4. Linked genes remain in their original combination during the course of inheritance.

The chromosome theory of linkage is widely supported from the cytological studies. It has helped in the construction of linkage maps of chromosomes (The distance between the genes is determined by the percentage of cross overs.

ARRANGEMENT OF LINKED GENES

In an individual, which is heterozygous for two pairs of linked genes, the linkage can be either of the two types:-

(i) The dominant genes of both the pairs are located in one member of the chromosome pair and their recessive alleles are located in the other chromosome of the pair. This arrangement is known as cis-arrangement**.** And the heterozygous with such arrangement (AB/ab) are known as cis - heterozygotes –COUPLING PHASE

(ii) The dominant gene of one pair and the recessive gene of other pair are located on one chromosome of the pair and the recessive gene of the first pair and dominant gene of the second pair are located in the second chromosome pair (Ab/aB). This arrangement of a dominant and recessive gene in the same chromosome of the chromosome pair is known as rans-arrangement and heterozygotes with such arrangement are called trans-heterozygotes. REPULSIVE PHASE

Diagram exhibiting arrangement of

the trans and cia genes

Types of linkage

Completely linked genes: any two genes on a particular chromosome is very close to each other and move together to the gametes and no recomination/cross overs/chiasma between these two loci. Eg. Male drosophila

Incompletely linked – any two genes on the same chromosome but show moderate level of cross overs

Tightly linked genes – show very little frequency of recombination

Advantages of linkage

- Linkage provides a way to map genes on chromosomes. (Until modern gene mapping techniques linkage was the only way to do this)
- .Even today linkage is important when used with other techniques to determine the location of genes and also for diagnosis of disease
- the presence of linkage complicate the evolutionary behavior of genes in populations

Lecture 21. Crossing over – significance of crossing over; cytological proof for crossing over - Stern's experiment; Factors controlling crossing over.

Crossing over

Crossing over is the exchange of strictly homologous segments between non sister chromatids of

homologous chromosomes during pachytene stage of prophase I of meiosis I.

Each event of crossing over produces

- two recombinant chromatids (involved in the crossing over) – crossover chromatid
- Two original chromatid (non cross over chromatid)

The term crossing over was first used by Morgan and Cattell in 1912.

The main features of crossing over are given below :

1. Crossing over takes place during meiotic prophase, *i.e.*, during pachytene. Each pair of chromosome has four chromatids at that time.
2. Crossing over occurs between non-sister chromatids. Thus one chromatid from each of the two homologus chromosomes is involved in crossing over.
3. It is universally accepted that crossing over takes place at four strand stage.
4. Each crossing over involves only two of the four chromatids of two homologus chromosomes. However, double or multiple crossing over may involve all four, three or two of the four chromatids, which is very rare.
5. Crossing over leads to recombinations or new combinations between linked genes. Crossing over generally yields two recombinant types or crossover types and two parental types or non-crossover types.
6. Crossing over generally leads to exchange of equal segments or genes and recombination is always reciprocal. However, unequal crossing over has also been reported.
7. The value of crossover or recombinants may vary from 0-50%.
8. The frequency of recombinants can be worked out from the test cross progeny. It is expressed as the percentage ratio of recombinants to the total population (recombinants + parental types) Thus,

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

Differences between crossing over and linkage

	Crossing over	**Linkage**
1.	It leads to separation of linked genes.	It keeps the genes together.

2.	It involves non-sister chromatids of homologous chromosomes.	It involves individual chromosome.
3.	Frequency of crossing over can never exceed 50%.	Linkage groups can never be more than haploid chromosome number.
4.	It increases variability by forming new gene combinations.	It reduces variability.
5.	It provides equal frequency of parental and recombinant types in test cross progeny.	Provides higher frequency of parental types than recombinant types in test cross progeny.

Chiasma and Crossing over

The point of exchange of segments between non-sister chromatids of homologous chromosomes during meiotic prophase is called chiasma (pleural chiasmata). It is thought to be the place where crossing over takes place. Chiasma was first discovered by Janssens in 1909. Depending on the position, chiasma is of two types, *viz.*, terminal and interstitial. When the chiasma is located at the end of the pairing chromatids, it is known as terminal chiasma and when it is located in the middle part of non-sister chromatids, it is referred to as interstitial chiasma. Later on interstitial chiasma is changed to terminal position by the process of chiasma terminalization. The number of chiasma per bivalent may vary from one to more than one depending upon the length of chromatids. When two chiasmata are formed, they may involve two, three or all the four chromatids.

Chiasma Terminalization

The movement of chiasma away from the centromere and towards the end of tetrads is called terminalization. The total number of chiasmata terminalized at any given stage or time is known as coefficient of terminalization. Generally, chiasma terminalization occurs between diplotene and metaphase I.

TYPES OF CROSSING OVER

Depending upon the number of chiasmata involved, crossing over may be of three types, *viz.,* single, double and multiple as described below :

Single Crossing Over

It refers to formation of a single chiasma between non-sister chromatids of homologous chromosomes. Such cross over involves only two chromatids out of four.

Double Crossing Over

It refers to formation of two chiasmata between non-sister chromatids of homologous chromosomes. Double crossovers may involve either two strands or three or all the four strands.

Multiple Crossing Over

Presence of more than two crossovers between non-sister chromatids of homologous chromosomes is referred to as multiple crossing over. Frequency of such type of crossing over is extremely low.

CYTOLOGICAL PROOF OF CROSSING OVER – STERNS EXPERIMENT ON DROSOPHILA –CYTOLOGICAL PROOF FOR 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 conducted with **Drosophila.** He used cytological markers in his studies. He selected a female fly in which one X-chromosome was broken into two segments. Out of these two segments, one behaved as X-chromosome. The other X-chromosome had small portion of Y-chromosome attached to its one *end.* Thus, both the X-chromosomes in the female had distinct morphology and could be easily identified under microscope. In female fly, the broken X-chromosome had one mutant allele (carnation) for eye colour and another dominant allele (B) for bar eye shape. The other X-chromosome with attached portion of Y chromosome had alleles for normal eye colour (red eye) and normal eye shape (oval eye). Thus, phenotype of female was barred.

A cross of such females was made with carnation male (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 crossover types (car+ and B+). The male flies produce only two types of gametes (car+ and Y), because crossing over does not take place 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, means there will be four females and four males

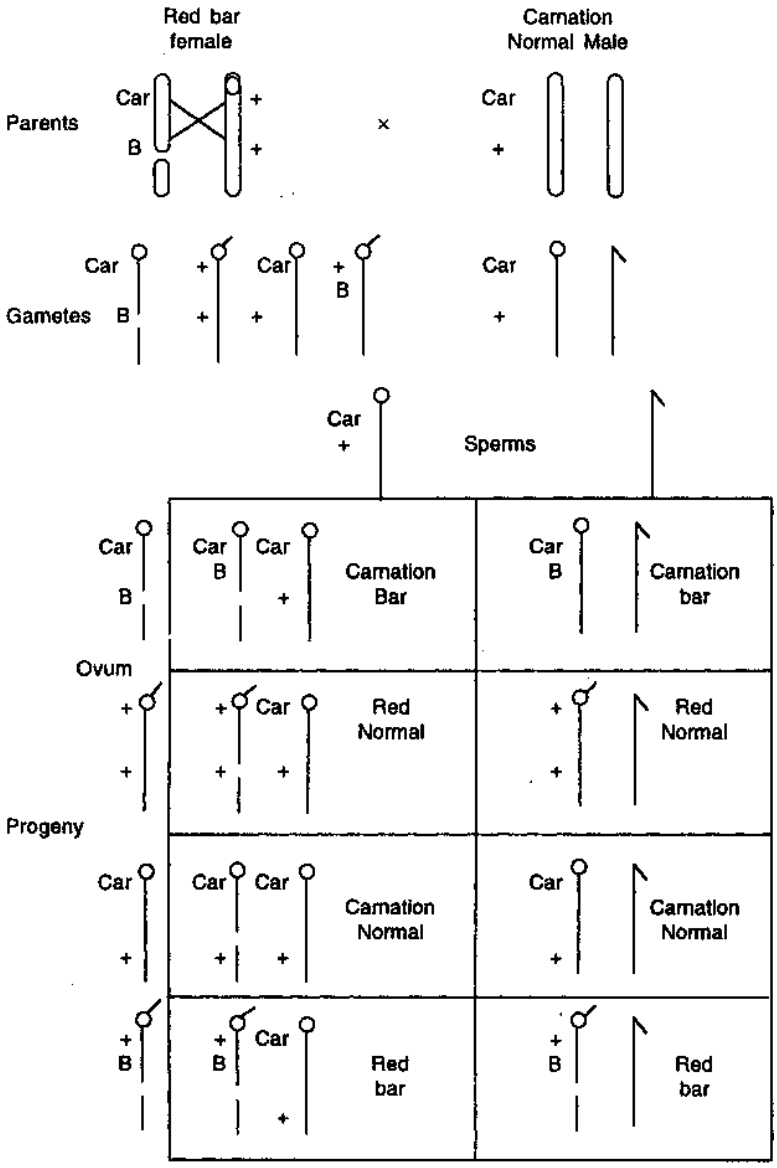


Fig. 9.4. *Cytological proof of crossing over in* ***Drosophila.***

Stern examined the chromosomes of recombinant types, *viz.*, red bar and carnation normal under microscope. He observed that in carnation normal females both the X-chromosomes were of equal length.

In red bar flies, one X-chromosome was normal and other was fragmented. The fragmented X-chromosome also had attached part of Y-chromosome. Such chromosome combination in red bar is possible only through exchange of segments between non-sister chromatids of homologous chromosomes. This has proved that genetic crossing over is the result of cytological crossing over. Similar proof of cytological crossing over was provided by Creighton and McClintock in maize.

Cytological evidence for crossing over in the autosomes (chromosomes other than sex determining chromosomes) is available in maize (n = 10), in which Creighton and McClintock (1931) identified a mutant. The mutant had a satellite (knob) at the terminal end of the ninth chromosome, which also had a segment of the eighth chromosome translocated to it. The genes for coloured or colourless aleurone and starchy or waxy endosperm were located on the 9^{th} chromosome. Recombinations due to crossing over were recognized in the progeny of a cross between the female parent having the aberrant 9^{th} chromosome with its normal homologue and a male parent with normal 9^{th} chromosome. The crossover could be cytologically detected, thus establishing the cytological basis for genetic crossing over.

FACTORS AFFECTING CROSSING OVER

The frequency of crossing over is influenced by several factors which are briefly discussed below :

1. **Distance,** The distance between genes affects the frequency of crossing over. Greater the distance between genes higher is the chance of crossing over and vice versa.
2. **Age.** Generally crossing over decreases with advancement in the age in the female **Drosophila.**
3. **Temperature.** The rate of crossing over in **Drosophila** increases above and below the temperature of 22°C.
4. **Sex.** The rate of crossing over also differs according to sex. There is lack of crossing over in

Drosophila male and female silk moth.

5. **Nutrition.** Presence of metallic ions like calcium and magnesium in the food caused reduction in recombination in **Drosophila.** However, removal of such chemicals from the diet increased the rate of crossing over.
6. **Chemicals.** Treatment with mutagenic chemicals like alkylating agents was found to increase the frequency of crossing over in **Drosophila** female.
7. **Irradiation.** Irradiation with X-rays and gamma rays was found to enhance the frequency of crossing over in **Drosophila** females.
8. **Structural Changes.** Structural chromosomal changes especially inversions and translocations reduce the frequency of crossing over in the chromosomes where such changes are involved.
9. **Centromere Effect.** Generally genes that are located adjacent to the centromere show reduced frequency of crossing over.
9. **Cytoplasmic Genes.** In some species cytoplasmic genes also lead to reduction in crossing over. For example, Tifton male sterile cytoplasm in pearl millet.

SIGNIFICANCE OF CROSSING OVER

Crossing over is useful in three principal ways, *viz.,* (1) creation of variability, (2) locating genes on the chromosomes, and (3) preparing linkage maps as described below:

1. **Creation of Variability**: Crossing over leads to recombination or new combination and thus is a potential genetic mechanism for creating variability which is essential for improvement of genotypes through selection.
2. **Locating genes** : Crossing over is a useful tool for locating genes in the chromosomes.
3. **Linkage maps :** Crossing over plays an important role in the preparation of chromosome maps or linkage maps. It provides information about frequency of recombination's and sequence of genes which are required for preparation of linkage maps.

Lecture 22. Strength of linkage and recombination; Two point and three point test cross.

Linkage studies revealed the following:

1. Genes that assort at random are unlinked genes. Genes that do not segregate at random are linked genes. They are situated in the same pair of chromosomes and are transmitted in unitary groups.
2. The linked genes are arranged in a linear fashion on the chromosome. Each linked gene has a definite and constant order in its arrangement.
3. **The distance between the linked genes determines the degree of strength of linkage. Closely located genes show stronger linkage than the widely located genes**.

Two-point Test Cross -A cross of dihybrid with its homozygous recessive parent

Three point testcross: A three point test cross is a cross of a trihybrid (F_t differing in three genes) with its homozygous recessive parent. The three point test cross provides useful information on two important aspects, *viz.*, (1) about the sequence of genes, and (2) about the recombination frequencies between genes. This information is essential for mapping of chromosomes.

Two-point Test Cross

Estimation of strength of linkage from test cross data

The value of linkage can be determined from the test cross data by dividing the total number of individuals with the parental combinations divided by the total number of progeny and multiplying it by 100.

Let us calculate the value of linkage from the results of the test cross:

P	Coloured full **CS/CS**	X	Colourless shrunken **Cs/cs**
F_1		Coloured Full **CS/cs**	
Test cross	Coloured full **CS/cs**	X	Colourless shrunkle **Cs/cs**

Test cross progeny		
Coloured full	**CS/cs**	4032
Coloured shrunken	**Cs/cs**	149
Colourless full	**CS/cs**	152
Colourless shrunken	**Cs/cs**	4035
Total number of progeny		8368
Parental combinations		
Coloured full		4032
Colourless shrunken		4035
Total		8067
Percentage		8067/8368 * 100 = 96.4
Recombinations		
Coloured shrunken		149
Colourless full		152
Total		301

Percentage	100 - 96.4 = 3.6
Linkage value	96.4%
Cross over value	3.6%

Estimation of linkage from F_2 data

Linkage values can be calcualted from the F_2 data by the additive method or the product method or the maximum likelihood method.

We shall now illustrate the calculation of the linkage value from the F_2 data.

Assume that a cross **CS/CS * cs/cs** gives in F_2

Coloured full (a)	7300
Coloured shrunken (b)	200
Colourless full ©	200
Colourless shrunken (d)	2300

i) Additive method:

The formula applied is $p^2 = E - M / N$

Where p = linkage value; E = sum of end classes; M = sum of middle classes and N = total number of progeny.

$P^2 = (7300 + 2300) - (200 + 200) / 10000$

$P^2 = 0.92$

P = 0.9592

Linkage value = 95.92%

Cross over value = 4.08%

ii) Product ratio method:

Product ratio (P) = ad / bc

P^2 = (P + 1) - root (3P + 1) / P - 1

Where P = linkage value,

P = product ratio,

A and d=No. of F_2 progeny in the parental combinations and b and c = No. of F_2 progeny in the recombinations.

Substituting the figures,

P = 7300 * 2300 / 200 * 200 = 419.75

P_2= (419.75 + 1) - root (3 * 419.75) - 1 / 419.75 - 1

= 0.920072

p = 0.9592 1 - p = 0.0408

Linkage value = 95.92%

Cross over value = 4.08%

iii) Square root method:

Percentage of non-cross over gametes = Frequency of double recessives

= square root(2300 / 10000)

= 0.9592

Linkage value = 95.92%

Cross over value = 4.08%

Chiasmata

- The points at which the chromosomes actually cross over are called chiasmata (singular chiasma),
- They involve large, multi-enzyme complexes that cut and join the DNA.
- There is always at least one chiasma in a bivalent, but there are usually many, and it is the chiasmata that actually hold the bivalent together.
- The chiasmata can be seen under the microscope and they can give the bivalents some strange shapes at prophase I.
- There are always equal amounts crossed over, so the chromosomes stay the same length.

- Ultimately, crossing over means that **maternal and paternal alleles can be mixed**, even though they are on the same chromosome i.e. **chiasmata result in different allele combinations**.

The probability of chiasma occurring between two linked genes

- Chiasma % = 2 (crossover %)
  - or
- Crossover % = 1/2 (chiasma %)
- Chiasma frequency is twice the crossover frequency because when a chiasma forms between two genes, only half of the meiotic products will be of the crossover type.

Lecture 23. Double cross over, interference and coincidence; genetic map, physical map.

Genetic map(cross over map or chromosome map or Linkage map)

CHROMOSOME MAPPING

Chromosome map refers to a line diagram which depicts various genes present on a chromosome and recombination frequency between them. Such maps are also known as genetic maps or linkage maps. The process of assigning genes on the chromosomes is known as

chromosomal mapping. The mapping of chromosomes is done with the help of three point test cross. A three point test cross is a cross of a trihybrid (F_t differing in three genes) with its homozygous recessive parent. The three point test cross provides useful information on two important aspects, *viz.,* (1) about the sequence of genes, and (2) about the recombination frequencies between genes. This information is essential for mapping of chromosomes.

Morgan postulated that genes are arranged in a linear order along the length of the chromosome, each gene having a fixed place on the chromosome and its allele, a corresponding position on the homologous chromosome.

In mapping genes, a unit of distance must be used and this unit is called a **map unit** which is the space within which one per cent of crossing over takes place. If the percentage of cross over between two linked genes is 1 per cent, it means that the map distance between these two linked genes is one unit of map distance or one map unit, or one centimorgan.

The chromosome map may be defined as a line, on which the genes are represented by points, separated by distances proportional to the amount of crossing over.

The chromosome maps are also referred to as cross over maps since they are sketched by the amount of crossing over.

The percentage of crossing over is directly proportional to the distance of the alleles showing crossing over in the chromosome.

The chromosomes maps are the graphic representation of the genes in a chromosome.

The percentage of crossing over is calculated by test crosses. In mapping the genes, a unit of distance is used and it is called as ***map unit*** or ***Morgan unit***.

The first chromosome map was made in 1911 by ***Sturtevant*** and soon after additional maps were made by ***Bridges*** and others.

Drosophila is the earliest material used by the scientists, for constructing maps.

Procedure for the chromosome mapping

In fact genes are plotted on the chromosome on the basis of crossing over results between different pairs of linked genes. The actual distance between two genes is said to be equivalent to the percentage of crossing over between these genes.

When the % of crossing over between two genes is 5, then the distance is 5 units. For example five genes A, B, C, D and E are to be plotted on a chromosome. If cross over results

indicate that genes A and E have the highest percentage of crossing over, it means that these should be placed at the maximum distance.

In this example, the gene A can be taken as a starting point in the chromosome and can be represented by O.

Now if the gene A and B exhibit 7% crossing over, the gene B can be placed on the chromosome at a distance of 7 units.

If the gene C shows 8% crossing over with gene B and about 15% crossing over with gene A, it can be plotted on the chromosome at a distance of 15 units from gene A.

Similarly if gene A and E exhibit 20% and 30% crossing over with gene D and 5% and 10% with gene C these, are located on the chromosome 5 and 10 units away from the gene C respectively.

Construction of Chromosome map in Drosophila

In Drosophila the chromosome map is constructed with the help of test cross. In Drosophila grey colour is dominant over black colour; and the long wing is dominant over vestigial wing.

The F_1 female hybrid is ***test crossed***. Four types of individuals are formed. Out of four types, two types are parental type (G:L & B:V) and other two are non parental type (G:V & B:L) due to crossing over. Non – parental type is 17%. So the percentage of crossing over is equivalents to 17%. The distance between the two genes (G-L) is equivalent to the percentage of crossing over or percentage of non parental combination. So the distance between the gene G & L is equivalent to 17 morgan units.

Percentage of non parental combination	=	17%
So the percentage of crossing over	=	17
So the distance between the Gene G & L	=	17 map unit

In another experiment the F_1 female grey red is test crossed with black cinnabar. The experiment shows 9% non parental combination individuals. So the distance between the Gene G & Cn is equavalent to 9 map unit.

In the same way the F_1 female red long is test crossed with cinnabar vestigial. The experiment shows 9.5% non-parental combination individuals. So the distance between the gene Cn is equivalent to 9.5 map unit.

According to the first experiment the distance between G & L is equivalent to 17 map unit. But the second and third experiment show 18.5 map units between the two genes. To find out the actual reason for this difference in the distance, conduct a 3 point cross.

Three Point cross

In the three point cross all the three pairs of genes are considered in the experiment. The F_1 hybrid female is test crossed. They produce 8 different types of individuals. Out of 8 types, two types are parental. Remaining six are non-parental.

	Male	Female
Parent :	Grey Red Long	Black cinnabar Vestigial
	G Cn L x	g cn l
	Y-chromosome	g cn l
		Normal
F_1 :		G Cn L
		g cn l
Back cross :	Female	Male
	Normal	Recessive
	G Cn L x	g cn l
	g cn l	Y – chromosome

Chromosome Maps of Drosophila

The chromosome maps of Drosophila include four linkage groups corresponding to four chromosome pairs. The genes present in the X chromosome constitute the first linkage group, those present in 2nd and 3rd chromosome constitute 2nd and 3rd linkage groups and those on the fourth chromosome form fourth linkage group. The fourth linkage group is the smallest of all.

Chromosome maps of maize

Chromosome maps of maize have been drawn by R.A. Emerson. As there are 10 pairs of chromosome 10 chromosome maps are seen.

THREE POINT TEST CROSS

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

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

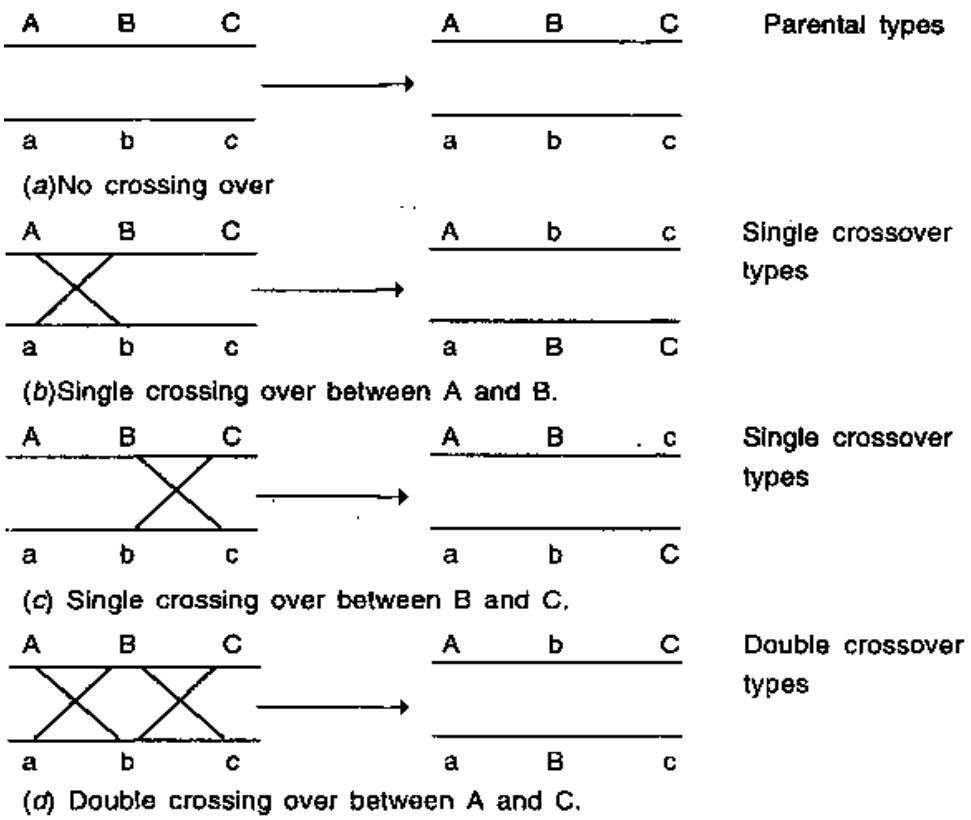


Fig. 9.3. *Single and double crossing over between three linked genes.*

Factors affecting the mapping

Chromosome map can be constructed only with the help of crossing over percentage. The crossing over percentage is highly modified by the interference and coincidence.

INTERFERENCE

The term interference was coined by Muller which refers to the tendency of one crossover to reduce the chance of another crossover in its adjacent region. Interference is affected by gene distance on the chromosome. Lesser the gene distance greater is the interference and vice versa. Generally, it is observed that crossing over in one region of chromosome may check the crossing over in the second region.

Sometimes, presence of recombination in one region enhances the chance of recombination in another adjacent region. This is termed as negative interference. This type of situation has

been observed in some lower organisms, *viz.*, Aspergillus and bacteriophages. Coefficient of interference is estimated as follows :

Coefficient of interference (%) = 1-Coefficient of coincidence x 100

Positive and negative interference differ from one another in three main aspects .

Differences between positive and negative interference

Positive Interference	**Negative Interference**
1. One crossover reduces the chance of another crossover in the adjacent region.	One crossover enhances the chance of another crossover in the adjacent region.
2. Observed in both eukaryotes and prokaryotes	Found in some lower organisms like Aspergillus and bacteriophages.
3. In this case coefficient of coincidence is less than one.	In this case coefficient of coincidence is always more than one.

COINCIDENCE

This term was also coined by Muller to explain strength or degree of interference. The coefficient of coincidence is the percentage ratio of observed double crossovers to the expected double crossovers. The greater the coincidence, lesser will be the interference and vice versa. Thus,

$$\text{Coefficient of coincidence (\%)} = \frac{\text{Observed double crossovers}}{\text{Expected double crossovers}} 100$$

Coefficient of coincidence is a measure of the intensity of interference, because it has negative association with interference. The value of the coefficient of coincidence is less than 1 for positive interference, greater than 1 for negative interference, 1 for absence of interference and zero for complete or absolute interference.

Lecture 24. Sex determination: Autosomes and sex chromosomes - chromosomal theory of sex determination - different types – sex determination in human, fowl, butterfly, grasshopper, honey bee, fumea; Genic balance theory of Bridges, quantitative theory, hormonal theory, barr bodies, metabolic differentiation theory; Gynandromorphs – sex reversal in chicken

Sex differentiation in living organisms into male and female causes morphological, physiological and behavioral differentiation between the two sexes and this phenomenon is called sexual dimorphism. In a large number of species of animals and a small number of species of plants, eggs and sperms are produced by different individuals, viz., females and males respectively.

MONOECIUOS

- Many plants and some animals (earthworms and hydra) have both male and female sex organs in the same individual and produce both male and female gametes (sperm and egg, respectively). These organisms are monoecious. (maize)

DIOECIOUS

- organisms come in two sexes, ie., male and female in separate individuals, and each individual will produce only one type of gamete (papaya, palm)

HERMOPHRODITES

- Plants/flowers where both the male and female organs occur together (paddy, Hibsicus)

GYNANDROMORPHS

- In Drosophila some individuals show male characteristics in a part of their body, while their remaining parts show the female phenotype, such individuals are known as gynandromorphs. This is mostly caused by irregularities in cell division during embryonic development

A **sex-determination system** is a biological system that determines the development of sexual characteristics in an organism.

• Most sexual organisms have two sexes. In many cases, sex determination is genetic:

- males and females have different alleles or even different genes that specify their sexual morphology.
- In animals, this is often accompanied by chromosomal differences. In other cases, sex is determined by environmental variables (such as temperature) or social variables (the size of an organism relative to other members of its population

How sex is determined in animals?

sex is determination by

1. sex chromsome
2. Genic balance
3. environment

History

- Henking (1891) first observed the X chromosome in male insects
- McClung also observed extra chromosomes in in males of grasshopper
- Wilson (1909) – named X chromosome
- Stevens (1909) – named Y chrosome

SEX CHROMOSOME (ALLOSOME)

Heteromorphic chromosomes whose distribution in a zygote determines the sex of the organism (usually designated as X or Y)

AUTOSOME

chromosome other than a sex chromosome and there is no difference in autosomes in male and female

Sex chromosomes

- X and Y are the two sex chromosomes named in animals and plants
- The **X chromosome** is one of the two sex-determining chromosomes in many animals Species,
- The X chromosome was named for its unique properties by early researchers,
- Y chromosome was named so as it it was discovered later and comes next to X in the alphabet

• Usually X chromosomes are larger than Y with some exception in plants

• X chromosmes carry more genes than Y

Barr body is the inactive X chromosome in a female cell ie., out of two X chromsomes of human beings one of the X chromosome is inactivated and called **BARR Body**

• rendered inactive in a process called **Lyonization** (in cells with multiple X chromosomes, all but one is inactivated during mammalian embryogenesis)

SEX DETERMINATION BY SEX CHROMOSOME – Chromosomal mechanisms of sex determination

• The different mechanisms of chromosomal sex determination may be grouped into five classes

• 1. XX female, XY male

• 2. XY female, XX male

• 3. XX female , X- male

• 4. X- female, XX female

• 5 diploid female and haploid male

usually most of the animals have one pair of sex chromosomes. However, in Ascaris incurva, there are 8X chromosomes in females and one Y chromosome in males; In case of Blaps polychresta the males have 12 X and 6 Y chromosomes while the females have 12 pairs of X chromosomes alone

1. XX-XY system

• XX female - XY male

• The **XY sex-determination system** is a well-known sex determination system.

• The XY sex determination system was first described independently by Nettie Stevens and Edmund Beecher Wilson in 1905

• It is found in humans, most other mammals, some insects (*Drosophila*) and some plants (*Ginkgo*).

• In the XY sex-determination system, females have two of the same kind of sex

chromosome (XX), and are called the homogametic sex.

• Males have two distinct sex chromosomes (XY), and are called the heterogametic sex

2. XY-XX sex-determination system -found in birds and some insects and other organisms.

• Females have two different kinds of chromosomes (XY), and heterogametic

• Males have two of the same kind of chromosomes (XX), Homogametic

3. XX - X0 sex-determination system

• in this system only one type of sex chromosome viz., the X chromsome is present

• in this system in one sex two X chromosome are present in another sex there is only one sex chromosome, X_(XO)

• XX female- X0 male

• found in grasshoppers, crickets, cockroaches, and some other insects

• In this system, there is only one sex chromosome, referred to as X. Males only have one X chromosome (X0), while females have two (XX).

• The zero (sometimes, the letter O) signifies the lack of a second X chromosome.

• Female gametes always contain an X chromosome, so the sex of the animals' offspring is decided by the male.

• Its sperm normally contain either one X chromosome or no sex chromosomes at all.

• In a variant of this system, certain animals are hermaphroditic with two sex chromosomes (XX) and male with only one (X0). The model organism *Caenorhabditis elegans* — a nematode frequently used in biological research — is one such organism.

4. XO-XX system

• In fumea this kind of sex determination is found

• XO- females

• XX- males

5. Haplodiploid sex-determination system

• The **Haplodiploid sex-determination system** determines the sex of the offspring of many Hymenopterans (bees, ants, and wasps), and coleopterans (bark beetles).

• In honeybees

females – diploid

males – haploid

II. SEX DETERMINATION BY GENIC BALANCE THEORY

• In *Drosophila*, the greater the number of X chromosomes relative to the autosomes, the more likely the individual will be female

Sex balance theory or genic balance theory states that the **X chromosome determines** the sex of the individual and that sex is a dosage phenomena,

• where the **ratio** of the amount of the X relative to the autosomes determines the sex.

• In addition, environmental effects can influence the development of the intersex flies.

• sex index = $\frac{\text{number of X chromosomes}}{\text{number of autosomes}}$

• If the ratio is =1 - females

= >1.0 - super females

=0.5 - male

= <0.5 – super males

= 0.5 – 1.0 - intersex

Genic Balance theory of sex determination (Bridges 1921)

A number of lines of evidence indicate that even in dioecious species, all individuals have genes for both sexes. To quote Bridges, `Both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female and the other to produce the characters called male. `Which sex actually develops is decided by the balance, i.e., by the preponderance of the female-determining or of the male-determining genes. The sex chromosomes are merely vehicles of genes which help in tilting the balance in one direction or another.

Support for the **balance theory of sex determination comes from the work of Bridges(1921)** on Drosophila. Bridges observed some females of Drosophila melanogaster with three X chromosomes and three sets of autosomes (i.e, triploids). When he crossed them with normal (diploid) males, he found that some of the progeny had one or more chromosomes less or more than the normal flies(i.e., aneuploids). His results are given below:

	X + A	Y + A
2X + 2A	3X + 3A Female	2X + Y + SA Intersex
X + A	2X + 2A Female	X + Y + 2A Male
2X + A	3X + 2A Superfemale	2X + Y + 2A Female
X + 2A	2X + 3A Intersex	X + Y + 3A Supermale

Bridges found intersexes, superfemales and supermales among the progeny. Intersexes are sterile individuals intermediate between females and males but are different from gynandromorphs which are typically female in certain portions of the body and typically male in others. Superfemales and supermales are sterile individuals which are very weak and very poor in viability.

This is shows that in Drosophla the X chromosomes carry genes that are predominantly female-determining.

Flies with one X, one Y and two sets of autosomes are normal males but flies with one X, one Y and three sets of autosomes are supermales.

1X + 1Y + 2A	Male
1X + 1Y + 3A	Supermale

This shows that the autosomes carry genes that are predominantly male-determining.

That individuals with two X, one Y and two sets of autosomes are female in spite of the presence of the Y chromosome shows that the Y chromosome plays no positive role in sex determination. That the Y chromosome does not determine maleness in also shown by the fact that flies with one X chromosome and two sets of autosomes (i.e., XO flies) are males in spite of the absence of the Y chromosome. They are, however, sterile showing thereby that the Y chromosome contains male fertility genes necessary for the production of a fertile male.

Bridges interpreted these results as follows:

Sex in Drosophila melanogaster is determined by the X chromosomes as well as by the autosomes, the ratio of the number of X chromosomes to the number of sets of autosomes being the deciding factor

Phenotype	**chromosomal complement**	**number of X chromosomes/ number of autosomal sets**
normal female	XX + 2N autosomes	1.00
normal male	XY + 2N autosomes	0.50
superfemale	XXX + 2Nautosomes	1.50
superamale	X + 3N autosomes	0.33
intersex (sterile)	XX + 3N autosomes	0.67

III. Environment –sex determination

- Many other exotic sex-determination systems exist. In some species of reptiles, including alligators, some turtles, and the tuatara, sex is determined by the temperature at which the egg is incubated.
- some hormones produced by the proboscis of female Bonelia induces larvae to differentiate into males
- in Dinophilus(sea worm) , animals from larger sized eggs are females and from smaller size are males
- Other species, such as some snails, practice sex change: adults start out male, then become female.

- In tropical clown fish, the dominant individual in a group becomes female while the other ones are male.
- In some arthropods, sex is determined by infection. Bacteria of the genus *Wolbachia* alter their sexuality;
- some species consist entirely of ZZ individuals, with sex determined by the presence of *Wolbachia*

Lecture 25. Sex linked inheritance – cris cross inheritance – reciprocal difference; holandric genes; sex influenced and sex limited inheritance.

SEX LINKAGE- location of gene on X chromosome or sex chromosome

Morgan crossed a red eyed female with a white eyed male and found that all F1 flies of both sexes were red eyed. In F2, 3 red and 1 white eyed. So, it is due to an allelic Pair of genes of which red is dominant.

Red female X White male

F1 Red female Red male

F2 Red female Red female Red male White male

A reciprocal cross was made between white eyed female and red eyed male. It was found that among the F1 off spring, all the females were red eyed and all the males were white eyed. The results were quite unexpected firstly, because the phenotypes of F1 females and male were different.

White female X Red female

F1 Red female White male

F2 White female Red female White male Red male

The different results from the reciprocal crosses could be explained only on the assumption that the gene for eye colour is located on 'X' chromosomes and that 'Y" chromosome has no gene for colour of the eyes. A white eyed female crossed with a red-eyed male produces red eyed females and whtie eyed males, this method of inheritance, is often referred to as 'criss-cross inheritance'. The F2 consisted of red eyed and white eyed individuals in equal numbers in both sexes.

Morgan concluded that the gene for eye colour is located on the X chromosome and that the Y chromosome carries no gene for eye colour.

Sutton and Boveri hypothesised that genes are borne on chromosome (i.e, the chromosome theory of heredity) but it was **Thomas Hunt Morgan (1910)** who first associated a particular gene (i.e., the gene for eye colour) with a particular chromosome (i.e., X chromosome) visible in microscopic preparations and showed that the gene for eye colour follows exactly the transmission of the X chromosome.

Criss cross inheritance:- A sex linked gene passes from male to female then back to male. The gene for eye colour is located on 'X' chromosome, it is called **'X' linked gene**. This pattern of inheritance is called '**Sex linkage'**.

There are genes located on 'Y' chromosomes and its allels absent in X chromosome. Such gene are called **'Y linked' or Holandric genes**. The gene responsible for hypertrichosis causing hairy prina (ear lobes) in human beings is a Y linked gene. There are certain homologons regions on X and Y chromosomes in which both the allels of a gene may be present as in the case of bobbed bristles (b) and its allele (b+) for normal bristle. Such genes are present both in X and

Y chromosomes are called **XY linked genes.** Eg. Genes for colour blindness, Xerodermia pigmentosum (pigmentation on the skin), Retinitis pigmentosa (pigmentation on the eye retina), Nephritis etc., in human beings are XY-linked.

Sex-influenced characters: are characters which may be expressed differently in the two sexes even when their genotypes are identical. The mere influence of the sex of the individual may be sufficient to alter the phenotypic expression of a gene. The most common expression of sex influence is that dominance is reversed between the sexes. Genes determining sex influenced characters are borne on autosomes.

A typical example of a sex-influenced character is the presence of horns in sheep. Both sexes of Dorset sheep are always horned while both sexes of Suffolk sheep are always hornless. If Dorset and Suffolk are crossed, the F_1 females are hornless while the F_1 males are horned. One interbreeding the F_1 sheep, an F_2 is obtained in which the females show a ratio of 3 hornless : 1 horned and the males show a ratio of 3 horned : 1 hornless. Presence of horns may therefore be said to be recessive character in females but a dominant character in males.

P	Horned female **HH**	X	Hornless male **Hh**
F_1		**Hh** Females, hornless Males, horned	

F_2	**1 HH**	**: 2Hh**	**: 1hh**
Female	Horned	Hornless	Hornless
Male	Horned	Horned	Hornless

Reciprocal crosses show no differences because the gene is carried by the autosome. Baldness in human beings is a sex-influenced character which is recessive in females and dominant in males.

Sex-limited character

Sex-limited inheritance is an extreme type of sex influence in which a particular phenotype can be expressed only in one sex. As genes for sex-limited characters are borne on autosomes, all genotypes should occur with identical frequencies in both sexes but the physiological differences between the sexes are such that certain genotypes can be expressed only in one sex. Unlike sex influenced characters in which gene is dominant in one sex and recessive in the other, sex-limited characters are controlled by genes which have no visible influence at all in one sex-either as a homozygote or as a heterozygote.

In domestic poultry, cock-feathering is a character limited to the male sex. Hen-feathering is due to a dominant gene **H** and cock-feathering is due to its recessive allele **h**, but females with genotype **hh** are hen-feathered. The genotypes and their corresponding phenotypes are as follows:

Genotype	Phenotype	
	Female	Male
HH	Hen-feathered	Hen-feathered
Hh	Hen-feathered	Hen-feathered
Hh	Hen-feathered	Cock-feathered

Removal of the ovaries in hens with genotype **hh** results in cock-feathering. This indicates that the female sex hormone inhibits the production of cock-feathering in hens with genotype **hh**.

Sex reversal

In several species of plants that ar normally bisexual, suppression of the male or female structuers has been observed in nature. The androecium getting converted into petals in ornamental plants or carpels as in carrot and cabbage or pistils as in maize, papaya and primrose has been observed. When the stamens get converted into rudimentary organs without the pollen sac and pollen, they are called staminodes and a similar convertion of the pistil into ononfunctional rudimentary organ is called the pistillode. The phenomenon in which there is

suppression of one sex at the expense of the other is called sex reversal. The sex reversals are mostly due to physiological and biochemical alterations involving sex hormones.

In maize, rarely it is observed that the male inflorescence called tassel bears seeds due to sex reversal. The recessive gene **`ba'** is responsible for barren plants and another recessive gene **`ts'** is responsible for tassel seed. Sex reversal in maize is due to the genetic constitution of the plants.

Lecture 26. Sex determination in plants – Melandrium, papaya, maize.

Lecture 27. Cytoplasmic inheritance and maternal effects – features of cytoplasmic inheritance, chloroplast, mitochondrial - plastid colour in *Mirabilis jalapa* - iojap gene of maize, cytoplasmic male sterility in rice, kappa particles of paramecium - plasmid and episomic inheritance.

Plastids and mitochondria, localized in the cytoplasm have been shown to be responsible for the extranuclear transmission of inherent qualities. Like the nuclear genes, they are capable of specific self-duplication. They are transmitted from generation to generation. They mutate and the mutant particles reproduce their own kind just as do mutated genes.

The totality of heredity transmitted through the cytoplasm is referred to as **plasmon**, and all cytoplasmic particles which manifest genic properties, viz., self-duplication, specificity and mutability are called **plasmagenes**.

Cytoplasmic inheritance: The transmission of hereditary characters by the cytoplasm.
Inheritance via genes found in cytoplasmic organelles.
A few traits are controlled by genes located in cell organelles in the cytoplasm

i.e. **cytoplasmic genes**,

- These genes are exceptions to the chromosome theory of inheritance
- The inheritance of **genes from the female parent** that are not in the nucleus but in organelles such as mitochondria that are found in the cytoplasm.

The cytoplasmic inheritance is known as extrachromosomal inheritance

Extra-chromosomal inheritance - controlled by non- nuclear genomes

- The inheritance of genes from the female parent that are not in the nucleus but in organelles such as mitochondria that are found in the cytoplasm.
- This type of inheritance is not controlled by Mendel's laws.
- Because the cytoplasm is usually contributed entirely by one parent,the characteristics encoded by these genes are usually inherited from only that parent.

Extrachromosomal inheritance

Cytoplasmic (maternal) Inheritance **maternal effect**

plastids **mitochondria** **symbionts like bacteria virus**

ORGANELLE DNA

Genes located outside of the nucleus (i.e., not on the major chromosomes)

- Mitochondrial genomes (mt DNA)
- Chloroplast genomes (cp DNA)
- extranuclear plasmids and other factors

• mtDNA and cpDNA are almost always **uniparentially inherited**, with only one sex (typically the female) transmitting the genomes to their offspring.

• The cytoplasm contributed by the female contain several components

Mitochondrial inheritance in petite mutants of Yeast

- Colonies smaller in size than the normal colonies are occasionally observed in the yeast (Saccharomyces cerevisiae) when grown on solid medium. These small colonies are called petite mutants.
- Petites have slow growth as they grow by gulcose fermentation anaerobically and hence inefficient in metabolism as compared to the normal colonies with aerobic metabolism.
- Among the petite mutants, there are segregational petites that exhibit Mendelian segregation when crossed with wild type and the petite is recessive to wild type.
- Another class of petite mutant is called neutral petite, which on crossing with wild type produces only wild type colonies and is thus uniparental in inheritance. The reason for such a differential behaviour seems to be that majority of neutral petites lack most or all mitochondrial DNA responsible for oxidative respiration.
- The petite phenotype is the result of large deletion in mitochondrial DNA. Thus the mitochondria is found to be responsible for inheritance in respiration defective mutants in yeast.

Inheritance of Kappa particles in Paramecium

- The presence of symbionts such as bacteria and viruses living in the cytoplasm was found to be responsible for a variety of cytoplasmically transmitted characters in eukaryotes.
- In Paramecium aurelia, the presence of bacterial inclusions known as kappa particles produce killer phenotype and its maintenance is dependent on a dominant nuclear gene **K**. the killer strain releases a substance lethal to many other strains of the protozoan.
- When conjugation occurs between the killer (**KK**) strain and the sensitive (**kk**) strain and if no cytoplasmic exchange takes place between them, the resulting progeny have killer and sensitive types in equal proportion eventhough the genetic constitution of both types is **Kk**. In the further progeny resulting form autogamy, the killer produces killer and sensitive types while the sensitive produces only sensitive types.

- On the other hand, when cytoplasmic exchange occurs between the killer (**KK**) and sensitive (**kk**) strains during conjugation, all the resulting progeny are killer (**Kk**) and no autogamy, each killer produces killer and sensitive types in equal number.
- They are diagrammatically presented below:
- When cytoplasmic exchange takes place between the killer and sensitive strains, the kappa particles in the cytoplasm of the killer strain mixes with the cytoplasm of the sensitive strain and in the presence of the dominant allele **K** in the heterozygote **Kk**, the kappa particles cause the killer phenotype. A
- fter autogamy, half of the progeny have cytoplasm with kapppa particles along with the dominant gene **K** and hence turn to be killers while the other half without both kappa particles and dominant gene turn to be sensitive. Thus an interaction between nuclear gene and cytoplasmic inclusion has been found to be responsible for inheritance of killer trait in Paramecium.

Maternal effect - Shell coiling in Snail

- There are instances in which the phenotype of the offspring is neither determined by the genotype of both its parents nor by the cytoploasmic or extra nuclear factors.
- In the snail Limnaea peregra, the direction of coiling of the shell is either dextral (right ward coiling) or sinistral (leftward coiling). When a dextral female is crossed to a sinistral male, the F_1 is dextral.
- On the other hand, when a sinistral female is crossed to a dextral male, the F_1 is sinistral. All the F_2 offspring produced by self fertilization (as the snail is hermaphroditic) are dextral in both the direct and reciprocal crosses. In this case, though, the F_1 behaviour resembles maternal inheritance, the behaviour F_2 does not conform to it. When the F_3 offspring obtained by self fetiliszation of the F_2 were examined, they segregate in the proportion of 3 dextral to one sinistral.
- Sinistrality is governed by the recessive allele **s** and dextrality by its dominant type allele +. Thus the parents in the direct cross are dextral female (+/+) and sinistral male (**s/s**) and

vice versa in the reciprocal cross. Yet the heterozygous F_1 (**s/+**) is dextral in the direct cross, but sinistral in the reciprocal cross. This is due to the maternal effect, which means that the genotype of the mother determines the phenotype of the offspring.

- Similarly the F_2 offspring possess the genotypes **+/+, +/s, +/s** and **s/s**. The F_3 offspring of **s/s** F_2 are sinistral while all other F_3 offspring are dextral. It has been proved that the orientation viz., dextrality or sinistrality is predetermined in the egg cytoplasm as a result of the mother's genotype, the mitotic spindles during the second cleavage division in the zygote determining the orientation. This is a case of maternal effect distinctly different from maternal inheritance.

Inheritance of plastids in Mirabilis

In a wide variety of plants, there occurs a type of leaf variegation in which the normal green leaves have pale green or white patches. These patches may be small or may extend throughout the leaves.

- The inheritance of plastids in the Four O' clock plant, ***Mirabilis jalappa*** was first described by **Correns (1908**). In *Mirabilis jalappa*, some of the branches may have normal green leaves, while in the same plant, some other branches may have only pale green or white leaves and still others may have variegated leaves.
- Flowers on branches with normal green leaves produce seeds that grow into plants with normal green leaves irrespective of whether they are pollinated by pollen from branches with normal green, variegated or pale green leaves.
- Flowers on pale green branches produce seeds that grow into plants with only pale green leaves regardless of the pollen parent.
- Flowers on variegated branches yield offspring of three kinds, viz., green, variegated and pale green in variable proportions irrespective of whether they are pollinated by pollen from branches with normal green, variegated or pale green leaves.

Progeny of a variegated four O' clock plant

Type of branch from which flowers are chosen for pollination	Type of branch from which pollen was obtained	Type of leaf in the progeny grown from seed

Green	Green Variegated Pale green	Only green Only green Only green
Variegated	Green Variegated Pale green	Green, variegated or pole Green, variegated or pole Green, variegated or pale
Pale Green	Green Variegated Pale green	Only pale green Only pale green Only pale green

It is clear that variegation is determined by agencies transmitted through the female and that it is not influenced by the type of pollen used. These agencies are the chloroplasts. They are easily visible under the microscope as particles localized in the cytoplasm. They are capable of self-duplication and are transmitted from generation to generation through the cytoplasm of the egg. Seeds borne on a green branch have therefore only green plastids, seeds borne on a pale green branch have therefore only pale green plastids and seeds borne on a variegated branch have green or pale green or a mixture of the two types of plastids.

Variegation is thus a hereditary character determined by stable, self-duplicating, extranuclear particles called plastids. Neither the nucleus of the female gamete not the male gamete is involved in the control of this type of hereditary character.

Maternal inheritance by 'iojap' gene in maize

Rhoades (1946) identified the iojap gene (**Ij**) in maize located in chromosome VII controlling plastid inheritance in the plant. The gene **Ij** is responsible for the normal green colour of the plant. When normal green plants with **Ij Ij** are used as female and pollinated by pollen from striped plants with **ij ij**, the F_1 plants with **Ij ij** are wholly green.

Green **Ij Ij**	X	Striped **Ij ij**

	F1 : Green **Ij ij**	

When striped plants with **ij ij** are pollinated by pollen from the normal green plants with **Ij Ij**, the F_1 plants, all of which have the same genotype, **Ij ij** are of three different phenotypes, viz., normal green, striped and white.

Striped **Ij ij**	**X**	Green **Ij Ij**
	F1 : Green, striped or white **Ij ij**	

When plants with the same genotype **Ij ij** have different phenotypes, viz., normal green, striped, or white, the differences can be attributed only to differences in plastids.

Cytoplasmic male sterility in maize

In several cases of cytoplasmic inheritance in plants, plastids have been shown to be the vehicles of heredity but in several other cases, cytoplasmic particles other than plastids have been identified as the basis for extranuclear transmission. Among these is a case of male sterility in maize. Most or all of the pollen grains of such male sterile plants are aborted. This character is transmitted only through the female and never by the pollen. When all of the chromosomes of the male sterile line were replaced with chromosomes of normal plants, the line still remained male sterile, showing thereby that male sterility is controlled by some genes in the cytoplasm. It was later recognized that cytoplasmic male sterility in maize results from alterations in the hereditary units in the mitochondria (mitochondrial DNA).

Lecture 28, 29, 30, 31,32 CHEMICAL

BASIS OF HEREDITY

Nature of the genetic material

A swiss biologist, Miescher (1869) identified a chemical compound in pus cells and salmon sperm in the large nuclei of these cells. The chemical was named **`Nuclein'.** As it was found to be acidic, it was called `Nucleic acid'. All plant and animal materials were found to contain nucleic acid. Nucleic acid was found associated with various proteins and along with the protein, it was called `Nucleoprotein'.

There are two kinds of **proteins** associated with nucleic acid and they are **prolamine and histone**. Prolamine consists mostly of linked groups of amino acids arginine. Histones are relatively complex in nature. Because of this complexity, proteins were originally thought as the genetic material. Proteins have long chemical chains consisting of many amino acids and they were considered to be capable of carrying many complex messages that cause variation in the biological material.

Considering the proportion of different constituents of cell, nucleic acid was found to be constant in volume in all the cells as compared to other cellular contents and hence it was inferred to be the hereditary material. There are **two types of nucleic acid, the deoxyribonucleic acid (DNA) and the ribonucleic acid (RNA)**. By staining nucleic acid, Feulgen (1924) found that the DNA was localized in the nucleus, while the RNA was found to occur outside the nucleus in the cytoplasm.

The **experiments of Griffith (1928)** with the pneumonia bacterium and the **interpretation of results by Avery, MacLeod and McCarty (1944) confirmed the DNA as the hereditary material.**

Bacterial Transformation

Giffith (1928) worked on the pneumonia causing spherical shaped bacterium, Diplococcus pneumoniae. Some of the strains of this bacterium have a smooth polysaccharide capsule which causes the disease and hence called virulent **S** strain. A mutant strain has no capsule and is avirulent or nonpathogenic and is called **R** strain. In agar medium, the virulent (**S**) strain produces smooth surfaced colonies, while the avirulent ® strain produces rough surfaced colonies. There are several types of these two strains, **S I, S II, S III, R I, R II, R III** etc. that

differ in the type of antigen they produce. The kind of antigen produced in genetically determined. The **S** type sometimes mutates to **R** type but not in the reverse.

Griffith injected the laboratory mice live **R II** bacteria and the mice did not get pneumonia as **R II** is a virulent. When injected with virulent **S III**, the mice suffered of pneumonia and died. When **S III** bacterial were heat killed at $65^{o}C$ and then injected into the mice, they did not suffer of the disease and lived. Later, the heat killed **S III** strain and live avirulent **R II** strain were mixed and injected into the mice. Contrary to expectations, the mice suffered of pneumonia and died. On analysing the blood sample of the affected mice, live **S III** and live **R II** bacteria were found in it. This could not be possible due to the mutation of the avirulent **R II** to virulent types. Evidently, some heat-stable component present in the heat killed, and hence, dead **S III** strain could have conferred the virulent nature to the live **R II** strain. Griffith designated this as the `transforming principle' that transformed the hereditary property of avirulent **R II** to virulent **S III**. This phenomenon is called **`Griffith effect'** or **`Bacterial transformation'**.

Griffith did not understand the cause of bacterial transformation. Avery, MacLeod and McCarty (1944) tested a fraction of the heat killed **S III** bacterial for the transforming ability. They removed proteins, lipids, polysaccharides and ribonucleic acid from **S III** extract by a variety of chemical and enzymatic methods without diminishing its ability to transfer **R II** into **S III** strain. They found that a cell-free and highly purified DNA extract of **S III** bacterial could bring about transformation of **R II** into **S III** and concluded that DNA is the transforming principle and hence the genetic material in bacteria.

Later studies on other bacteria such as Haemophilus influenzae, Bacillus subtilis, Escherichia coli, Shigella paradysenteriae and others revealed that they also undergo transformation.

Transformation is the process of adding a foreign DNA fragment from a donor genome into the genome of a recipient cell. The donor fragment passes through the cell membrane of the recipient cell of the same or different species and becomes incorporated into the genome of the recipient cell through recombination.

DNA as the genetic material in Viruses

Hershey and Chase (1952) provided direct proof that DNA is the genetic material in certain bacterial viruses.

Bacteriophage is a virus that infects or feeds on certain specific bacteria. T_2 bacteriophage that infects the colon bacteria, Escherichia coli was involved in the studies.

Bacteriophage is eletron microscopic. It has a head and a tail. Inside the head, there is a long chain of DNA molecule. The phage attaches itself by its tail to the bacterial and injects the DNA into the bacillus. It dictates the cell to produce many copies of the viral DNA.

Bacteriophages are used in many finer analyses of the genetic material since they are haploid organisms and there is no hiding of mutant effect. As there is no differential sex, there is no need for two different individuals to unite for reproduction. They multiply enormously and have a short life span. Recombination's and mutations, even if in a very low frequency, could be recognised with relative ease. When a population is raised from a single phage all the descendents will be identical. But occasionally, through errors in copying of genetic material, rare mutants appear and such mutants are called `**copy error**'.

In a chemically defined cultural medium, known quantities of radioactive isotopes of phosphorus $\mathbf{P^{32}}$ and sulphur $\mathbf{S^{35}}$ were added. Escherichia coli were grown in the medium and the labelled E. coli cells were used as hosts for unlabelled T_2 bacteriophage. The virus progeny that multiplied inside the bacteria could be traced in the culture medium on lysis (cell wall breakage) of the bacteria.

The viral DNA was labelled with $\mathbf{P^{32}}$ and the viral capsid (protein coat) with $\mathbf{S^{35}}$, since DNA contained **P** and the capsid protein contained **S**. then the labelled viruses were allowed to infect unlabelled E. coli and get multiplied. Later the viruses were separated from the bacterial host cell by agitation and the content of $\mathbf{P^{32}}$ and $\mathbf{S^{35}}$ of the virus and bacteria was assessed. $\mathbf{P^{32}}$ could be traced in the infected bacterial cells. Hershey and Chase inferred that DNA of the virus entered the bacterium and played a role in viral multiplication, whereas the protein of the virus did not play any role in the intef cellular replication of the virus. Thus it was established that the genetic material of the virus was DNA.

RNA as the genetic material in some viruses

Ribonucleic acid was found to be the genetic material in Tobacco mosaic virus, Turnip yellow mosaic virus, Poliomyelitis, Foot and Mouth virus, Influenza virus, Reovirus, Rous sarcoma virus and some of the bacteriophages such as MS_2.

Properties of the genetic material

The biological information properties of the genetic material enable the capability to provide, from one generation to another, the information that gives each species its ability to reproduce its own kind accurately. These include structural gene properties and regulatory gene properties.

The genetic material must be capable of specifying the structure of other molecules. The genetic material should be capable of accurately coding for a large number of different polypeptide amino acid sequences during protein synthesis. The genetic material should regulate the programmed of embryological development and organ differentiation for a given species, which differ from one species to another. Thus the genetic material should be able to regulate gene action and function.

The genetic material has the replicative properties. During mitosis, each daughter cell receives a copy of all the genetic information from the parent cell. During meiosis, each gamete receives a copy of half the genetic material.

Mutational properties of the genetic material provide a fundamental source of variation essential for evolution of species.

Chemical composition of DNA

DNA is a complex macromolecular or polymeric chemical compound which contains four kinds of monomers (small building blocks) called Deoxyribonucleotides. Each deoxyribonucleotide is made up of 1) a phosphoric acid molecule, biologically called phosphate, discovered by Levene (1910), 2) a pentose sugar called 2 -deoxyribose, also discovered by Levene (1910) and 3) four major kinds of nitrogen bases, two heterocyclic and two ringed purines, adenine (A) and guanine (G) and two one ringed pyrimidines, cytosine (C) and thymine (T), discovered by Fischer (1880).

A nucleotide results from covalent bonding of a phosphate and a nitrogen base to the pentose. That part of each nucletide which contains a nitrogen base and deoxyribose is called Deoxyribonucleoside.

The four kinds of deoxyribonucleosides and deoxyribonucleo tides are as follows:

Nucleosides and nucleotides of DNA

Nitrogen base	N base + Deoxyribose = Deoxyribo nucleoside	Deoxyribo nucleoside + Phosphoric acid = Deoxyribo nucleotide	Nucleotide
Adenine	Deoxyadenosine	Deoxyadenylic acid	Deoxy-adenosine mono-phosphate (dAMP)
Guanine	Deoxyguanosine	Deoxy-guanylic acid	Deoxy-guanosine nono-phosphate (dGMP)
Thymine	Deoxythymidine	Deoxythymidylic acid	Deoxythymidinemon o phosphate (dTMP)
Cytosine	Deoxycytidine	Deoxycytidylic acid	Deoxycytidine mono-phosphate (dCMP)

Two organic chemists, Levene of the Rockefeller Institute and Todd of Cambridge (1910) demonstrated that the components of DNA were joined together to form a long chain of alternating deoxyribose and phosphoric acid units with side chains of the nitrogen bases.

Double helical model of DNA

Based on the findings of **Chargaff (1950)** that the total amount of purines equalled the total amount of pyrimidines (A + G = T + C), that the amount of adenine equalled the amount of thymine (A = T) and the amount of guanine equalled the amount of cytosine (G = C) and, that the ratio between total purines and total pyrimidines was always not far from one, (A + G) : (T + C) = 1, as well as the crystallographic evidences and X-ray differentiation photographs (Astbury,

1947, Wilkins and Franklin, 1953), the **double helical model of DNA was constructed by Watson**, an American biologist **and Crick**, a British physicist in 1953.

The DNA molecule was conceived as a two stranded structure coiled like a rope, and hence called plectonemic, so that if the ends are permitted to revolve freely, the complementary strands could easily separate. The coil was proposed to be helical and conceived to resemble a circular staircase, maintaining the same diameter throughout and having a constant width between steps. The steps are connected on either side by a railing.

The helix has a diameter of 20 A^o and makes a complete turn at every 34 A^o along its length. The distance between nucleotides is 3.4 A^o. Each complete turn has a stack of 10 nucleotides. The helix contains two polynucleotide chains or two stacks of 10 nucleotides each per turn.

Each complementary strand is only half the circular staircase, either side consisting of approximately half the width of the step. Each half step is connected by a railing or backbone. The railing consists of phosphate - sugar linkages which are repeated without change. The half step of one strand extends to meet the half step of the complementary strand. Each half step has either a purine or pyrimidine base. Each step consisting of two half steps is together called **base pair**.

The fit between the bases is determined by hydrogen bonding. The bonding involves the ability of the H atom with positive charge (H^+) to be placed between an O atom with weak negative charge (O^-) and a N atom with a light negative charges (N^-) from opposite strands. Adenine pairs with thymine with two H bonds (A = T) and guanine with cytosine with three H bonds (G = C). These N bases are connected to each other by deoxyribose and phosphoric acid.

Hydrogen bonds are generally weaker than other chemical bonds. But there are several of them, two between A and T (A = T) and three between G and C (G = C) that give rigidity and stability to the molecules.

The position of purines, pyrimidines, deoxyribose and phosphoric acid in DNA is presented in Fig. 17-4.

Chemical composition of RNA

In RNA, there are four nitrogen bases, the purines consisting of adenine and guanine and the pyrimidines consisting of cytosine and uracil. Thymine in DNA is replaced by uracil in RNA.

For uracil, the ribonucleoside is uridine and ribonucleotide is uridylic acid or uridine mono phosphate (UMP). The ribonucleosides for adenine, guanine and cytosine are adenosine, guanosine and cytidine respectively and the ribonucleotides are adenylic acid (or adenosine monophosphate - AMP), guanylic acid (or guanosine monophosphate - GMP) and cytidylic acid (or cytidine mono phosphate -CMP) respectively.

The structure of ribose sugar (as different from deoxyribose in DNA) and uracil (U) are as follows:

The methyl group at position 5 in the thymine is replaced by a H atom in uracil.

RNA is made up of many ribonucleotides. The ribose sugar and phosphoric acid remain linked by phosphodiester bonds.

Organisms which have only RNA employ their RNA in their genetic mechanism. Such RNA is called genetic RNA. Organisms having DNA along with RNA, use the RNA for carrying the orders of DNA and such RNA is called nongenetic RNA. Ribosomal RNA (rRNA), transfer RNA (tRNA), heterogeneous RNA (htRNA) and messenger RNA (mRNA) are nongenetic RNAs. rRNA and mRNA are single stranded, while tRNA and htRNA are doubled stranded. The genetic RNA present in most of the viruses is single stranded while that in reterovirus is double stranded.

24. REPLICATION OF DNA AND RNA

Replication of DNA

DNA has two important functions as the carrier of genetic information.

1) When DNA directs the synthesis of DNA itself, or in other words, when DNA replicates, such a function is autocatalytic.
2) When DNA directs the synthesis of chemical molecules other than itself, such as synthesis of RNA, protein etc., such a function is heterocatalytic.

The double helical model of DNA proposed by Watson and Crick provides a template system for self-replication. Because of the specificity of base pairing, A with T and G with C, the sequences of bases along one chain automatically determines the base sequence along the

other. Hence each chain of the double helix can serve as a template for the synthesis of the other.

Replication involves disruption of H bonds, followed by a rotation and separation of the two-polynucleotide strands. Each purine and pyrimidine base of each polynucleotide strand attracts a complementary free nucleotide available for polymerisation in the cell and holds it in place on the paternal template chain. The free nucleotides are sewn together by formation of phosphate di ester bonds for linking adjacent deoxyribose, thus forming a new polynucleotide molecule. Thus two double helical molecules, identical to each other are formed.

Three theories were proposed for DNA replication. They are semiconserative theory, conservative theory and dispersive theory.

Semiconservative theory

According to this theory, both the complementary strands in a DNA molecule separate and each strand functions as a template or mould to prepare its replica.

Conservative theory

The strands are not supposed to separate from each other, but a new double helix appears within the old double helical strands.

Dispersive theory

Each of the strands of the double helix breaks into pieces and these pieces duplicate. The broken and duplicated pieces are reconstructed into two double helices consisting of strands containing both old and new pieces.

Proof for semiconservative DNA replication - Meselon and Stahl's (1958) experiments with Escherichia coli by labelling the DNA with heavy isotopes of N viz., N^{14} and N^{15} and distinguishing the density difference in a cesium chloride gradient by using sedimentation equilibrium centrifugation and observing the band on ultra violet tube proved that DNA replication occurs by the Semiconservative methods.

Cairns (1963), by autoradiography, studied the DNA replication of E. coli, which has a single large duplex circular chromosome, and suggested that the chromosome replicated as an intact ring and the model of replication was semiconservative.

A rolling circle model of DNA replication is suggested for bacteria and viruses.

Taylor, Woods and Hughes (1957) demonstrated that eukaryotic DNA replicates semi conservatively in studies conducted with *Vicia faba.*

Some of the nucleic acid enzymes play an important role in DNA replication. They are: 1) Nucleases that catalyse the breakdown of particular bonds leading to fragmentation of nucleic acid. They may be exonucleases that attack nucleic acid at its terminal nucleotide only or endonucleases that react only with those bonds that occur within the interior of the nucleotide chain to cut in into pieces, 2) Ligases that join broken ends of two DNA chains by catalysing the synthesis of a phosphodiester bond between 3'- hydroxyl group and 5' - phosphate group and restore an intact DNA duplex, 3) Restriction enzymes that produce breaks only within sequences which have two identical bases in adjacent position, 4) Swivelases that allow unwinding of one of the two strands of DNA to allow free rotation within DNA molecule and 5) Polymerases that are involved in the synthesis of nucleic acids by addition of bases to a growing nucleotide chain.

The speed of DNA replication was studied in vivo and in vitro by Kornberg et al. (1967) using ø X 174 phage. The speed was 500 to 1000 nucleotides per minute in vitro and as high as 100,000 nucleotides per minute in vivo.

Replication of RNA

The genetic RNA of viruses is self-replicating. Its model of replication is called `RNA dependent RNA synthesis'. RNA polymerase enzyme mediates in the replication mechanism keeping the parent RNA as the template and synthesizing a complementary RNA chain.

25 & 26. PROTEIN SYNTHESIS AND GENETIC CODE CONCEPT

One gene - one enzyme hypothesis

When we consider the phenotype of an organism, we should understand that it is the result or end product of many complicated actions and interactions within and between genes. Phenogenesis is the mechanism by which the phenotype of an organism is produced under the control of DNA in a given environment, which includes not only external factors such as temperature, intensity and quality of light but also internal factors such as enzymes and hormones. The phenotype is the result of various embryological and biochemical activities involving enzymatic proteins. Enzymes catalyse in separating or uniting different cellular

molecules. A precursor is transformed into an end product through the production of many intermediate products, each aided by an enzyme produced by a gene and this constitutes the biosynthetic pathway.

DNA itself does not have enzymatic character and does not directly involves in the biosynthesis pathway. The immediate gene product is a kind of RNA called messenger RNA (mRNA) which controls the amino acids to form enzymes at the surface of the cytoplasmic ribosomes. Thus, DNA transcribes mRNA which translates protein that ultimately produces a phenotypic trait.

Beadle and Tatum (1941), the Nobel Laureates of 1958, working on the bread mould, Neurospora, analysed the biochemical effects of genes by studying wild type prototrophs (strains that grow one minimal medium) and auxotrophs (nutritional mutants that grow on supplmented media). They found that ultra violet mutants produced defect in enzyme or loss of specific enzyme. This concept is known as "one gene - one enzyme hypothesis".

Protein and Enzymes

Enzymes are proteins that composed of subunits called polypeptides, which can be further broken into amino acids. The amino acids are united by peptide linkage. Though there are 35 different amino acids in biological systems, most of the biological proteins contain only 20 amino acids.

Amino acids consist of four sub units viz., 1) a carboxyl group with a potential negative charge, 2) an amino group with a potential positive charge, 3) a hydrogen (H) subunit and 4) an R subunit which differentiates the amino acids.

Proteins have several structural levels

1. primary structure consists of the linear sequence of amino acids in a polypeptide chain.
2. Secondary structure consists of section of primary poly peptide chain twisted or coiled into a helix.
3. Tertiary structure consists of very long spiral chains compressed in a globular form with extensive folding over and bending of helices.
4. Quarternary structure consists of two or more independent polypeptide chains linked together by interchain bonds to produce complex structure.

One gene - one polypeptide concept

Investigations by **Ingram (1957)** brought to light that each gene controls the production of a single polypeptide chain of protein molecule. The studies were on hamoglobin pigment of the red blood corpuscles that consist of four polypeptide chains viz., α, β, δ, γ varying in number and arrangement of amino acids and four iron containing ring compounds (heme). Hemoglobin of adult and foetus and abnormal haemoglobins **Hb**^{3} and Hb^3 were studied electrophoretically to understand the control of polypeptide by gene.

Crick (1958) proposed that the sequence of nucleotides in DNA and of amino acids in proteins is colinear. This means that there is a direct correspondence between the base pairs sequence in DNA and the amino acid sequence in the corresponding protein.

Nongenetic RNAs involved in protein synthesis

RNA is in intermediary between DNA base pair sequence and amino acid sequence. Several kinds of RNA have been identified.

RNAs differ from one another in molecular weight, structure and role in protein synthesis. The macromolecules are measured in Svedberg (S) units, determined by the rate of sedimentation or the molecule in a density gradient under a standard centrifugal force. The rate of sedimentation depends on the size and shape of the molecule.

1. Messenger RNA (mRNA)

Messenger RNA is made up of single stranded molecule consisting of nucleotides ranging from 300 to 12000. In Escheichia coli, mRNA has 900 to 1500 nucleotides. It has a molecular weight of $4 * 10^6$. It is constituted of the bases adenine, guanine, uracil or cytosine.

In eukaryotes, RNA molecules of variable length with sedimentation coefficient of 20 S to 100 S are involved. This is called heterogeneous nuclear RNA (hnRNA). It is synthesized in the nucleus and is present in the nucleoplasm outside nucleus. The hnRNA has two fractions, one with coding sequences that produce mature messenger RNA (mRNA) and another with non coding sequences that are degraded within the nucleus. The coding sequences of hnRNA constitute **exon** and the non coding sequences constitute **intron**. Polyadenylic acid (poly A) consisting of about 200 nucleotides is added to hnRNA by the action of polysynthetase enzyme and after polydenylation, selective degradation takes place by the action of nucleases and the final product poly A + mRNA reaches cytoplasm to get attached to ribosomes.

Functionally mRNA molecules code the sequence of amino acids in protein synthesis. Eventhough mRNA is single stranded, base pairing occurs between its segments by forming hair pin loops to produce secondary structures.

Jacob and Monad (1961) first proposed the existence of mRNA.

The life span of mRNA is 2 minutes at 37^{o} C in E. coli, 1 to 4 hours extending to 2 days in eukaryotes, 6 hours Bacillus cerus and several months and years in the dormant seeds of plants and animal eggs.

The process by which the information in the nucleotids sequence of DNA is transferred to a complementary sequence of mRNA is called transcription.

2. Transfer RNA (tRNA)

Transfer RNA molecules are smaller than mRNA molecules and contain 70 to 80 nucleotides. TRNA has a molecular weight of $2.5 * 10^{4}$ and a sedimentation coefficient of 4 S. tRNA molecules have a completely folded tertiary structure due to hydrogen bonds between the constituent bases and due to the presence of a number of unusual bases such as A - U - G - C situated in the curves of the molecules where no base pairing occurs.

Holley (1968) suggested a clover leaf model of alanine tRNA in yeast with the following structural peculiarities.

1. An amino acid attachment site with a terminal sequence of CCA at 3' - OH end of the polynucleotide chain. Adenylic acid (A) is the last residue at 3' end. Its complementary strand aligned by folding has 5' - P end.
2. T ψ C arm consisting of a loop with seven unpaired bases including pseudouridine and is involved in the binding of tRNA molecule to the ribosome.
3. DHU arm consisting of dihydro uridine loop having 8 to 12 unpaired bases and functioning as the site for recognition of amino acid activating synthetase enzyme.
4. Codon recognition site or anti-codon arm consisting of one nucleotide triplet which is complementary to the corresponding triplet codon of mRNA.
5. A short extra arm when the chain is long.

A three -dimentional L-shaped configuration was proposed by Kim et al. (1972) for phenyl alanine tRNA molecule of yeast cells.

3. Ribosomal RNA (rRNA)

Ribosomes are ribonucleoprotein molecules present in all types of cells. They occur as 60 S units in mitochondria, 70 S units in bacteria and chloroplast and 80 S units in eukaryotes. Their constitution is as follows.

Prokaryotic ribosome	70 S	50 S Sub unit 30 S Sub unit	5 S rRNA, 23 S rRNA + 34 proteins 16 S rRNA + 21 proteins
Eukaryotic ribosome	80 S	60 S Sub unit 40 S Sub unit	5 S rRNA, 7 S rRNA 28 S eRNA + 50 proteins 12 S rRNA + 30 proteins

RRNA is the insoluble RNA that constitutes tha largest part, even up to 80 per cent of the total cellular RNA. It has four major RNA bases A - G - U - C with a slight degree of methylation.

RRNA molecules are single polynucleotide stranded, unbranched, and flexible and behave as a random coil or show helical regions with base pairing between A - U and G - C. rRNA has a definite role in protein synthesis.

Protein Synthesis

Central dogma of molecular biology

The process of protein synthesis involves one of the central dogma of molecular biology, **postulated by Crick (1958) according to which genetic information flows from nucleic acid to protein.**

Protein synthesis involves two steps viz., transcription and translation. Transcription involves a sequential flow of information from DNA to mRNA. This doesnot involve a change of code since DNA and mRNA are complementary. Translation involves a change of code from nucleotide sequences to amino acid sequences.

Generally the flow of information is one way, from DNA to RNA and from RNA to protein.

Transcription		**Translation**
DNA	**mRNA →**	**Protein**

In certain viruses, the existence of an enzymes `RNA dependent DNA polymerase' (also called inverse transcriptase) was reported and this enzyme could synthesize DNA from a single stranded RNA template. This **finding of Baltimore** (1970) and others gave rise to the concept of **`central dogma reverse'**. According to this, the sequence of information flow is not necessarily from DNA to RNA to protein, but can also take place from RNA to DNA.

Transcription		**Translation**	
DNA → **←** **inverse transcription**	**RNA**	**→**	**protein**

Transcription

The process by which the information in the nucleotide sequence of DNA is transferred to a complementary sequence of RNA is known as transcription.

Transcription occurs throughout interphase and continues up to early prophase of cell division. `DNA dependent RNA polymerase' or `transcriptase' is the enzyme involved in transcription. The locations of transcription are 1) the nucleolus where genes from rRNA are transcribed and 2) the remaining chromatin where hnRNA (mRNA) is transcribed.

The system for in vitro RNA synthesis contains 1) ribonucleotide triphosphates (**ATP, CTP, GTP and UTP**), 2) enzyme RNA polymerase, 3) **Mg^{++} or Mn^{++}** and 4) template DNA. The enzyme links the ribonucleotides together by catalysing the formation of 3' - 5' phospho diester bonds that pin the nucleotides. Consequently, RNA is synthesized and pyrophosphate is released. The enzyme RNA polymerase acts only in the presence of DNA, against which the

correct sequence of ribonucleotides is arranged and they are linked together by the enzyme. That is why the enzyme is known as `DNA - dependent RNA polymerase'.

N : r A - P ~ P ~ P (ATP) N : r U - P ~ P ~ P (UTP) N : r G - P ~ P ~ P (GTP) N : r C - P ~ P ~ P (CTP)	RNA Polymerase + Template DNA $\rightarrow$ Mg^{++} or Mn^{++}
Template DNA +	N r A - P r U - P + 4n P - P r G - P r C - P

The site of transcription in cistron is called the promotor site. The template strand is called sense strand, while its complemetary strand is known as antisense strand. When only one strand of DNA is transcribed for a given region, it is called asymmetrical transcription. When both the strands of the DNA are transcribed, it is known as symmetrical transcription.

The details of the transcription process are the following: the enzyme RNA polymerase attaches itself at the promotor site. The DNA molecule unwinds over a short region. Then the free bases in the template strand of DNA determine the sequence of ribonucleotides in the newly formed mRNA.

The RNA polymerase enzyme joins the nucleotides together to produce RNA transcript. After the transcript becomes detached, the DNA template strand re-forms H-bonds with its complementary strand rewinds to form the double helix.

RNA polymerase enzyme has five subunits of polypeptide chains viz., α, β, β' σ and ω subunits form the core of the enzyme and catalyse the linkage of ribose nucleotides by phosphodiester bonds. The σ - factor recognises the start signal in the promotor region of DNA.

Translation

As soon as the mRNA is formed, it leaves the nucleus and reaches the cytoplasm where translation takes place.

Before the process of protein synthesis, the ribosomes occur in dissociated and inactive state. The mRNA binds with 30 S ribosomal subunit in the presence of a protein factor called Initiation Factor (IF). The mRNA carries triplet codons for the synthesis of proteins. Protein synthesis involves mRNA, ribosomes, amino acids and their specific tRNAs.

Translation process involves the following steps:

1. Attachment of mRNA with 30 S ribosomes and formation of polyribosomes

The mRNA transcribed inside the nucleus moves to the cytoplasm and binds itself with 30 S subunit of the ribosome in the presence of Initiation Factor. Then the tRNA, present in the cytoplasm binds itself with the first triplet codon 5' - AUG - 3' called the chain initiation codon of mRNA to form the `Initiation Complex'. Later, the 30 S subunit of ribosome unites with 50 S subunit to form 70 S ribosome, in the presence of Mg^{++} ions. The message in the mRNA is not deciphered by one ribosome but many ribosomes are involved in the process and hence they are called polyribosomes.

2. Activation of the amino acids

Amino acids present in the cytoplasm ared in a domant stage. Each amino acid is activated by an activating enzyme called aminoacyl synthetase, beside the energy rich adenosine triphosphate (ATP). The free amino acids react with ATP to produce aminoacyl adenylate and pyrophosphate (PP).

Amino acid + ATP + Amino acyl synthetase enzyme→	Amino acid - AMP - Enzyme(aminoacyl adenylate - enzyme complex) + PP

The aminoacyl adenylate enzyme complex bound together by a mono covalent bond attaches itself with the specific tRNA molecule. As the enzyme is specific for specific amino acid, the concerned amino acid gets attached without error.

3. Attachment of activated amino acid to tRNA

The aminoacyl adenylate remains bound with the enzyme till it is hooked to the tRNA molecule. The dihydrouridine (DHU) loop of tRNA recognises the synthetase enzyme. Then the amino acid residue of the aminoacyl adenylate is transferred to the amino acid attachment site of

tRNA, where its carboxyl group forms linkage with 3 - OH group of the ribose of the terminal adenosine at - CCA end of tRNA.

As a result, adenosine monophosphate (AMP) and the enzyme are released and aminoacyl tRNA is formed. Then the aminoacyl tRNA moves towards the ribosome.

AA - AMP - Enzyme + tRNA → AA - tRNA +AMP + Enzyme

4. Initiation of the polypeptide chain

In the mRNA, the first triplet codon is AUG at its 5' end. AUG codes for methionine. Hence protein synthesis commences with coding for methionine. The peptide chain formation starts in 5' end and proceeds towards 3' end and this helps in the correct sequence of protein synthesis.

The mRNA moves across the ribosome. A new codon of mRNA is brought in position. A new tRNA charged with specific amino acid is brought in position in such a way that the anticodon of tRNA pairs with the codon of mRNA. The attachment of two amino acids by polypeptide linkage involves enzymes translocase and peptidyl transferase along with energy rich GTP, and tRNA is released.

This process of movement of mRNA from 5' to 3' direction and addition of aminoacids to polypeptide chain continues till mRNA is no longer translated.

5. Termination of the polypeptide chain

Any one of the three terminating codons in mRNA, viz., UAA, UAG or UGA can signal the temination of chain elongation.

After chain termination, the enzyme peptidyl transferase hydrolyses the ester bond between the chain and tRNA releasing the polypeptide chain, the last tRNA and mRNA.

Thus a polypeptide chain with a specific series of amino acids is formed which results in synthesis of a specific protein that involves in a specific phenotypic expression in the organism.

Genetic Code

In the DNA and RNA, there are four types of nucleotides or bases viz., A, G, T, C and A, G, U, C respectively. If it is assumed that each base codes for one amino acid, then only four amino acids can be coded. If two bases together are responsible for production of one amino acid, then they will code for $4^2 = 16$ amino acids. If three bases together code for an amino acid, then

4^3 = 64 amino acids could be coded. As the essential amino acids in a biological system are 20 in number, the possibility of one or two bases coding for each amino acid is remote.

Crick and Brunner (1961) suggested that the **genetic code might be a triplet code, involving three nucleotide bases to code for an amino acid.**

Further investigations by Nirenberg and Mathhaei (1961), Nirenberg (1961), Khorana (1964) and others lead to the construction of a complete genetic code dictionary.

First base (5'end)	**Second base**				**Third base (3' end)**
	U	**C**	**A**	**G**	
U	Phe	Ser	Tyr	Cys	**U**
	Phe	Ser	Tyr	Cys	**C**
	Leu	Ser	NS	NS	**A**
	Leu	Ser	NS	Trp	**G**
C	Leu	Pro	His	Arg	**U**
	Leu	Pro	His	Arg	**C**
	Leu	Pro	Gln	Arg	**A**
	Leu	Pro	Gln	Arg	**G**
A	Ileu	Thr	Asn	Ser	**U**
	Ileu	Thr	Asn	Ser	**C**
	Ileu	Thr	Lys	Arg	**A**
	Met	Thr	Lys	Arg	**G**
G	Val	Ala	Asp	Gly	**U**
	Val	Ala	Asp	Gly	**C**
	Val	Ala	Glu	Gly	**A**
	Val	Ala	Glu	Gly	**G**

The pattern of genetic code indicates the following:

1. Codons for the aromatic amino acids begin with Uracil

UUU UUC	Phenyl alanine (Phe)
UAC UAC	Tyrosine (Tyr)
UGG	Tryptophan (Trp)

2. Codons for amino acids that form amides begin with Guanine and Adenine.

GAU GAC	Asparagin (Asp)
GAA GAG	Glutamin (Glu)

3. For many of the synonymous codons specifying the same amino acids, the first two bases of the triplet code are constant while the third varies, being less specific.

GCU GCC GCA GCA	A l a n i n e (Ala)	CUU CUC CUA CUG	Leucine (Leu)
GUU GUC GUA GUG	V a l v i n e (Val)	CCU CCC CCA CCG	Proline (Pro)
GGU GGC GGA GGG	G l y c i n e (Gly)	CGU CGC CGA CGG	Arginine (Arg)
ACU		UCU	

ACC ACA ACG	Threonine (Thr)	UCC UCA UCG	Serine (Ser)

According **to Crick (1966),** the third base tends to wobble or is unsteady and he proposed the **wobble hypothesis**.

The genetic dictionary of mRNA codons reveals the following features of the triplet code

General feature of genetic code

1. some of the nucleotides have to code for more than one amino acid and hence called **ambiguous code.** For example, UUU codes for phenyl alanine and in the presence of streptomycin, it may code for isoleucine, leucine or serine.
2. The code contains many synonyms and hence called **degenerate code**. Almost all the amino acids are represented by more than one codon. For example, arginine, serine and leucine have six synonymous codons.

   For many of the synonymous codons specifying the same amino acid, the first two bases of the triplet are constant whereas the third varies, as for alanine, valine, glycine, leucine, proline, arginine, threonine and serine. This flexibility in the third base of codon minimises the consequences of errors.
3. The code is read continuously without interruption and no codon is reserved for punctuation. Hence it is called a **comma a less code**.
4. There is no overlapping of base sequences specifying for different amino acids and no single base in a triplet can take part in the formation of more than one codon. Hence it is called a **non overlapping code**. For example, in a polynucleotide chain, UCAGAA, UCA codes for serine and GAA for glutamine, but over-lapping as UCA, CAG, AGA etc. does not occur to code for other amino acids.
5. As the same code applies for all living systems, it is called an **universal code.** However, a few codons in some organelle DNAs were found to have different meanings than those in nuclear DNAs. For example, AUA that normally codes for isoleucine in nuclear tRNA

codes for methionine in mitochondrial tRNA and UGA codes for tryptophan in mt tRNA instead of being a termination codon.

6. Among the triplet codons, AUG is the chain initiation codon as it initiates the synthesis of polypeptide chain. Codons UAA, UAG and UGA are the terminating codons as they terminate translation of the polypeptide chain. As these three codons do not specify any amino acid, they are called **nonsense codons**.

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