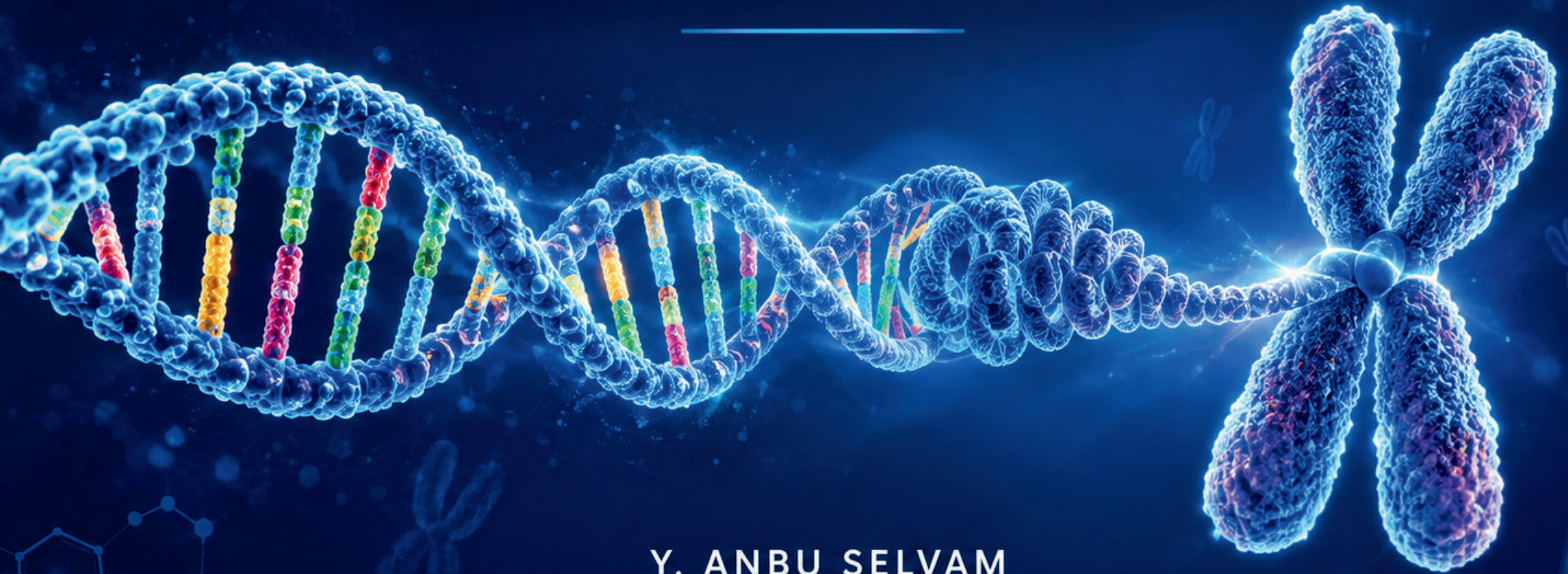


A UNIVERSITY TEXTBOOK

ELEMENTS OF GENETICS

A — T
G — C
T — A
C — G

GENES
CHROMOSOMES
HEREDITY
VARIATION
EVOLUTION



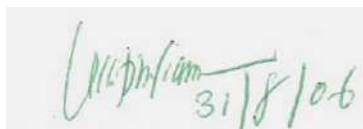
Y. ANBU SELVAM
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FOREWORD

This text aims to provide a comprehensive set of basic notes in Genetics and Cytogenetics. Knowledge of Genetics is very essential for overall progress in Agriculture, Biology and Medicine. The genetic laws are similar for all the several million species on earth and have consequences at all levels of life. Since the discovery of double helical model of DNA in 1953, the progress in molecular genetics is admirable. Nowadays, cytogenetics becomes increasingly molecular in approach which generated many exciting avenues of enquiry.

The chromosome, which carries the genetic material, is the centre of all biological systems. Therefore, manipulation of the biological systems meaningfully presupposes an understanding of the chromosomes and their behaviour.

In this textbook the sequence of presentation is very unique. The presentation is simple, lucid with essential photographs and illustrations which would be of great help to the Genetics students. The authors have taken great efforts to include all the essential requirements necessary for College text. I am confident that this book will be adequately catering to the learning requirement of the students of Genetics. In this regard, the authors are to be commended for bringing out very useful and standard text book on Genetics and Cytogenetics.



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Preface to the second edition

During the last four decades the knowledge on the science of genetics has exploded manifold and has been helpful in solving the needs of mankind. Genetics represents one of the greatest achievements of mankind, possibly even greater than those of the space programmes, for it is here it has come close to marshalling the techniques which may well govern the evolutionary destinies of all the biological entities of the world. The research especially at molecular level has opened up the possibility of genetic manipulation at will. The transfer of nitrogen fixing genes to the crop plants and health and desired genes into embryos and tissues of human beings have increase the hope of a human race free from sufferings.

Realising the manifold importance of its knowledge, genetics is taught as an important discipline to undergraduate and post graduate students.

This text book on genetics and cytogenetics has been prepared to provide the students adequate but concise matter pertaining to these aspects. Due care has been taken to include the fundamentals which are very essential for understanding the basic concept. The subject matter has been divided into 29 chapters. We have made sincere efforts to provide a simple and lucid account of each chapter in the light of latest informations available from different sources.

Each chapter contains suitable examples and well illustrated diagrams. A glossary has been included in the end so as to learn the meaning and origin of the word used in the text.

The authors acknowledge with thanks the University authorities for granting permission to write and publish this book. Since the help of the books of various authors has been taken in preparing this book, we do not claim our authenticity. We always welcome and entertain the critical and constructive suggestions whole heartedly.

Authors

CONTENTS

Chapter 1. Earlier Concepts of Heredity	1
Spontaneous generation	
Reproduction in animals	
Reproduction in plants	
Preformation	
Epigenesis	
Inheritance of acquired characters	
Pangenesis	
The Germplasm theory	
Hybridization in plants	
Chapter 2. Cell Ultrastructure	6
Size and shape of the cell	
Cell wall	
Plasma membrane	
Nucleus	
Nuclear membrane	
Nucleoplasm	
Nucleolus	
Cytoplasm	
Cell organelles	
Genetic organelles	
Mitochondria	
Chloroplast	
Non-genetic organelles	
Endoplasmic reticulum	
Ribosomes	
Golgi complex	
Lysosomes	
Microbodies	
Sphaerosomes	
Glyoxysomes	
Peroxisomes	
Lomasomes	
Centrosome	
Other plastids	

Other substances	
Vacuole	
Cytoskeleton	
Microtubules	
Ergastic substances	
Cilia and flagella	
Cell theory	
Types of cells	
Prokaryotic cell	
Eukaryotic cell	
Differences between prokaryotes and eukaryotes	
Chapter 3. Chromosomes I. Morphology	22
Chromosome structure	
Centromere	
Telomere	
Sat-chromosome	
Nucleolus Organiser Region (NOR)	
Isochromosome	
Karyotype	
Idiotypic	
Chromosome number	
Chapter 4. Chromosomes II. Structure and Function	30
Recent model of chromosome structure	
Folded-fiber model	
Organization of chromatin fibers	
Nucleosome-Solenoid model	
Special chromosomes	
Lampbrush chromosomes	
Giant chromosomes	
Supernumerary or Accessory or Extra chromosomes	
Chapter 5. Cell Division I. Mitosis	41
Mitosis	
Importance of mitosis	

Stages of mitosis	
Interphase	
Cell cycle	
Prophase	
Metaphase	
Anaphase	
Telophase	
Cytokinesis	
Chapter 6. Cell Division II. Meiosis	49
Significance of Meiosis	
Stages of Meiosis	
First Meiotic division	
First Prophase	
Leptotene	
Zygotene	
Pachytene	
Diplotene	
Diakinesis	
First Metaphase	
First Anaphase	
First Telophase	
Cytokinesis	
Interphase	
Second Meiotic division	
Second Prophase	
Second Metaphase	
Second Anaphase	
Second Telophase	
Differences between Mitosis and Meiosis	
Chapter 7. Chromosomal Aberrations I. Structural Aberrations	62
Deletion	
Duplication	
Inversion	
Translocation	
Chromosome pairing	

Chapter 8. Chromosomal Aberrations II.**Numerical Changes****70**

Aneuploidy

Nullisomy

Monosomy

Trisomy

Tetrasomy

Euploidy

Monoploidy and haploidy

Autopolyploidy

Allopolyploidy

Chapter 9. Mendelian Inheritance**91**

Mendel's work

Reasons for Mendel's success

Highlights of Mendel's paper

Inheritance of seed shape in pea

Mendel's Laws of heredity

Mendel's first law or Law of Segregation

Mendel's second law or Law of

Independent Assortment

Chapter 10. Physical Basis of Heredity**114**

Chromosomal theory of inheritance or

Sutton-Bovery hypothesis

Evidence for chromosomal theory of inheritance

Behaviour of genes and chromosomes

during segregation and Independent

assortment

Sex chromosomes

The white eye gene of *Drosophila*

(Sex Linkage)

Non-disjunction of chromosomes

(Sex Linkage)

Linkage and crossing over

Structural Chromosomal changes

Numerical Chromosomal changes

Holandric characters

Chapter 11. Dominance and Lethal Genes**107**

Principles of dominance

Complete dominance

Incomplete dominance

Co- dominance

Over dominance

Lethal genes

Recessive lethals

Dominant lethals

Conditional lethals

Balanced lethals

Gametic lethals

Semilethal genes

Subvital genes

Vital genes

Supervital genes

Chapter 12. Gene Interaction**119**

Typical Dihybrid ratio / Collaboratory gene

Interaction (9:3:3:1)

Duplicate gene Interaction / Duplicate

dominant epistasis (15:1)

Complementary gene Interaction / Duplicate

recessive epistasis (9:7)

Supplementary gene Interaction / Recessive

epistasis (9:3:4)

Inhibitory gene Interaction / Dominant

recessive epistasis (13:3)

Masking gene Interaction / Dominant epistasis

(12:3:1)

Polymeric gene action / Duplicate with

cumulative effect (9:6:1)

Modifying genes

Pleiotropism

Chapter 13. Polygenic Inheritance	132
Multiple factor hypothesis	
Studies on seed coat colour in wheat	
Studies on <i>Nicotiana longiflora</i>	
Environmental effects on character development	
Penetrance	
Expressivity	
Phenocopy effect	
Chapter 14. Linkage and Crossing Over	137
Linkage	
Experiment on Pea	
Experiment on Maize	
Incomplete linkage	
Crossing Over	
Crossing over in four stranded stages	
(Experiments on <i>Neurospora</i>)	
Cytological basis of crossing over	
Crossing over between three linked genes	
Chromosomal theory of linkage	
Linkage map / chromosome map / genetic map	
Coefficient of coincidence	
Coefficient of interference	
Chapter 15. Physical Mapping of Chromosomes	150
Heteroduplex mapping	
Restriction mapping	
Chapter 16. Sex Determination	153
Chromosomal sex determination	
XX – XO type	
XX – XY type	
ZO – ZZ type	
ZW – ZZ type	
Genic balance theory of sex determination	
Sex limited inheritance	
Sex influenced inheritance	

Chapter 17. Sex Linkage	162
Inheritance of white-eye in <i>Drosophila</i>	
Characteristics of sex-linked inheritance	
Criss-cross inheritance	
Inheritance of holandric / Y-linked genes	
Chapter 18. Multiple Alleles I.	167
Classical Examples	
Fur colour in rabbits	
Inheritance of blood group in man	
Self-incompatibility in plants	
Chapter 19. Multiple Alleles II.	172
Fine structure of genes	
Modern concept of gene	
Fine structure of gene in prokaryote	
The R ^{II} locus	
Complementation test	
The ultimate fine structure of gene	
Chapter 20. Genetic Material I. Chemical Nature	176
Properties of genetic material	
Identification of DNA as genetic material	
Experiment of Griffith	
Experiments of Avery, Macleod and McCarty	
Experiments of Hershey and Chase	
Chapter 21. Genetic material II. Primary and Secondary Structures	184
Phosphoric acid	
Pentose sugar	
Organic bases	
Pyrimidines	
Purines	
Nucleosides	
Nucleotides	
DNA structure	
Genetic material	

Modern concept of gene	
Double helical model of DNA	
The A, B and Z forms of DNA	
Chapter 22. Genetic material III. Replication	194
Semi conservative model	
Replication of bacterial DNA	
Chapter 23. Genetic material IV. Protection and Repair Systems	199
DNA modification	
The Type ^{II} enzyme system	
DNA Repair Systems	
Single base changes	
Structural distortions	
Chapter 24. Ribonucleic Acid (RNA)	204
Chemical composition of RNA	
RNA as genetic material	
Types of RNA	
Messenger RNA (mRNA)	
Transfer RNA (tRNA)	
Ribosomal RNA (rRNA)	
Genetic and non-genetic RNA	
Functions of RNA	
Chapter 25. Gene action	211
The Genetic Code	
The Coding Dictionary	
Chapter 26. Gene Expression / Protein Synthesis	214
Central Dogma	
Protein synthesis	
Requirement of Protein synthesis	
Site of Protein synthesis	
Mechanism of Protein synthesis	
Transcription of mRNA	
Activation of aminoacids	

Attachment of activated aminoacids to tRNA	
Translation	
Elongation of polypeptide chain	
Termination of polypeptide chain	
Processing of polypeptide chain	
Polyribosome	
Rate of synthesis	
Protein synthesis and house construction	
Inhibitor of protein synthesis	
Protein synthesis in non-nucleated cells	
Chapter 27. Regulation of Gene Expression in Prokaryotes	226
The Operon concept	
The Operon hypothesis	
Structure of Operon model	
Mechanism of Operon model	
Chapter 28. Regulation of Gene Expression in Eukaryotes	231
Gene and enzyme sequences	
One gene one enzyme hypothesis	
One gene one polypeptide hypothesis	
Genetic control of protein	
The translation process	
Chapter 29. Mutation	234
Characteristics of mutation	
Classification of mutation	
Based on size	
Based on cell type	
Based on quality	
Based on origin	
Based on direction	
Based on magnitude of phenotypic effect	
Mutagens	
Physical mutagens	

Chemical mutagens	
CIB Technique	
Chapter 30. Cytoplasmic Inheritance	250
Characteristics of cytoplasmic inheritance	
Maternal effect	
Plastid inheritance	
Nuclear Cytoplasmic inheritance	
Chapter 31. Sexual Reproduction	259
Flower	
Sporogenesis	
Microsporogenesis	
Megasporesogenesis	
Gametogenesis	
Microgametogenesis	
Megagametogenesis	
Fertilization	
Significance of sexual reproduction	
Chapter 32. Effects of the Environment on the Development of Human Characters	264
Identical and Fraternal Twins	
Chapter 33. Eugenics and eugenics	268
Eugenic measures	
Negative eugenic measures	
Regulation of marriages	
Sexual separation of defectives	
Consanguineous marriages or marriages in blood relations	
Positive Eugenic measures	
Selective mating	
Removal of Social Hindrances	
Early Marriage and prevention of loss or wastage of good germplasm	
Preservation of good Germplasm	
Eugenic consultation or genetic counseling	

Consanguineous Marriage	
Euthenic Measures	
Good nature and nurture	
Education	
Euphenics	
Chapter 34. Mobile genetic elements	275
siRNA and RNAi	
Metagenomics	
Transcriptomics	
Metabolomics	
Phenomics	
Plant genome structure	
DNA Methylation	
Functional Genomics	
Meaning of Developmental Genetics	
Glossary	288
Index	309

EARLIER CONCEPTS OF HEREDITY

Spontaneous generation

Many biologists believed that some primitive organisms originated from non-living matter e.g., decaying organic matter. Such a fresh origin of living organisms from non-living materials is known as spontaneous generation.

Aristotle (384 – 322 B.C) the greatest biologists of early days believed that not only plants but animals like fleas, mosquitoes and snails originated from decaying organic matter. But Redi (1621 – 1697) and Spallanzani (1729 –1799) presented experimental evidence against spontaneous generation.

Reproduction in Animals

In 1673, de Graff proved that the tiny vesicles on the surface of mammalian ovaries were eggs. He also noted that progeny had the characteristics of both their parents. On this basis, he suggested that both parents contribute to heredity.

Reproduction in Plants

As early as 700 B.C, Babylonians and Assyrians were aware of the fact that female palm trees produce fruits only when their flowers were dusted with pollen, and that the source of pollen had a marked effect on the quality of fruits produced by female plants. This effect is known as metaxenia.

O. Hurting in 1875 presented a conclusive proof for the fusion of sperm and egg. He studied fertilization in sea urchin and observed the fusion of sperm and egg nuclei. Grew described stamens in flowers and suggested that they were the male reproductive parts.

Camerarius (1694) showed that maize plants did not set seed when pollen was not applied to their pistil. He also presented the first description of reproductive organs of a plant (maize). The first artificial hybrids in plants were produced by Thomas Fairchild in 1717 when crossed sweet William with carnation. Subsequently, many scientists, Linnaeus (1707 –1778), Koelreuter (1733 – 1806), Gaertner (1772-1850) produced artificial hybrids in plants and noted equal contribution by both the pollen (male) as well as the seed (female) parents to the characters of progeny.

Amici (1830) described the entry of pollen tubes into the ovary. Strausberger (1884) described fertilization in angiosperms and noted the fusion of sperm and egg nuclei. These discoveries had clarified the role of male and female gametes in sexual reproduction.

Preformation

Dutch scientist Swammerdam propounded the preformation theory. This theory holds that the sex cells (sperm and ova) had the miniature copy of adults and the development of embryo was actually only the enlargement of parts that were already present in the sperm or egg. Some of the scientists of this time Hartsoeker (1694) and Dalempotius (1699) even imagined that they could see a miniature human being homunculus.

Epigenesis

In the early stages of embryonic development, embryonic tissues are composed of similar (in structure and most likely, function) cells. These cells progressively differentiate into the adult tissues and organs. This concept is known as Epigenesis, and the credit for this concept goes to Wolff (1738 –1794).

Inheritance of Acquired characters (Lamarckism)

Lamarck (1744 –1829) proposed that characters acquired by individuals of one generation is transmitted

to those in the next generation. The idea of the inheritance of acquired characters was originally suggested by Hypocrates about 400 B.C. An acquired character refers to the intensity and form of a character which differs from those in the parents of an individual and which develops primarily due to the effects of environmental factors. According to this, if a person develop strong muscles by exercises, his children will inherit this character.

Pangenesis

Charles Darwin published his classic origin of species in 1859. He proposed the theory of evolution according to which two or more different species evolved or differentiated from a parent species primarily due to the effect of natural selection on the variation present in the species. According to Darwin natural selection allows only the fittest individuals of a species to survive, this idea is commonly known as survival of the fittest due to natural selection.

Darwin proposed that each organ of individual produce very small, almost invisible identical copies of itself called gemmules or pangenes. This will be transmitted through the gametes to its progeny and will produce a similar modification in the corresponding organ of the progeny. This idea known as pangenesis, is of only historical interest without any scientific validity.

The Germplasm Theory (Weismannism)

In order to test the validity of the hypotheses of the inheritance of acquired characters (Lamarck) and of pangenesis (Darwin), Weismann cut off the tails of a group of experimental mice for 22 successive generations. According to the hypothesis of the inheritance of acquired characters, the tailless condition of mice, produced by cutting off their tail, should be transmitted to their progeny. Similarly if the hypothesis of pangenesis were correct, the gemmule for tail would be absent in the

gametes of the tailless mice thus produced and as a result their progeny should be tailless.

But Weismann did not obtain a single tailless progeny although he kept on excising the tail of these mice for as long as 22 generations. This finding conclusively disproved the ideas of inheritance of acquired characters and of pangenesis. Weismann proposed the germplasm theory to explain these and other findings. According to this theory, the body of an individual is composed of two different types of tissues 1. Somatoplasm and 2. Germplasm.

Somatoplasm consists of all the body tissues which, although necessary for survival and proper functioning of the organism, do not contribute to sexual reproduction. Therefore, changes occurring in the somatoplasm cannot be transmitted to the next generation and are confined to the concerned individuals only. The germplasm, on the other hand produces gametes that are the basis of sexual reproduction. The changes taking place in the germplasm are therefore transmitted to the next generation. But the basic contribution made by Weismann that acquired characters are not inherited is entirely valid.

Hybridization in plants

Thomas Fairchild is credited to have produced the first artificial hybrid in plants when he crossed carnation and sweet William in 1717. The hybrid produced from this cross was known as Fairchild's mule.

Koelreuter made extensive crosses in Nicotiana between 1761 and 1767. He noted the uniformity and heterosis in F_1 and the appearance of a larger variation in F_2 . He also concluded that both male and female parents make equal contribute to the characteristics of progeny since hybrids attained from reciprocal crosses were identical.

John Knight (1759-1835) developed commercial varieties of apple, pear, apricot, and grapes through hybridization.



CELL ULTRASTRUCTURE

The cell is a fundamental, structural and functional unit of living organism. Robert Hook first time observed the presence of cells in 1665.

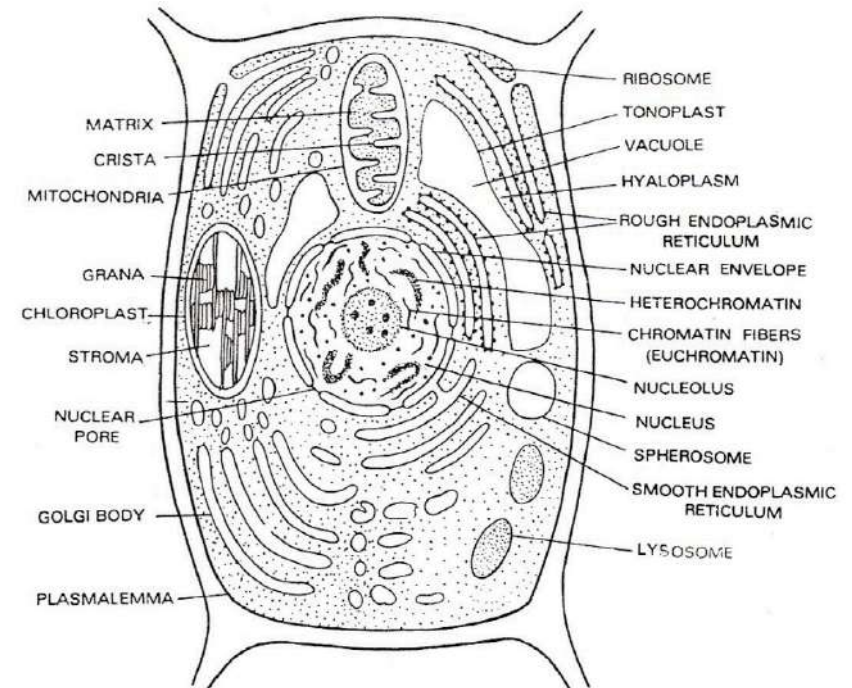


Fig. 2.1 Ultrastructure structure of eukaryotic cell (plant cell)

Size and Shape of a Cell

1. Cells may be spherical, cylindrical, rod, hexagonal or irregular in shape.
2. The size of the cells ranges from 0.2-0.5 microns in bacteria to 75mm in case of Ostrich eggs.

- Most of the cells are cylinders of 15-30 microns in diameter.
- The number of cells in an individual ranges from one (bacteria, protozoans) to 6×10^{12} in human beings.
- A new born baby has 2×10^{11} cells. The weight of an average cell is 1×10^{-8} g.

Cell wall

A cell wall surrounding the plasma membrane gives strength and rigidity to cell. It is primarily composed of cellulose. It consists of three parts namely,

- Middle lamella
- Primary wall
- Secondary wall

In plants the walls of immediate neighbour cells are joined by middle lamella. Primary cell wall is deposited after middle-lamella formation. Secondary cell wall lies between primary cell wall and plasma lemma (Fig. 2.1).

Plasma membrane:

The membrane enclosing the cytoplasm of cell are known as plasmalemma or ectoplast or plasma membrane. It is a lipid layer through which extra cellular substances e.g., nutrients, water enter the cell and waste substances or secretions exit the cell. Passage of substances may require expenditure of energy (active transport) or may be passive (diffusion) without energy.

Nucleus

It is the master control of cellular contents via its genetic material (DNA). This governs both the growth and development. It was discovered by Robert Brown in 1833.

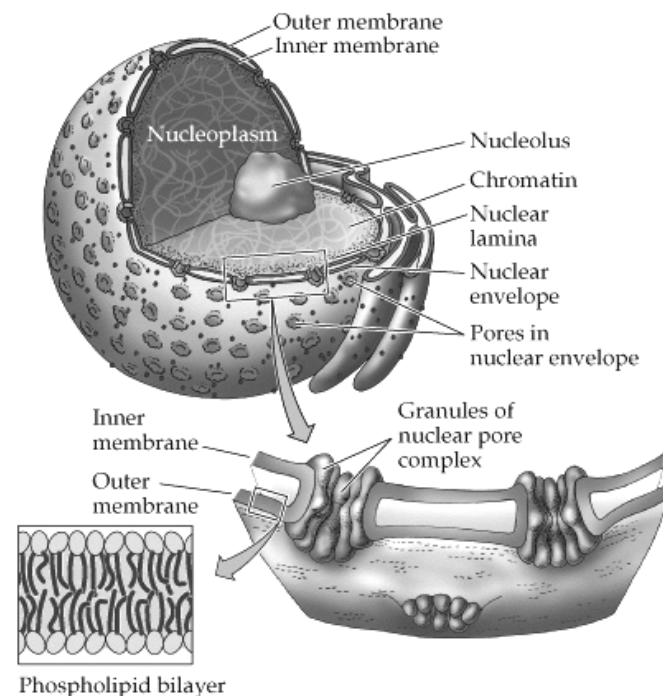


Fig. 2.2. STRUCTURE OF NUCLEUS

Nuclear membrane

Double membrane which controls the movement of materials between the nucleus and cytoplasm. It consists of pores that communicate with endoplasmic reticulum called annulus (Fig. 2.2).

Nucleoplasm

Nucleus consists of a granular substratum called nucleoplasm or nuclear sap which contains chromatin and one or more nucleoli. The chromatin fibres condense during cell division and forms into chromosomes. It consists of nucleoprotein. Only the DNA component is heritable.

Perinuclear space

The two nuclear membranes are separated from each other by a space of 400-700 Å wide space known as perinuclear space or perinuclear cisterna.

Nucleolus

The nucleoplasm contains of one or more dense spherical bodies called nucleoli. They disappear during cell division. It is rich in R-RNA and protein. It is composed of two regions

1. A dense mass of filaments known as 'plasamorphs'.
2. A fibrillar coarse thread like component known as 'nucleonema'.

Cytoplasm

1. Kolliker in 1843 recognized that a jelly like substance of cell, except the nucleus and vacuole is surrounded by cytoplasm.
2. It consists of membranous, sub cellular organelles and non membranous hyaloplasm or ergastic substances. It contains the multiple structural and enzymatic systems of (Glycolysis & Protein synthesis).

CELL ORGANELLE

An organelle may be defined as a specific sub-structure of a cell with a specific structure and function. These are classified into genetic and non genetic based on DNA.

Genetic Organelles

1. Mitochondria and
2. Chloroplasts

Both mitochondria and chloroplasts are the two organelles in which DNA is present. Hence called as Genetic organelles. Most of the organelles are too small to be seen with the ordinary microscope. But they can be studied with electron microscope.

Mitochondria

Distribution

Mitochondria was discovered by Altmann and Benda in 1894. Mitochondria are uniformly distributed throughout the cytoplasm. They move freely in the cytoplasm carrying ATP wherever needed. They usually constitute 30-35 percent of the total protein content of the cell. A typical cell has 200-300 mitochondria.

Shape and Size

They are small spheres, spheroids, rod shaped or dumb-bell shaped bodies varying in size from 0.2µm-5µm.

Structure

Mitochondria is bounded by double osmophilic membrane of 185 Å thick. There are number of invaginations in the stroma or matrix (interior of mitochondria) which are finger shaped called as cristae or villi. There are a number of spherical bodies attached to the membrane by a stalk. This stalked units are called as F₁ particles or oxysomes. These particles are identified as molecules of ATP-ase enzyme responsible for A.T.P synthesis. Chemically mitochondria contains 75% protein, 25% lipids small amounts of DNA and t-RNA (Fig. 2.3).

Functions

1. Mitochondria controls the oxidation of respiratory substrates.
2. They contain about 70 enzymes and a dozen of coenzymes responsible for electron transport chain and phosphorylation.

3. This has the capacity of converting potential energy of different food materials into the form of energy that can be used by the cell to carryout its various activities.

Mitochondria owing to all its functions is aptly termed as the power house of the cell.

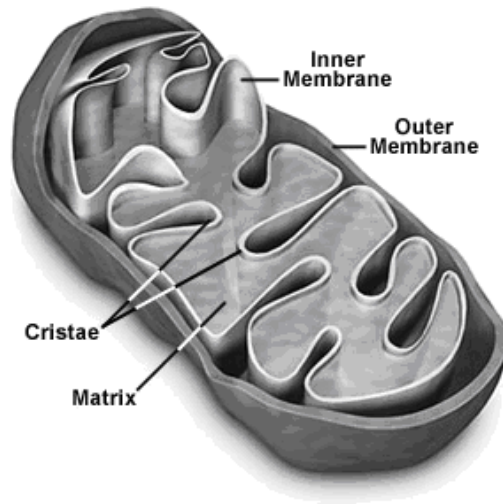


Fig. 2.3 Structure of mitochondria

Chloroplast

Distribution

They occur mainly in palisade and spongy parenchyma tissue of leaves and cortical cells of young stems.

Shape and Size

The chloroplasts are disc flat structures measuring 4-10 μm in length and 1-3 μm in thickness. The size and shape however change in light and darkness. The plants growing in shade have longer chloroplasts than the ones

growing in sunlight. There are about 20-40 chloroplasts per cell.

Structure of chloroplast

Each chloroplast is enclosed by a double membrane of lipoprotein which is 40-60 $\text{\AA}$ thick. The space enclosed by this membrane is called as stroma which is filled with proteinaceous matrix. Starch grains and osmophilic droplets are distributed within the stroma. The inner membrane of chloroplast extends to form a lamellar system. At several points these paired lamella are stacked on top of each other. These ball like stack are around 0.3 μm in diameter called grana. 40-60 grana will be present in a chloroplast (Fig. 2.4).

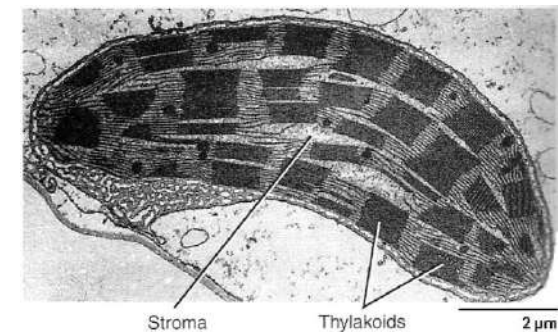
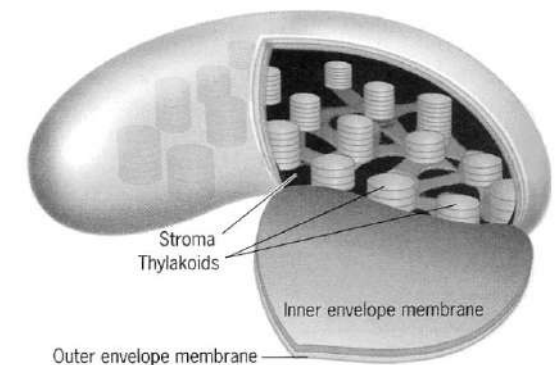


Fig. 2.4. Internal Structure of A Typical Chloroplast

Each granum is composed of 5-25 discs and each disc encloses a space called Thylakoid. The thylakoid of one grana is connected to the thylakoid of other grana by fret channel. The inner surface of the lamellar membrane contain small spherical structures called quantosomes. The chemical composition of chloroplast is protein 30-35%, phospholipids 20-30%, chlorophyll 5-10, 0.5% DNA and 5% R.N.A.

Development

Von Wettstein discovered that chloroplasts are developed from small, membranous, undifferentiated bodies called proplastids. In light proplastids develop into chloroplasts, which latter on invagination produces other structures within that.

Functions

1. Specialized to harvest light energy and utilise it in the fixation of carbon and manufacture the high energy molecules involved in cellular energy metabolism.
2. These are the site in a cell in which the photosynthesis takes place.

Non-genetic Organelles

All the cell-organelles which does not contain DNA within them are termed as Non-genetic organelles. Organelles other than chloroplasts and mitochondria are non-genetic organelles.

(e.g.) Endoplasmic reticulum, ribosomes, golgibodies, lysosomes, microbodies, microtubules, other plastids, centrosomes, cilia and flagella etc.

Endoplasmic reticulum

This is a network of tubes extending from nuclear membrane to cell surface and even to the neighbouring cells. The membrane of ER is lipo-protein in nature (Fig. 2.5).

K.P. Porter first described ER as a semi viscous fluid of cytoplasm traversed by membrane bound vesicular system. The ER may be granular (rough) or agranular (smooth). When granular, it is associated with ribosomes mainly concerned with protein synthesis, when the ER is not associated with ribosomes it is called agranular. Each thickness of cisternae varies from 400-700Å.

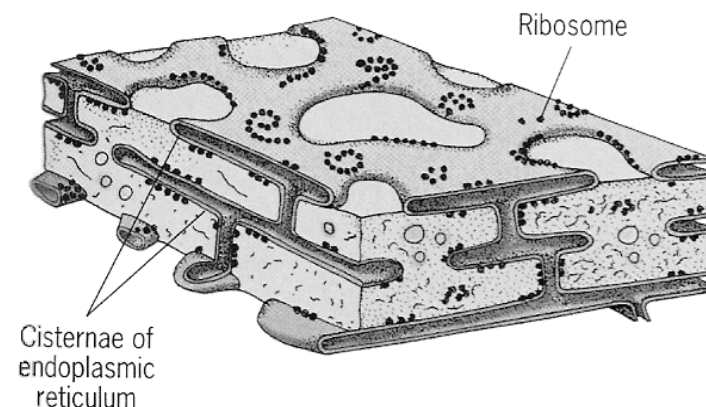


Fig. 2.5. Endoplasmic reticulum

Functions

1. ER gives mechanical support to the cytoplasm
2. Owing to its osmotic properties it is involved in intracellular exchange of materials.
3. The ER serves as a circulatory system for the transportation of cellular molecules and helps in the storage of synthesized molecules.
4. This plays a vital role in the synthesis of new nuclear membrane during cell division.
5. It is involved in metabolism of cholesterol and steroid hormones.
6. It causes detoxification of many endogenous and exogenous compounds.

Ribosomes

Palade in 1953 discovered ribosomes. The ribosomes are small granules associated with ER or free floating in cytoplasm. The ribosomes are $150\text{\AA}$ - $230\text{\AA}$ in diameter, with a sedimentation co-efficient of 70 S (Svedberg unit) in prokaryotic cells and 80 S in eukaryotic cells (Fig. 2.6).



Fig. 2.6. A Ribosome Showing Two Sub-Units

Each ribosome is made up of two sub-units. Ribosomes of prokaryotic cells is composed of 50 S + 30 S sub units, whereas the eukaryotic cells is of 60 S + 40 S. The association of sub-units depends upon the Mg^{++} concentration.

The outer boundary of each ribosome is an envelope of protein which surrounds r-RNA. It consists of 60% r-RNA and 40% proteins. The ribosomes often found in clusters or groups to which a RNA is attached, and is called as poly ribosomes or ribosomes.

Function

It's major function is to provide template for the synthesis of protein.

Golgi Complex

This was discovered by Camillo Golgi in 1898. This is a membrane system with almost disc shaped structure which occupies a definite position between the nucleus and pole of the cell (Fig. 2.7).

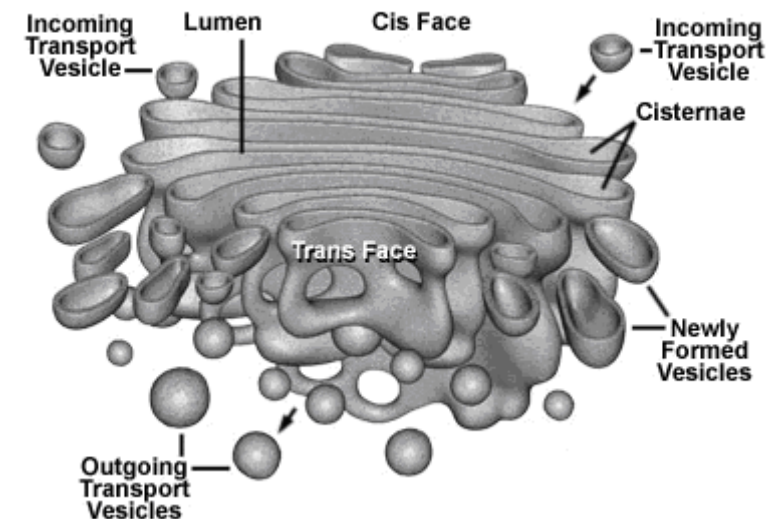


Fig. 2.7. Golgi apparatus

This golgiosome or dictyosome consists of stacked discs, each composed of a flattened sac like cisternae bounded by two cytomembranes. Around the edge of these cisternae are small globular bodies or vesicles, which appear to be released from dialated margins or discs. These vesicles are of two types smooth walled and sculptured.

Function

1. They are also involved in the secretion and transport of materials out of the cell.
2. They are also involved in cell-plate formation.

Lysosomes

It was discovered by C. de Duve. It is otherwise called as Suicidal bags. These are the vesicles containing several hydrolytic enzymes. The main enzyme present in the lysosome is the acid phosphatase. Other enzymes present in lysosomes are acid-DNAase, acid RNAase, β -galactosylase etc. The membrane surrounding lysosome has unit membrane organization. They digest the food particles and release the digested food particles into the cytoplasm.

The membrane of lysosome structure enzymes are released, causing its own disintegration called as Autolysis (self killing). It aids in intracellular digestion of bacteria and other foreign bodies. They may cause cell destruction, if absorbed.

Microbodies

There are certain cellular organelles which are large in number, tiny, and membrane-bound called microbodies. They are of several types namely sphaerosomes, peroxisomes, glyoxysomes and lomasomes.

Sphaerosomes

These are vesicles containing about 98% lipids and little quantity of acid phosphatase. They are found in plant cells only. They are surrounded by a membrane and are identical to lysosome. Their function is lipid storage.

Glyoxysome

It was discovered by Beevers from *Casuarina* endosperm. They are spherical bodies surrounded by a single membrane. They contain granular stroma and the

key enzymes of glyoxylate cycle, such as iso-citrate & malate synthetase. They are usually found in tissues where fats are actively converted into sugars as in germinating seeds.

Peroxisomes

It was discovered by Tolbert *et al.*, from spinach leaf. They are similar in shape and size to glyoxysomes. Like glyoxysomes they also contain granular stroma but the key enzymes of glycolate metabolism and photolysis phase.

Lomasomes

These are vascular and membranous structure usually present between the cell-wall and plasma-membrane of the plant cells. They appear to be originated from golgibodies. Their definite function is not yet known but probably they will help in cell wall elongation.

Centrosome or centriole

The term centrosome was coined by T. Boveri in 1888 to represent a specialized portion of cytoplasm present adjacent to the interphase nucleus. It consists of two bodies, centrioles and the kinoplasm. It consists of structural protein, tubulin and lipid molecules. It also shows the presence of ATP-ase enzyme.

Function

The function of centrioles is to form the spindle of microtubules at the time of cell division.

Other Plastids

Leucoplasts are colourless plastids, which function as storage organelles. Leucoplasts when contain starch called as Amyloplasts and if oil, then they are elaioplasts. If the leucoplasts contain protein then they are aleuroneplasts. Plastids containing chlorophyll are chloroplast. Plastids containing carotenoids are chromoplast.

Other substances

Vacuole

It is present in the centre of the matured cell. It is surrounded by a membrane called tonoplast. It is filled with a watery solution of organic and inorganic substances and certain gases which is altogether called a cell sap. It creates turgor pressure which makes the cell turgid. Thus it maintains an osmotic relation of the cell. Colour of the majority of the flowers are chiefly due to the presence of anthocyanin pigments dissolved in the cell sap.

Cytoskeleton

It contributes to the shape, division and motility of a cell also the ability to move and arrange its components. It consists of micro tubules, micro filaments and intermediate filaments.

Microtubules

Microtubules are made up of protein tubulin. They are primarily found in dividing cells in the form of spindle fibres. They are responsible for the movement of chromosome, flagella and cilia, they may also found in non-dividing cells and provide some degree of stability to the structures present in the cytoplasm.

Ergastic substances

It is otherwise referred to as hyaloplasm or cytosol which are non living. It contains water, minerals, ions, sugars, amino acids and other nutrients for building the macro molecules, bio polymers such as nucleic acid, proteins, lipids and large carbohydrate such as starch and cellulose. It also include tannins, resins, gums, oils, latex, alkaloids and mineral salts in the form of cystolith (crystals).

Cilia and Flagella

These are not universal cell organelles but found in many plant and animal cell. Cilia is a short, motile and present in large numbers covering the small cells. Flagella are large, less numerous as well as more complex in their movement. The main function of both cilia and flagella are locomotion.

CELL THEORY

Schlieden and Schwann (1838 and 1839) formulated the cell theory which states that all living organisms are composed of cells which consist of a kern (nucleus) and a cytoplasm (cytoplasm). In 1846, Nageli provided the logical extension of cell theory; he postulated that all cells originated from pre-existing cells. Virchow (1853), presented an elaborate version of this hypothesis along with considerable evidence in its support. It is referred to as cell lineage theory.

Types of cells

There are two basic types of cells depending on the degree of internal organisation.

Prokaryotic cell

Pro-Primitive; karyon-Nucleus. In this type of cell DNA is not enclosed in a nuclear membrane but it is distributed throughout the cell. e.g. Cells of bacteria (Fig. 2.8) and blue green algae.

Eukaryotic cell

Eu-True; karyon-Nucleus. In this type of cell, there is a nucleus with a nuclear membrane and DNA is located in the chromosomes e.g., Cells of fungi, animals, etc. The difference between prokaryotic and eukaryotic cell is given in Table 2.1.

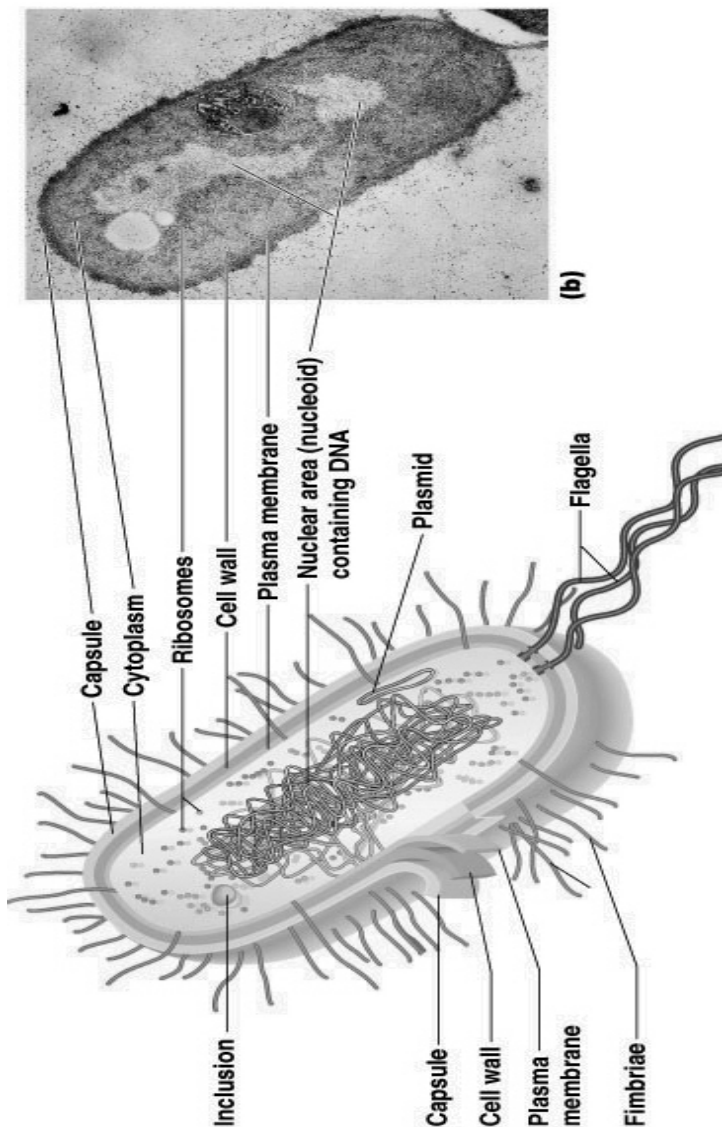


Fig. 2.8. Structure of prokaryotic cell (bacterial cell)

Table 2.1 Difference between Prokaryotes and Eukaryotes

Sl. No.	Character	Prokaryotes	Eukaryotes
1.	Cell size and shape	Small, rod or spears	Large, mostly spheroid
2.	Nucleus	Nucleoid without nuclear membrane commonly multiple nucleoid per cell	With nuclear membrane. Mostly single nucleoid per cell, rarely more.
3.	Chromosomes	No chromosomes individual DNA molecules	Multiple chromosomes
4.	Cell division	No mitosis divide by fission	Mitosis, meiosis and amitosis takes place
5.	Endo-membrane	Absent	Present
6.	Mitochondria	Absent	Present
7.	Chloroplast	Absent	Present
8.	Cell-wall	Non-cellulosic	Cellulosic
9.	DNA	Not histone bound shorter & circular. DNA rejoins rapidly after denaturation DNA polymerase sensitive to ethinobromide	Histone bound long & linear DNA rejoins slowly after denaturation. DNA polymerase not sensitive to ethinobromide.
10.	RNA polymerase	Sensitive to Vifamycin	Not sensitive to Vifamycin
11.	Ribosomes	70S (50S + 30S)	80S (60S + 40S)
12.	Ribosomal RNA (r RNA)	16S, 23S	18S, 28S
13.	Transfer RNA (t RNA)	Formyl methionyl t RNA present	Absent
14.	Protein-synthesis	Inhibited by chloramphenicol	Inhibited by cyclohexinoide
15.	Cholesterol	Either absent or very low	Present
16.	Locomotion	Flagella with a single fibril is only present	Both cilia and flagella are present
17.	Exo and endocytosis	Absent	Present
18.	Example	Bacteria, blue green algae	Fungus, animals, human beings

CHROMOSOMES I. MORPHOLOGY

Cytogenetics originated as a result of the realization, which brought together two different branches of biology, namely cytology and genetics. "A study of internal structure and functional organisation of different structures within a cell is known as Cytology".

In Cytogenetics, structure and function of the organelle containing genes, i.e., chromosome is studied.

Chromosome Structure

Chromosomes are the rod-shaped, stained bodies seen during metaphase stage of mitosis when the cells are stained with a suitable basic dye and viewed under a light microscope (Fig. 3.1).

Chromosome was first described by Strausberger in 1875, and the term chromosome was first used by Waldeyer in 1888. Chromosomes are clearly visible as distinct bodies during the stages of cell division only, and their number can be counted with relative ease only during mitotic metaphase. These chromosomes are composed of thin chromatin threads called Chromanemata.

Centromere

Each metaphase chromosome appears to be longitudinally divided into two identical parts each of which is known as chromatid. The two chromatids of a chromosome appeared to be joined or 'fused' together at a point called centromere i.e., the region where the two sister chromatids of a chromosome appear to be joined or 'held together' during mitotic metaphase is known as centromere. Centromeres are the first part of chromosomes to be seen moving towards the opposite poles during anaphase; the remaining, regions of

chromosomes lag behind and appear as if they were being pulled by the centromere. Therefore, it appears as if the anaphase chromosome movement is due to the centromeres of the chromosomes, hence they are known as kinetochores.

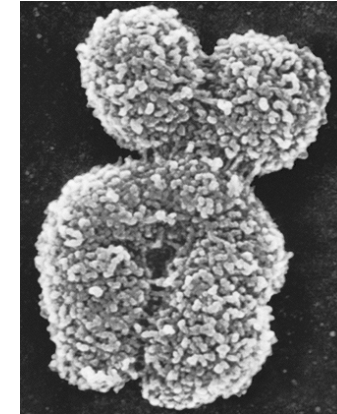


Fig. 3.1. Mitotic Metaphase Chromosome

Types of chromosomes based on the position of centromere

- | | |
|-----------------------|---------------------|
| (i) Metacentric | (ii) Submetacentric |
| (iii) Acrocentric and | (iv) Telocentric |

i) Metacentric chromosome

In this, the centromere is located in the centre of the chromosome i.e., the centromere is median, so that the two arms of such chromosomes are equal, and the arm ratio is 1:1. Metacentric chromosomes appear 'V' – shaped during anaphase.

(ii) Submetacentric

The centromere is located on one side of the central point i.e., the centromere is submedian; such chromosomes are known as submetacentric.

(iii) Acrocentric

Centromeres located close to one end of chromosomes are referred to as subterminal, and chromosomes having them are called subhelocentric or acrocentric.

iv) Telocentric

In some chromosomes, however, centromeres appear to be located at one end of the chromosome. Such centromeres are called terminal and the chromosomes having them are telocentric chromosomes.

Telomere

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

Sat – chromosomes

In some chromosomes, a second constriction in addition to that due to centromere (the primary constriction) is also present, this additional constriction is known as secondary constriction. Generally, secondary constrictions are located in the short arm of chromosomes near one end, but in many chromosomes they are located in the long arm and / or nearer to the centromere than to telomere. The chromosome region distal to the secondary constriction i.e., the region between the secondary constriction and the nearest telomere, is known as satellite.

Therefore, chromosomes having secondary constrictions are called satellite chromosomes or sat – chromosomes. It may be noted that sat – DNA is not related to sat – chromosomes, sat – DNA is presented in the centromeric regions of all the chromosomes.

Nucleolus Organiser Regions (NOR)

Nucleolus is always associated with the secondary constrictions of sat-chromosomes. Therefore, secondary constrictions are also called nucleolus organiser regions (NOR) and sat-chromosomes are often referred to as nucleolus organiser chromosomes (NOC). NOR of each sat-chromosome contains several hundred copies of the gene coding for ribosomal RNA (rRNA).

Isochromosomes

A metacentric chromosome in which the two arms are identical in gene content.

Karyotype

The general morphology i.e., the size of chromosomes, the position of centromeres, the presence of secondary constrictions, the size of satellite bodies of the somatic chromosome complement of an individual constitutes its karyotype (Fig. 3.2).

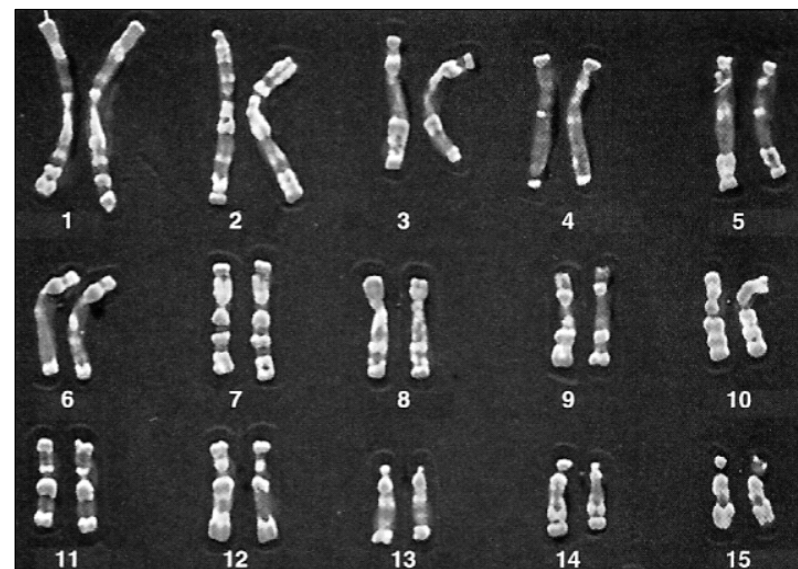


Fig. 3.2. Karyotype of a human male

Idiotype

The karyotype of a species may be represented diagrammatically showing all the morphological features of the chromosomes; such a diagram is known as idiotype. Ordinarily, idiotypes are prepared for the haploid chromosome complement of a species. An idiotype gives the same amount of information as the concerned karyotype.

Heterochromatin and Euchromatin

The material of which chromosome are composed is called chromatin. Chromatin was classified into two groups by cytologists on the basis of stainability with basic dyes at various stages of cell cycle. **Heterochromatin** takes up deep stain during interphase and prophase, while during metaphase it is stained lightly. In contrast, **Euchromatin** takes up little stain during interphase, stains only lightly during prophase, particularly early prophase, but is deeply stain during metaphase. The distribution of heterochromatin in chromosomes has been extensively investigated by analyzing early and mid-prophase chromosome. During these stages, there are three deeply staining structures in chromosomes. (i) chromomeres (ii) centromeric regions and (iii) Knobs. Centromeric regions invariably contain heterochromatin: in salivary glands, these regions of all the chromosomes fuse to form a large heterochromatin mass called **chromocentre**.

Knobs are spherical structures usually several times the diameter of the concerned chromosomes present in certain chromosomes of some species *e.g.*, maize: knobs are more clearly observable during pachytene stage in maize. where present; knobs serve as valuable chromosome markers. Heterochromatin is classified into two groups: (i) constitutive and (ii) facultative.

Constitutive heterochromatin remains permanently in the heterochromatic state, *i.e.*, it does not revert to the euchromatic state, *e.g.*, centromeric regions. In contrast, facultative heterochromatin is essentially euchromatin that has undergone heterochromatinization (conversion into heterochromatin). Heterochromatinization may involve a segment of a chromosome, a whole chromosome (*e.g.*, one X chromosome of human females and females of other mammals), or one whole haploid set of chromosome (*e.g.*, in some insects, such as mealy bugs, the full paternal set of chromosomes undergoes heterochromatinization).

Genetic studies revealed that *constitutive heterochromatin is largely genetically inactive, while almost all of the genes are located in euchromatic regions of chromosomes. However, heterochromatin may not be completely devoid of genes or genetic functions.*

Chromosome number

Each species has a definite and generally, a constant somatic and gametic chromosome number.

Somatic chromosome number is the number of chromosomes found in somatic, more specifically meristematic, tissues of a species and is represented by $2n$. Ordinarily, somatic cells contain two copies of each chromosome (except sex chromosomes). The two copies of a chromosome are usually identical in morphology, gene content, and gene order *i.e.*, location of different genes, and they are known as homologous chromosomes.

Gametic chromosome number is precisely one half of the somatic number, is represented by n , and denotes the number of chromosomes found in the gametes of a species. It is important to note that gametes of a species ordinarily contain only one copy of each of the different chromosomes of that species.

In contrast, in some animals, the complete somatic complement (somatic number) may be present only in the reproductive tissues, while some chromosomes or portions of chromosomes may be eliminated in somatic tissues this phenomenon is known as chromosome diminution.

Chemical Composition of Chromosomes

Chromatin is composed of DNA, Protein and RNA. Purified chromatin isolated from interphase nuclei consists of about 30-40% DNA, 50-65% Protein and 1-10%RNA; there is a considerable variation due to species and tissues of the same species. The chemical composition of metaphase chromosomes differs markedly from that of interphase chromatin; they contain relatively less DNA and more protein and RNA than the latter. Metaphase chromosomes contain, on an average, 15-20%DNA, 10-15% RNA and 65-75% protein.

Chromosome Banding

A chromosome band may be defined as a section of a chromosome, which shows relatively darker or lighter stain as compared to the neighbouring sections of the same chromosome. Bands appear in metaphase chromosomes when they are prepared according to specific staining procedures developed for the purpose. When chromosomes are stained with some quinacrine dyes, they exhibit fluorescent bands under ultraviolet light; the bands so obtained are called **Q-bands**. Certain staining techniques with Giemsa produce **G-bands** in chromosomes, while certain other techniques with the same stain (viz., the reverse staining techniques) generate **R-bands**. Another staining techniques, the C-staining technique, yields **C-bands** in which centromere and other heterochromatic G-band contains approximately 10⁶ - 10⁷ base pairs; clearly, a G-band does not represent a single gene.

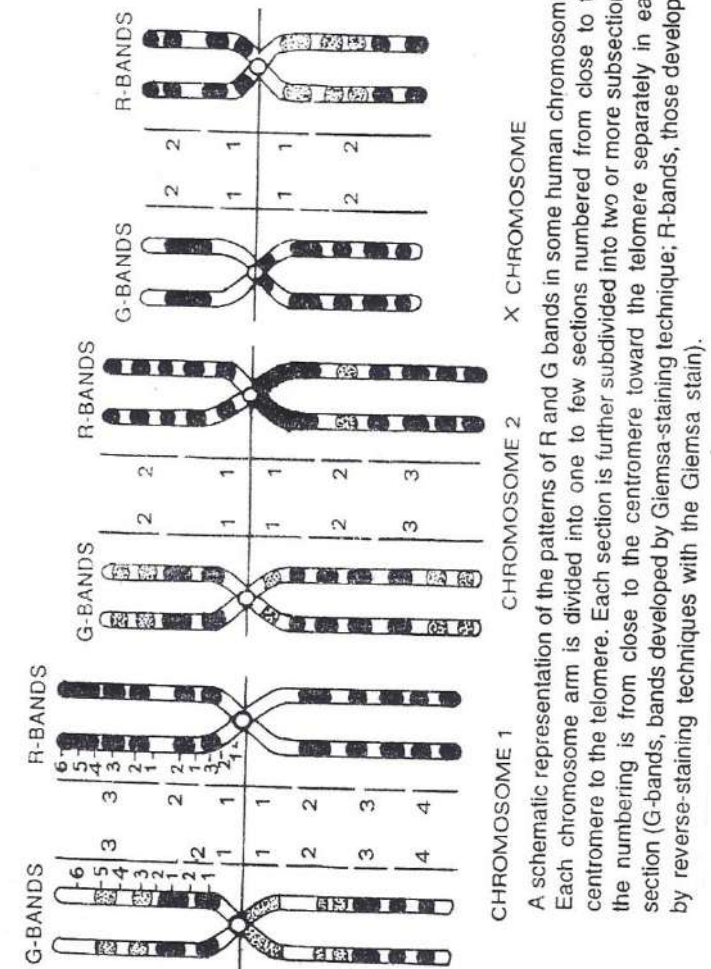


Fig.3.3 Types of chromosome Banding

CHROMOSOMES II. STRUCTURE AND FUNCTION

Recent models of chromosome structure

Folded fiber model

It was proposed by Du Praw in 1965. According to this model the chromosomes are made up of chromatin fibers of about 230 – 300 Å diameter. Each chromatin fiber contains only one DNA double helix in a coiled state. Each chromatin contains a single enormously long chromatin fiber. Thus the 300 Å chromatin fiber is produced by coiling of a single DNA double helix. The DNA of this chromatin fiber replicates during interphase, producing two sister chromatin fibers. It remains unreplicated in the centromeric region, so that the two sister fibers remain joined in this region. Also, replication (0.3 %) takes place in centromeric region during zygotene phase, so that the sister chromatin fiber separate in the centromere region.

The two sister chromatin fibers undergo extensive folding separately in an irregular manner to give rise to the two sister chromatids. Folding of sister chromatin fiber reduces their length and increases their thickness and stainability. Most of the available evidence supports this model. Electron micrographs of metaphase chromosomes are in perfect agreement with this model (Fig. 4.1).

Organisation of chromatin fibers

Nucleosome-Solenoid Model

It was proposed by Kornberg and Thomas in 1974. Here, the chromatin fibrils (nucleosome fibers) of 10 nm diameter, coiled to give rise to the chromatin fiber of ~ 30 nm. The chromatin fibers consist of discrete particles called nucleosomes which form a bead-like structure. The thread/string is made up of DNA molecules wound

around the beads, but does not pass through the beads. A bacterial enzyme, micrococcal nuclease, has the ability to cut DNA between nucleosomes but cannot cleave the DNA wound round the histone octamer. The stretch of DNA between two nucleosomes (DNA digested/cut by micrococcal nuclease) is called linker DNA or spacer DNA.

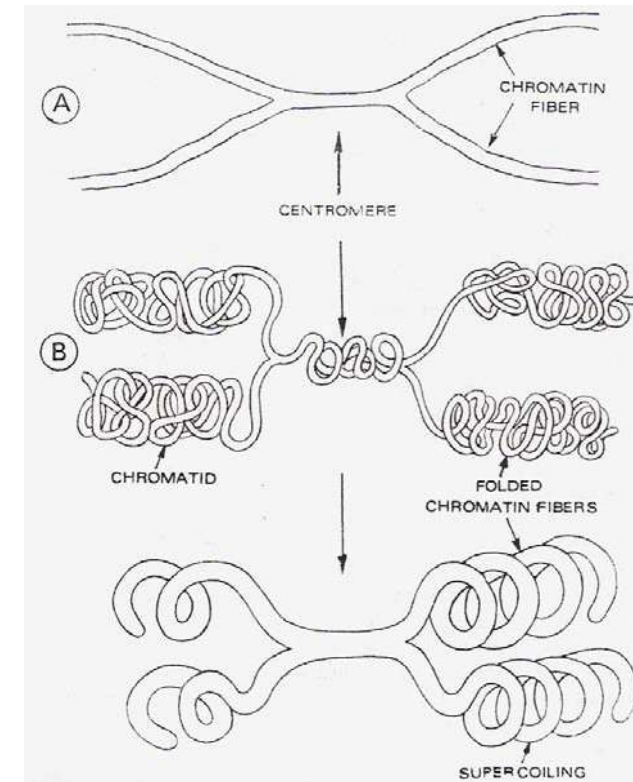


Fig. 4.1. Folded fiber model of chromosome organisation

Nucleosome

It consists of (1) Nucleosome core, (2) Linker DNA and (3) H₁ Histone

Nucleosome core

It consists of a histone octamer composed of two molecules of each of histones H₂a, H₂b, H₃ and H₄. About 200 bp DNA is associated with the histone octamer. These associated 200 bp DNA and the histone octamer together constitute the core particle or nucleosome core. The micrococcal nuclease produces nucleosome monomers by cleaving the DNA between the core particles, leaving atleast 146 bp DNA that associate with the protein octamer. Thus the core DNA (DNA bound to core particles) is actually 146 bp in length and it is nuclease resistant. The remaining part of the DNA is called linker DNA which joins neighbouring core particles of the chromatin fibers.

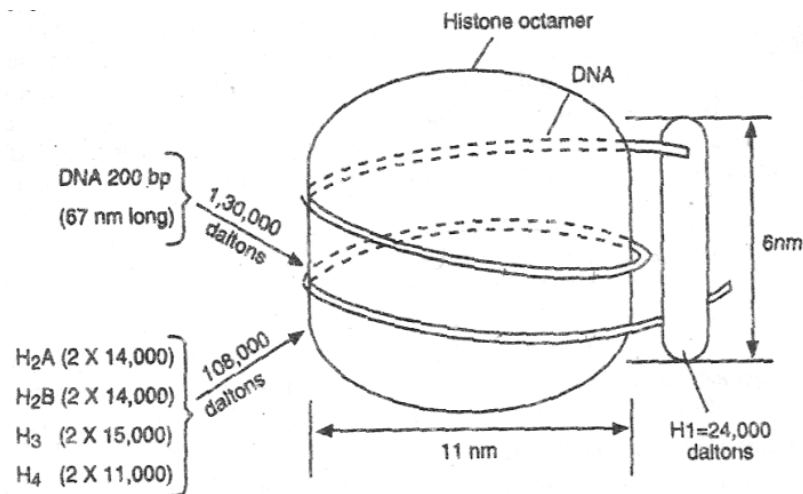


Fig. 4.2 Nucleosome

Linker DNA

The size of linker DNA varies from 8-114 bp depending on the species. This DNA forms the string part of the chromatin fiber. It can be cleaved by micrococcal

nuclease, i.e., nuclease susceptible. The linker DNA joins two neighbouring nucleosomes.

H₁ Histone

Each nucleosome contains on an average of one molecule of H₁ histone. It is not clear, that the distribution of H₁ histone is associated on the outside of each nucleosome core, and that one H₁ histone stabilizes about 166 bp long DNA. Other studies suggest that, the H₁ molecules are involved in stabilizing the supercoils of nucleosome chromatin fibers.

Nucleosome

The nucleosome is cylindrical in shape (ellipsoidal) with 11 nm in diameter and 6 nm high. Thus the circumference of a nucleosome is about 34 nm. Under some condition, the nucleosome pack together without any detectable linker DNA produce 10 nm thick chromatic fiber called nucleosome fiber. The nucleosome fiber then supercoil to give rise to 30 nm thick chromatin fiber seen in electron micrographs of metaphase chromosomes. The supercoiled nucleosome fiber is called solenoid. The length of 146 bp DNA associated with the core particle is sufficient to wound around the particle about 1.8 turns. The width of DNA double helix is 20 Å, thus it occupies 4 nm of the cylindrical length of the histone octamer/beads due to its two turns. One complete turn of the DNA double helix around the histone octamer involves 80 bp. Therefore, the two points (or the two bp) come close to one another on the core is the 80th bp (Fig. 4.2).

Thus the hierarchy of organization of DNA associated with protein can be summarized as follows.

1) DNA double helix has a diameter of 2 nm; single DNA molecule runs through the entire length of the chromosome (Fig. 4.3).

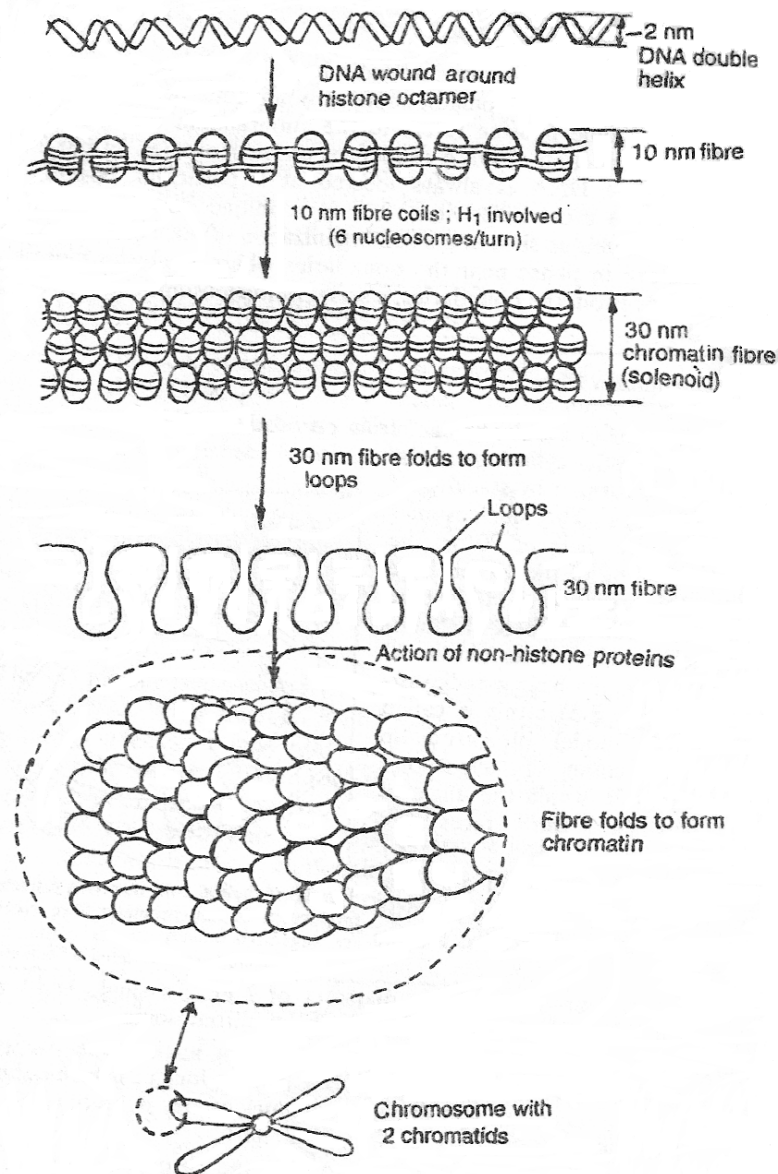


Fig. 4.3 Nucleosome solenoid model of chromatin fiber organisation

2) DNA is associated with histones to form nucleosome particles. One nucleosome contains 200 bp DNA (67 nm long); it forms the fiber of 10 nm diameter. The 10 nm fiber looks like a "string on beads". The shape of nucleosome is more or less a flat cylinder. The packing ratio of DNA is about six (67/10). The nucleosomes are filled at an angle of 200° of the fiber axis.

3) Coiling of the 10 nm fiber occurs in the presence of H₁ histone to give rise to 30 nm diameter chromatin fiber. The number of nucleosomes per turn is six which have a radial organization; this is called the solenoid model of chromatin structure. The packing ratio of DNA is about 40.

4) The folding of 30 nm fiber gives rise to a packing ratio of about 1000 in euchromatin.

5) In mitotic metaphase chromosomes, the packing ratio comes about 10,000.

Special Chromosomes

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

Lampbrush chromosomes

They have been the most studied in amphibian oocytes and observed during the prolonged diplotene stage. During diplotene, a lampbrush chromosome consists of two homologous chromosomes that are in contact with each other at several points (chiasmata). From each chromosome, a pair of loops emerge in the opposite directions, vertical to the main chromosomal axis. These lateral loops give these chromosomes the appearance of a lampbrush, which is the reason for their name 'Lampbrush chromosomes'. These chromosomes

are extremely long, in some cases being 800-1000 μ in length.

The main chromosomal axis consists of two sister chromatids of a chromosome, while the loops themselves represent single chromatids. The loops are made up of fully extended single DNA double helix since RNAase and protease digestion of loops generates DNAase susceptible 20 A° thick fibers. All the available evidence has been interpreted as follows: The pair of loops are produced due to uncoiling of the two chromatin fibers (hence the two sister chromatids) present in a highly coiled state in the chromosomes; this makes their DNA available for transcription (RNA synthesis). Thus each loop represent one chromatid of a chromosome and is composed of one DNA double helix (Fig. 4.4).

One end of each loop is markedly thinner (thin end) than the other end (thick end). There is extensive RNA synthesis at the thin end of loops, while there is little or no RNA synthesis at the thick end. The chromatin fiber of the chromomere is progressively uncoiled towards the thin end of a loop; the DNA in this region supports active synthesis but later becomes associated with RNA and markedly thicker.

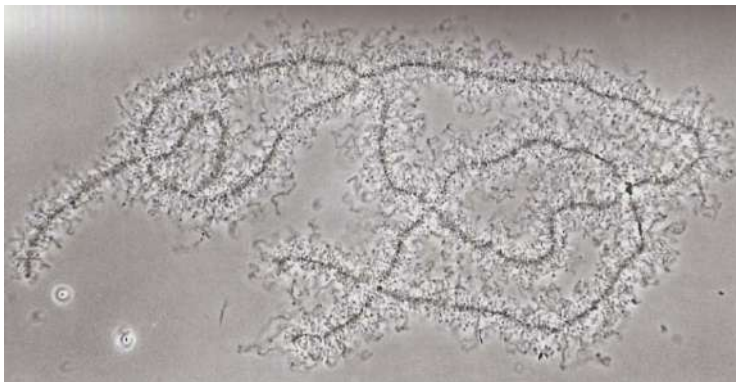


Fig. 4.4 Looped chromatin domains of a lampbrush Chromosomes isolated from an amphibian oocytes

The DNA (hence chromatin fiber) at the thick end of a loop is progressively withdrawn and reassembled into the chromomere. Thus the DNA axis of loops moves progressively from the thin end to the thick end of a loop.

Clearly, loops represent the sites of gene action (transcription) and the function of lampbrush chromosomes is to produce large numbers and quantities of proteins and RNA's stored in eggs.

Giant Chromosomes

These chromosomes are very long (upto 200 times their size during mitotic metaphase in the case of *Drosophila*) and hence very thick, hence they are known as giant chromosomes. They were first discovered by Balbiani in 1881 in dipteran salivary glands, giving them the commonly used name salivary gland chromosomes.

Giant chromosomes have also been discovered in suspensors of young embryos of many plants, but these do not show the bands so typical of salivary gland chromosomes. Homologous chromosomes pair to form the giant chromosomes (somatic pairing), so that the number of different giant chromosomes is only 'n'. In *Drosophila melanogaster* salivary glands, the giant chromosomes radiate as five long and one short arm from a deeply staining and more or less amorphous structure, called chromocentre (Fig. 4.5).

The chromocentre is formed by fusion of the centromeric regions of all the chromosomes and in males of the entire Y chromosome as well. Giant chromosomes are made up of several dark staining regions called bands separated by relatively light or non-staining interband regions. The total number of bands in the four giant chromosomes of *D. melanogaster* is estimated as more than 5000. Some of these bands are as thick as 0.5 μ , while some may be only 0.05 μ thick (visible only in electron micrographs).

Each giant chromosome is composed of numerous strands, each strand representing one chromatid. Therefore these chromosomes are also known as polytene chromosomes, and the condition is referred to as polyteny. The numerous strands of these chromosomes are produced due to repeated replication of the paired chromosomes without any nuclear or cell division (endo reduplication) so that the number of strands (chromatids) in a chromosome doubles often every round of DNA replication.

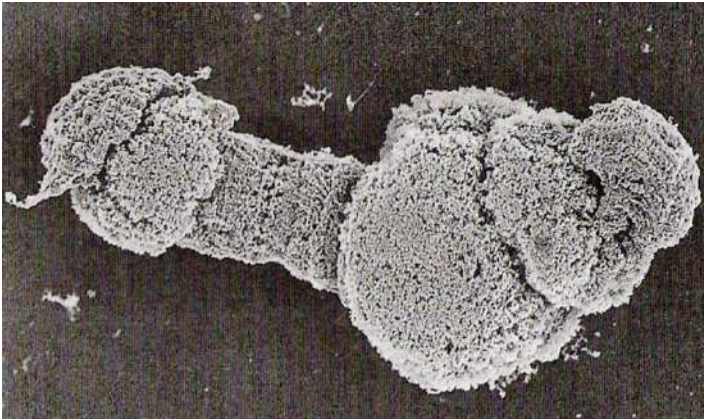


Fig.4.5 Giant chromosomes of *D. melanogaster* salivary glands

In *Drosophila*, the location of many genes is correlated with specific bands in the concerned chromosomes. It is believed that genes are normally located within bands, and that interband regions do have at least functional genes. The pattern of banding in different chromosomes and the location of specific bands within a chromosome is constant and highly characteristic. During certain stages of development, specific bands and interband regions associated with them greatly increase in diameter as compared to the rest of the chromosome; structures so produced are known as puffs or Balbiani rings. The puffs are believed to be produced due to uncoiling of chromatin fibers present in

the concerned chromomeres (forming the bands); these uncoiled chromatin fibers extend outside the chromosome in the form of loops. The puffs are sites of active RNA synthesis, the rate of RNA synthesis in the puffs is several fold higher than that in the other regions of giant chromosomes. Some hormones, eg., ecdysone, induce puffs in specific bands during specific period of development.

Since transcription (RNA synthesis based on DNA) is the first step in gene action, the sites of loops and puffs are indicative of activity of the genes located in those sites of the concerned chromosome.

Supernumerary chromosomes/Accessory chromosomes/Extra chromosomes

In many species, one to many extra chromosomes in addition to the normal somatic complement are found; these extra chromosomes are called as accessory chromosomes, B-chromosomes or supernumerary chromosomes.

These chromosomes are broadly similar to the normal somatic chromosomes in their morphology, but they have some peculiar functional aspects. One of the most important features of these chromosomes is that their number may vary considerably among individuals of the same species; in maize as many as 25-30 B chromosomes may become accumulated in some individual without affecting their phenotype. However, the presence of several B-chromosomes often leads to some reduction in vigour and fertility in maize.

Accessory chromosomes are generally smaller in size than the chromosomes of the normal somatic complement, but in some species they may be larger (e.g., in *Sciara*). They are believed to be genetically inactive, but they may not be completely devoid of genes.

In some animal species, they may arise due to fragmentation of heterochromatic Y chromosome. In maize, morphological features and pairing behaviour of

supernumeraries clearly show that they do not have any segment which is homologous to a segment of any chromosome of the normal somatic complement. They are relatively unstable. In many species they tend to be eliminated from somatic tissues due to lagging and non-disjunction and they frequently change their morphology through fragmentation. They are maintained in the population even though they do not appear to confer any selective advantage to the individuals carrying them.



CELL DIVISION I. MITOSIS

Cell is the structural and functional unit of all living organism and all cells originate through division of pre-existing cells. The division of chromosomes and cytoplasm of a cell into two cells is known as cell division. The cell that undergoes division is known as parent cell, while the cell derived from the division of a parent cell are known as daughter cells. The main function of cell division is to produce two daughter cell from a single parent cell so that repeated cell division generate a multitude of cells.

There are mainly two types of cell division (1) Mitosis and (2) Meiosis.

Mitosis

The term mitosis was first used by Flemming in 1882. In mitosis the two chromatids of each chromosome separate and move to the opposite poles of a cell. As a result the two daughter nuclei produced by mitotic division of a nucleus contain the same number and kind of chromosomes as the parent cell. Somatic cells of all the organism divide through mitosis.

Importance of mitosis

Development of embryos from zygotes involves repeated mitotic division in the initial stages, all the cell of young embryo undergo cell division. But as the development of embryo progresses, cell division becomes confined to some specific organs and tissues. In plants mitosis is confined to the meristematic tissues of root and shoot tip, young leaves, flower buds and cambium. In adult animals mitosis is confined with the production of blood cell, outer skin and gut epithelium. Healing of wounds and replacement of damaged organs in both plant and animals is based on mitotic cell division.

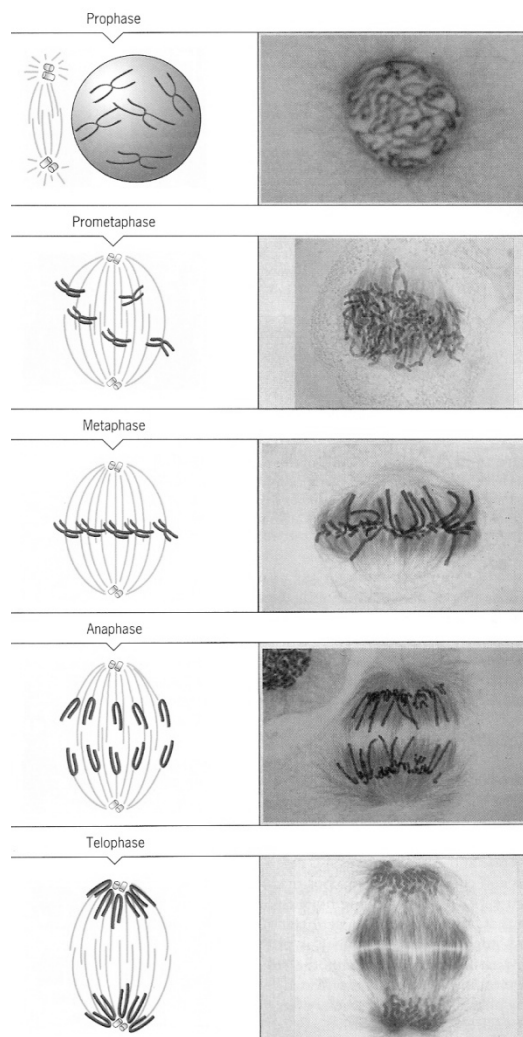


**Fig. 5.1 Different phases of mitotic cell division
in a plant cell with $2n=10$**

Stage of Mitosis

On the basis of changes in the morphology of nucleus and of chromosome the events in a mitotic cell division are grouped in five stages (Fig. 5.1).

1. Interphase
2. Prophase
3. Metaphase
4. Anaphase and
5. Telophase

Interphase

It is the stage of cell following the telophase stage of the previous mitotic division and before the onset of prophase of the next one. During interphase, chromosomes are fully extended and uncoiled so that they do not take up sufficient stain to be visible under light microscope.

Nuclei are surrounded by a well defined nuclear envelope and nucleoli are present. Since chromosomes do not undergo any cytological observable change during interphase, it is often not regarded as a stage of cell division and early cytologists signified this by referring to it as resting stage.

But interphase is not a resting phase since chromosome replication (based on DNA replication) and protein and RNA synthesis takes place during this stage. Interphase is the longest stage and of 24 hr, the interphase occupy up to 23 hr, while rest of the division or mitotic phase may take up only one hr.

The cell cycle

The life cycle of a cell begins with the formation of daughter cells at the end of telophase. These daughter cells are smaller than the parent cells. Moreover, their DNA content is just half of the parent nucleus. These cells synthesize new cytoplasmic and nuclear material till the total volume of each cell become four times of its original volume and the DNA content gets doubled by the replication of DNA. After this the cell is again ready to

undergo division (Fig. 5.2). It means the life-cycle of a cell involves two distinct phases:

1. Interphase (Non-dividing period)
2. Cell division or Mitosis (period of division)

Interphase

DNA replication occurs during the middle part of interphase. This provides the basis for classifying interphase into three substages:

1. G_1 (first gap)
2. S-phase (synthesis of DNA) and
3. G_2 (second gap)

G_1 phase

G_1 substage is the period between the beginning of interphase and the beginning of DNA synthesis (S phase). The duration of G_1 is highly variable. It may constitute 25-50% of the total interphase duration. In cells preparing for cell division, there is a marked protective and RNA synthesis during G_1 . The enzymes and substrates necessary for DNA synthesis during S phase are also synthesised during this phase. Ribosome production in nucleoli during G_1 is somehow necessary for the entry of cells into mitosis as inhibition of their production delays the entry of the cells into mitosis.

S-phase

S phase is the period of interphase during which DNA synthesis takes place as a result of which the chromosomes replicate. Each chromosome consists of a single chromatid during telophase and G_1 . DNA synthesis during S leads to the production of two sister chromatids from the single chromatid of each of the chromosomes in the nucleus. As a result, at the end of S phase each chromosome is composed of two morphologically and genetically identical sister chromatids. The duration of S phase is about 35-40% of the interphase time.

G_2 Phase

It is the period between termination of DNA synthesis and beginning of prophase of the next division. The duration of G_2 is generally between 1-4 hr, but in some cases it may be as long as 12 hr. There is considerable RNA and protein, particularly components of microtubules, synthesis during G_2 ; some proteins synthesised during this period are essential for the entry of cells into mitosis. Some messenger RNAs produced during G_2 are essential for the initiation of mitosis.

At the end of G_2 mitosis is initiated, consequently, a meristematic cell cyclically passes through mitosis, G_1 , S, G_2 and mitosis repeatedly; this phenomenon is termed as cell cycle.

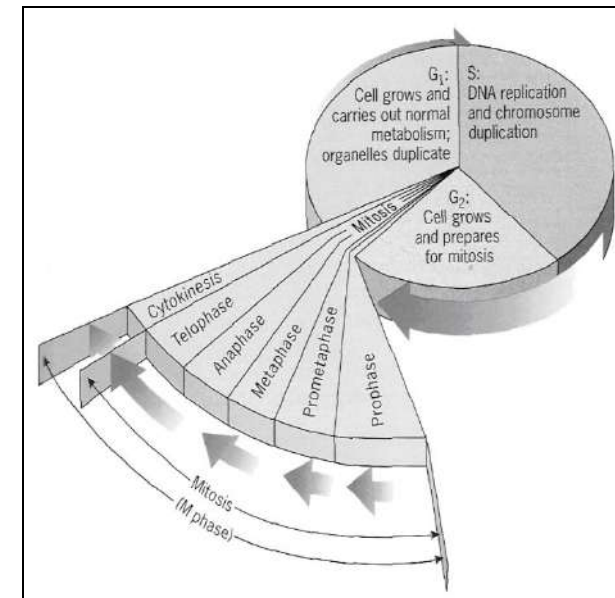


Fig. 5.2 Mitotic Cell Cycle. M. Mitotic phase, G_1 . First gap, S. DNA synthesis phase, and G_2 Second gap, G_1 , S and G_2 are the three sub stages of interphase

Prophase

The appearance of definite thread like structure in nuclei is most important event of prophase. In the beginning chromosomes appear as a loose ball of thin wool.

1. As prophase progresses, chromosomes become increasingly shorter and thicker due to increased condensation. By mid-prophase, the two chromatids of each chromosomes become visible. By the end of prophase, all the chromosomes become considerably shorter and thicker.
2. During prophase, nucleolus and nuclear membrane remains present.
3. Spindle apparatus is not yet organized. Chromosome condensation, i.e., decrease in length and accompanied increase in thickness is mainly due to coiling of chromosomes. The two sister chromatids of each chromosome are coiled in relation to each other; this is referred to as relational coiling.

Metaphase

At the end of prophase, four important events take place. 1. Disappearance of nucleolus 2. Breakdown of nuclear envelope and distribution of its component in the endoplasmic reticulum 3. Appearance of spindle apparatus and 4. Arrangement of the chromosome on a single plane called equatorial plate. At metaphase, centromeres of all the chromosomes lie on the equatorial plate, while their arms may extend outside this plate. The movement of chromosome to and their orientation on the equatorial plate is termed as metakinesis.

This is the shorter and the thickest i.e., more condensed state of chromosome. Absence of relational coiling between sister chromatid is noticed.

Anaphase

During anaphase the two sister chromatids of each chromosome separate and migrate towards the opposite poles of the cell.

Anaphase begins when the centromeres of chromosome appear to divide longitudinally so that the sister chromatids separate from each other and ends with the reaching of chromosome to the opposite poles. Centromere is the first portion of each of the chromosome to begin to move towards the poles and away from the equatorial plate after the two arms of each of the chromatids seem to be dangling from the centromere towards the equatorial plate.

Chromosome movement during anaphase is due to spindle fibres which are composed of microtubules.

Telophase

Anaphase ends and telophase begins when sister chromatids of all the chromosomes of a cell reaches the opposite poles. During telophase the following events takes place.

1. The chromosome uncoil so that they become very long and thin and appear to be coiled like a loose ball of fine thread.
2. Nucleolus reappears.
3. Nuclear membrane is organized around each group of chromosome.
4. At the end of telophase appears at the equatorial plate of the cell

Nucleolus dissociates at the end of prophase, nuclear materials are most likely distributed on to the chromosome.

Cytokinesis:

Ordinarily, cytokinesis is complete by the end of telophase. In plants, the separation of cytoplasm begins at the centre of the cell and gradually extends outwards

on each side in a plane perpendicular to the axis of the spindle.

The two daughter cells produced by mitosis contain one nucleus each, which has the same number of chromosomes as the parent cell. Each daughter cell enlarges in size till it becomes comparable to the parent cell.



CELL DIVISION II. MEIOSIS

Meiotic division of one diploid ($2n$) cell gives rise to four haploid (n) daughter cells. The reduction in chromosome number of the daughter cells is mainly due to a single 'S' phase during the premeiotic interphase, followed by two successive nuclear divisions.

In general, meiosis takes place during gamete formation and hence it is confined to reproductive cells only.

Significance of Meiosis

As a consequence of meiosis, gametes contain only half (n) of the somatic chromosome number ($2n$).

Therefore, union between one male and one female gamete during fertilization restores the chromosome number to the diploid ($2n$) state. Thus the chromosome number of a species remains constant from one generation to the next generation by sexual reproduction.

The various functions of meiosis may be summarized as follows:

1. Production of haploid (n) gametes so that fertilization restores the normal somatic ($2n$) chromosome number.
2. Segregation of the two alleles of a gene due to pairing between the two homologues of each chromosome and their separation at the first anaphase, since the two alleles of a gene are located in the two homologues of the same chromosomes.

- Independent segregation of the alleles of different genes located in separate chromosome due to independent orientation of different bivalents at the first metaphase.
- Recombination between linked genes due to crossing over during pachytene stage.
- Generation of genetic variation through segregation, independent assortment and recombination.

Stages of Meiosis

Prophase I (PI), Metaphase I (MI), Anaphase I (AI), Telophase I (TI) and Prophase II, Metaphase II, Anaphase II and Telophase II (Fig. 6.1).

First Meiotic Division

The most significant events of the first meiotic divisions are

- Pairing between homologous chromosomes
- Crossing over between them during prophase I and
- Separation of homologous chromosomes and their migration to the opposite poles of a cell during AI.

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 to as the reduction division. There are four stages in the first division:

- Prophase I (PI)
- Metaphase I (MI)
- Anaphase I (AI) and
- Telophase I (TI)

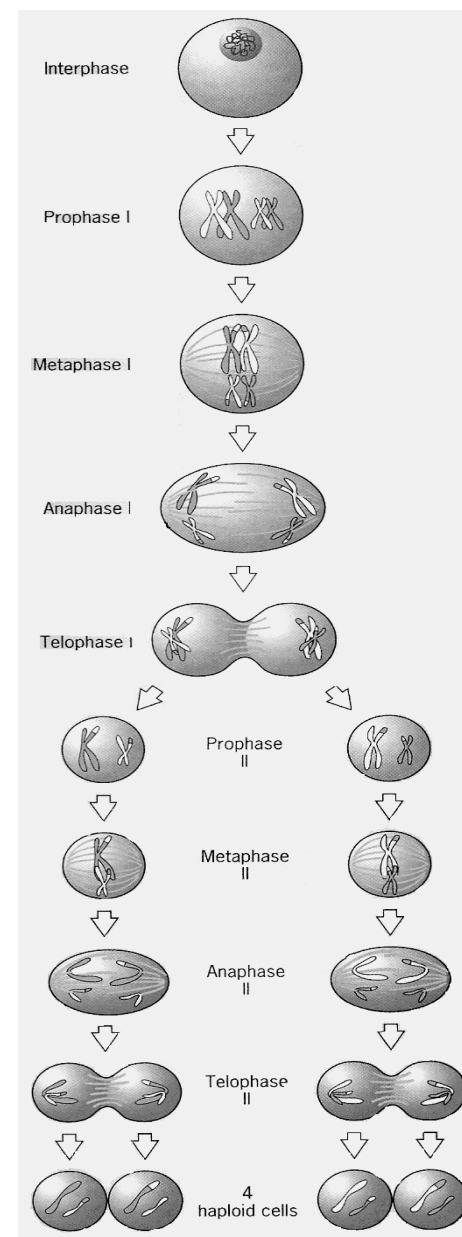


Fig.6.1 Meiotic cell division in a plant cell having 2n=8 chromosomes

Prophase I

This stage is divided into five substages.

1. Leptotene, 2. Zygotene, 3. Pachytene, 4. Diplotene and 5. Diakinesis (Fig. 6.2).

Leptotene:

During leptotene,

1. There is a marked increase in the nuclear volume.
2. There is chromosome condensation so that they become visible as fine threads; all the chromosomes in a nucleus appear wound around each other in a manner similar to a loose ball of knitting wool. Each chromosome consists of two chromatids since the chromosomes are already replicated during the premeiotic interphase.

In some species, chromosomes may show chromomeres distributed throughout the length at specific positions; chromomeres represent highly condensed regions of chromosomes.

3. There is RNA synthesis as a result of which nucleolus increases appreciably.
4. Proteins required for chromosome condensation are also synthesised.

Zygotene

The main events are,

1. Pairing between homologous chromosomes
2. Completion of replication of the remaining 0.3% DNA of each nucleus (this DNA does not replicate during the premeiotic interphase, and is referred to as Z-DNA or Zygotene – DNA)
3. Synthesis of a specific nuclear protein.
4. Development of the synaptenemal complex and

5. Progressive condensation of chromosomes.

Pairing between homologous chromosomes is extremely precise so that only the homologous segments of the two chromosomes pair with each other. Pairing between homologous chromosomes is often referred to as synapsis.

Pachytene

Pachytene begins when synapsis comes to an end and it ends, when the homologous chromosomes begin to move away from each other. The main events that occur during this stage are as follows:

1. There is further condensation of chromosomes so that chromosome pairs become shorter and thicker.
2. As a consequence of synapsis between homologous chromosomes, the number of observable chromosomes is only half of the somatic chromosome number i.e., only 'n'. This haploid number of chromosome pair commonly referred to as bivalents, becomes distinctly visible during this stage only.

Since, each of the two chromosomes of a bivalent consists of two sister chromatids, each bivalent has four chromatids or strands; therefore bivalents are said to be in the four strand or tetrad stage.

3. Chromosomes are easily recognizable during this stage.
4. The nucleolus is distinct and quite large.
5. Crossing over between homologous chromosomes takes place during this stage.

Diplotene

The main events taking place during diplotene are as follows:

1. Homologous chromosomes of each bivalent begin to move away from each other. As a consequence, the two

chromosomes constituting each bivalent become clearly visible.

2. The two homologues of each bivalent appear to be attached with each other at one or more points, these attachments are known as chiasma.

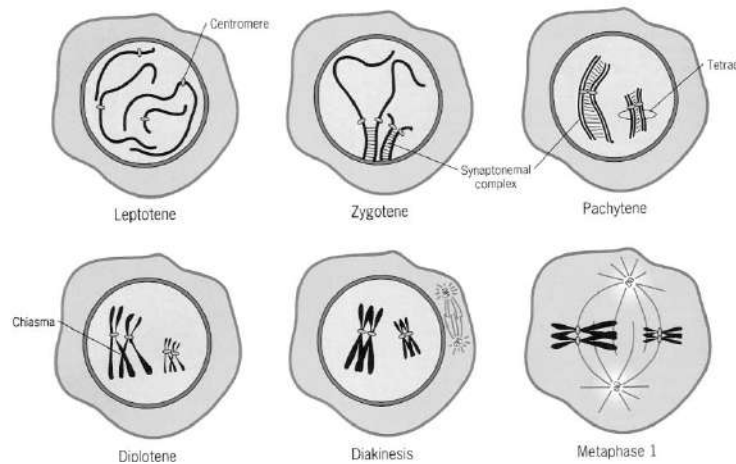


Fig.6.2. The stages of prophase I

3. As diplotene progresses, chiasmata slowly move towards the ends of the homologous chromosomes, this movement is referred to as chiasma terminalization. Chiasma terminalization occurs mainly due to the movement of homologous chromosomes away from each other.

4. Finally, there is further condensation of chromosomes so that they become progressively shorter and thicker.

Diakinesis

The end of chiasma terminalization marks the beginning of this stage. The main events of diakinesis is as follows:

1. Chiasma terminalization is completed first before this stage so that the two homologous chromosomes of each bivalent are now attached at or close to one or both the telomeres only.
2. Chromosomes become shorter and thicker due to further condensation.
3. Bivalents move away from each other and spread towards the periphery of cells, towards the end of diakinesis.
4. Nucleolus and nuclear envelope disappear.
5. The spindle apparatus is organised.

The bivalent now migrates to the equatorial plate of cells, this marks the end of diakinesis.

Metaphase I

During metaphase,

1. Bivalents are arranged at the metaphase plate.
2. The centromeres of the two homologous chromosomes of each bivalent lie on either side of the equatorial plate.
3. The part of the bivalents lying on the equatorial plate is the central part of each bivalent, which consists of the telomeres of the two homologous chromosomes attached almost end-to-end with each other.
4. The centromere of each chromosome is structurally divided into two, but these two parts functionally behave as a single centromere. Metaphase stage terminates as soon as homologous chromosomes begin to separate from each other and to migrate to the opposite poles of the cell.

Anaphase I

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

2. One chromosome from each bivalent begins to migrate to one pole, while the other migrates to the opposite pole.
3. At the end of anaphase I, one chromosome from each of the bivalent gather about one pole, while the second chromosome from each of the bivalents aggregate about the opposite pole.

As a result, the number of chromosomes at each of the two poles of a cell is exactly half (n) of the somatic chromosome number ($2n$) and each pole receives one homologue from each of the bivalents present in a cell. Thus the reduction in chromosome number is not only a quantitative but a qualitative one as well. Thus at the end of AI, each homologous chromosome pair present in somatic cells is effectively and precisely separated into two identical groups.

4. During this phase, the two sister chromatids of each chromosome seem to repel each other so that they are closely observable as constituents of each chromosome. The sister chromatids of a chromosome are, however, attached with each other at the centromere. This stage comes to an end when the homologous chromosomes reach the opposite pole.

Telophase I

During meiotic telophase I,

1. The chromosomes uncoil only partially, while a complete uncoiling takes place during meiotic telophase.
2. Nuclear envelope becomes organized around the two groups of chromosomes.
3. Nucleolus also reappears.

Cytokinesis

In many species, e.g., maize, the cytoplasm of each cell divides into two halves by the end of first telophase so that in each of the two halves of a cell a single haploid

nucleus is present. The two halves of each cell do not separate but they stay together; this two-celled structure is known as a Dyad.

Interphase

It is important to note that there is no DNA synthesis during this interphase. In addition, chromosomes do not uncoil any further than they already have at the end of telophase I.

Second Meiotic Division

During the second division of meiosis the two haploid nuclei of each dyad divide synchronously. The two sister chromatids of each chromosome separate and migrate to the opposite poles. As a result, the number of chromosomes in each of the haploid nuclei remains the same (i.e., haploid) at the end of this division. Therefore, the second division of meiosis is often referred to as equatorial division. The second meiotic division is also divided into four stages. 1. Prophase-II, 2. Metaphase-II, 3. Anaphase – II and 4. Telophase – II.

Prophase II

1. During this stage there is no relational coiling between sister chromatids as a result of which
2. The two sister chromatids of each chromosome are clearly visible.
3. The chromosomes are relatively much more condensed, hence they appear shorter and thicker than in mitosis of the same species.
4. There is further condensation of chromosomes so that they become thicker and shorter. At the end of this stage, nucleolus and nuclear envelope disappear and spindle apparatus is organized. Following this, chromosomes migrate to the equatorial plate.

Metaphase II

This stage begins when the chromosomes become arranged on the equatorial plate. During this stage:

1. Nucleolus and nuclear envelope are absent.
2. Spindle apparatus is present.
3. Centromeres of all the chromosomes are arranged in a single plane at the equatorial plate.
4. The two sister chromatids of each chromosome are distinctly separated from each other due to repulsion between them, but they remain attached at the centromere.
5. Chromosomes become more condensed, thicker and shorter.
6. This stage is relatively quite short in duration.

Anaphase II

In this stage centromeres of chromosomes observably divide longitudinally and the two sister chromatids of each chromosome begin to separate and move away to the opposite poles.

Telophase II

This stage begins when sister chromatids of chromosomes reach the opposite poles. During this stage:

1. The chromatids uncoil so that they assume the appearance of a loose ball of thread.
2. Nuclear envelope is reorganized from the elements of endoplasmic reticulum.
3. Nucleolus reappear.

Cytokinesis

By the end of telophase – II, the cytoplasm of each of the two cells of a dyad divides into two parts. As a

result, one parent cell produces four haploid daughter cells after the completion of the two meiotic division. The four daughter cells from a parent cell are present together and are known as a Tetrad. Cytokinesis occur at the end of telophase – II dividing each cell into four daughter cells each of which contains a single haploid nucleus.

Differences between mitosis and meiosis

Characteristic	Mitosis	Meiosis
Location	Somatic and reproductive tissues.	Reproductive tissues only
Shape of cells	Generally rectangular.	Generally spherical.
Daughter Cells	One cell produces daughter cells.	Four daughter cells are produced from one cell.
	Chromosome number of daughter cells is the same as that of the parent cell.	Chromosome number of daughter cells is half of that of the parent cell.
Genetic variation	Maintains genetic uniformity among daughter cells.	Generates genetic variability due to segregation and recombination.
S phase	Occurs in every interphase.	Occurs only in the premeiotic interphase; there is no S phase in the interphase following the first division of meiosis.
	The total DNA present in a nucleus is replicated during S phase.	About 0.3% of the DNA is not replicated during S phase.
Number of nuclear divisions	Each nucleus divides once after every S phase.	After the premeiotic S phase, nucleus undergoes two divisions.
<i>First Prophase</i>		
Prophase	Homologous chromosomes do not pair; 2n chromosome number can be seen.	Homologous chromosomes pair; 2n chromosome pair. As a result, only n chromosome pairs can be

Characteristic	Mitosis	Meiosis
	There is no crossing over between homologous chromosomes and chiasma cannot be seen.	seen. There is crossing over between homologous chromosomes, and chiasma are observable.
	There is no DNA synthesis.	About 0.3% of the DNA is synthesized during zygotene and pachytene.
<i>Second Prophase</i>		
	Chromosomes are 2n in number.	Chromosome number is only n.
	Sister chromatids show relational coiling. In early prophase, chromosomes are very long; later they become smaller due to progressive condensation.	Relational coiling between sister chromatids is absent. Chromosomes are highly condensed and small from the very beginning.
	It is quite long in duration.	It is of relatively short duration.
	<i>First metaphase</i>	
Metaphase	Each chromosome is unpaired.	Homologous chromosomes are paired to form bivalents.
	2n chromosomes are clearly seen.	The number of bivalents (chromosome pairs) is n.
Metaphase	Centromeres of all the chromosomes lie on the equatorial plate.	Centromeres of the chromosomes lie on the either side of equatorial plate.
	Each chromosome has two chromatids.	Each bivalent has four chromatids.
	<i>Second Metaphase</i>	
	The number of chromosomes is 2n.	The chromosome number is n.
	The two sister chromatids of a chromosome do not exhibit repulsion.	Repulsion between sister chromatids is clearly seen from prophase itself.

Characteristic	Mitosis	Meiosis
	Chromosomes are relatively longer	Chromosomes are much smaller.
<i>First Anaphase</i>		
	The two sister chromatids (of each chromosome) move to the opposite poles.	Homologous chromosomes from each bivalent move to the opposite poles.
	Centromere of each chromosome divides longitudinally.	Centromeres do not divide.
	<i>Second Anaphase</i>	
	Chromosome number is 2n.	Chromosome number is n.
	Generally a single nucleus divides in a cell.	In all the stages of second meiotic division, two nuclei divided in parent cell.
<i>First Telophase</i>		
Telophase and Cytokinesis	Chromosomes almost completely uncoil.	Chromosomes do not uncoil completely.
	In all the species, cytokinesis takes place	In some species, cytokinesis does not take place.
	Cytokinesis involves the formation of middle lamella and primary cell wall.	Cytokinesis consists of membrane formation and middle lamella is not deposited.
	Telophase and interphase are found in all species	In some species, first telophase and the following interphase are absent.
	Each cell produces two daughter cells.	Each cell gives rise to 4 haploid cell; the resulting structure is called tetrad.
	Daughter cells are 2n.	Daughter cells are n.
	Daughter cells generally differentiates as body cells.	Daughter cell generally differentiate into spores or gametes.

CHROMOSOMAL ABERRATIONS

I. STRUCTURAL ABERRATIONS

Occasionally, spontaneous variations in chromosome number or structure do arise in nature. These variations are called chromosome aberrations.

Chromosome aberrations may be grouped into two broad classes : (1) structural and (2) Numerical.

There are aberrations in the sequence or the kind of genes present in chromosomes.

There are four types (1) Deletion (2) Duplication (3) Inversion and (4) Translocation.

Deletion

Loss of a chromosome segment is known as deletion (or) deficiency. When the missing segment contains a telomere of the affected chromosome, it is called as terminal deletion. In most deficiencies, however, the missing segment does not contain a telomere. Such deletions are known as interstitial or intercalary (Fig. 7.1).

The deleted portion without the centromere will have no power of movement in anaphase and will be lost. The portion of the chromosome with the centromere functions as a deficient chromosome.

If the genes of a normal chromosome are represented as ABCDEF and if two genes D and E are lost, the deficient chromosome will be ABCF.

In individual heterozygote for a deficiency, the deficient chromosome meets the normal one in synapsis and since the pairing occurs only between strictly homologous segments, the portion of the normal chromosome which contains the segments missing in the chromosome with an interstitial deficiency forms an unpaired loop.

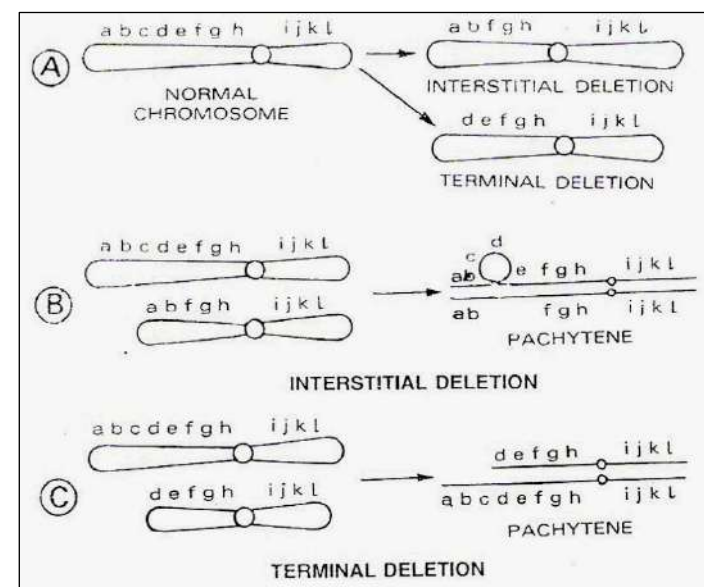


Fig. 7.1 Deletional aberration

A summary of different structural changes in chromosomes

Term	Type of aberration	Remarks
Deletion	Loss of a segment of a chromosome.	Produces pseudo-dominance for recessive alleles located in the lost segment.
1. Terminal deletion	Lost segment includes telomere. A segment between telomere and centromere is lost.	Believed to be rare.
2. Interstitial deletion	A segment between telomere and centromere is lost	Most deletions are of this type.
Duplication (Repeat)	A chromosome segment present in more than two copies in the same nucleus.	According to Ohno (1970), duplications are the source of all new genes, and thus the basis of organic evolution.

1. Tandem duplication	The additional chromosome segment located just after the normal segment, gene sequence being the same.	
2. Reverse duplication	Same as above but the gene sequence of the additional segment is inverted.	
3. Displaced duplication	The additional segment is located in the same chromosome but away from the normal segment.	
4. Translocation duplication	The additional segment located in a non homologous chromosome.	
Inversion	A chromosome segment contains genes in a sequence which is reverse of the normal gene sequence	Produces characteristics dicentric bridge and eccentric fragments at AI in inversion heterozygotes.
1. Paracentric inversion	The inverted segment does not contain centromere.	
2. Pericentric inversion	The inverted segment contains centromere.	May change centromere location.
Translocation	Chromosome segment(s) integrated into non-homologous chromosome(s).	The main mechanism for changes in chromosome number and morphology in nature.
1. Simple translocation (shift)	A segment of a chromosome integrated into a nonhomologous chromosome.	May lead to translocation duplication.
2. Reciprocal translocation (Exchange)	A segment of chromosome integrated into a nonhomologous chromosome from which a segment is integrated into the first one.	Characteristic ring or chain of four or more chromosomes at MI of translocation heterozygotes

Since a deficiency involves the loss of genes, they often have deleterious effects on the individuals in which they occur. Even deficiencies of a very few genes existing

in a heterozygous condition produce detectable morphological abnormalities. Deficiency homozygotes (i.e., individuals in which certain genes are wholly missing) are mostly lethal.

E.g. 1. The first deficiency to be recorded is the “notched wing” type in *Drosophila*, discovered by Bridges.

2. Terminal deficiency is recorded in Maize.

Duplication

The presence of an additional chromosome segment, as compared to the normally present in a nucleus is known as duplication. In a diploid organism presence of a chromosome segment in more than two copies is called duplication. The extra chromosome segment may be located immediately after the normal segment in precisely the same orientation (i.e., having the same gene sequence), producing tandem duplication. When the gene sequence in the extra segment of a tandem duplication is in the reverse order i.e., is inverted (eg., e d c in the place of c d e) it is known as reverse tandem duplication. In some cases, the extra segment may be located in the same chromosome, but away from the normal segment, such cases are termed as displaced duplications. In translocation duplications, the additional chromosome segment is located into a non homologous chromosome. i.e., the extra segment is translocated into a non homologous chromosome (Fig. 7.2).

Cytology

Relatively larger duplications are detachable due to the presence of loops formed by the duplicated segment during pachytene stage. In the case of terminal reverse tandem repeats the affected end of the duplicated chromosome may fold-back and may pair (1) with the additional segment of the chromosome or (2) with the corresponding segment of the homologous chromosome.

Genetic effect

In general duplications do not produce drastic consequences as deletions in terms of both phenotype and survival. But some duplications may have distinct phenotypic effects and may be regarded as a mutant allele e.g., Bar duplication in *Drosophila*.

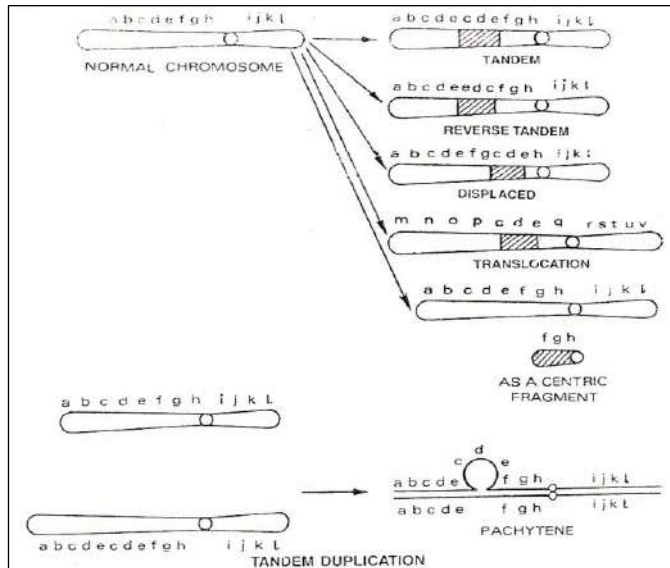


Fig.7.2 Duplicate aberration

Inversion

When a segment of a chromosome is oriented in the reverse direction, such segment is said to be inverted and the phenomenon is termed as inversion.

Obviously, the gene sequence in an inverted segment is exactly the opposite of that in its normal homologous (non-inverted) segment.

They are classified into two groups on the basis of the presence or absence of centromere within the inverted segment (Fig. 7.3).

Paracentric inversion

Here the centromere is located outside the inverted segment.

Pericentric inversion

Here the centromere is within the inverted segment.

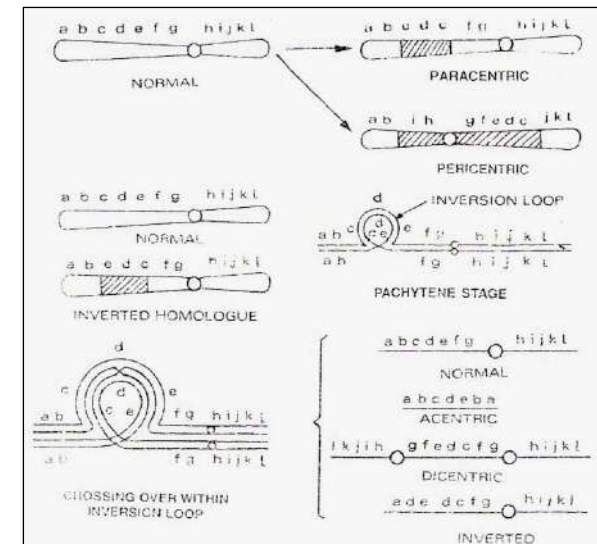


Fig. 7.3 Inversional aberration

Inversion loops produced during synapsis in inversion heterozygotes are similar in both the cases. However, the consequence of crossing over within the inversion loops are quite different in the two types of inversions.

Further, paracentric inversions do not alter the morphology of the affected chromosomes, while pericentric inversions may alter the centromeric position.

Translocation

Integration of a chromosome segment into a non homologous chromosome is known as translocation. In a simple translocation, terminal segment (telomere) of a chromosome is integrated at one end of a non homologous chromosome.

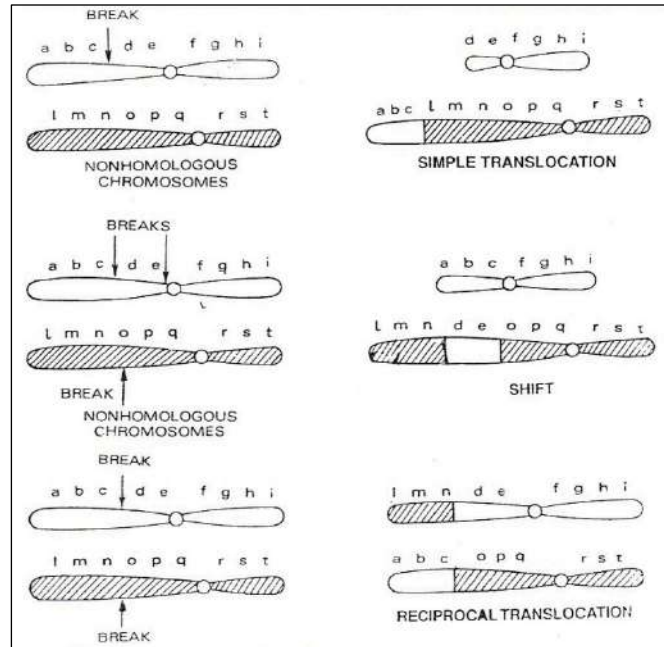


Fig.7.4. Translocational aberration

In the case of shift, (intercalary) the segment (not including telomere) of a chromosome is integrated in an intercalary position of a non homologous chromosome. Such translocations are known in natural populations of *Drosophila*, *Neurospora*, etc.

The most common and as a consequence, the most widely studied translocations are reciprocal translocation.

A reciprocal translocation is produced when two non homologous chromosomes exchange segments i.e., a segment from a chromosome is translocated into a non

homologous chromosome from which a segment is reciprocally transferred to the first chromosome (Fig. 7.4).

Genetic effects

1. The shift, in *Drosophila* the concerned translocation produced phenotypic effect as well as lethality.
2. Alteration in the genetic maps of the affected chromosomes; genes present in the translocated segments are linked with the genes present in those chromosomes into which they are now integrated.

Chromosome pairing

Translocation homozygote

In this, the two homologues of each of the two translocated chromosome are identical in their gene content. As a result they form normal bivalents.

Translocation heterozygote

In this, one member from each of the two chromosome pair is involved in reciprocal translocation while the remaining chromosome of the two concerned pair is normal. Chromosome pairing in a translocation (reciprocal) heterozygote produces a peculiar (+) shaped pachytene configuration due to point-per-point precision during pairing. The point of the 'cross (+)' i.e., the point where the four chromosome converge at pachytene represents the break points in the two reciprocally translocated chromosomes.



CHROMOSOMAL ABERRATIONS – II. NUMERICAL CHANGES

In somatic cells of a diploid organism, two copies of the same genome are present i.e., ($2n = 2x$), while their gametes contain a single genome i.e., ($n = x$).

A deviation from the diploid state ($2n = 2x$) represents a numerical chromosome aberration which often is referred to as heteroploidy; individuals possessing the variant chromosome numbers are known as heteroploid.

There are two categories of heteroploids:

(1) Aneuploids and (2) Euploids.

Aneuploidy

A loss or gain of one or few chromosomes as compared to the somatic chromosome number of a species is known as aneuploidy.

In somatic cells of all plants and animal species, each chromosome of a genome is represented by two homologous chromosomes. This condition is called disomic.

The terminology for aneuploid variation has been developed with reference to the disomic state for each of the chromosome of a genome. Thus the deficit of a chromosome from the somatic number of a species ($2n - 1$) is known as monosomic condition, while the absence of a chromosome pair ($2n - 2$) is termed as nullisomic condition (one copy of one chromosome pair of the genome present). However, if the two missing chromosomes are non homologous ($2n - 1 - 1$), they do not represent the loss of a chromosome pair, such a condition is known as double monosomic state.

A summary of various changes in chromosome number

Term	Type of change	Symbol
Heteroploid A) Aneuploid	Change from the $2x$ state. One or a few chromosomes extra or missing from $2n$.	$2n \pm \text{few}$
Nullisomic	One chromosome pair missing,	$2n - 2$
Monosomic	One chromosome missing,	$2n - 1$
Double monosomic	Two nonhomologous (each from a different pair) chromosomes missing.	$2n - 1 - 1$
Trisomic	One chromosome extra.	$2n + 1$
Double trisomic	Two nonhomologous (each from a different pair) chromosomes extra	$2n + 1 + 1$
Tetrasomic	One chromosome pair extra.	$2n + 2$
B) Euploid	Number of genomes different from two	
Monoploid	Only one genome present.	x
Haploid	Gametic chromosome number of the concerned species present.	n
1) Autopolyploid	More than two copies of the same genome present.	
Autotriploid	Three copies of the same genome.	$3x$
Autotetraploid	Four copies of the same genome.	$4x$
Autopentaploid	Five copies of the same genome.	$5x$
Autohexaploid	Six copies of the same genome.	$6x$
Autooctaploid	Eight copies of the same genome.	$8x$
2) Allopolyploid	Two or more distinct genomes; generally each genome has two copies.	
Allotetraploid	Two distinct genomes; each has two copies.	$(2X_1 + 2X_2)$
Allohexaploid	Three distinct genomes; each has two copies.	$(2X_1 + 2X_2 + 2X_3)$
Alloctaploid	Four distinct genomes; each has two copies	$(2X_1 + 2X_2 + 2X_3 + 2X_4)$

Individuals possessing one extra chromosome in excess of the $2n$ number i.e., $2n + 1$ are termed as trisomics while those having an additional chromosome pair ($2n + 2$) are referred to as tetrasomics. But if the two additional chromosomes are non-homologous ($2n + 1 + 1$), such individuals are known as double trisomics.

Nullisomy ($2n - 2$)

The absence of one chromosome pair has strong and deleterious effect on the organism so that nullisomic are viable only in polyploid eg. hexaploid bread wheat and oat. They are commonly obtained afresh in each generation from selfed progenies. Monosomic plants occur in a low frequency i.e., 3%. Consequently they are of relatively little value in practical aneuploid programme.

Cytology

Chromosome pairing in nullisomics is ordinarily normal and at MI $n-1$ bivalents are formed. These bivalents disjoin normally to produce all $n-1$ gametes. Therefore selfing in those nullisomics that are partially fertile produces only nullisome progeny. But in certain nullisomics regular pairing is disturbed and multivalent associations are present at MI, e.g., in nullisome 5B of wheat. This is because chromosome 5B of wheat has the normal allele of gene *Ph* that suppresses pairing between homeologous chromosome. In nulli - 5B plant the gene is absent so that they show homeologous chromosome pairing leading to multivalent association at MI.

Homologous chromosomes

Partially homologous chromosomes, generally show reduced or lack of pairing.

Monosomy ($2n - 1$)

The loss of one whole chromosome creates a major genetic imbalance which is generally not tolerated by diploid species. Monosomics of polyploid species e.g.

wheat, oats, cotton and tobacco are fully viable and a complete set of monosomics is available in these crops.

Cytology

Since in a monosomic, one chromosome of the haploid complement does not have a pair, it remains unpaired during meiosis and is present as a univalent (unpaired chromosome) at MI, the remaining chromosome of the diploid complement ordinarily pair normally and produce $n-1$ bivalents at MI (Fig. 8.1). During AI, the $n-1$ bivalents disjoin normally, while the single univalent may follow any one of the following courses (1) move to one of the two poles, (2) lag behind at the metaphase plate and ultimately be lost or (3) divided into two chromatids, which move to the opposite poles. As a consequence of the irregular behaviour of the univalent during AI and AII, monosomics produce $n-1$ gametes in excess of the expected 50%. In wheat, about 75% of the female gametes in monosomic plants are $n - 1$.

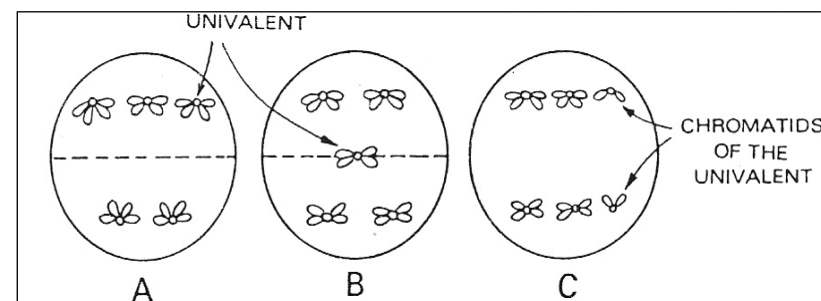


Fig. 8.1. Behaviour of univalent at AI in a monosomic

Univalent shift

When monosomics are crossed to normal plants during monosomic analysis, it is expected that in the progeny all monosomic plants will be monosomic for the same chromosome as that of the monosomic parents involved in the cross. But sometimes, some monosomic progeny plants are monosomic for a different

chromosome as compared to the monosomic parent of the cross; this phenomenon is known as Univalent shift.

Turner syndrome

The Turner syndrome was first described by H.H. Turner in 1938; it is associated with monosomy for X chromosome and has a frequency of about 1 per 2,500 live female births. More than 90 per cent of XO zygotes abort spontaneously. Adults with Turner syndrome are females who have virtually no ovaries and whose secondary sex characters are poorly developed. Such females have short stature, low-set ears, webbed neck and a shield-like chest but generally do not show mental retardation. Some persons suffering from Turner syndrome are mosaics having XO and XX cells in different parts of their body. Such individuals show fewer anomalies than XO females, and sometimes may even be fertile. Some other Turner syndrome individuals have one normal X and one deleted X in which the short arm is missing. It may be noted that persons having one normal X and one deleted X from which the long arm is missing do not show as many symptoms of Turner syndrome as do those in which the short arm of one X chromosome is missing. Some other patients of Turner syndrome contain a Y chromosome; such persons are generally mosaics having XO and XY (normal Y chromosome) cells in different tissues. XY persons having a deleted Y chromosome (lacking the short arm) are normal females with rudimentary ovaries. These findings clearly show that male-determining genes are present in the short arm of Y chromosome, while those preventing Turner syndrome are located both in the long arm of Y and the short arm of X chromosomes.

Trisomy ($2n + 1$)

In general an extra chromosome does not produce so striking an effect as a missing one. In wheat, $2n = 43$ occur but they are nearly indistinguishable from normal

plants (i.e. disomics with $2n = 42$). Blakslee has isolated 12 different trisomics in *Datura stramonium* ($2n = 24$), each having in triplicate a single different chromosome of a normal set. These trisomics differ morphologically from one another and from diploid form. Trisomics are generally grouped into three categories (Fig. 8.2). 1) Primary trisomics; the additional chromosome is an unaltered number of the haploid chromosome complement of the species. 2) Secondary trisomics; the additional chromosome is an isochromosome i.e., a Metacentric chromosome in which the two arms are identical in gene content. 3) Tertiary trisomics; the additional chromosome is a translocated chromosome.

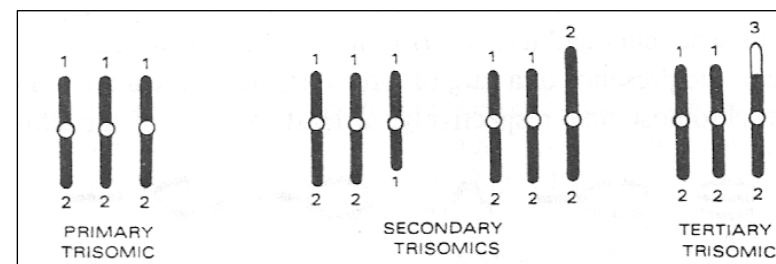


Fig. 8.2. The chromosome constitutions of primary, secondary and tertiary trisomics

Although, trisomics give rise to two kinds of gametes, one kind with n chromosome and other kind with $n + 1$ chromosome they tend to be somewhat more stable than monosomics.

Klinefelter's syndrome

Another trisomic condition for the sex chromosome (XXY) produces Klinefelter's syndrome, first described by H.F. Klinefelter in 1942. Such individuals are phenotypically males but with some tendency toward femaleness, particularly in secondary sex characteristics. They may have enlarged breasts, less body hair, small testes and small prostate glands.

XXY is the most common karyotype found in patients of this syndrome, but whenever more than one X is present with a Y chromosome this syndrome is produced. Other karyotypes found in Klinefelter patients are, XXYY, XXXY, XXXYY, XXXXY, XXXXYY and XXXXXY. Patients having more than two X chromosomes show mental retardation in addition to the other symptoms of syndrome. Studies of Turner and Klinefelter syndromes made it obvious that in human beings Y chromosome determines maleness.

Tetrasomy (2n + 2)

One chromosome of the complement is present in four copies i.e., has four homologues. In general, gain of an chromosome pair is more deleterious than that of one chromosome lost. Tetrasomics are viable in polyploid species e.g., wheat, where they have been used in genetic studies particularly in conjugation with nullisomy. Those chromosomes which compensate for the deficit of each other in nullisomic – tetrasomic ($2n - 2 + 2$) condition are termed as homeologous chromosome.

Cytology

The tetrasomic plant produce an appreciable frequency of n gametes and 2n and 2n + 1 zygotes in addition to 2n + 2 zygotes.

Production and uses of Aneuploid

1. They may be obtained by meiotic irregularities like non-disjunction or lagging of one chromosome, occur spontaneously in low frequencies and produce n+1 and n-1 gametes. When such gamete unite with normal (n) gametes, 2n+1 and 2n-1 individuals are obtained.
2. Triploid plants are best sources of aneuploids as they produce a high frequency of aneuploid gametes.
3. Many univalents are regularly present at MI of asynaptic and desynaptic plants. Consequently, most of their gametes are aneuploids.

4. The ring of four in translocation heterozygotes may disjoin 3:1; aneuploid gametes thus produced would generate trisomics and monosomics.
5. Progeny from a cross between tetrasomic ($2n+2$) and disomic plants show a high frequency of trisomics and monosomics.

Uses

1. They have been used to determine the phenotypic effects of loss or gain of different chromosome.
2. They have been used to produce chromosome substitution lines which yield information on the effects of different chromosome of a variety on various plant characteristics when they are placed singly on the same genetic background.
3. They are used to produce alien addition and alien substitution lines which are useful in gene transfer from one species into another.
4. It prevents the location of a gene as well as a linkage group onto a specific chromosome.
5. They are useful in identifying the chromosome involved in translocation.

Euploidy

Euploids have one or more complete genomes which may be identical with or distinct from each other. The most common condition of Euploid is the diploid state, in which two copies of the same genome are present in a cell; it is represented as 2x.

Euploid variations are designated with reference to the diploid (2x) state. These variations may be grouped into two broad categories (1) Monoploids, including haploids and (2) Polyploids.

Monoploidy and Haploidy

Monoploidy denotes the presence of a single copy of a single genome, and is represented by x .

Haploidy represents the gametic chromosome number of a species irrespective of whether it is a diploid or a polyploid species and is represented by n .

Euhaploids derived from diploid species are known as monoploids, while those obtained from polyploid species are called polyhaploids.

In haploids, none of the chromosome has two homologous, hence all chromosomes are unpaired during meiosis and are present as n univalents at MI. During AI, usually a random sample from the n univalents passes to one pole, while the remaining univalents move to the opposite pole. But, sometimes all the n chromosomes may pass to the same pole and thereby, give rise to normal (n) viable gametes. Union between such rare male and female gametes produces normal $2n$ progeny. Consequently, haploids are highly sterile, but they do produce few $2n$ progeny.

Production

Haploid plants have to be produced afresh since they cannot be maintained except through clonal propagation.

1. Haploids are spontaneously produced in many plants in low frequencies. Eg : Maize
2. Haploid plants are generally developed from unfertilized egg cells. But in some cases, they have been reported to develop from sperms (parthenogenesis).
3. The frequency of haploid production may be improved through delayed pollination, pollination with incompatible pollen, temperature shock and X-ray treatment.

4. Colchicine treatment of the shoot apical meristem of normal disomic plants is reported to induce a same proportion of haploid plants in same species. Eg. : Sorghum.
5. A more reliable technique for haploid production is through anther or pollen culture. Pollen grains of many species e.g., *Brassica*, rice, tobacco, *Triticale*, etc., when cultured in appropriate stage divide to yield haploid plants in appreciably large numbers.

In general, haploids (n) show a marked reduction in vigour and size of various vegetative and reproductive organs as compared to normal disomic ($2n$) plants. For e.g., haploids of tobacco show smaller leaves, flowers, seeds, stomata, pollen grains and over-all plant size.

But some haploids ($2x$) of potato are as vigorous and yield as much as the normal disomic ($4x$) strains from which they are derived.

Uses

1. Chromosome doubling of haploids produces homozygous disomic ($2n$) plants in first two years as compared to 6-7 generations of selfing.
2. Selection at the haploid level may be more efficient than that at the diploid level. E.g. Lucerne.

Polyploidy

Presence of more than two genomes in an individual is known as polyploidy. If all the genomes present in an individual are identical, it is called as Autopolyploids.

But in allopolyploidy, two or more distinct genomes are present.

Autopolyploidy

Autopolyploids may have three (triploid-AAA), four (tetraploid-AAAA), five (pentaploid-AAAAA), six (hexaploid-

AAAAAA), seven (heptaploid-AAAAAAA), eight (Octoploid-AAAAAAA) or more copies of the same genome.

Cytology

1. Autopolyploids are characterised by the presence of multivalents configuration by pairing among more than two homologous chromosomes at MI. In triploids, the three homologues for each chromosome form either a trivalent or a bivalent and a univalent. During AI, disjunction of bivalent and distribution of univalents is irregular resulting in production of aneuploid gametes. It produces trisomic, double trisomic etc. Most of the triploids are unbalanced and inviable as a result of which they are sterile. Eg : Banana, watermelon etc.
2. In Autotetraploids, the four homologous of each chromosome may associate as a quadrivalent or a trivalent and a univalent or two bivalents at MI. The disjunction is irregular, while quadrivalents may often dissociate as 2:2. As a result the variable proportion of the gametes produced by tetraploids are unbalanced and hence inviable which accounts for their variable fertility.

Production

They are produced through chromosome doubling of a species.

- a. Occasionally unreduced ($2n$) gametes are produced spontaneously; union between such gametes would yield tetraploid zygotes.
- b. Sometimes, chromosome doubling may occur in somatic cells giving rise to tetraploid buds.
- c. Heat or cold treatments and irradiation of seeds of plants with X-rays or gamma rays may produce polyploid progeny in low frequency.

- d. A variable frequency of plants regenerated from tissue cultures of many species, e.g., *Nicotiana*, *Datura*, Rice etc., are polyploids.
- e. Some chemicals, such as acenaphthene, 8-hydroxy-quinoline and nitrous oxide, induce chromosome doubling.
- f. The most efficient method of chromosome doubling is the treatment of seeds, seedlings or shoot-tip with colchicine.

Colchicine is an alkaloid extracted from the bulbs of *colchicum autumnale*. It interferes with the development of spindle apparatus, as a consequence of which the sister chromatids of chromosomes are unable to migrate to the opposite poles during anaphase. Therefore, all the chromatids are included in the same restitution nucleus leading to chromosome doubling. Colchicine is generally applied as a 0.2% aqueous solution or as a mixture with lanolin paste. The specific concentration may vary considerably with plant species.

Application in Crop Improvement

- (i) Triploid watermelons are produced by crossing tetraploid ($4x$, female) and diploid ($2x$, male) lines. These watermelons produce only rudimentary seeds which are not objectionable when chewed. They have been commercially grown in Japan.
- (ii) Triploid sugarbeets produce larger roots and more sugar per unit area than diploids. It has been grown in Europe.
- (iii) Autotetraploid varieties of some forage crop have been successful. E.g., Tetraploid red clover and rye grass.
- (iv) Many ornamentals are autotetraploids. They generally have large flowers and larger flowering duration than diploids.

- (v) The only autotetraploid commercial crop variety is rye. E.g. Double steel and Tetra petkus.

Allopolyploidy

Allopolyploids contain two or more distinct genomes, obviously, derived from different species; almost all natural allopolyploids have two copies of each of the genomes they contain. They show regular bivalents at MI as if they were essentially diploid. In view of these fact they are known as amphidiploids.

Origin

Natural allopolyploids most likely originated through chromosome doubling of F₁ hybrids produced through chance natural hybridization between two distinct species of the same genus or from different genera. Such F₁'s would be generally sterile since the chromosomes of their two parental species are expected to be differentiated and are not likely to pair during meiosis. Chromosome doubling may have occurred in somatic cells of F₁ hybrids due to irregular mitosis; this would give rise to doubled sectors either in the apical meristem or in axillary buds (later producing branches). Sexual progeny from such branches would be amphidiploid, i.e., having two copies of each genome (as compared to only one in the F₁). Alternatively, irregular meiosis may lead to the production of unreduced gametes which may unite to produce amphidiploid progeny. Unreduced gametes may be produced due to the inclusion of all the chromosomes in a single nucleus at telophase I, following a complete failure of chromosome pairing in above F₁. Both the pathways are known to occur in experimental populations. For example, in the F₁ from *Primula verticillata* x *P. floribunda* some branches were highly fertile, while the F₁ was highly sterile; these fertile branches invariably had doubled chromosome number. The amphidiploid thus obtained was designated as a new species, *P. kewensis*. On the other hand, Raphanobrassica, the allopolyploid from the cross

Brassica oleraceae (cabbage) x *Raphanus sativus* (radish) was first obtained by the Russian geneticist Karpechenko in 1927 through fusion of spontaneously produced unreduced gametes.

Synthesis

Experimental production of allopolyploids is achieved through chromosome doubling with the help of colchicine (or some other agent) of distant hybrids (hybrids) between two distinct species); such allopolyploids are often known as synthetic allopolyploids. Thus synthesis of allopolyploids involves two steps: (1) production of F₁ hybrids by crossing two distinct species and (2) chromosome doubling of such F₁ hybrids. For example, Triticale (Fig. 8.3), a synthetic allopolyploid, is produced by crossing tetraploid wheat, *Triticum turgidum*, AABB (hexaploid wheat, *T. aestivum*, AABBDD, may also be used) with rye (*Secale cereale*, RR). The F₁ from this cross has 21 chromosomes (ABR) and is completely sterile due to a lack of pairing among the chromosomes of A, B and R genomes. Doubling of the chromosome number of this hybrid produces *Triticale hexaploide*, the allohexaploid Triticale (AABBRR), which is highly fertile. Use of *T. aestivum* in place of *T. turgidum* in this scheme yields octaploid Triticale (AABBDDRR).

Allopolyploids may also be developed through the recently developed technique of somatic hybridization. In this technique, walls of somatic cells (usually leaf mesophyll cells, since they are uniformly 2C) of the concerned species are enzymatically removed leaving virtually naked cells, called protoplasts, enclosed by plasma lemma only. Protoplasts of different species are mixed in equal proportions, and they are induced to fuse by an agent, polyethylene glycol (PEG). PEG is nonspecific in that it induces fusion between protoplasts of the same as well as of different species. The hybrid protoplasts (derived from fusion of one protoplast each of the two species) are selectively induced to divide on a specifically

devised culture medium to yield calli, which are later induced to regenerate plantlets.

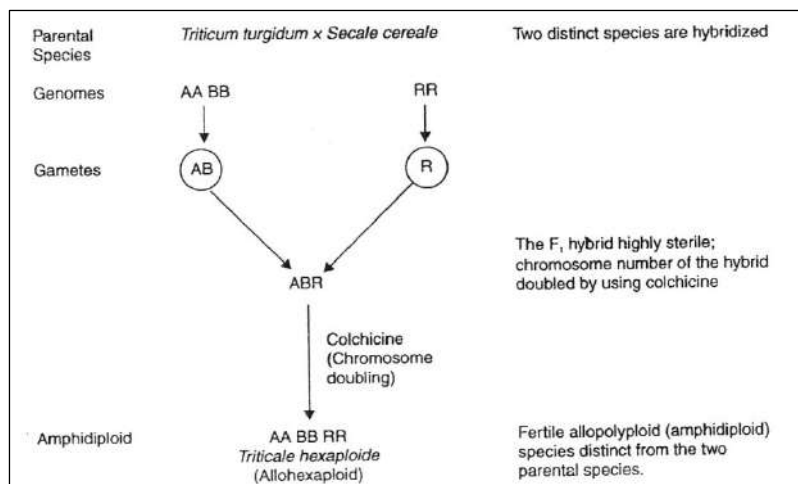


Fig. 8.3. Production of Triticale (an allohexaploid)

This technique has been successfully employed to produce the interspecific hybrid from a fusion of the protoplasts; of *Nicotiana glauca* with those *N. langsdorfii*, hybrids between two mutant lines of *N. tabacum*, two sexually compatible species of *Petunia* etc. Such hybrids are denoted by a + ('plus') = 1 sign between the names of the two parental species, e.g., *N. glauca* + *N. langsdorfii*. The general applicability of this technique is limited by the lack of a suitable technique for selection of hybrid cells, and for plantlet regeneration from hybrid calli; attempts are being made to develop refined techniques.

Genetic Effects

(1) Allopolyploids generally combine the morphological and physiological features of the parent species, but it is very difficult to predict the precise combination of the parental characters that would appear

in the allopolyploid. An interesting example is provided by *Raphanobrassica* which was synthesized with a view to combine the leaves of cabbage with the roots of radish, but it turned out to possess the roots of cabbage and the leaves of radish. In contrast, Triticale has a combination of the desirable features of both the parental species, that is, yield and quality of wheat with the hardiness (tolerance to stresses) of rye. The difficulty in prediction of the features of allopolyploids may be appreciated in view of the fact that they contain two or more genomes that have evolved independent of each other. (2) In general, allopolyploids are more vigorous than diploids, but this is not true in all the cases. (3) Allopolyploids appear to differ in their adaptability from the parental species; often they are hardier than the parents but rarely they are able to displace their parental species from the areas of their adaptation. (4) Many of the allopolyploids, particularly in grasses, are apomictic, which is an efficient mechanism of countering the sterility in distant hybrids without doubling their chromosome number.

Cytology

The successful hybridization between two distinct species indicates some degree of relatedness between them; hence their chromosomes may be expected to be partially homologous or homeologous. As a result, a majority of newly synthesized or raw allopolyploids show variable frequencies of multivalents depending upon the degree of homeology between their parental genomes; this leads to variable sterility, and both cytogenetic and genetic instability. Sterility is a common feature of raw allopolyploids, which in many cases, is believed to be due to physiological disturbances. Fertility of allopolyploids can be improved through hybridization and selection; this not only improves chromosome behaviour during meiosis but also selects for desirable gene combinations. The natural polyploids show regular bivalent formation at MI, hence they are said to have undergone diploidization.

Diploidization involves selection of such genes or gene combinations which prevent multivalent formation by suppressing pairing between homeologous chromosomes. In hexaploid wheat, this is achieved by a single gene, Ph, located on the short arm of chromosome 5B; there is evidence for similar genetic systems in other natural allopolyploids like tobacco, oats, etc.

Role of Evolution

Allopolyploids have been more successful as crop species than autopolyploids. Many of our present-day crops, e.g., wheat, tobacco, cotton, *Brassica* sp., sugarcane, oats etc., are allopolyploids. However, it may be pointed out that allopolyploid *per se* does not ensure the success of a species as a crop; in fact many allopolyploids are noxious weeds. Allopolyploid has greatly contributed to the evolution of plants, a fact attested to by its widespread occurrence in natural populations: according to one estimate, about one-third of angiosperms are allopolyploids. In 1917, Winge postulated that allopolyploidy was important in evolution, which has been substantiated by subsequent studies. The possible evolutionary history of many crop species has been traced through systematic investigations. The identification of parental diploid species is primarily, based on pairing between the chromosomes of the diploid and the allopolyploid species in question. Additional evidence is obtained by comparing the features of synthetic allopolyploids with the natural ones, from banding patterns of the chromosomes of the concerned species and from biochemical data, particularly those on electrophoretic patterns of proteins and enzymes, and now on affinity for specific DNA probes.

Origin of Wheat

The common or bread wheat (*Triticum aestivum*) is an allohexaploid having two copies each of the genomes A, B and D; its somatic complement is AABBDD. The

sources of A and D genomes are more or less unanimously accepted as *Triticum monococum* (AA) and *Aegilops squarrosa* (DD), respectively. There is considerable doubt about the source of B genome. According to one hypothesis, *Aegilops speltoides* may be the source of this genome, but recent evidences do not support this. The amphidiploid AABB was initially produced which gave rise to the tetraploid wheats, e.g., *T. turgidum*, *T. dicoccum* etc. This amphidiploid (AABB) subsequently outcrossed with *Ae. squarrosa* to ultimately yield the hexaploid wheats (AABBDD).

Origin of Cotton

The upland cotton, *Gossypium hirsutum*, is an allotetraploid; it has 13 large and 13 small chromosomes in its haploid complement ($n = 26$). Available evidence is interpreted to suggest that *G. hirsutum* is an amphidiploid of the hybrid between *G. herbaceum* var. *africanum* (old world diploid cotton, $n = 13$, small chromosomes) and *G. raimondii* (American diploid cotton, $n = 13$, large chromosomes).

Origin of Tobacco

Similarly, *Nicotiana tabacum* and *Nicotiana rustica* are also allotetraploids, each having 24 chromosomes in their haploid complements ($2n=48$). Available evidence indicates that *N. tabacum* is an amphidiploid derived from the cross *N. sylvestris* x *N. tomentosa*, each of the parental species having 12 chromosomes in their haploid complements.

Origin of Brassica sp.

Several of *Brassica* sp., e.g., *B. juncea*, *B. napus* and *B. carinata*, are allotetraploids. It is believed that *B. juncea* is an amphidiploid from the cross *B. nigra* x *B. campestris*, *B. napus* is an amphidiploid from the cross *B. oleracea* x *B. campestris*, while *B. carinata* is an amphidiploid from the cross *B. nigra* x *B. oleracea*.

Applications in Crop Improvement

At one time it was hoped that since many successful crop species were allopolyploid, man could create new crop species as well. Experience with a large number of synthetic allopolyploids has had a sobering effect; in fact, *Triticale* is the only synthetic allopolyploid that has succeeded as a crop species. Varieties of *Triticale* have been released for commercial cultivation in several countries, e.g., Australia, Canada, Italy, Portugal, Mexico, etc.; in India TL 419 has been released and several promising lines are being evaluated. More than fifty years of intensive research effort was required before *Triticale* varieties became commercially attractive. Newly synthesized triticales are low yielding due to high sterility, poor seed set and poor and variable development of grains. They also show genetic and cytogenetic instability due to meiotic irregularities. These defects were connected through hybridization and selection for which elaborate breeding strategies have been devised. Some other promising synthetic allopolyploids that may succeed as crop are, Raphanobrassica (as a fodder crop), Festuca-Lolium hybrids.

Allopolyploids have some other applications in crop improvement. They may be synthesized to widen the genetic base of allopolyploid crops which have a rather narrow genetic base. For example, the genetic base of *B. napus* is sought to be widened by synthesizing it from the parental diploid species (*B. oleracea* and *B. campestris*).

Some synthetic allopolyploids serve as bridging species between two diploid species which do not cross with each other, or those which do cross but their F₁ hybrid is sterile. For example, the F₁ hybrid from the cross *N. sylvestris* x *N. tabacum* is sterile, but the amphidiploid *N. digluta* produced by chromosome doubling of the F₁ is reasonably fertile. The hybrid from the cross *N. digluta* x *N. tabacum* partially fertile and can

be easily backcrossed to *N. tabacum*. This manner, resistance to tobacco mosaic virus was transferred from *N. sylvestris* to *N. tabacum*. In this programme, the synthetic amphidiploid served as a bridging species through which the genes were passed on from *N. sylvestris* to *N. tabacum*.

Turner syndrome

The Turner syndrome was first described by HH. Turner in 1938; it is associated with monosomy for X chromosome and has a frequency of about 1 per 2,500 live female births. More than 90 per cent of XO zygotes abort spontaneously. Adults with Turner syndrome are females who have virtually no ovaries and whose secondary sex characters are poorly developed. Such females have short stature, low-set ears, webbed neck and a shield-like chest but generally do not show mental retardation. Some persons suffering from Turner syndrome are mosaics having XO and XX cells in different parts of their body. Such individuals show fewer anomalies than XO females, and sometimes may even be fertile. Some other Turner syndrome individuals have one normal X and one deleted X in which the short arm is missing. It may be noted that persons having one normal X and one deleted X from which the long arm is missing do not show as many symptoms of Turner syndrome as do those in which the short arm of one X chromosome is missing. Some other patients of Turner syndrome contain a Y chromosome; such persons are generally mosaics having XO and XY (normal Y chromosome) cells in different tissues. XY persons having a deleted Y chromosome (lacking the short arm) are normal females with rudimentary ovaries. These findings clearly show that male-determining genes are present in the short arm of Y chromosome, while those preventing Turner syndrome are located both in the long arm of Y and the short arm of X chromosomes.

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MENDELIAN INHERITANCE

Mendel's Work

Selection of Pea as Experimental Material

1. In the pea varieties available commercially, several characters had two contrasting forms which was easily distinguishable from each other.

Sl. No.	Character	Dominant form	Recessive form
1.	Seed shape	Round	Wrinkled
2.	Seed coat colour	Grey	White
3.	Cotyledon colour	Yellow	Green
4.	Pod colour	Green	Yellow
5.	Pod shape	Full	Constricted
6.	Position of flowers	Axial	Terminal
7.	Length of stem	Tall	Dwarf

This permitted an easy classification of F_2 and F_3 progeny from various crosses into clear-cut classes on the basis of the contrasting forms of different characters.

1. The flower structure of pea ensured self-pollination. This was experimentally verified by Mendel. This greatly facilitated the production of F_2 and F_3 progeny as well as avoided contamination by foreign pollen.
2. Pea flowers are relatively large. Therefore emasculation and pollination of pea flowers is quite easy, which allows easy artificial hybridization in pea.
3. The duration of pea crop is single season. As a result, every year one generation of pea can be grown.
4. Pea seeds are large and present no problem in germination.

Reasons for Mendel's Success

1. At first, Mendel studied the inheritance of only one pair of contrasting character at a time. This allowed him to classify all the F_2 and F_3 progeny into two clear-cut groups.
2. He selected pea varieties that had clearly different forms of one or more characters.
3. Mendel was able to formulate appropriate hypotheses on the basis of the experimentation, he offered for his experimental findings. Further, he proceeded to test these hypotheses experimentally to prove the correctness of his explanations.
4. Further, Mendel was undoubtedly lucky, because,
 - a. The seven characters selected by Mendel showed qualitative inheritance and not a single one was inherited quantitatively.
 - b. The contrasting forms of each of the seven characters were governed by a single gene and in each case one form was completely dominant over the other.
 - c. Of the seven characters studied by Mendel, the genes for two were located in one chromosome and others were present in another chromosome.

Highlights of the Mendel's Paper

- 1) Mendel concluded a complete fusion of male and female gametes and equal contribution of the two parents to develop the characters of the hybrid (F_1).
- 2) He postulated the existence of factors, now the genes, which are responsible for the development of various characters. It is these factors (genes) that are transmitted from one generation to the next, and not the characters themselves.

- 3) He made a clear distinction between the appearance (phenotype) and the genetic makeup (genotype). He classified the F_2 individuals with the dominant phenotype into pure and hybrid forms on the basis of the character forms present in the F_2 progenies produced by them.
- 4) Mendel introduced the concept of dominance and recessiveness and also gave these terms.
- 5) Mendel elucidated the laws of segregation and independent assortment.

Inheritance of Seed Shape in Pea

Mendel began his studies on a total of 34 distinct varieties of pea (*Pisum sativum* L.). For two consecutive years, he grew each of these varieties in separate plots.

1. To check for their purity (mechanical mixtures and heterozygosity) and
2. To assess the stability of their characters i.e., how much the expression of characters was influenced by the environment.

Finally he selected 22 of these varieties for hybridization. Each of these varieties differed from the other with respect to one or more characters. A character is any morphological, anatomical, biochemical or behavioural feature of an organism. Varieties differing from one another for one or more characters and used in a hybridization programme are known as parents.

Crossing or hybridization consists of placing active pollen grains from flowers of one of the two parents (male parent) on to the stigma of the emasculated flowers of the other parent (female parent). Emasculation is the removal of all the immature anthers (or even androecium) of a flower to prevent self pollination.

The progeny obtained by crossing (hybridization) are known as hybrid or F_1 generation. Self-pollination in

F₁ produces F₂ while that in F₂ generates F₃ and so on. When one parent (parent A) is first used as female in a cross with another parent (parent B) and then it (parent A) is used as male in another cross with the same parent (parent B) such cross are known as reciprocal crosses. A cross between two parents differing for a single character is termed as monohybrid cross, while those between parents differing for two and three characters are known as dihybrid and trihybrid crosses respectively.

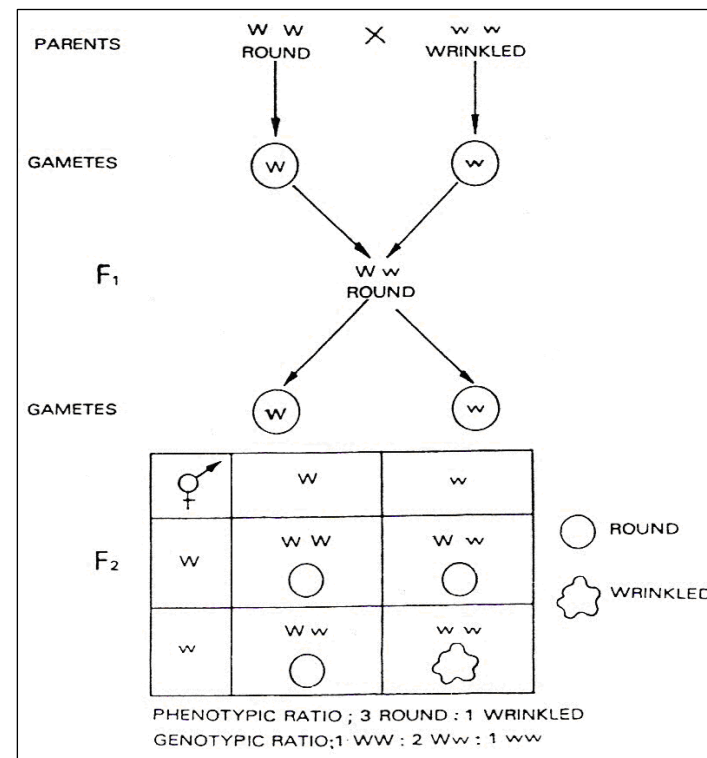
Each gene has two alternative forms. These forms are known as alleles. Each somatic cell of an organism has two copies of each gene.

An individual may have two copies of the same allele, e.g., WW or ww; such an organism is called homozygous and the situation is known as homozygosity. But, when an individual has two different alleles of the same gene, e.g., Ww, it is referred to as heterozygous, and the condition is termed as heterozygosity. Mendel used the terms pure and hybrid for homozygous and heterozygous respectively. The allele which expresses itself i.e., produces the character governed by it, in the heterozygous state is known as dominant, while unable to do so is called recessive.

The appearance of an individual with respect to a specific character or all of its characters is referred to as phenotype. But the kind of alleles of a specific gene or all of its genes carried by an individual is known as its genotype or genetic constitution.

“A zygote is produced by the fusion of one male and one female gamete, and that the contribution of male and female gametes is equal”. The appearance of 3:1 ratio in F₂ may be explained as follows:

The two alleles, W and w, present in the hybrid (Ww) separate and go into different gametes, since each gamete is required to contain only one copy of a gene. The hybrid would thus produce two types (W and w) of male and female gametes in equal frequency.



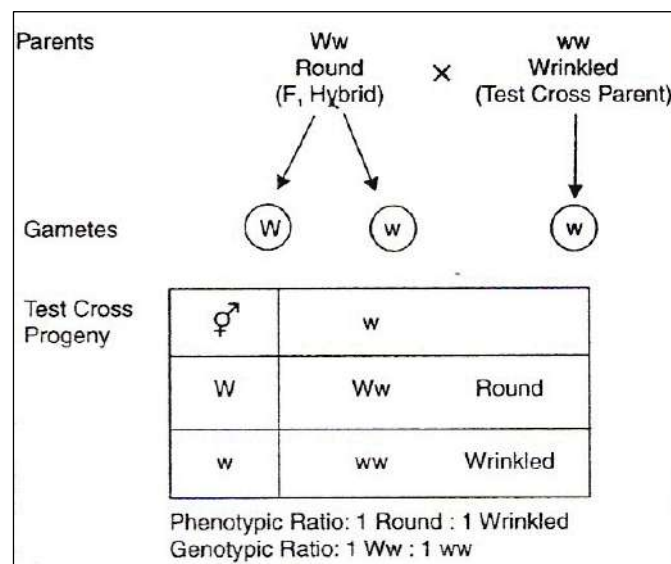
Half of the gametes (of each sex) produced by a hybrid will be W and the remaining half will be w. A random union of the male gametes with the female gametes of the hybrid would yield four types of zygotes with respect to this gene.

Random union of gametes implies that a W male gamete has the same chance to unite with a given female gamete as a w male gamete and vice-versa. The four kinds of zygotes produced in F₂ would be WW, Ww, Ww, ww.

and ww and their frequencies will be equal. The first three zygotes would develop into round seeds due to the presence of W allele, while the fourth (ww) would produce wrinkled seed. This would give rise to the 3:1 ratio obtained by Mendel and others.

As a result, “the two alleles present in the F₁ are able to separate and pass into different gametes in their original form producing two different types of gametes in equal frequencies; this is known as segregation”.

A random union between male and female gametes produces the typical 3:1 ratio, often referred to a Monohybrid ratio since it is observed in the F₂ generation of crosses segregating for a single character or gene.



Test Cross

A cross between F₁ hybrid and a variety or strain with the recessive form of a character is known as test cross. The purpose of a test cross is to present evidence that segregation for the alleles of a single gene in the F₁ hybrid produces two types of gametes in equal frequencies.

Mendel's Laws of Heredity

This law is the basis of the science of genetics and Mendel is aptly known as the Father of Genetics.

Mendel's First Law or Law of Segregation

“Allelic genes in a hybrid do not blend or contaminate each other but segregate and pass into different gametes”.

Monohybrid Ratio

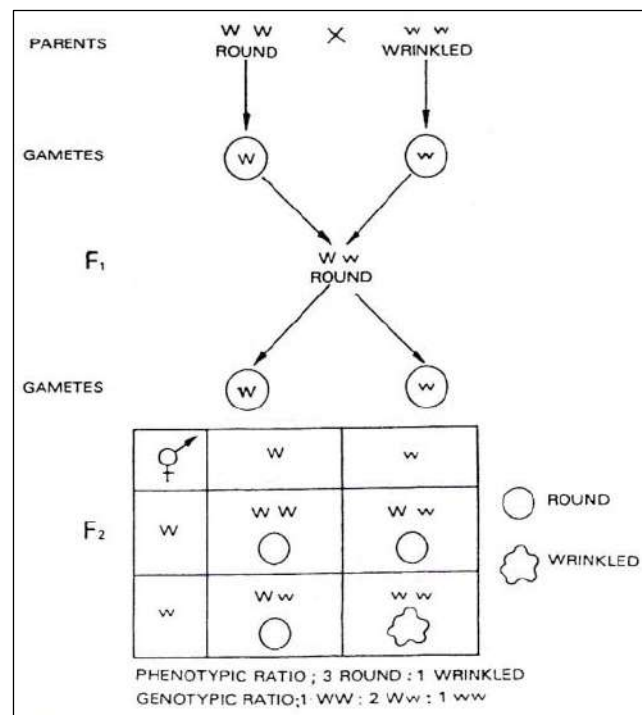
In Mendel's cross between the round-seeded and wrinkled-seeded peas, both the parents belong to true-breeding varieties.

The true breeding round seeded parent RR forms gametes, all of which are alike and have 'W' and the true-breeding wrinkled seeded parent ww forms gametes, all of which are alike and have 'w'.

When pollen from the wrinkled seeded parents is dusted on the emasculated flowers of round-seeded parent, an egg with 'W' is fertilized by a sperm with 'w'. The hybrid seeds are therefore Ww and all of them are round because 'W' is dominant to 'w'.

Though, there is no visible indication of the presence of the gene 'w' in the F₁, the alleles 'W' and 'w' do not blend or fuse with each other while they are together in the F₁. The alleles 'W' and 'w' do not also contaminate or affect each other even though all the hybrid seeds are only round.

When the hybrid seeds are sown and when these plants form eggs and sperms, the genes 'W' and 'w' segregate so that two kinds of eggs, one with 'W' and other with 'w', and two kinds of sperms, one with 'W' and other with 'w' are produced in approximately equal numbers.



During fertilization, the gametes combine at random. The F₂ therefore consists of four combinations viz., WW, Ww, Ww and ww in approximately equal numbers.

The genotypic ratio in F₂ is therefore 1 WW : 2Ww : 1ww. Since 'W' is dominant over 'w' the genotypes WW and Ww have the same phenotype viz., round and the phenotypic ratio is 3 round : 1 wrinkled.

Cytological Interpretation of Mendel's First Law

Allelic genes in an individual segregate according to Mendel's first law only because they are carried on homologous chromosomes that separate at meiosis and go to different gametes.

Let us presume that the gene 'W' responsible for the rounded seed coat in pea is situated at a particular

locus in a chromosome. The true breeding round-seeded parent has a pair of these chromosomes and each of these will have a gene for round-seed coat. At meiosis, the two homologous chromosome separate and consequently the two genes 'W' and 'W' will also separate. Each gamete has only one homologous chromosome and consequently will have only one 'W' gene. A homozygous round-seeded plant, thus, produces gametes, all of which are alike in having a single chromosome with the gene 'W'. Similarly for gene 'w' responsible for wrinkled seeds, similarly for the hybrid Ww. During meiosis in their flowers, the two homologous chromosomes separate, go to opposite poles, and enter into different gametes. Each gamete has only one homologous chromosome and consequently will have only one allele, either 'W' or 'w', but never the both.

During fertilization, the union of like eggs and sperms occurs with the same frequency as the union of unlike eggs and sperms. The F₂ therefore consists of four combinations in equal number WW, Ww, Ww and ww.

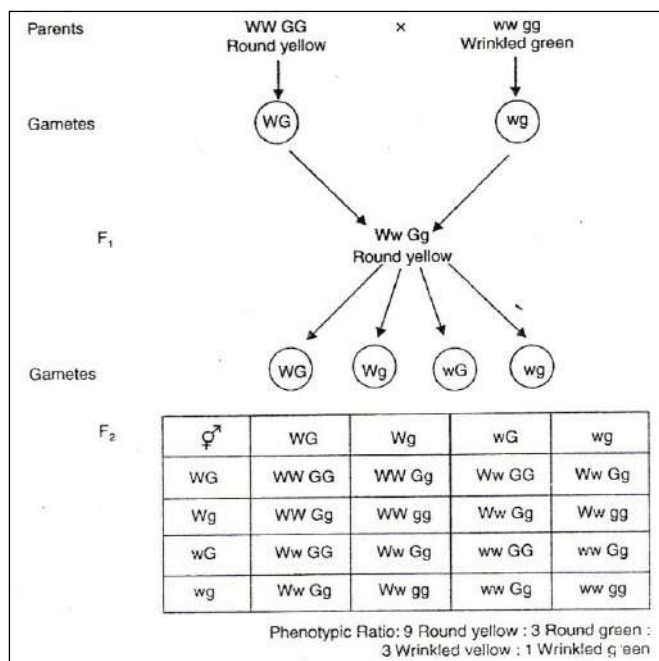
Mendel's Second Law or Law of Independent Assortment

"If the inheritance of more than one pair of character (two pairs or more) is studied simultaneously, the factors or genes of each pair of characters assort out independently of the other pairs".

Dihybrid ratio

The two varieties (round yellow and wrinkled green) used as parents in the dihybrid cross. The genotypes are WW, GG and ww, gg respectively. They have to be homozygous for both the genes since they produced same genotype on selfing. But, the gamete will contain one copy of each of W and G in WW and GG and one copy of w and g in the case of ww and gg, parent. The union between WG and wg gametes from the two parents will produce Ww Gg progeny in F₁ and all the F₁ seeds will have round (due to W allele) and yellow (due to G allele) phenotype.

In F_2 both Ww and Gg genes will segregate simultaneously. The segregation of Ww alone produces two types of gametes, W and w in equal frequencies and like that G and g allele possess two different gametes, with equal frequency. A random union between four types of male and female gamete will produce 16 zygotic combinations.



The first four genotypes have at least one W and one G and as a result will produce round yellow phenotype. Thus there will be 9 round yellow seeds out of every 16 F_2 seeds. The next two genotypes have W but homozygous for g , a total of 3 out of 16 seeds will be round green. Similarly, the next two genotypes have at least one G allele but are homozygous for w , these 3 seeds out of 16 are wrinkled yellow. The last genotype homozygous for w and g , therefore the single F_2 seeds

having this genotype will be wrinkled green. This in summary given 9:3:3:1 ratio.

Genotype	Genotypic Frequency (probability)	Phenotype	Phenotypic Frequency (probability)
$WWGG$	1	Round yellow	9
$WWGg$	2		
$WwGG$	2		
$WwGg$	4		
$WWgg$	1	Round green	3
$Wwgg$	2		
$wwGG$	1	Wrinkled yellow	3
$wwGg$	2		
$wwgg$	1	Wrinkled green	1

Test cross in a dihybrid

In a test cross, the F_1 hybrid ($Ww\ Gg$) is crossed with a variety or strain having the recessive forms of both the characters (double recessive) i.e. wrinkled green. The purpose of a test cross is to verify the ratio in which the different gametes of the F_1 hybrid are produced. According to independent segregation of the two genes (Ww and Gg), the F_1 should produce four kinds of gametes. viz., WG , Wg , wG and wg in equal frequencies i.e., in the ratio of 1:1:1:1. The double recessive parent ($ww\ gg$) used in the test cross, on the other hand will produce only one kind of gamete, viz., wg .

The union of wg gamete from the double recessive or test cross parent with the four gametes produced by F_1 is expected to produce four kinds of zygotes: $Ww\ Gg$, $Ww\ gg$, $ww\ Gg$ and $ww\ gg$. These zygotes produce round yellow, round green, wrinkled yellow and wrinkled green seeds respectively in the ratio of 1:1:1:1. This produces sufficient evidence for independent segregation of the concerned genes.

Test cross $Ww Gg$ $\times$ $ww gg$
 Round yellow Wrinkled green

Test cross
gametes and
progeny

wg	
WG	$Ww Gg$ (Round yellow)
Wg	$Ww gg$ (Round green)
wG	$ww Gg$ (wrinkled yellow)
wg	$ww gg$ (wrinkled green)

PHYSICAL BASIS OF HEREDITY

Chromosomal Theory of Inheritance (or) Sutton – Boveri hypothesis

Correns (1900) supposed the genes to be located in chromosomes and presented a beads-on-a-string drawing of chromosomes. The idea of chromosomal basis of segregation and independent assortment was put forth by Sutton in 1902.

The hypothesis that genes are located in chromosomes was designated as Sutton-Boveri hypothesis or more commonly as the Chromosomal Theory of Inheritance.

Evidence for Chromosomal Theory of Inheritance

It is a known fact that the genes are located in chromosomes and it is not regarded as a theory or hypothesis. However it would be rewarding to examine the various direct and indirect evidences that indicate or establish the location of genes in chromosomes.

Behaviour of genes and chromosomes during segregation and independent assortment

1. It is clear from the work of Mendel and of subsequent workers that each somatic Cell contains two copies of each gene. Similarly each somatic cell has two copies of homologues of each chromosome. Clearly, each somatic cell produced during embryonic and subsequent development faithfully receives the two copies of each gene present in the zygote.
2. Gamete contains only one copy of a gene or only one allele of a gene. This phenomenon is termed as segregation. Similarly, each gamete receives only one of the two homologues of each chromosome pair i.e.,

each gamete receives only one haploid set of chromosome.

Obviously, there is enough parallelism between gametes and chromosomes. It is assumed that the two alleles of a gene are located in the two chromosomes of a homologous pair (one allele in each homologue) the separation of two homologous chromosomes during anaphase I of meiosis will adequately account for segregation of the two alleles.

3. In Mendel's and several subsequent studies, two or more genes assorted independently to yield typical dihybrid, trihybrid etc ratios. Similarly, different chromosome pairs on bivalents orient themselves at metaphase I independent of each other. As a result, the members of each homologous pair of chromosome assort independently in that one member of a homologous pair has equal opportunity of migrating to a pole at anaphase I with any one of the two members of another homologues chromosome pair.

Sex chromosomes

4. In 1901, Mc Clung discovered the accessory or X chromosome of grasshopper and postulated that this chromosome was involved in sex determination. This was the first demonstration that a character (in this case sex) was associated with a specific chromosome. Therefore, specific chromosome is involved in sex development, the genes governing this trait may be located on the X chromosome.

The white eye gene of *Drosophila* (Sex linkage)

5. In 1901, Morgan presented reports on the peculiar inheritance of white eye (w) gene of *Drosophila*. The pattern of transmission of white eye gene was identical with that of X chromosome of *Drosophila*. This prompted Morgan to postulate that the gene w was located in X chromosome.

Nondisjunction of chromosomes (Sex linkage)

6. In 1913, Bridges observed an unusual transmission of a sexlinked gene in *Drosophila*. The unusual transmission of the gene was explained as being due to non disjunction of the X chromosome in some of the oocytes so that half of the eggs produced from such oocytes received two X chromosome while the remaining half of the eggs received no X chromosome. This demonstrated that presence or absence of a specific chromosome.
7. In 1922, L.V. Morgan noted that X chromosome of female fly showed a regular non-disjunction and postulated that the two X chromosomes of this fly should be attached with each other

Linkage and crossing over

8. In 1910, Morgan described the phenomenon of linkage and crossing over between two sex-linked genes in *Drosophila*.
9. Morgan also explained the recombination between linked genes to be due to exchange of chromatin material between homologous chromosomes. In McClintock devised brilliant experiment in this and become rather direct demonstration that genes are located in the chromosomes.

Structural chromosomal changes

10. Structural changes in chromosomes are always accompanied by corresponding changes in linkage groups (i.e., position of genes), genes must be located in chromosomes.

Numerical chromosomal changes

11. Numerical chromosome changes also furnish evidence in favour of the chromosome theory of heredity. e.g., Monosomy, Trisomy.

12. Autopolyploids, allopolyploids having more than two copies of the concerned genes.

Holandric characters

13. Some genes, e.g., H-Y gene in humans are located in the Y chromosome. Such genes are transmitted from father to son and are absent in females. They are called as holandric characters. This indicates that H-Y gene is located in human Y chromosome.

Location of DNA

14. It is accepted beyond any reasonable doubt that DNA is the genetic material in eukaryotes and in most of prokaryotes (In some prokaryotes, e.g., most plant viruses and some bacteriophages, RNA is the genetic material). It, therefore, follows that the organelle containing DNA must contain genes and vice-versa. In fact, the presence of DNA is the acid test to demonstrate the presence of genes in an eukaryotic organelle. By far the vast majority of DNA occurs in chromosomes of eukaryotes. It is, therefore, self-evident that the genetic information or genes must be an integral part of eukaryotic chromosomes. A small amount of DNA occurs in chloroplast (plastids) and mitochondria as well; this DNA is the basis for cytoplasmic inheritance in eukaryotes.



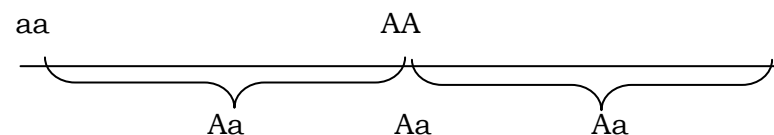
DOMINANCE AND LETHAL GENES

Principles of Dominance

It was noted by many workers even before Mendel that many characters of the F_1 hybrid were exactly the same as those of one or the other parent of the hybrids. The phenomenon of a F_1 hybrid being identical to one of its parents for a character is termed as dominance.

The form of a character that is seen in the F_1 hybrids is known as dominant; while that form of the concerned trait which does not appear in F_1 is known as recessive. These two terms were first used by Mendel.

Gaertner observed that the dominant form of a character always appears in the hybrid irrespective of it being contributed by the male or the female parent of the hybrid.



Location of the heterozygote Aa in relation to the two homozygotes (aa and AA) in complete, incomplete and over dominance relations. The alternative forms of a character are governed by the alternative forms or alleles of the same gene.

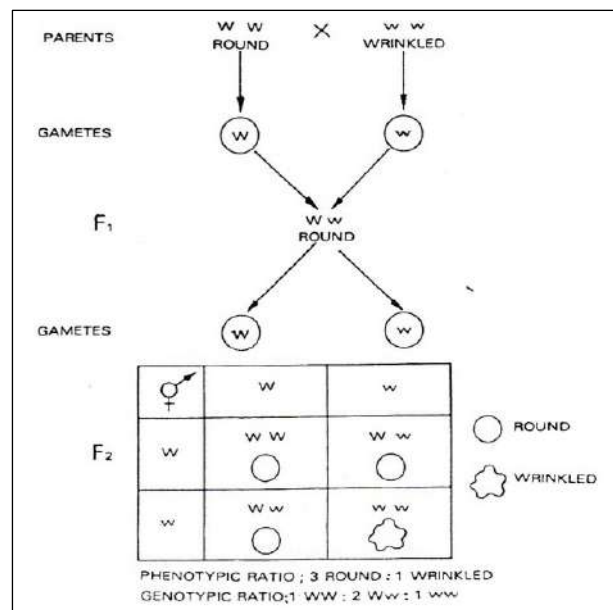
In F_1 hybrid, both the allele of a gene are present together in heterozygous state. Mendel found that in heterozygous state only one of the two alleles of a gene is able to express itself i.e., produce the character; this allele is referred to as the dominant allele. The recessive allele, on the other hand is unable to express itself in heterozygous state. The phenomenon of dominance

describes the relationship between two alleles of the same gene. The alleles of different genes may show different patterns of dominance relationships as indicated above.

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 as completely or fully dominant.

An example of complete dominance occurs for seed shape in peas, first studied by Mendel and subsequently by many later workers.



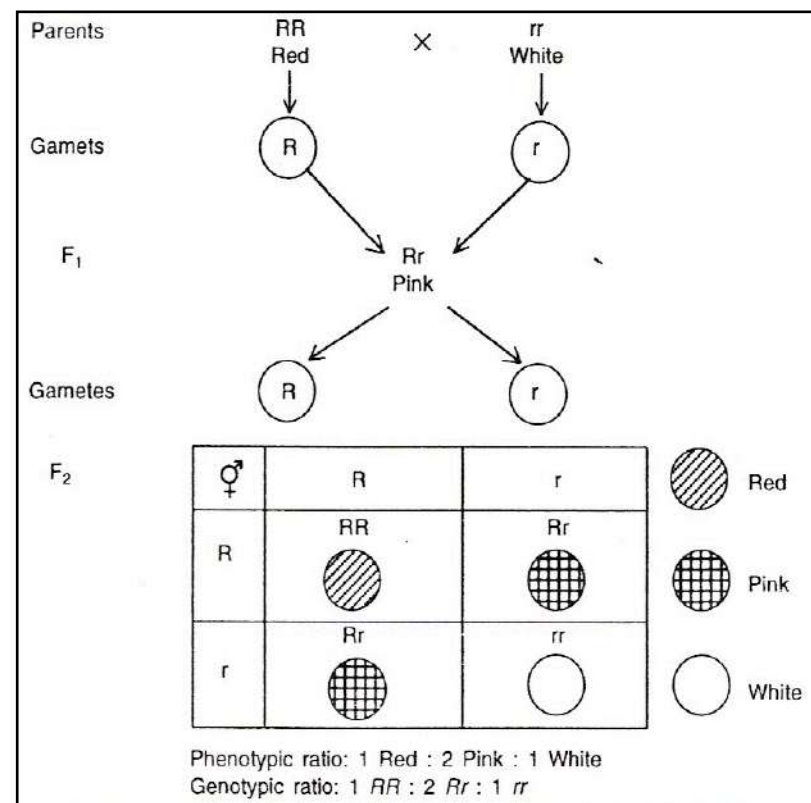
Round seed shape is produced by the dominant allele W, while wrinkled shape is determined by its recessive allele w. Seeds having the genotype Ww are round and indistinguishable from those having the genotype WW. As a result, character showing complete dominance yield the typical 3:1 monohybrid ratio in F₂.

Incomplete Dominance

“In several crosses, dominant is incomplete and the hybrids resembles neither parents exactly but are more or less intermediate between the two”, such a situation is known as incomplete or partial dominance.

E.g. : In Mirabilis, a red-flowered plant crossed with white-flowered one gives F₁ which are all pink in colour and segregates in the F₂ ratio into 1 red : 2 pink : 1 white.

The intensity of phenotype produced by heterozygotes is less than that produced by homozygotes for the concerned dominant allele.



Co-Dominance

In this, both the genes of an allelomorphic pair express themselves equally in F_1 hybrids. It means a heterozygote for codominant genes exhibit both the characters side by side.

E.g. Coat colour in Cattle :Gene R-stands for red coat colour, r-stands for white colour.

When red cattle (RR) are crossed with white cattle (rr), the F_1 hybrids have roan coloured skin (Not intermediate). The roan colour is actually expressed by a mixture of red and white hairs, which develop side by side in the heterozygous F_1 hybrid. In F_2 generation, red, roan and white appear in the ratio of 1:2:1.

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. Over dominance is not the property of an allele; it is the consequence of the heterozygous state of the concerned gene.

E.g. The white eye (W) gene of *Drosophila* exhibits over dominance for some of the eye pigments e.g. Sepiapteridine and himmelblaus.

Allele w produces white eye in the homozygous state (ww) while its completely dominant allele W gives rise to the normal dull red eye colour both in homozygous and in heterozygous states (WW and Ww). Both the eye pigments are present in low concentrations in ww homozygotes while WW homozygous have relatively higher concentration of these pigments. However, flies heterozygous for this gene (Ww) have an appreciably higher concentrations of these two pigments than the two homozygotes (WW and ww).

The white eye gene of *Drosophila* is a sex-linked gene and as a result of this, it would not exhibit the typical

ratio in F_2 , but autosomal genes (genes not located in X-chromosome) showing over dominance would give rise to a 1:2:1 ratio in F_2 as long as the two homozygotes have distinctly different levels of expression of the character.

LETHAL GENES

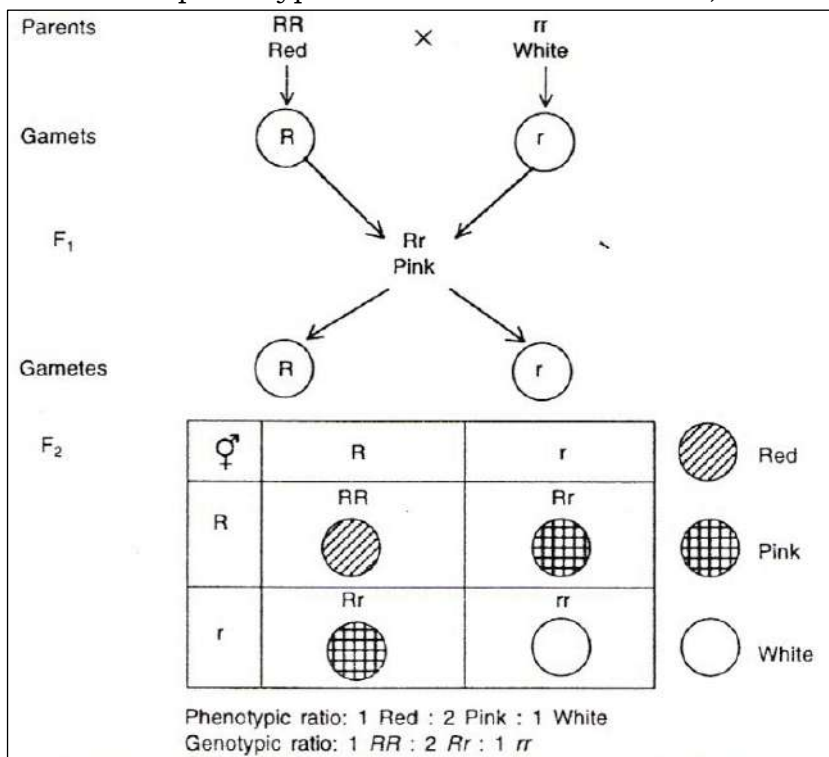
Some genes affect the survival of those zygotes or individuals in which they are present in the appropriate genotype; this effect ranges from an increase in survival, through no effect to death of all the individuals carrying the gene. All such genes may be grouped into the following five categories: (1) lethal, (2) semi lethal, (3) sub vital, (4) vital and (5) super vital.

A lethal gene causes death of all the individuals carrying this gene in the appropriate genotype before these individuals reach adulthood. The appropriate genotype for an allele would depend on its dominance relationship with its other allele(s). For an allele producing a recessive effect on survival, the appropriate genotype would be the homozygous state, while for an allele having a dominant effect on survival both homozygous and heterozygous states would be the appropriate genotype. Lethal genes may be grouped into the following five categories: (1) recessive lethals, (2) dominant lethals, (3) conditional lethals, (4) balanced lethals and (5) gametic lethals.

Recessive Lethals

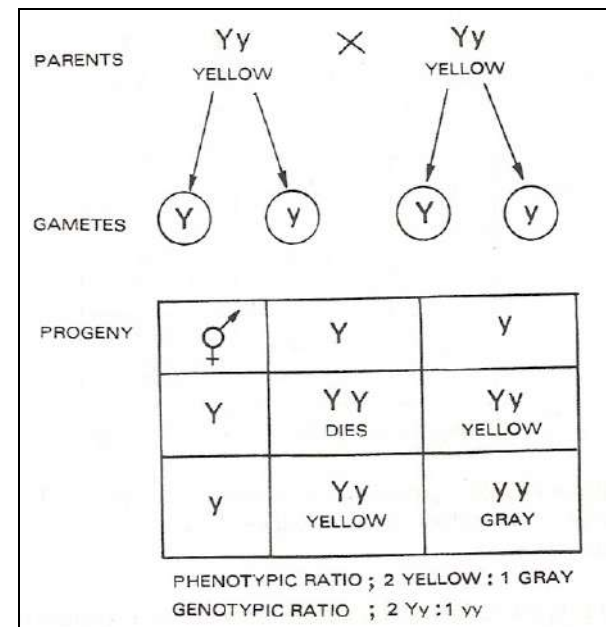
Most of the lethal genes are recessive lethals since their lethal effect is expressed only when they are in the homozygous state and the survival of heterozygotes is unaffected. Many recessive lethals affect some other character in addition to their effect on survival and generally their phenotypic effects are dominant over those of their normal alleles. The gene Y has now been redesignated as the dominant allele N in the agouti (A) series that affects fur colour in mice.

Thus the inheritance of yellow coat colour in mice may be explained as follows. The dominant allele Y is a recessive lethal so that YY embryos do not survive. As a result, all the yellow mice are heterozygous (Yy) for this gene. Therefore, matings of yellow females with yellow males would produce the following four zygotes: (1) one-fourth YY, (2) one-half Yy and (3) one-fourth yy. Homozygous YY embryos will die at an early stage in the uterus itself. Heterozygous Yy embryos will develop normally and give rise to yellow mice, while yy embryos will develop into grey individuals. Thus the phenotypic ratio obtained in yellow x yellow matings will be 2 : 1 in place of the typical monohybrid ratio of 3 : 1. This 2 : 1 ratio is the typical ratio obtained due to segregation for a recessive lethal gene. Obviously, the Y gene in mice has a dominant phenotypic effect on coat colour, but is



recessive lethals. The two characteristic features of recessive lethals are given below.

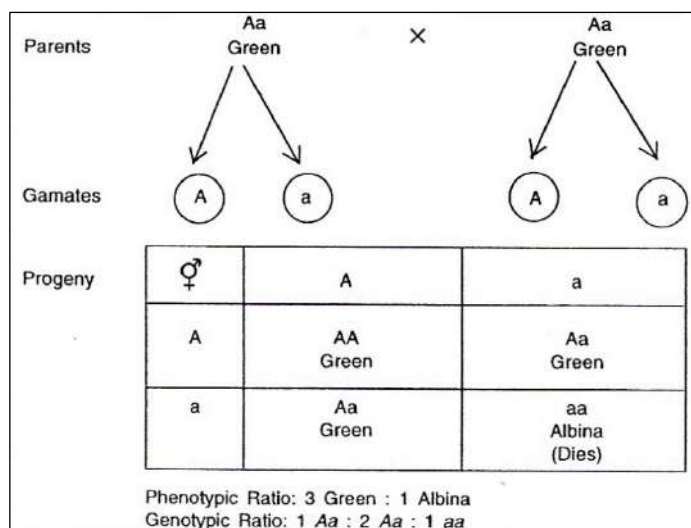
1. They are always present in the heterozygous state since their homozygotes do not survive.
2. A cross between the heterozygotes for a recessive lethal gene yields a 2 : 1 ratio (instead of the typical 3 : 1 ratio).



But many genes are recessive both in their phenotypic as well as lethal effects. In such cases, heterozygous individuals have normal phenotype as well as normal survival and they cannot be differentiated from normal homozygotes. An example of such a gene are the genes producing albina seedlings in plants, such as, barley.

Albina character is governed by recessive alleles of several genes in barley; when anyone of these alleles is in homozygous state, the seedlings are near-white and totally devoid of chlorophyll. Albina seedlings survive only

as long as the food stored in seeds is available since they are not able to carry out photosynthesis. The heterozygotes, however, are normal green and are identical with the normal homozygotes in their phenotype as well as survival. Segregation for such genes yields 3 green: 1 albina, if the seedlings are scored within a week from germination. However, if the plants are scored several weeks after germination or at maturity, there will be only green plants in the progeny.



The lethal genes described so far reduce the survival of zygotes that carry them in the appropriate genotype. Such lethals are known as zygotic lethals and constitute the vast majority of lethal genes. The stage of development at which a lethal gene produces its lethal effect varies considerably from one gene to the other. Some genes cause the death of embryo very early in development, e.g., the Y gene in mice, while others allow survival and development close to the reproductive age, e.g., epiloia in man.

Dominant Lethals

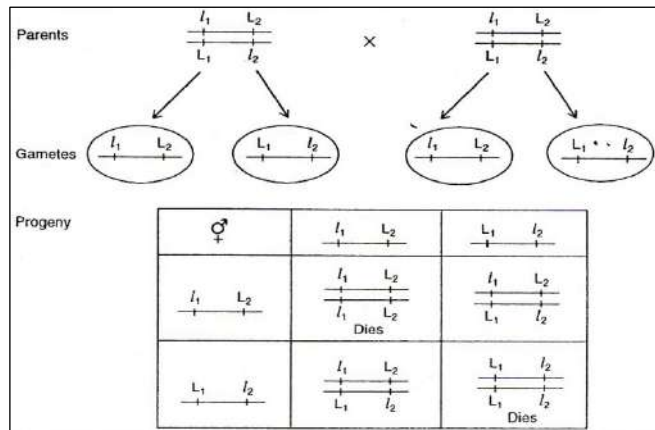
Some lethal genes reduce viability in the heterozygous state as well; such genes are known as dominant lethals. An example of a dominant lethal is the epiloia gene in human beings. This gene causes abnormal skin growths, severe mental defects and multiple tumors in the heterozygotes so that they die before reaching adulthood. Dominant lethals, therefore, cannot be maintained in the population, while recessive lethals are maintained in the heterozygous state. Thus dominant lethals have to be produced in every generation through mutation.

Conditional Lethals

Some lethal genes allow normal development and survival, except under a specified condition, which is necessary for their lethal effect; such lethal genes are termed as conditional lethals. The environment under which conditional lethals produce their lethal effect is known as non-permissive environment, while under permissive environment they have no adverse effect on survival, i.e., they behave as the normal allele. Many mutants of *Drosophila*, *Neurospora*, barley, maize, and many other organisms are termed as temperature sensitive mutations since each of them requires a specific, generally elevated, temperature to exhibit their lethal action. An example of a temperature sensitive lethal gene is the Kidney-eyed mutant of the wasp *Bracon hebetor* (previously, *Hebrobracon juglandis*). This gene allows normal development and survival at lower temperatures, but is lethal at 30°C. Similarly, a chlorophyll mutant of barley permits normal chlorophyll development at a temperature of 19°C or above but produces albina seedlings at temperatures below 8°C. This conditional lethal in barley requires a lower temperature to exert its lethal effect.

Balanced Lethals

In progeny from the matings between heterozygotes for a lethal gene, about 1/3 individuals are homozygous for the normal allele, while the remaining 2/3 are heterozygous for this gene. But in a balanced lethal system all the surviving progeny are heterozygous for the lethal genes and the homozygotes for their normal alleles are not obtained. In such a system, two nonallelic recessive lethals are linked in the repulsion phase. In repulsion phase linkage, recessive allele of one gene and dominant allele of the other gene are present in the same chromosome. In the example of a balanced lethal system shown in Fig., the recessive allele (l_1) of the first lethal gene and the normal allele (L_2) of the second lethal gene are present in one chromosome, while the homologous chromosome carries normal allele (L_1) of the first gene and recessive lethal allele (l_2) of the second gene. Lethal genes arranged in this manner are known as balanced lethals and the arrangement itself is called balanced lethal system. Individuals heterozygous for both the recessive lethals l_1 and l_2 are fully viable.

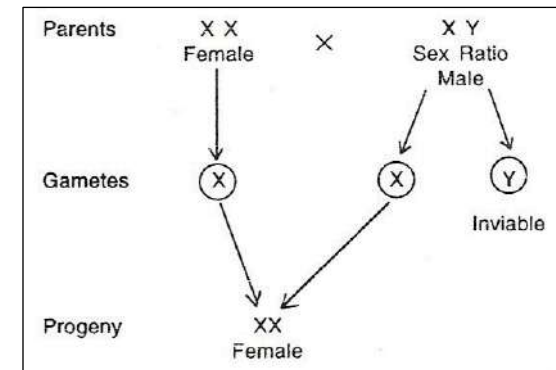


Matings between individuals heterozygous - for these balanced lethals will produce four types of zygotes.

One-fourth of the zygotes will be homozygous for the recessive lethal l_1 (l_1l_2/l_1l_2) and will not survive. Another $\frac{1}{4}$ of the zygotes will be homozygous for the other recessive lethal l_2 (L_1l_1/L_1l_2) and will die. The only surviving progeny will be the heterozygotes for the two recessive lethals (l_1l_2/L_1l_2). Thus a balanced lethal system maintains the genes closely linked to the lethal genes in a perpetual heterozygous state.

Gametic Lethals

Some genes lead to inviability of a class of gametes or make them incapable of fertilization; such genes are termed as gametic lethals. Gametic lethals lead to a drastic departure from the typical ratios expected in segregating generations; this phenomenon is commonly known as segregation distortion or meiotic drive.



Semilethal Genes

Semilethal genes do not lead to death of all the individuals that carry them in appropriate genotype. However, they cause death of more than 90 per cent of the individuals; only less than 10 per cent of the individuals survive. Certain xantha mutants of many plants are semilethal in the homozygous state.

Subvital Genes

Many mutant genes reduce viability of the individuals carrying them in appropriate genotype as compared to that of the normal individuals. Such genes kill less than 90% of the individuals in which they are present in appropriate genotype. Most of the mutant genes are subvital in their effect, e.g., miniature wings in *Drosophila*, some viridis mutants of barley etc.

Vital Genes

Those genes, which do not affect survival of the individuals in which they are present are known as vital genes. Such genes neither enhance nor reduce the viability of individuals carrying them in appropriate genotype. Wild type alleles of all the genes of an organism are regarded as vital genes; they serve as the reference point in determining the effect of mutant alleles of these genes on survival.

Supervital Genes

Some mutant alleles enhance the survival of those individuals that carry them in appropriate genotype as compared to that of the wild type allele; such genes are known as supervital genes. Genes for disease resistance in a crop species may be regarded as supervital genes as they protect the individuals carrying them against the concerned disease and thus increase the chances of their survival in the presence of the concerned disease. Similarly, genes conferring resistance/tolerance to the various abiotic stresses, e.g., salinity, alkalinity, high temperature, low temperature, drought etc., may also be regarded as supervital genes in that they enhance the fitness of plants in presence of the concerned stress.



GENE INTERACTION

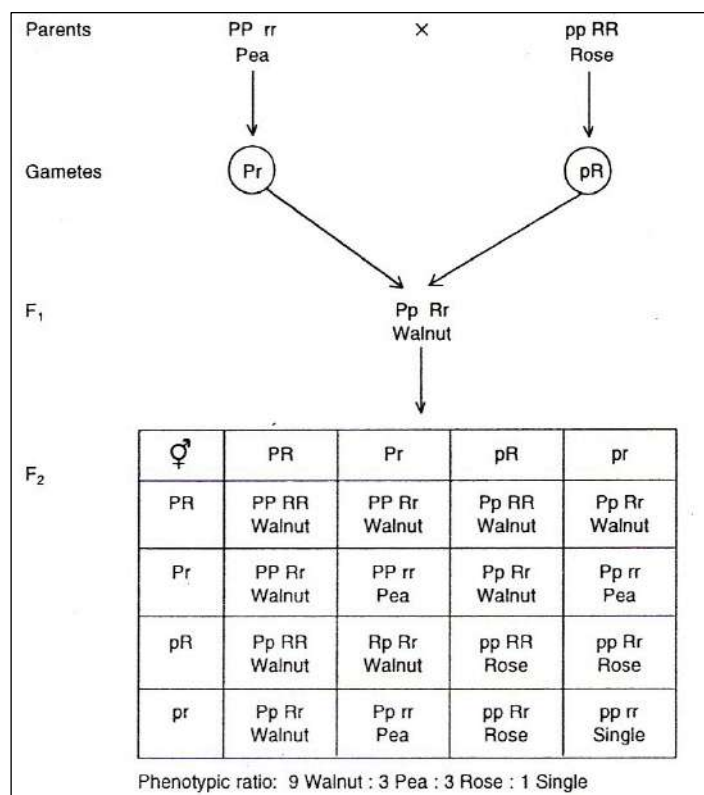
The phenomenon of two or more genes affecting the expression of each other in various ways in the development of a single character of an organism is known as gene interaction.

Typical Dihybrid ratio or Collaboratory gene interaction (9:3:3:1)

This interaction produces the typical dihybrid ratio of 9:3:3:1 in F_2 for a single character. Obviously the concerned character is controlled by two genes exhibiting full dominance. The dominant allele of each of the two genes produces a different form of character when they are alone, i.e., when the dominant allele of one gene is present with the homozygous recessive allele of other locus. But when the dominant alleles of both the genes are present together, they produce a distinct phenotype, the homozygous recessive state at both the loci giving rise to get another phenotype.

In chickens, comb shape is governed by two genes, P and r. The dominant allele of gene P (P) alone (with rr) produces pea comb. While that of gene r (R) alone (with pp) produces rose comb. But when both the dominant genes P and R are present together, e.g., PPRR, such individuals have walnut comb. Recessive condition at both the loci, e.g., pp rr, give rise to a distinct comb called single. When a breed of poultry homozygous for pea comb (PP rr) is crossed with another breed homozygous for rose comb (pp RR), the F_1 (PpRr) has walnut comb as it has the dominant allele of both the genes P & R. Segregation of the two gene produces 16 possible zygotic combinations in F_2 . Nine of the combinations have at least one P and one R allele; having walnut comb. Three have one P or two P alleles; but they are homozygous rr,

as they have pea comb. Three other zygotes are homozygous for p (pp) have one or two R alleles. So, they develop rose comb. The remaining one individual has the genotype pp rr and develop single comb. Since it is homozygous recessive for both the genes p and r. Thus the interaction between two dominant genes controlling the development of a single trait generates 9:3:3:1 ratio in F₂ for a single character.



Duplicate gene interaction or Duplicate dominant epistasis (15:1)

Characters showing duplicate gene action are determined by two completely dominant genes. These

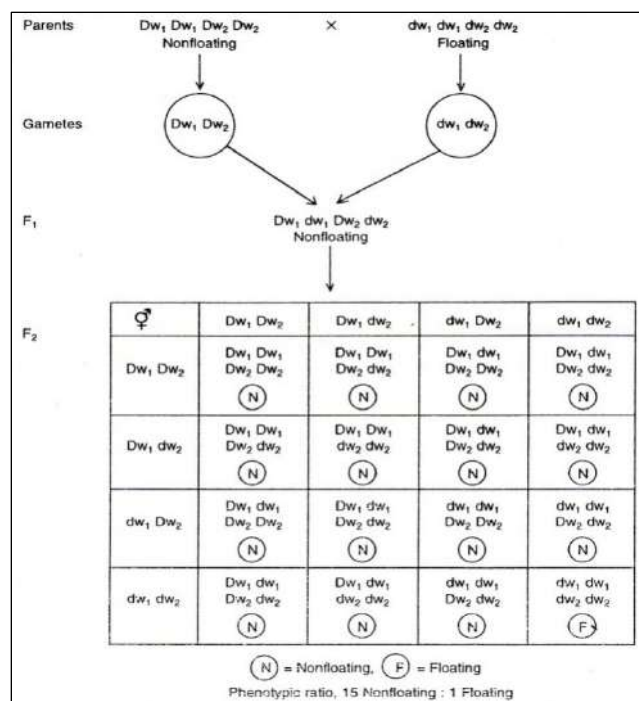
dominant genes produce the same phenotype whether they are alone i.e., with the recessive allele of the other gene or together; the contrasting phenotypes is produced only when both the genes are in homozygous recessive state.

The non floating habit in rice is controlled by two dominant genes DW₁ and DW₂. Genes DW₁ and DW₂ alone, e.g., DW₁DW₁dw₂dw₂ and dw₁dw₁DW₂DW₂, as well as together, e.g., DW₁DW₁DW₂DW₂, produce the same phenotype, viz., non floating. The floating habit is obtained, only when both these genes are in the recessive state, viz., dw₁dw₁dw₂dw₂.

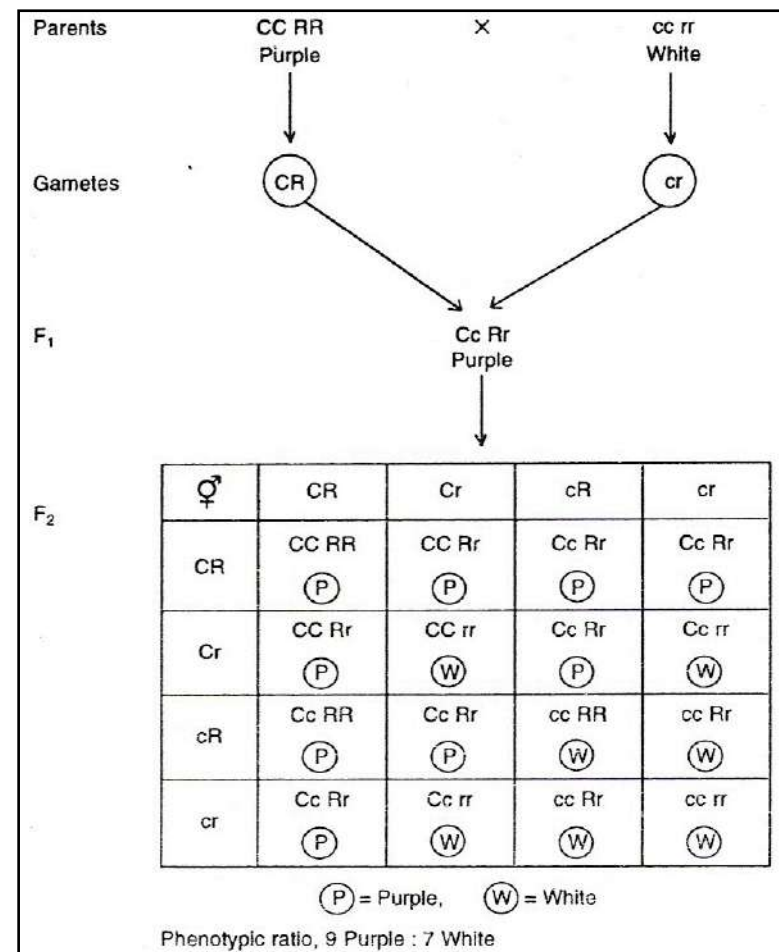
When a non-floating rice strain with the genotype DW₁DW₁DW₂DW₂ is crossed with a floating strain (dw₁dw₁dw₂dw₂) the F₁ (DW₁dw₁DW₂dw₂) is non-floating. In the F₂ generation of this cross, on an average 9 plants will have at least one dominant allele of both the genes, 3 plants will have at least one dominant allele of one of the two genes (but will be homozygous recessive for the other gene) and 3 individuals will have one or two dominant allele of the other gene; all these individuals will have non-floating habit. Only one of the 16 possible zygotic combinations will be homozygous recessive for both the genes dw₁ and dw₂ and hence will exhibit the floating habit.

Complementary gene interaction or Duplicate recessive epistasis (9:7)

In this type of interaction, the production of one of the two phenotypes of a trait requires the presence of dominant allele of both the genes controlling the concerned trait. When any one of the two on both the genes are present in the homozygous recessive state, the contrasting phenotype is produced. Thus anyone of the two dominant genes is unable to produce the phenotype when alone, but they complement each other to produce the concerned phenotype when they are together.



In sweet pea, the development of purple (or coloured) flowers requires the presence of two dominant genes, C and R, e.g., CCRR. When either C (e.g., ccRR) or R (e.g., CCrr) or both the genes (e.g., ccrr) are present in homozygous recessive condition, purple flower colour cannot be produced as a result of which white flowers are obtained. When a purple flowered variety of sweet pea (CCRR) is crossed with a white flowered variety with a genotype (ccrr), the F₁ (CcRr) has purple flowers. In F₂ generation, on average 9 plants will have at least one dominant allele of both the genes C & R; these plants, therefore will produce purple flowers. Three out of the 16 will have dominant C but will be homozygous rr, three others will have dominant R but will be homozygous cc, while one plant will have both the genes in homozygous recessive state (ccrr); all these (total 7) plants will have white flowers.

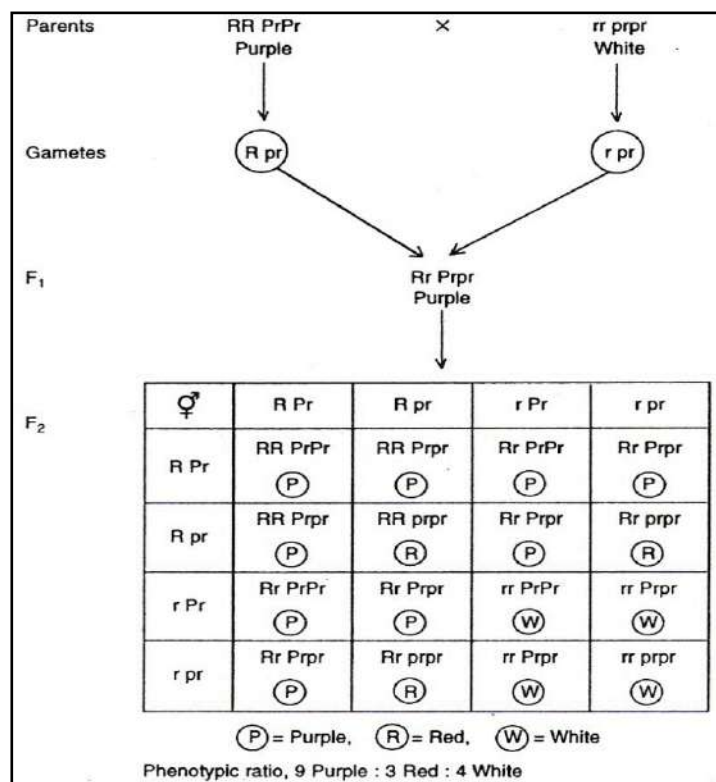


Supplementary gene interaction or Recessive epistasis (9:3:4)

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 the first gene. Thus in supplementary gene action, the

dominant allele of one gene is necessary for the development of the concerned phenotype, while the other gene modifies the expression of first gene.

For e.g., The development of aleurone or grain colour in maize is governed by two completely dominant genes R and Pr. The dominant allele R is essential for colour (red) production; homozygous state of the recessive allele r (rr) prevents the production of red colour. The gene Pr is unable to produce any colour on its own, but it modifies the colour produced by the gene R from red to purple. The recessive allele pr has no effect on grain colour.



A maize inbred with purple grains (RRPrPr) when crossed with an inbred having white grains and the genotype rrprpr, the F₁ (RrPrpr) plants produce purple grains. In F₂, 9 out of the 16 zygotic combinations will have dominant allele of both the genes R and Pr; they will therefore, develop into purple grains. Three out of the 16 zygotes will have dominant allele of the gene R but will be homozygous for Pr (e.g., RR prpr); these grains will develop red colour since the recessive allele pr has no effect on colour production. Three other zygotes will be homozygous rr but will have the dominant allele of pr (e.g., rrPrPr), these seeds will be white since rr is unable to produce any colour and Pr does not produce any colour. The remaining one zygote will be homozygous recessive for both genes (rrprpr) and will produce white seed. As a result the 9:3:3:1 ratio is modified into 9 purple : 3 red : 4 white.

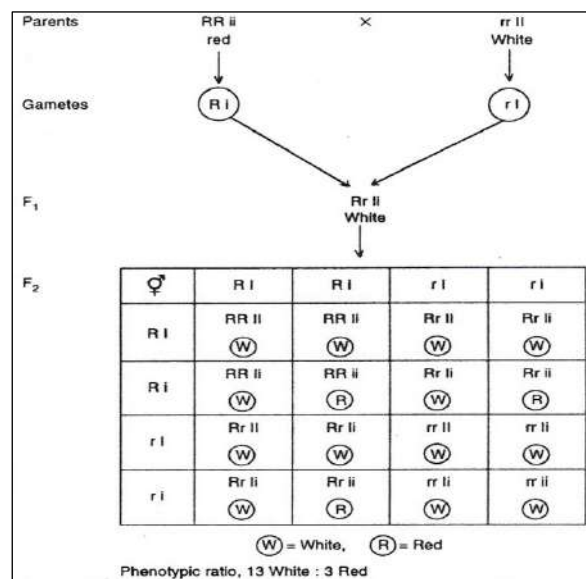
A similar gene interaction occurs in the development of agouty (gray) coat colour in mice.

Inhibitory gene interaction or Dominant recessive epistasis (13:3)

In inhibitory gene action, one of the two completely dominant genes produces the concerned phenotype or the character, while its recessive allele in homozygous state produces the contrasting phenotype. The second dominant gene has no effect of its own on the character in question; however, it has the ability to stop the expression of the dominant allele of the first gene. As a result, when the two dominant genes are present together, they produce the same phenotype as that produced by the recessive homozygote of the first gene. The recessive allele of the second gene does not affect the development of character in any way. Thus, in inhibitory gene action, one dominant gene is capable of producing a character only if its expression is not prevented by another dominant gene known as inhibitory gene and

denoted by I. The 9:3:3:1 F_2 ratio is modified to 13:3 ratio in this case.

An example of inhibitory gene interaction occurs in the development of aleurone colour in maize. A dominant gene R produces red colour, while its recessive allele r produces no colour. Another dominant gene I does not produce any colour by itself.



It only prevents the colour production by R when both I and R are present together. The recessive allele i, does not affect in any way, the colour production in maize aleurone. As a result, red colour in aleurone is produced only when R is present with the homozygous recessive state of the inhibitory locus (e.g., $RRii$). [When a maize inbred with red aleurone ($RRii$) is crossed with another inbred having the genotype $rrII$ and white grains, the F_1 ($RrIi$) has white grains. This is because the gene I stops the colour production by R. In F_2 generation on an average nine out of sixteen seeds will have at least one dominant allele of both R and I; these seeds will develop

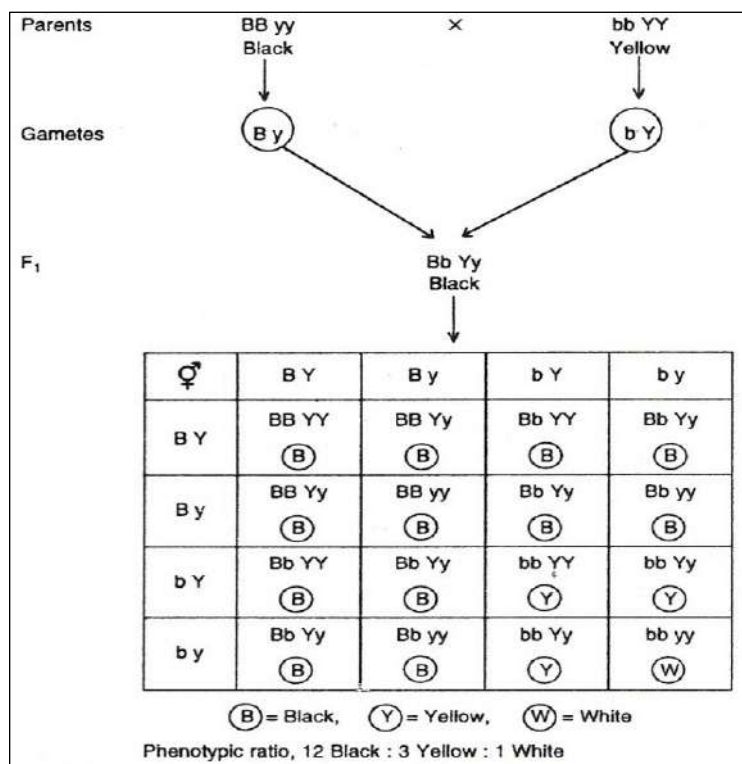
no colour due to inhibitory action of I on colour production by R and will be white in colour. Three seeds (out of 16) will have the dominant allele R, either in homozygous ($RRii$) or in heterozygous state ($Rrii$), but will be homozygous for the recessive allele of i. These seeds will develop red colour since the gene R is allowed to function in the absence of the dominant gene I. Three other seeds will be homozygous rr , but will have one or two dominant alleles of I, while the remaining one seed will be homozygous recessive for both the genes ($rrii$); these seeds will be white simply because the colour producing gene is in the recessive state (rr).]

Masking gene interaction or Dominant – epistasis (12:3:1)

In this interaction, the two genes affecting the same character produce distinct phenotypes when they are alone. But when both the gene are present together, the expression of one gene masks the expression of the other. When both the genes are present in recessive state, a different phenotype is produced (In fact, both the genes express themselves when they are present together, but the expression of one gene is so intense or strong that the expression of the other gene cannot be observed. That is why such a gene interaction has been named as Masking gene action.) In barley, seed coat colour is governed by two dominant genes B and Y. Gene B leads to the development of black colour; while its recessive allele b produces white seeds. The other gene Y produces yellow seed coat, while its recessive allele (y) also gives rise to white seed coat colour.

When the dominant allele B and Y are present together, both the genes express themselves. However, the black colour produced by gene B is so intense that it does not permit the detection of yellow colour produced by the Y gene. [When a barley strain having black seed coat and the genotype $BByy$ is crossed with another strain having yellow seed coat $bbYY$ the F_1 ($BbYy$) seed

have black seed coat. In F_2 , on an average 9 seeds will have atleast one dominant allele of both the genes B and Y; these will develop black seed coats since the presence of yellow colour in their seed coats cannot be detected. Three of the sixteen zygotic combinations will have a dominant allele of B but will be homozygous for recessive allele y (BByy, Bbyy). These seeds will also have black seed coat due to presence of B. In three other seeds, recessive allele b will be in homozygous state, while one or two dominant allele of Y gene will be present (bbYY, bbYy). These seeds will have yellow seed coats due to Y gene since gene B is in the recessive state. The remaining one combination (bbyy) is homozygous recessive for both the genes and will develop while seed coat color.

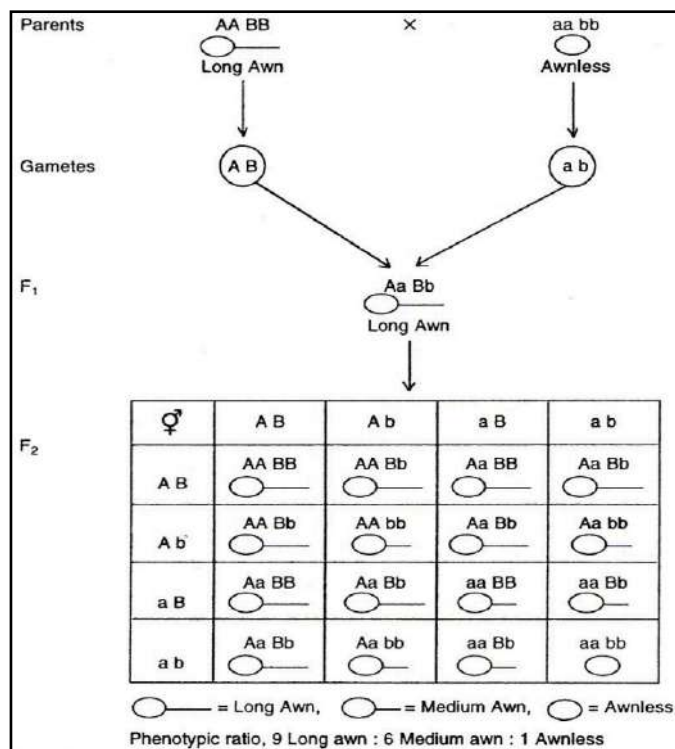


Polymeric Gene Action or Duplicate with cumulative effect (9 : 6 : 1)

In polymeric gene action, the two genes controlling a character produce identical phenotypes when their dominant alleles are alone (that is, with the homozygous recessive condition of the other gene). But when dominant alleles of both the genes are present together, their phenotypic effect is enhanced as if the effects of the two genes were cumulative or additive

It should be noted that in this case both the genes show complete dominance. If the two genes showing polymeric gene action also show an absence of dominance, an additive gene action will be the consequence.

In barley, two completely dominant genes A and B affect the length of awns, the thin needle like extensions of lemma. Gene A or B alone (e.g., AAbb and aaBB respectively), gives rise to awns of medium length; the magnitude of effect of A is the same as that of B. But when both the genes A and B are present together, they produce long awns indicating that the effects of A and B on awn length are added together. Individuals homozygous recessive for both these genes are awnless. When a long awned (AA BB) variety of barley is crossed with an awnless (aa bb) one, the F_1 (Aa Bb) has long awns due to the cumulative effect of both the genes A and B. In the F_2 generation, on an average, nine plants (out of 16) will have dominant alleles of both the genes A and B; as a result, these plants will produce long awns. Three plants will have at least one dominant allele of A but will be homozygous for b (AA bb, Aa bb), while three others will be homozygous for a, but will have atleast one dominant allele of B (aaBB, aaBb). All these six plants will have medium-sized awns since they have either A or B (but not both). The remaining one plant (out of the 16 zygotic combinations in F_2) will be homozygous recessive for both the genes (aa bb) and will, therefore, be awnless.



Thus polymeric gene interaction changes the typical 9 : 3 : 3 : 1 F₂ ratio into a 9 : 6 : 1 ratio. Some other examples of this interaction are also known, e.g., fruit shape in summer squash etc.

Modifying Genes or Modifiers

Modifying genes are a group of genes which enhance or reduce the phenotypic effect of a major genes.

Characteristic Features

1. Alteration of dominance relationship at a locus
2. Generation of quantitative variation produced by a major gene.
3. Suppression of mutant alleles of same genes.

Pleiotropism

“When a gene causes changes (or) affect two or more characters that are not obviously related, the gene is called a pleiotropic gene and the condition is known as pleiotropy”.

E.g., (1) In cotton, the Punjab hairy lintless gene, *lic* produces seeds which are without lint. This gene also causes incomplete lacination of the leaf, reduction in the number and length of internodes and reduction in boll size and fertility.

(2) In plants a gene may produce a red pigment in several organs such as flowers, stem and leaves but it is not quite correct to say that the gene is pleiotropic because the gene has only one general effect, the production of pigment.



POLYGENIC INHERITANCE

Multiple factor hypothesis (Polygenes / Minor genes / Polygenic inheritance)

The existence of several genes with small cumulative effect governing a single trait; such genes are known as polygenes (or) minor genes

Based on this, multiple factor hypothesis was proposed by Nilsson-Ehle in 1908. According to this, “A character is governed by several genes; the positive alleles of all the genes governing the trait are similar to each other in their action (effect) and that their effects are additive or cumulative in nature. This is the essence of the multiple factor hypothesis”.

Studies on seed colour in wheat

The inheritance of seed colour in crosses showing 1:4:6:4:1 ratio in F_2 may be explained as follows. The seed colour in such crosses is governed by two genes, say R_1 and R_2 (positive allele), r_1 and r_2 being the negative allele of those genes. If the two parents of such a cross have the phenotypes dark red and white, the genotypes are $R_1R_1R_2R_2$ and $r_1r_1r_2r_2$ respectively. They produce the gamete R_1R_2 and r_1r_2 respectively. They unite to give rise to $R_1r_1R_2r_2$ in F_1 .

Since F_1 seeds have only two positive alleles, their colour will be intermediate between these of the true parents i.e., Medium red. In F_2 , one zygote out of 16 will have four positive allele ($R_1R_1R_2R_2$). They produce dark red colour. In four, 3 positive alleles will be present ($R_1R_1R_2r_2$ and $R_1r_1R_2R_2$). These seeds will be lighter in colour than dark red but darker than F_1 seeds, they shows medium dark red. Six out of 16, will have only two positive alleles each ($R_1r_1R_2r_2$, $R_1R_1r_2r_2$, $r_1r_1R_2R_2$), they

are similar to F_1 and show medium red colour. In four others, only one positive allele will be present and will be light red, i.e., intermediate between F_1 seeds and white parental seeds. The remaining one seed will have no positive allele ($r_1r_1r_2r_2$) and they show white colour. Thus it produces 1:4:6:4:1 ratio in F_2 of wheat.

Parents		$R_1R_1R_2R_2$ Dark Red		X	$r_1r_1r_2r_2$ White	
Gametes		R_1R_2			r_1r_2	
F_1		$R_1r_1R_2r_2$ Medium Red				
F_2		R_1R_2	R_1r_2	r_1R_2	r_1r_2	
R_1R_2		$R_1R_1R_2R_2$ Dark red	$R_1R_1R_2r_2$ Medium dark red	$R_1r_1R_2R_2$ Medium dark red	$R_1r_1R_2r_2$ Medium red	
R_1r_2		$R_1R_1R_2r_2$ Medium dark red	$R_1R_1r_2r_2$ Medium red	$R_1r_1R_2r_2$ Medium red	$R_1r_1r_2r_2$ Light red	
r_1R_2		$R_1r_1R_2R_2$ Medium dark red	$R_1r_1R_2r_2$ Medium red	$r_1r_1R_2R_2$ Medium red	$r_1r_1R_2r_2$ Light red	
r_1r_2		$R_1r_1R_2r_2$ Medium red	$R_1r_1r_2r_2$ Light red	$r_1r_1R_2r_2$ Light red	$r_1r_1r_2r_2$ White	

Characteristic features

1. They show continuous variation
2. They had marked influence on the character and their expression
3. They won't show dominance relationship.

“A character is governed by several genes; the positive alleles of all the genes governing the trait are similar to each other in their action and their effects

are additive or cumulative in nature. This is the essence of multiple factor hypothesis”.

Studies on *Nicotiana longiflora*

In 1916, East reported his studies on the inheritance of corolla length in *Nicotiana longiflora*, which presented a conclusive evidence for polygenic inheritance of this trait. He crossed two inbred lines with contrasting corolla lengths of 40 and 93 mm. The F_1 from the cross was intermediate with mean corolla length of 63 mm. In F_2 , a much larger variation for corolla length than that present in the parents on the F_1 was observed, this variation was continuous as well.

The main features of his study are summarised below:

1. The two parents were inbreds (obtained by selfing for several generations) and almost homologous. But there was variation for corolla length within each of the two parents. Obviously, this variation cannot be genetic in nature since all the plants within a parental line are expected to have the same genotype since they are progeny of a (almost) homologous plants. The variation for corolla length within each of the parents is, therefore, clearly due to environmental factors.
2. The F_1 mean was intermediate between those of the two parents. This is precisely what would be expected if corolla length was governed by multiple factors (polygenes) with cumulative effect.
3. The amount of variation in F_1 was comparable to that in the parents. Again all the F_1 plants will have the same genotype. Therefore the variation for corolla length in F_1 also was produced by the environment was not of genetic nature.
4. The F_2 mean was close to that of F_1 and intermediate between those of the two parents. This is precisely what is expected in case of polygenic inheritance. In addition, there was a much greater variation for

corolla length in F_2 than in either of the parents or in F_1 . Segregation and recombination of polygenes governing corolla length is expected to generate genetic variation in F_2 . This together with the variation produced by the environment will account for the increased variation in F_2 .

Environmental Effects on Character Development

Penetrance

“The ability of gene to express itself in an individual that carry them is called penetrance”.

Complete penetrance

In general, “genes express themselves in all the individuals in which they are present in the appropriate genotype. This is known as complete penetrance”.

E.g., In pea plants the red and white flower colour, tall and dwarf character, the seed colour and shape all exhibit complete penetrance.

Incomplete penetrance

“Many genes do not produce the concerned phenotype in all the individuals which carry them in the appropriate genotype is called Incomplete penetrance”.

E.g., The recessive gene producing partial chlorophyll deficiency in the cotyledonary leaves of lima bean shows 10 % penetrance.

Expressivity

It is the ability of a gene to produce itself uniformly in all the individual with same genotype.

Complete or uniform expressivity

A gene that produce itself uniformly in all the individual that carry it. E.g. All Mendelian genes.

Incomplete or variable expressivity

“The genes which do not have similar expression in all the individuals possessing them”.

E.g. The gene producing chlorophyll deficiency in Lima bean (*Phaseolus lunatus*) exhibit variable expressivity in addition to incomplete penetrance. In some seedlings, the complete cotyledonary leaves are without chlorophyll, in some only the leaf tip lacks the chlorophyll, while in other the margins of the leaves have no chlorophyll.

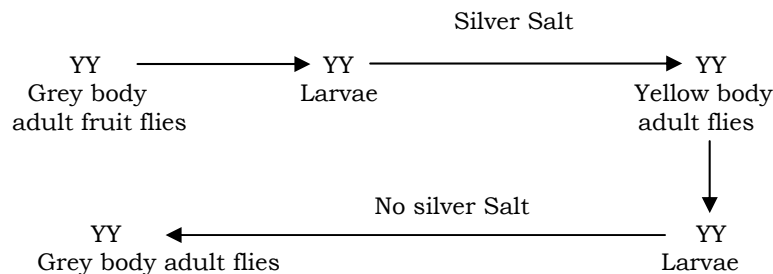
Phenocopy Effect

Certain specific environments change the expression of a gene to such an extent that the resulting phenotype is the same as that produced by another allele of the same gene. This type of effect due to environment is referred to as phenocopy effect, while individuals possessing such altered phenotypes are called phenocopies.

The phenocopy effect represents an alteration in the phenotype due to a modification of the expression of the concerned gene and it does not represent a change in the gene itself, i.e., gene mutation.

Consequently, a phenocopy effect is confined to the individuals and the generation in which it is produced and is not transmitted to the next generation unless the environment producing the phenocopy is also present in the next generation.

Example



LINKAGE AND CROSSING OVER

Linkage

The tendency of two or more genes to stay together during inheritance is known as linkage. It is the consequence of the concerned genes being located in the same chromosome.

Linked genes do not show independent segregation; as a consequence, the ratios obtained in F_2 and test-cross generations are significantly different from the expected ratios of 9:3:3:1 and 1:1:1:1 (in two linked genes).

Experiment on Pea

A significant deviation from the expectations due to independent segregation was first reported by Bateson and Punnet in 1905. They studied the inheritance of flower colour and pollen shape in pea. A dominant gene B produces blue flowers, while its recessive allele b determines red flowers. Another dominant gene L governs elongate pollen grains, whereas its recessive allele l is responsible for normal round pollen grains. When the F_1 from the cross blue elongate (BB LL) x red round (bb ll) was test crossed (with red round, bb ll), the progeny showed the ratio 7 blue elongate (Bb Ll) : 1 blue round (Bb ll) : 1 red elongate (bb Ll) : 7 red round (bb ll) in place of the expected 1 : 1 : 1 : 1 ratio. It appears as if the two dominant genes (B and L) have an affinity for each other so that they tend to stay together during inheritance; Bateson and Punnet called this situation as coupling phase. But when the F_1 from the cross blue round (BB ll) x red elongate (bb LL) was test crossed (with red round, bb ll), the progeny exhibited the ratio 1 blue elongate (Bb Ll) : 7 blue round (Bb ll) : 7 red elongate (bb Ll) : 1 red round (bb ll). It appears as if, in this case, the two

dominant genes (B and L) repel each other so that they tend to stay away from each other during inheritance. This situation was referred to as repulsion phase.

Bateson and Punnet failed to advance a suitable explanation for these findings. They suggested that there was a selective multiplication (through mitosis) of some types of gametes, *viz.*, of BL and bl in the coupling phase, and of Bl and bL in the repulsion phase, after meiosis; this gave rise to the 7 : 1 : 1 : 7 and 1 : 7 : 7 : 1 testcross ratios in place of the expected 1 : 1 : 1 : 1. This hypothesis is untenable as it is contrary to the known facts. But the terms coupling phase and repulsion phase are widely used to denote linkages between two (or more) dominant genes, and between one (or more) dominant and one (or more) recessive genes, respectively.

Experiment on Maize

In maize, a dominant gene 'C' produces coloured seeds, while its recessive allele, 'c' determines colourless seeds. Another dominant gene 'Sh' governs full seeds, whereas its recessive allele 'sh' gives rise to shrunken seeds.

When plants having coloured full seeds (CC ShSh) were crossed with the having colourless shrunken seeds (ccshsh), F₁ seeds were coloured full (Cc Shsh). Out of the 8,368 seeds obtained from the test cross Cc Shsh X ccshsh, 4,032 (48.2%) were coloured full 4035 (48.3%) were colourless shrunken, 149 (1.7%) were coloured shrunken and 152 (1.8%) were colourless full. Clearly, the four phenotypic classes are not presented in the expected ratio of 1:1:1:1. The phenotypic classes of the parental combinations are much higher than the expected. They are known as parental phenotypes (coloured full and colourless shrunken). The remaining two phenotypic classes, coloured shrunken and colourless full are far less frequent than expected (25%). These two character combinations are called recombinant

phenotypes, since they are generated by reshuffling of the characters of the two parents of the F₁ used in the test cross.

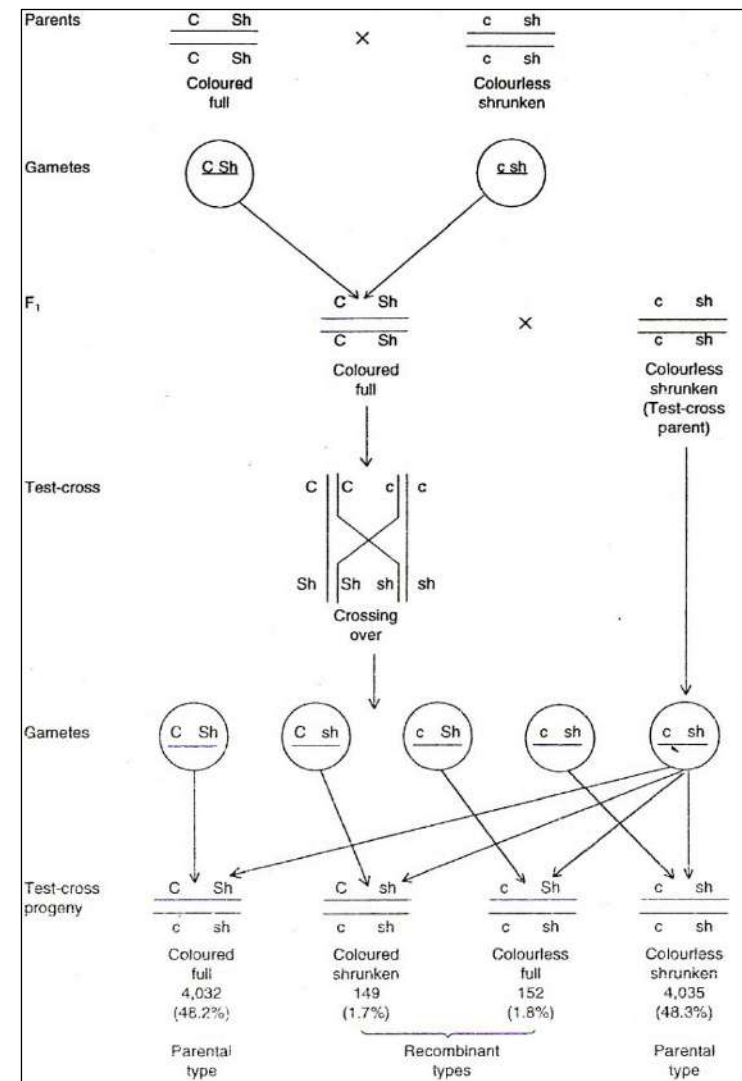


Fig. 14.1 Coupling Phase Linkage in Maize

In the above example, it appears as if the two dominant gene C and Sh have a strong affinity for each other so that the frequencies of coloured full and colourless shrunken phenotypes are greater than expected. This situation is referred to a coupling phase, (Fig 14.1) and is due to the presence of genes C and Sh in the chromosome.

Similarly, when plants having coloured shrunken seeds (CC shsh) were crossed with those having colourless full seeds (ccShSh), the F₁ seeds were coloured full (CcShsh). But when F₁ plants were test-crossed (CcShsh) x (ccshsh), 47.9 % of the seeds were coloured shrunken, 49.1 % were colourless full, 1.4 % were coloured full and 1.5 % were colourless shrunken. In this case also, the parental types (colourless full and coloured shrunken) were more frequent while the recombinant types (coloured full and colourless shrunken) were less frequent than expected. It appears as if the dominant genes C and Sh dislike each other so that the phenotypic classes coloured shrunken and colourless full are much more frequent than expected. This situation is referred to as repulsion phase (Fig 14.2); it is due to the presence of the dominant allele of one gene, e.g., C, with the recessive allele of the other gene, e.g., sh, in the same chromosome. Obviously, coupling and repulsion phases are only the two situation of linkage.

Incomplete Linkage

When only parental character combinations, are recovered in the test cross progeny, it is called complete linkage. Sometimes, their alleles recombine to produce recombinant gametes, e.g., Csh and cSh when such recombinant (due to crossing over) types are also recovered, in addition to the two parental types, in the test cross progeny, it is called incomplete linkage.

Crossing Over

The exchange of precisely homologous segments between non-sister chromatids of homologous chromosomes is known as crossing over.

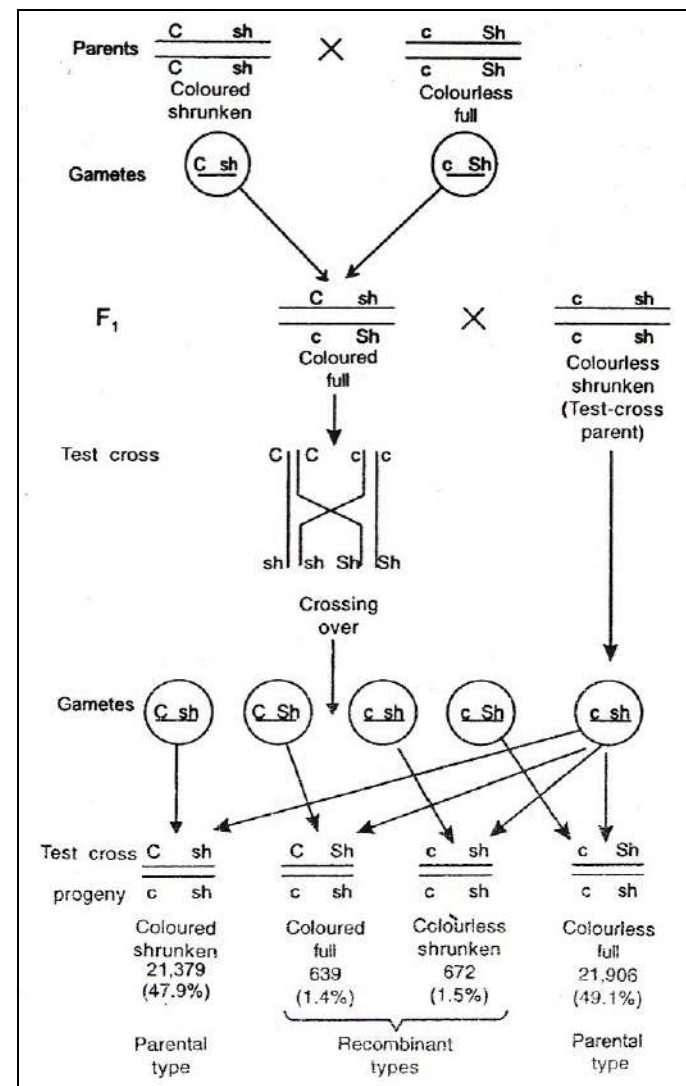


Fig. 14.2 Repulsion Phase Linkage in Maize

. It is responsible for recombination between linked genes. Crossing over takes place during pachytene, i.e., after the homologous chromosome undergone pairing and before they begin to separate. During pachytene, each chromosome of a bivalent (chromosome pair) has two chromatids so that each bivalent has four chromatids or strands (four stranded stage).

Generally, one chromatid from each of the two homologous of a bivalent is involved in crossing over (Fig 14.3). The point of exchange of the homologous segment of the non-sister chromatids of homologous chromosome is known as chiasma.

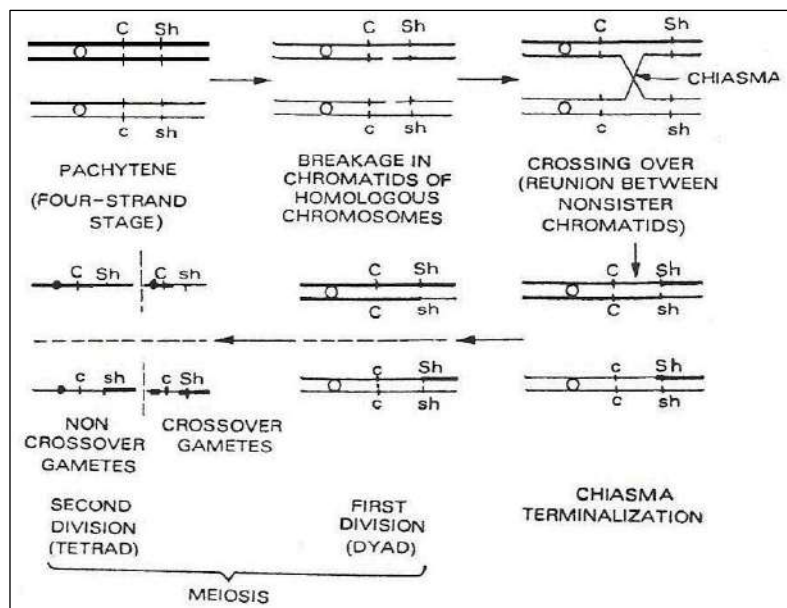


Fig. 14.3 Crossing Over Between Non-sister Chromatids

Obviously, each event of cross over produces two recombined chromatids, called crossover chromatids, and two original chromatids referred to as non-crossover chromatids. The frequency of crossing over between two genes can be estimated as the frequency of recombinant

progeny from a test cross for these genes. It is expressed in percentage.

$$\text{Frequency of crossing over (\%)} = \frac{\text{Number of recombinant progenies from a test cross}}{\text{Total number of progenies}} \times 100$$

Crossing over in four-strand stage (Experiments on *Neurospora*)

Studies with light microscope showed that during diplotene the chromosomes were four-stranded, some times during pachytene stage. Does crossing over takes place in four-stranded stage ?

This question was answered through genetic studies in *Neurospora* (ascospores colour) and *Drosophila* (using attached X chromosome).

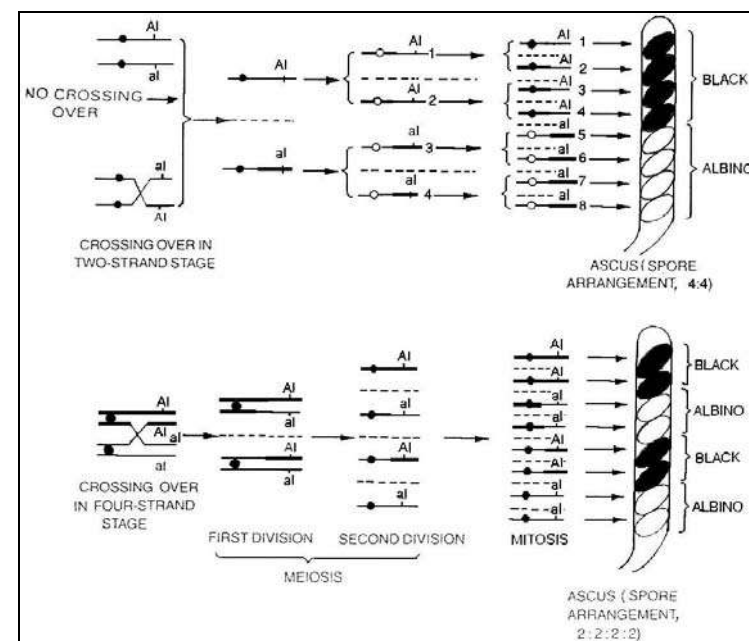


Fig. 14.4 Crossing Over in Four Stranded Stage in *Neurospora*

In *Neurospora*, each zygote undergoes meiosis to yield four haploid nuclei; these nuclei undergo mitosis to yield eight haploid (n) nuclei. They are arranged in a linear order; each nucleus gives rise to one ascospore (Fig 14.4).

The first meiotic division in the zygote Aa would produce two nuclei A and a; the four haploid nuclei obtained A, A, a and a ; and the eight nuclei generated after the mitotic division would lie in the order Al,Al,Al,Al, al,al,al,al. Thus the arrangement of ascospore in a mature ascus may be used to deduce the arrangements of the concerned homologous chromosomes and their chromatids during the meiotic and mitotic division.

The dominant gene 'Al' of *Neurospora* produces black ascospores, while its recessive allele 'al' determines albino ascospores. The regular arrangement of ascospores makes it possible to predict the consequence of crossing over between this gene and its centromere on the spore arrangement.

If there is no crossing over between the gene and the centromere or if crossing over takes place between two-strand stage, the ascospores obtained from the heterozygous Alal, zygotes would show 4 black: 4 albino spore arrangement. However, if the crossing over occurred in four strand stage, the spores would show a 2 black : 2 albino : 2 black : 2 albino or 2 :4:2 arrangement.

The asci from Alal heterozygotes show 4 : 4, 2 : 4 : 2 as well as 2 : 2 : 2 : 2 spore arrangement. The 4 : 4 arrangement may be due to no crossing over or due to crossing over in two-strand stage. But 2 : 4 : 2 and 2 : 2 : 2 : 2 arrangement in some of the asci of Alal heterozygotes clearly show that crossing over takes place in four-strand stage.

Cytological basis of crossing over

Since linked genes are located in the same chromosome, they would show recombination only when

there is exchange of the concerned segments between the homologous chromosome i.e., crossing over.

Curt Stern used a *Drosophila* female in which one 'X' chromosome was shorter than the normal; this chromosome had the recessive gene car (carnation eye colour) and the dominant gene B (Bar eye shape). The other 'X' chromosome of this female was of normal length, but a segment of 'Y' chromosome was translocated into its short arm; this chromosome had the dominant gene car⁺ (wild type allele of car, producing dull red eye colour) and the recessive gene B⁺(wild type allele of B, producing normal ovate eye shape). Stern, test crossed this female to a Car B⁺male. As expected, the following four types of flies were recovered in test-cross progeny; red normal (Car⁺B⁺), red bar (Car⁺B); carnation normal (Car B⁺); and carnationbar (Car B).

Two of these four phenotypes, viz., red normal and carnation bar, are non-cross over or non-recombinant types. Therefore the carnation bar individuals are expected to carry one short 'X', while the red normal over would have one long 'X' with a 'Y' segment. In contrast, the remaining two phenotypes, viz., red bar and carnation normal, are crossover or recombinant types.

If crossing over involves an exchange of homologous chromatid segments between homologous chromosomes, then one 'X' chromosome of these individuals would be the product of such an exchange (Fig. 14.5).

Therefore carnation normal (Car B⁺) flies are expected to have normal or long 'X' chromosome without the attached 'Y' segment, while red bar (Car⁺B) individuals will have one short 'X' with the attached 'Y' segment. Stern concluded that (1) During meiosis, there is exchange of precisely homologous chromatid segments between homologous chromosome, (2) Crossing over is responsible between linked genes.

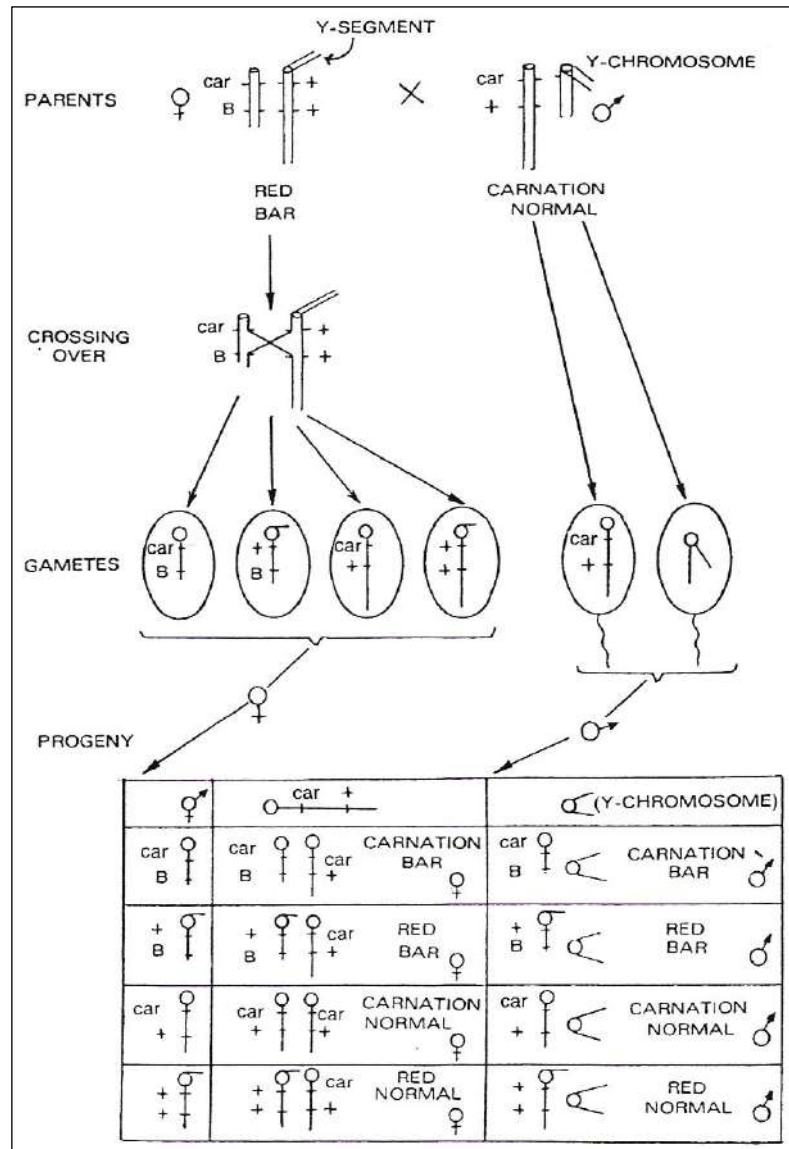


Fig. 14.5 Cytological Basis of Crossing Over in *Drosophila*

Crossing Over between three linked genes

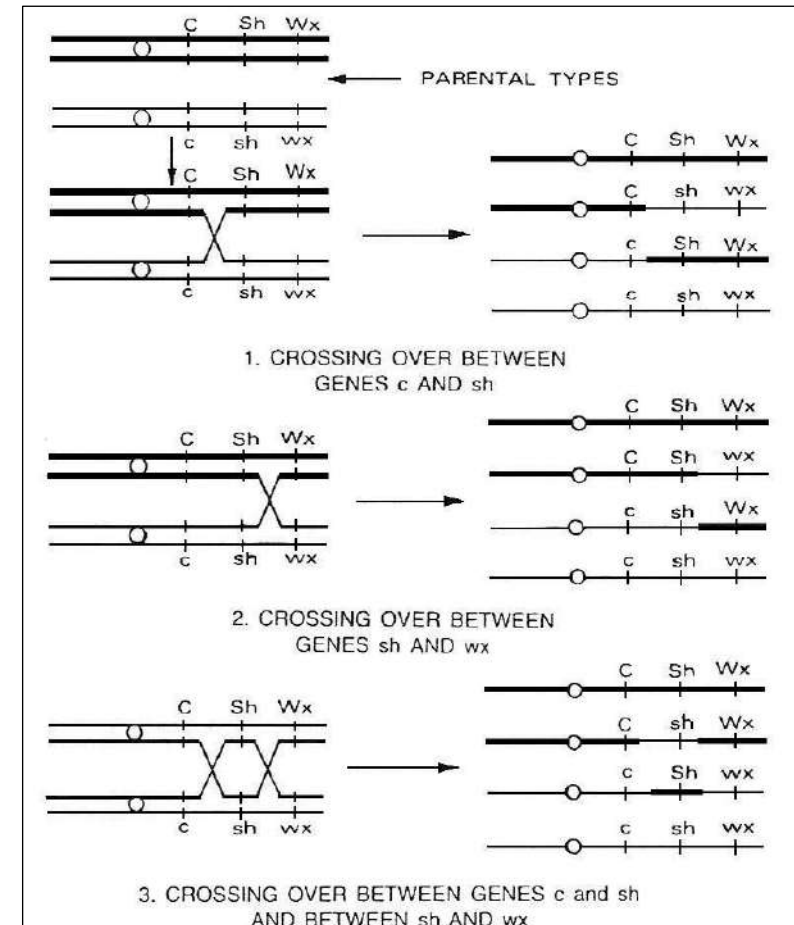


Fig. 14.6 Crossing over between three linked genes

A test cross for three linked genes eg., *c*, *sh* and *wx* in maize, yields eight types of gametes and phenotypes (Fig. 14.6). Two of these types i.e., CWxSh and cwxsh are the most frequent and represent the parental or non-recombinant types, while two others i.e., CwxSh and cWxsh are the least frequent and are double cross overs.

The remaining four types i.e., Cwxsh, cWxSh, cwXsh and CWxsh are produced by single cross overs between three linked genes, and the frequency is intermediate between the parental and double cross over types.

Chromosomal Theory of Linkage

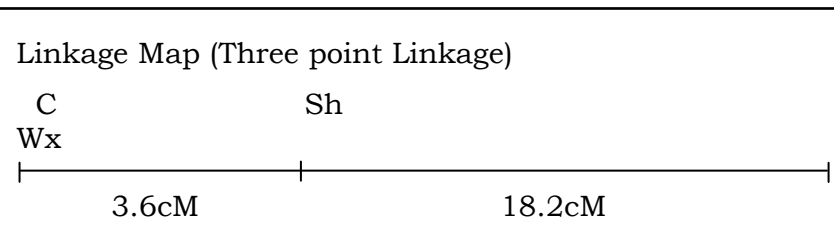
This theory was proposed by Morgan and Castle. Genes located in the same chromosome are inherited together and are said to be linked. They are arranged in a linear fashion on the chromosome. The degrees of linkage is determined by the distance between genes, closely located genes show strong linkage while genes widely located show weak linkage.

Linkage map (Chromosome map / Genetic map)

All the genes that are linked together form a linkage group. They are represented on a single straight line in the same order in which they are normally present in the concerned chromosome.

The line drawing depicting the linked genes and the recombination frequencies between them is known as linkage map or genetic map or chromosome map.

The recombinant frequencies between linked genes are determined by studying test crosses. The present frequencies are used as map units for linkage maps. A map unit is also called as Morgan. The member of different linkage groups is equal to its genetic chromosome number (n), for maize it is 10, for man 23. The frequency of recombination between two linked genes cannot exceed 50%.



The genes c, sh and wx in the order of C-Sh-Wx at the distance of 3.6 and 18.2 map units respectively.

Coefficient of Coincidence

The ratio between the observed and the expected frequencies of double crossovers is called coefficient of coincidence. Thus,

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

Coefficient of Interference

In the double cross over, the occurrence of crossing over in one region of a chromosome interferes with its occurrence in the neighbouring segments. This is called interference.

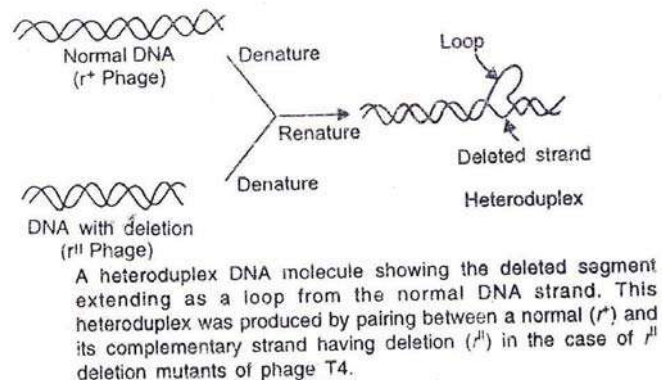
$$\text{Coefficient of interference} = 1 - \text{coefficient of coincidence}$$

$$\text{Coefficient of interference} = \frac{1 - \text{coefficient of coincidence} \times 100}{(\%)}$$



PHYSICAL MAPPING OF CHROMOSOMES

Physical maps of chromosomes depict such other recognition features *eg*, sites for restriction enzymes, sites and sizes of deletions, sites of A-T- rich regions, *etc*, of the genetic material, which is not directly connected with the production of a trait (*i. e.* genetic function). In contrast, when production of phenotypes is used for constructing the maps of chromosomes using recombination data, it is known as genetic map. Here two main types of physical mapping, *viz*, heteroduplex mapping and restriction mapping, are briefly described.



Heteroduplex mapping

A heteroduplex is a double-stranded nucleic acid molecule the two strands of which are not entirely complementary. For example, in a DNA heteroduplex one strand may contain one allele of a gene while the other strand may have another allele. Such heteroduplex molecules are prepared by mixing two denatured DNA preparations (*e.g.* DNA from a deletion mutant of the r^{II} locus of T₄ phage and that from the wild type phage) and

allowing them to renature. Many of the double-stranded molecules thus produced will be heteroduplexes *i.e.*, they will be composed of one strand from the wild type phage DNA and the other strand from the deletion mutant of the r^{II} locus. In such heteroduplexes, the segment of wild type strand, which is missing in the mutant strand will remain unpaired and can be seen as a loop under an electron microscope.

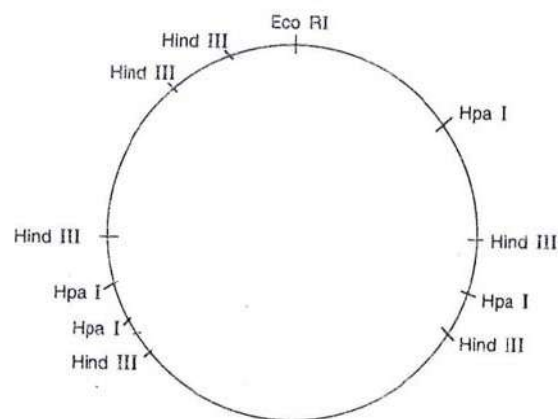
This technique is known as **heteroduplex mapping**. It permits determination of the size and location of a deletion of a gene. Using this technique, the presumed deletion mutants of r^{II} locus by Benzer were shown to really contain deletions in the r^{II} region. Further, the size of r^{II} locus was estimated as 2645 bp (cistron A 1800 ± 70 bp; and cistron B, 845 ± 50 bp). This technique is extended to DNA : RNA heteroduplexes, which has permitted the discovery and demarcation of introns and exons of eukaryotic genes.

Restriction mapping

A restriction map of a chromosome (or a segment thereof) depicts the sites where one or more restriction endonucleases produce cuts in the chromosome. Several approaches are used for restriction mapping. *e.g.*, comparison of the products of (i) partial digestion with one enzyme (ii) sequential digestion with two or more different restriction enzymes, (iii) digestion of a circular DNA molecule under conditions permit that only one cut by an endonuclease (in place of a complete digestion) followed by digestion of the linear segments thus produced by another endo-nuclease, which indicates only one cut at a specific site in the chromosome and several other approaches.

Restriction maps are also called **physical maps**. These maps are extremely useful in DNA sequencing experiments since they are helpful in the production of desired fragments of chromosomes. Physical maps are

generally combined with genetic maps permitting the location of specific genes in specific segments of chromosomes.



A physical map (restriction map) of the Simian Virus 40 (SV40) chromosome showing the recognition sites for three restriction endonucleases, viz., *EcoRI*, *HpaI* and *HindIII*. The recognition site for *EcoRI* has been arbitrarily placed; other recognition sites are mapped in relation to this recognition site.



SEX DETERMINATION

Chromosomal sex determination

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. In most of the dioecious organisms, females and males differ visibly in chromosome constitution. The precise form of the chromosomal difference between the sexes is not the same in different organisms. Four types have been organised and they are as follow:

XX - XO type

In the squash bug, *protenor* the females have 14 chromosomes and the males have only 13 chromosomes in their somatic cells. In the females, 7 bivalents are formed during meiosis. All the eggs have therefore 7 chromosomes each. In the males, one odd, unpaired chromosome and 6 bivalents are seen during meiosis. The unpaired chromosome passes undivided into one of the two daughter cells. Two kinds of sperms in equal number are therefore formed, one kind with 7 chromosomes each and other kind with 6 chromosomes each. An egg (with 7 chromosomes) fertilized by a sperm with 7 chromosome produces a female (14 chromosomes) and an egg (with 7 chromosomes) fertilized by a sperm with 6 chromosomes produces a male with 13 chromosomes. The odd chromosomes of the male thus determines the sex of the individual receiving it was consequently called the sex-determinar or sex chromosome or the 'X' chromosome. The other chromosomes, which are alike in females and males, are called autosomes (Fig. 16.1).

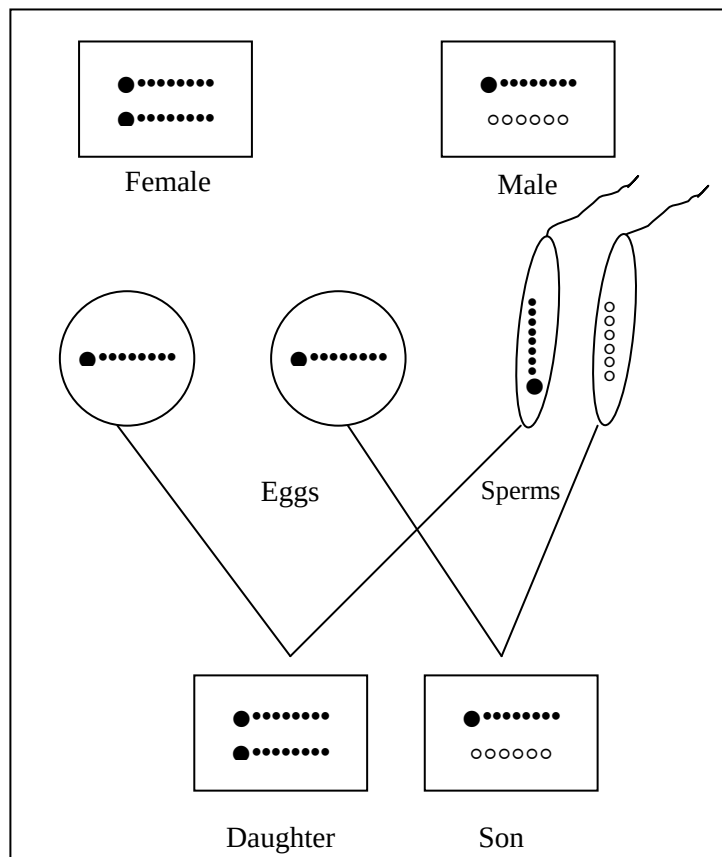


Fig. 16.1. XX-XO Type of Sex Determination

In grasshoppers and bugs, the female is thus XX and the male is XO (using O to indicate the absence of one X chromosome). Among plants, *Dioscorea sinuata* is an e.g. where female is XX and the male XO.

XX - XY type

In many animals and plants, females and males have the same even number of chromosomes, but whereas in the females the members of each pair of

chromosomes are alike, in the males the members of one pair of chromosomes are dissimilar in size and form.

In *Drosophila melanogaster* the female has four pairs of chromosomes as follows: (1) a pair of rod-shaped chromosomes (2) a pair of V-shaped chromosomes (3) a pair of slightly longer V-shaped chromosomes and (4) a pair of very short dot-like chromosomes (Fig. 16.2).

In the male *Drosophila*, there is only one rod shaped chromosome and other member of this pair being J-shaped (i.e., hook shaped or rod with a bent end). Wilion in 1905 discovered this in male and named it as 'Y' chromosome and the other member of this pair as 'X' chromosome.

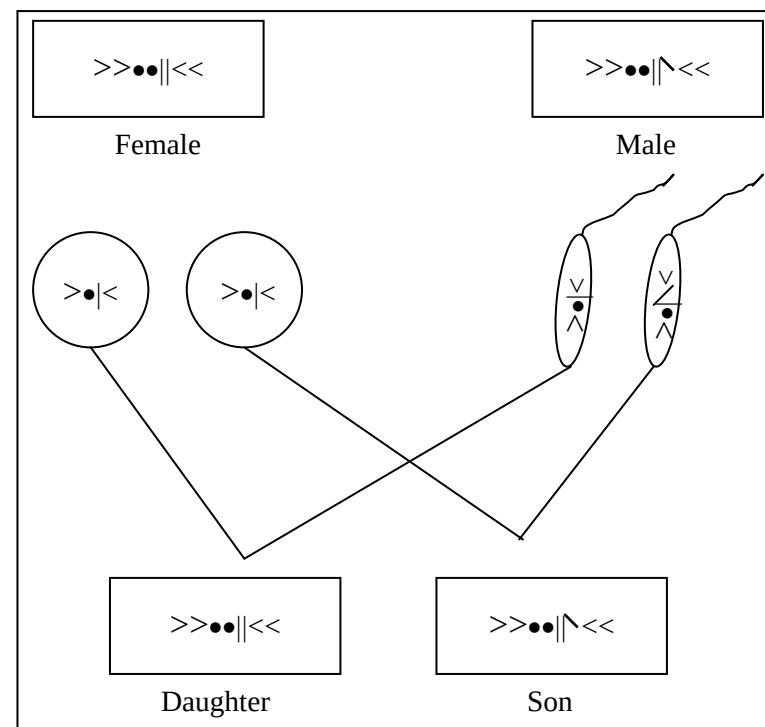


Fig. 16.2 XX-XY Type of Sex Determination

All the eggs have one X and 3 autosomes, each sperm are of 2 kinds, one kind with one X and 3 autosomes each and other kind with one Y and 3 autosomes each. Any egg fertilized by X-containing sperms produces a female and any egg fertilized by a Y containing sperms produces a male. It is found in *Helandrum allum* among plants.

In Human beings, 46 chromosomes are present in the somatic cells. Females have 22 pairs of autosomes and two X chromosomes. Males have 22 pairs of autosomes, one X-chromosome and one very short Y-chromosome, considerably smaller than the X, each egg carries 22 autosomes and an X sperms however are of 2 kinds, one kind with 22 autosomes and an X and the other kind with 22 autosomes and a Y. The sex of a child is determined at the time of fertilization by the kind of sperm that happens to meet and penetrate the egg, an X-bearing sperm producing a girl and Y-bearing one a boy.

ZO – ZZ type

In all the above instances, the female is the homologous sex because it produces eggs, all of which are alike and the male is the heterogametic sex because it produces two kinds of sperms. But there are instances where female is heterogametic and male is the homogametic one.

In a moth, *Talaeoporia*, the female have 59 chromosome and the males have 60 chromosomes in their somatic cells. The eggs are of two kinds. One kind with 29 chromosomes, and the other kind with 30 chromosomes. All the sperms have 30 chromosomes each. On fertilization an egg with 29 chromosomes gives rise to a female and an egg with 30 chromosome gives rise to male.

ZW – ZZ type

In this type including the domestic fowl, the females have an unlike pair of chromosome. ZW, and

forms eggs of two forms, one with a 'W' chromosome and the other with 'Z' chromosome. The male has like pairs of chromosomes. On fertilization an egg with a W chromosome give rise to female and egg with Z chromosome give rise to a male. Among plants, *Fragaria elatior* is one in which the female is ZW and male ZZ.

Genic Balance Theory of Sex Determination

In genic balanced theory of sex determination 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 character called females and the other to produce the character 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 balanced theory of sex-determination comes from the work of Bridges in *Drosophila*.

	X + A	Y + A
2X + 2 A	3 X + 3 A Female	2 X + Y + 3 A Female
X + A	2 X + 2 A Female	X + Y + 2 A Male
2 X + A	3 X + 2 A super female	2 X + Y + 2 A Female
X + 2 A	2 X + 3 A Inter sex	X + Y + 3 A Super male

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 lesser or greater than the normal flies (i.e., aneuploids).

Bridges found inter sexes, super females and super males 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. Super females and super males are sterile individuals, which are very weak and very poor in viability.

Flies with two X-chromosomes and two sets of autosomes are females, but flies with three X chromosomes and the same two sets of autosomes are super females.

2 X + 2 A – female

3 X + 2 A – super female

This shows, that in *Drosophila* 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 super males.

1 X + 1 Y + 2 A – Male

1 X + 1 Y + 3 A – Super male

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

Flies with two X-chromosomes and two sets of autosomes are females. So also, flies with two X, one Y and two sets of autosomes are females.

2 X + 2A – female

2 X + 1 Y + 2 A – female

That individuals with two X, one Y and two sets of autosomes are females in spite of presence of Y chromosomes show that the Y chromosomes plays no positive role in sex determination. That the Y chromosomes does not determine maleness is also shown

by the fact that flies with one X chromosome and two sets of autosomes (i.e., XO flies) are male in spite of the absence of Y chromosomes. They are however, sterile showing thereby that the Y chromosomes contains male fertility genes necessary for the production of a fertile male.

Ploidy	Number of X chromosome = (x)	Number of autosomal sets = (A)	Sex index (x/A)	Sex expression
2n	3	2	3/2 = 1.5	Super female
3n	4	3	4/3 = 1.33	Super female
4n	4	4	4/4 = 1.00	Female
3n	3	3	3/3 = 1.00	Female
2n	2	2	2/2 = 1.00	Female
4n	3	4	3/4 = 0.75	Inter sex
3n	2	3	2/3 = 0.67	Inter sex
2n	1	2	1/2 = 0.5	Male
4n	2	4	2/4 = 0.5	Male
3n	1	3	1/3 = 0.33	Super male

Sex – Limited Inheritance

Some autosomal genes may only come to phenotypic expression in one of the sexes (either male or female) either because of differences in the internal hormonal environment or because of anatomical dissimilarities. The phenotypic expression of such genes remain limited to the sex of the bearer, therefore are called sex – limited genes.

Many of the secondary sexual characters of man depend on sex limited genes i.e., the development of beard in man and development of mammary glands (breasts) in women. Though, man and woman both have genes for beard and mammary gland development but due to sex hormones the female lacks in beard and male lacks in developed breasts.

Anotger example is Plumage pattern or feathering in birds. Many birds display a marked sexual dimorphism

in plumage pattern. For instance, in domestic fowl of leghorn breed, males have long, pointed, curved, fringed feathers on tail and neck, but feathers of female are shorter, rounded, straighter and without the fringe. Thus, males are cock feathered and females are hen feathered.

It has been shown that hen feathering result from a single gene 'H' and cock feathering from its allele, 'h' as follows.

Genotypes	Phenotypes	
	Males	Females
HH	Hen-feathering	Hen-feathering
Hh	Hen-feathering	Hen-feathering
hh	Cock feathering	Hen-feathering

It has been found that the expression of gene H and h, depends upon the sex hormones, because where the ovaries or testes of chickens have been removed, all become cock feathered regardless of genotype. It shows that particular type of feathering depends upon specific combination of genotype and sex hormones and H gene produces hen feathering in the presence of either sex hormone; and cock feathering in the absence of any hormone. The 'h' gene produces cock feathering if female hormone is absent and hen feathering if female hormone is present.

Sex Influenced Inheritance

There are a number of known autosomal and sex – linked genes whose phenotypic expression will be either as a dominant, if the individual is male or as recessive, if the individual is female. Such genes are called sex – influenced genes. e.g., Inheritance of baldness in man

In man, in some families baldness is found to be an inherited trait. The gene B for baldness is found to be

dominant in males and recessive in females. In heterozygous conditions it expresses itself only in the presence of male androgenic hormone (in male sex).

Genotype	Phenotype	
	Man	Woman
BB	Bald	Bald
Bb	Bald	Non-Bald
bb	Non-bald	Non-bald



SEX LINKAGE

“Sex linkage is the consequence of a gene being located on the ‘X’ or sex chromosome which occurs in different numbers in the two sexes”.

Inheritance of White Eye in *Drosophila*

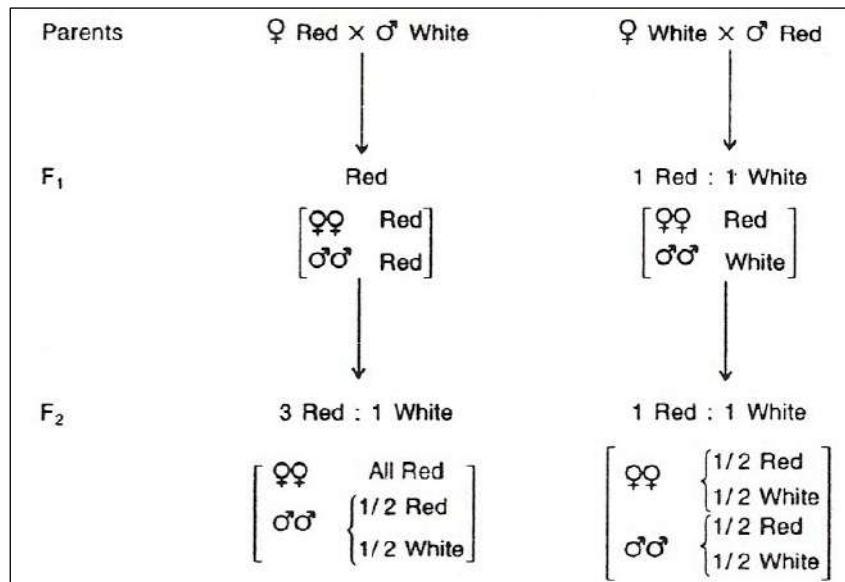


Fig. 17.1. Inheritance of White Eye in *Drosophila*

Morgan reasoned that white eye gene was located in ‘X’ chromosome of *Drosophila* and the inheritance pattern of this gene is closely parallel to that of the X chromosome. It also assumed that the Y chromosome did not carry an allele for this gene (Fig. 17.1).

Accordingly, female flies will have two copies of w gene (WW, Ww or ww) while male have only one copy (W or w). Thus the male *Drosophila* will be hemizygous

(having only one copy of a gene) for w gene and therefore a single copy of the recessive allele w will express itself in the hemizygous males for a simple reason that Y chromosome does not contain an allele for this gene.

The genotypes of red-eyed females and white-eyed males may be written as $X^W X^W$ and $X^W Y$, respectively. The location of w gene in the X chromosome is indicated by writing it as super script of X. All the eggs of the red-eyed female will be X^W , while half the sperms of the white eyed male will be X^w and the other half will be Y. A random union between male and female gametes will produce the following four zygotes, $X^W X^W$, $X^W X^w$, $X^W Y$ and $X^w Y$; the first two of these would produce red-eyed females, the third a red-eyed male and the fourth a white-eyed male.

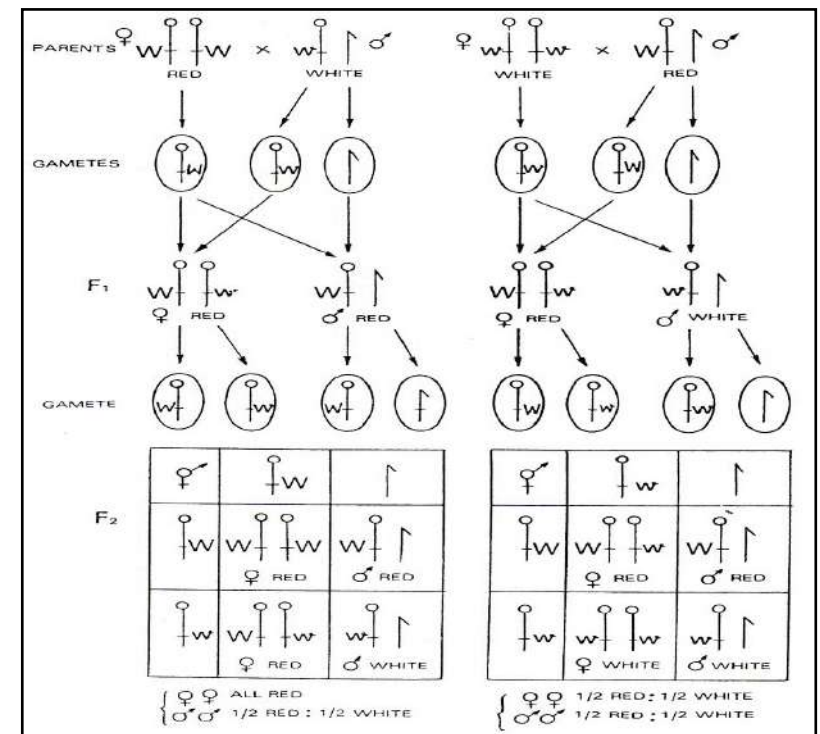


Fig.17.2. Inheritance of White Eye Gene in *Drosophila* Located in X chromosome

In the reciprocal cross, a white-eyed female ($X^w X^w$) was mated with a red-eyed male ($X^W Y$). All the eggs of the female will be X^w , while male will produce X^W and Y sperms in equal proportion. In F_1 , two types of zygote will be produced: $X^W X^w$ zygote will give rise to red-eyed females and $X^w Y$ zygotes will develop into white-eyed males. The F_1 females ($X^W X^w$) will produce X^W and X^w eggs, while F_1 males ($X^w Y$) will generate X^w and Y sperms. A random union between eggs and sperms from F_1 individuals will generate 4 zygotic combinations, $X^W X^w$, $X^w X^w$, $X^W Y$ and $X^w Y$. The first zygotic combination will give rise to red-eyed females, the second ($X^w X^w$) to white eyed females, the third ($X^W Y$) to red-eyed males and the fourth ($X^w Y$) to white-eyed males (Fig. 17.2).

Thus, the hypothesis that w gene is located in X chromosome and that it has no allele in the Y chromosome is adequate to explain the peculiar inheritance pattern of the white eye trait in *Drosophila*. This was the first conclusive demonstration that a specific gene was located in a specific chromosome of an organism.

Characteristics of sex-linked inheritance

The characteristic features of inheritance of a sex-linked trait may be illustrated as follows.

1. The frequency of individuals showing a recessive sex-linked trait is markedly higher in the heterogametic sex than that in the homogametic sex.
2. Ordinarily, genes governing sex-linked traits are not transmitted from male parents directly to their male progeny.
3. A male transmits its sex-linked genes to all its daughters, since females receive one of their two X chromosomes from their fathers. The daughters, in turn, transmit this gene to half of their male progeny. Thus a sex-linked recessive gene is transmitted from a

male to its female progeny and then to half the male progeny of such females. In other words, a sex-linked gene passes from male to female then back to male; such an inheritance pattern is known as **criss-cross inheritance**.

4. Sex-linked genes are not located in the Y chromosome. Consequently, the heterogametic sex (male humans, mice, *Drosophila* etc., and female birds) is hemizygous for such genes, i.e., it has only one allele of sex-linked genes. Therefore, recessive alleles of sex-linked genes express themselves in the hemizygous condition, while they have to be present in homozygous state to be able to express themselves in the homogametic sex. This is the reason why sex-linked recessive traits are more frequent in the heterogametic than in the homogametic sex.
5. It was noted that a region of the Y chromosome is homologous to a region in the X chromosome. For example, the long arm of human Y chromosome is homologous to the short arm of human X chromosome. Similarly, one-third of the *Drosophila* X chromosome (the region close to the centromere) is homologous to a region of the Y chromosome. Genes located in such regions of the X chromosome that are homologous to a segment of the Y chromosome do not show sex-linked inheritance if their allele is located in the Y chromosome. An example of such a gene is the bobbed (bb) gene of *Drosophila*. However, this region of both X and Y chromosomes is unusually genetically inactive and contains few known genes; in *Drosophila*, only gene bb is definitely known to be located in this segment.

Criss-cross inheritance or X linked genes

The X – linked genes are represented twice in female (because female has two X -chromosome) and once in male (because male has only one X chromosome). In

male single X-linked dominant or recessive gene expresses it phenotypically in contrast to females in which two X-linked genes are necessary for the determination of a single phenotypic trait related to sex.

The recessive X-linked genes have characteristic criss-cross inheritance i.e., when male, for example, transmits his X-linked recessive genes to his grand son (F_2) through this daughter (F_1).

Inheritance of holandric or Y – linked genes

The Y-linked genes inherit directly from male to male. In man, the Y-linked gene such as *Ichthyosis hystrixgravis*, *hypertrichosis* (excessive development of hairs on pinna of ear) etc, are transmitted directly from father to son.



MULTIPLE ALLELES I. CLASSICAL EXAMPLES

Many alleles of a single gene controlling different forms of a single character is known as Multiple allele.

- E.g. 1. Coat colour in rabbit.
2. Inheritance of blood group in man
3. Self incompatibility in plants

Fur Colour in Rabbits

The fur colour (colour of body hairs) in mammals shows considerable variation. The fur colour of rabbits is of several types; of these, the following four types are produced by alleles of a single gene c : normal or full colour, albino, himalayan and chinchilla (Table 18.1). Full fur colour is produced by the dominant allele C or c^+ of this gene: in the presence of allele C , the whole body of animals is uniformly coloured (the colour may be black if the recessive allele of another gene a is in the homozygous state, or it may be agouti or grey if the dominant allele of this gene, A , is present.

The fur colour of chinchilla animals is lighter than 'full colour', since their fur has a mixture of both coloured and colourless hairs. In the case of himalayan animals, the main body has colourless or white fur, while the tips or extremities of their feet, ears, tail and snout are coloured. The last category of fur colour, albino, has white or colourless hairs all over the body. The allele C for full colour is dominant over the alleles c^h (for himalayan); c^h (for chinchilla) and c (for albino); allele c^h is dominant over the alleles c^h and c , while allele c^h is dominant over allele c ; thus allele c is recessive to the other three alleles.

Table 18.1. The multiple allelic series controlling fur colour in rabbits

Allele	Genotype	Phenotype
c	cc	Albino (no pigment, white fur colour)
c ^h	c ^h c ^h , c ^h c	Himalayan (white body trunk; only tips of ears, feet, face and tail black)
c ^{ch}	c ^{ch} c ^{ch} , c ^{ch} c ^h , c ^{ch} c	Chinchilla (fur has a mixture of coloured and white hairs over the whole body; intensity of colour less than that in full coloured)
c ⁺ or C	c ⁺ c ⁺ , c ⁺ c ^{ch} , c ⁺ c ^h , c ⁺ c	Full coloured (entire body coloured black or grey).

Dominance relationship = c⁺ > c^{ch} > c^h > c

The allele c^h produces a temperature sensitive form of the enzyme tyrosinase, which is involved in the production of melanin (the skin and hair pigment) from the cyclic amino acid tyrosine. This mutant form of the enzyme is active at low temperature but is inactive at higher temperatures. This is easily demonstrated by plucking or shaving off the hairs from a part of the body, say back having white hairs of himalayan animals and keeping the shaven part cool while the hairs grow. The shaven part may be kept cool either by placing ice on the shaven part or by keeping the animal in a cold place. The new fur that grows in the shaven area of such animals is coloured rather than white. But if the animals were kept in a warm place, the new fur would be white as before. This explains why himalayan rabbits have coloured fur at the extremities of their body. Since these parts becomes cooler than the rest of their body, the temperature sensitive tyrosinase is able to function normally and produce melanin in these areas.

Inheritance of Blood Group in Man

The ABO blood group system is believed to be controlled by a single gene, generally designated as I. Gene I has three alleles ; I^A, I^B and I^o.

Allele I^A controls the production of antigen A, allele I^B determines antigen B, while allele i (I^o) does not produce any active antigen. These alleles are codominant so that individuals having the genotype I^AI^B have both the antigens A and B on their RBCs. (AB blood groups).

Individuals with the genotype I^AI^A or I^Ai produce antigen A and are classified into the blood group A; those with the genotype I^BI^B or I^Bi produce antigen B and belong to the group B; persons with the genotype I^AI^B have both the antigen A and B and are placed into the group AB; while those having the genotype ii (I^oI^o) produce neither A or B and are classified into the group O.

Self Incompatibility in plants

In a large number of plant species, self pollination may not lead to fertilization and consequently, seed set, although in these cases both the male and female gametes are functional and normal seed set is obtained if they are cross-pollinated are referred to as self incompatibility (SI). Self-incompatibility is generally classified into 2 types viz., Sporophytic and Gametophytic.

In the sporophytic system (Fig. 18.1), the SI reaction of pollen grain is determined by the genotype of the plant on which they are produced; as a result the SI reaction of all the pollen grains produced by a plant is identical irrespective of the genotype of the plant of the SI locus.

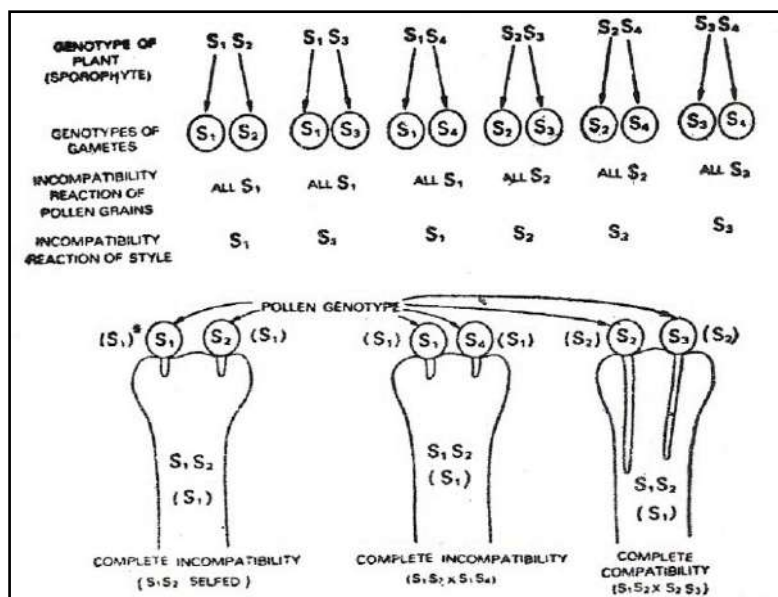


Fig. 18.1. Sporophytic incompatibility

In contrast, the SI reaction of pollen grains in the gametophytic system (Fig. 18.2) is determined by the genotype of the individual pollen grain so that the plants ordinarily produce pollen grains of two different SI reaction.

Pollen grains having the same SI reaction as that of the stigma on which they land will either fail to germinate on the growth of this pollen tubes or will be inhibited after they have germinated. Pollen grains with SI reactions different from that of the stigma will germinate normally and effect fertilization.

In most plant species, SI is governed by a single gene S which has several alleles in same cases the number of known S alleles has exceeded 30. The multiple allele of S are designated as S_1, S_2, S_3, S_4 etc. In some species, two genes (S and Z) are involved, while in

some other species there is evidence for a trigenic control of the SI reaction.

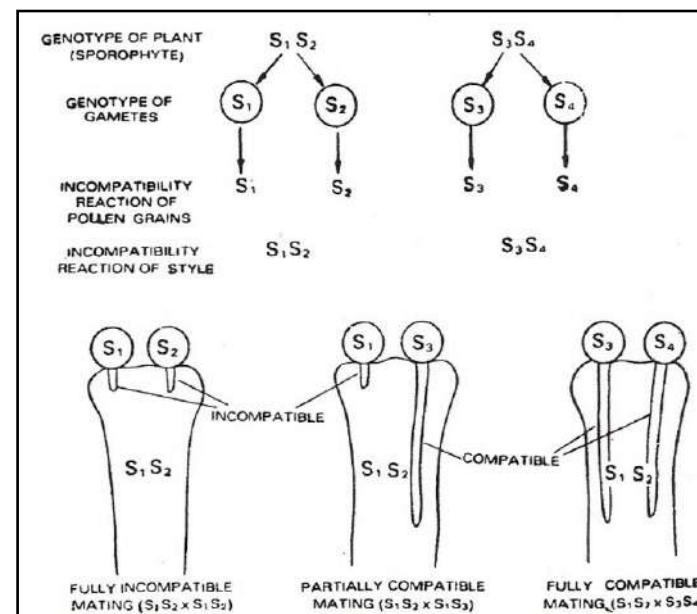


Fig. 18.2. Gametophytic incompatibility

Monogenic control of SI reaction is reported in the members of Solanaceae e.g., tobacco, tomato, potato, etc. Cruciferae, e.g., radish, *Brassica* sp., *Lactuca* etc. and many other plant species. In the species belonging to family Cruciferae it is generally believed that SI reaction is of simple sporophytic type governed by multiple allele of a single gene S. The SI reaction in Cruciferae is governed by multiple allele of two loci, one sporophytically operating locus S and one gametophytically operating locus G; the alleles of S and G loci complement the expression of each other.

MULTIPLE ALLELES II. FINE STRUCTURE OF GENES

Modern concept of gene (The changing concept of gene)

The fine structure of a gene is essentially the linkage map of its various mutant alleles. In this map, the sites of mutational changes in different alleles of the same gene are determined on the basis of the frequency of recombination among these alleles. Thus the fine structure map of a gene is essentially comparable to the linkage map of a chromosome [except for the number of genes involved (one in the case of gene fine structures and several in the case of chromosome maps)]. Understandably, the values of crossing over observed in the studies on fine structure of a gene are very low (e.g., 0.01 or even 0.0001%). Therefore extremely large progeny populations have to be effectively screened for efficient identification of the cross over products.

For this reason, fine genetic analysis is more convenient in prokaryotes than in eukaryotes.

The Fine Structure of a gene in a prokaryote

The most extensive fine structure map of a locus constructed to date is that of the *r* II locus of T4 phage of *E. coli* due to Seymour Benzer. T4 is similar to T2 in morphology, is an obligate parasite like all viruses, and contains a chromosome of about 200,000 bp (base pairs) long, which is packed within its head. When a T4 phage particle infects a cell of *E. coli* the bacterial cell lyses in about 20 – 25 minutes liberating 200 - 300 progeny phage particles.

When *E. coli* cells are plated (in an agar medium in petriplates) in sufficiently large numbers they produce a

uniform confluent growth or 'lawn'. If individual T₄ particles are placed on the surface of an agar medium seeded with a lawn of *E. coli* cells, each phage particle would initiate a chain of infection-lysis so that all the *E. coli* cells in the immediate vicinity of the phage particles will be lysed. This leads to the development of clear zones, called plaques, the lawn of bacterial cells. The plaques produced by the wild type T₄ particles are relatively small with fuzzy or turbid margins called halos. The halos are produced due to a phenomenon called lysis inhibition, which is a delay in the lysis of T₄-infected *E. coli* cells as a consequence of its subsequent infection by another T₄ particle.

The R^{II} Locus

Several mutants of T₄ do not exhibit lysis inhibition, and produce relatively large plaques with clear margins, these mutants are called rapid lysis mutants and are denoted by *r*. Most of the *r* mutants map in one of the three distinct loci called *r*^I, *r*^{II} and *r*^{III}. Mutants in the *r*^{II} locus are easily recognized due to their inability to multiply in *E. coli* strain K₁₂ (λ), which has the chromosome of phage λ integrated, in its chromosome. However, *r*^{II} mutants grow rapidly in other strains of *E. coli* e.g., strain B and K₁₂ (lacking the λ chromosome). Thus *r*^{II} mutants are conditional lethal as they are unable to grow in K₁₂ (λ); this property was exploited by Benzer for a genetic analysis of *r*^{II} locus.

Complementation Test

Benzer isolated over 3000 independent mutants of the *r*II locus and subject them to complementation test. For this purpose, cells of *E. coli* strain K12 (λ) were infected with a mixture of the two *r*II mutants to be tested. Free phage particles not involved in infection were removed from the suspension of bacterial cells through centrifugation. Many bacterial cells will be infected by particles of both the mutants. If there is complementation between the two *r*II mutants, the phages will multiply in

such host cells and lyse them releasing progeny phage particles. However, if there was no complementation, they will fail to multiply and no progeny phage particles will be released.

A suspension of *E. coli* K₁₂ (λ) cells infected in this manner is kept for about 90-120 minutes to permit phage multiplication and bacterial-cell lysis samples from the whole suspension or its supernatant are now placed on *E. coli* strain b lawn to detect the presence of phage particles. "If plaques develop on *E. coli* B lawn, it reveals complementation between the two r^{II} mutants used for co-infection; while the absence of plaques signifies a lack of complementation".

Benzer placed two r^{II} mutants which failed to complement each other in two arbitrary groups designated as A and B. Each of the r^{II} mutants showed complementation either with the mutant belonging to group A or with that belonging to group B, but never with both. Thus all r^{II} mutants could be classified into two clear cut groups (group A and B); mutants of one group showed complementation with the mutants of the other group, but mutants within a group failed to complement each other. Thus the r^{II} locus was divided into two cistrons, cistron A (r^{II} A, represented by the r^{II} mutant of group A) and cistron B (r^{II} B evidenced by the r^{II} mutants of group B) on the basis of complementation test.

The Ultimate Fine Structure of Gene

"The complementation test resolves a locus into distinct cistrons, while the recombination test maps different mutants within each of the cistrons". The recombination mapping, however, relates to only those regions of a gene that are involved in coding of amino acids of the concerned polypeptide (called exons for expressed sequences). But most eukaryotic genes have one or more intervening non-coding sequences (called introns) within their genes. Such features of the genetic fine structure are revealed only by partial or total

determination of the nucleotide sequence of a gene, which constitutes the ultimate fine structure of a gene. Introns and exons are collectively known as split genes since their coding sequences are split into several parts due to the introns.



GENETIC MATERIAL I. CHEMICAL NATURE

Properties of Genetic Material

It was postulated by Mendel in 1865 that the development of characters in peas (and other organisms) was controlled by particulate factors. These factors were later called genes. In 1903, Sutton and Boveri postulated that genes were located in chromosomes; this is commonly known as the chromosomal theory of inheritance.

The chemical of which genes are composed must possess the following properties.

- i) The genetic material must be replicated with high fidelity so that its flawless copies are produced. Further these copies must be distributed to the daughter cells with a very high precision so that genes are passed on to the various daughter cells and generation without alteration.
- ii) The genetic material must be able to express itself by exercising a control on the development of character it governs.
- iii) Therefore, the structure of genetic material must be able to store the highly variable information necessary for gene function.
- iv) The replication and/or the distribution of genetic material must allow errors in low frequency for the origin of new genetic variation through mutation.

Identification of DNA as genetic material

Genetic material is the chemical of which genes are composed. In eukaryotes, chromosomes are made up of proteins and nucleic acids. Nucleic acids were discovered

by Meischer in 1869; he named them as nuclein since they were isolated from nucleus of cells. There are two types of nucleic acids; deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA). We know that in eukaryotes genes are located in chromosomes. Therefore it is certain that they would be composed of either protein RNA or DNA. The process of identification of genetic material began in 1928 with the experiments of Griffith and concluded in 1952 with the study of Hershey and Chase. They took three brilliantly novel experiments to conclusively demonstrate that DNA is the genetic material and that protein does not perform genetic function.

Experiment of Griffith

Griffith discovered the phenomenon of transformation through his studies on *Diplococcus pneumonia*, which causes pneumonia in most of the mammals.

“Transformation is essentially a special type of recombination in which a segment from the transforming DNA replaces the homologous segment of the bacterial chromosome”.

Therefore transformation may be defined as a hereditary change in the traits, in a cell/organisms, brought about by an integration through recombination of a segment of a DNA molecule into the chromosome of that cell.

Different strains of *Diplococcus* form two types of colonies : (1) smooth and (2) rough. The cells of strains forming smooth colonies are enclosed in a polysaccharide capsule; such strains are able to produce pneumonia and are called virulent. But cells of strains producing rough colonies lack the polysaccharide capsule; such strains are termed as avirulent since they cannot produce pneumonia. Virulent strains of *Diplococcus* are classified into several types, e.g., II and III etc., On the basis of

antigenic properties of the polysaccharides present in their capsule.

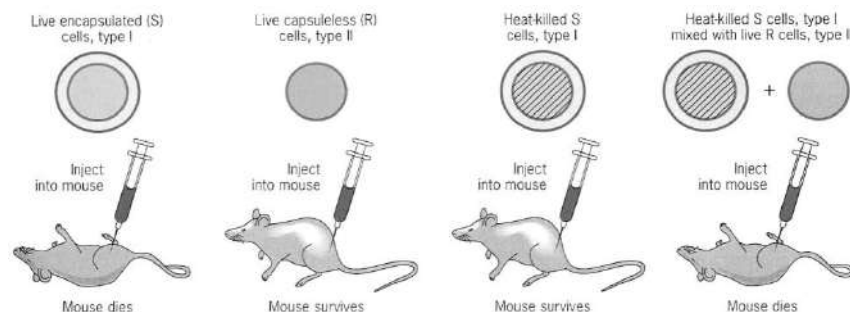


Fig.20.1. Griffith's Experiment with *Diplococcus pneumoniae*

The experiments of Griffith are briefly described that: when live cells of the avirulent strain IIR were injected into mice, they did not suffer from pneumonia and all the mice survived (Fig. 20.1).

On the other hand, when mice were injected with live IIIS (virulent) cells, all the mice died due to pneumonia. But mice injected with heat-killed cells of the virulent strain IIIS did not develop pneumonia. However, when mice were injected with a mixture of heat-killed IIIS cells and live IIR cells, some of them died due to pneumonia; *Diplococcus* cells isolated from the dead mice were of the type IIIS. Since all the cells of the heat-killed culture were dead, it was postulated that some of the cells of IIR changed into the IIIS type due to the influence of dead IIIS cells present in the mixture. This phenomenon was called transformation, and that component of IIIS cells which induced the conversion of IIR cells into IIIS cells were named the transforming principle.

The experiments of Griffith demonstrated transformation but they did not hint at the identity of the transforming principle. It was later shown by Avery and co-workers that DNA is the transforming principle.

The Experiments of Avery, MacLeod and McCarthy

Avery and associates carried out the experiments of Griffith *in vitro* (in test tubes) in place of mice (*in vivo*).

A culture of live IIR cells produce typical rough colonies, while plating of heat killed IIIS cells or of DNA isolated from IIIS cells did not yield any colony. But when live IIR cells were either mixed with heat-killed IIIS cells or with DNA isolated from IIIS cells and the mixture was plated on a culture medium containing antibodies specific for IIR cells (anti – IIR), some colonies of IIIS type were obtained (Fig. 20.2).

Anti – IIR were used for inactivating IIR cells so that the few transformed cells of IIIS type may not be over run by them. These findings clearly show that DNA is the transforming principle. But as DNA preparations do contain traces of protein and RNA, it is not established beyond a reasonable doubt.

In their experiments, Avery, Mac Leod and Mc Carthy treated IIIS DNA with RNase (the enzyme which digest RNA) or proteases before it was mixed with IIR cells. In both the experiments, some IIIS type of colonies were obtained. These findings clearly show that the traces of RNA and proteins present as impurities in IIIS DNA preparations were not responsible for transformation.

In another experiment, they treated the IIIS DNA with DNase, the enzyme which digest DNA, before it was mixed with IIR cells; in this experiment, IIR cells were not transformed into IIIS cells. This establishes beyond any doubt that DNA is the transforming principle.

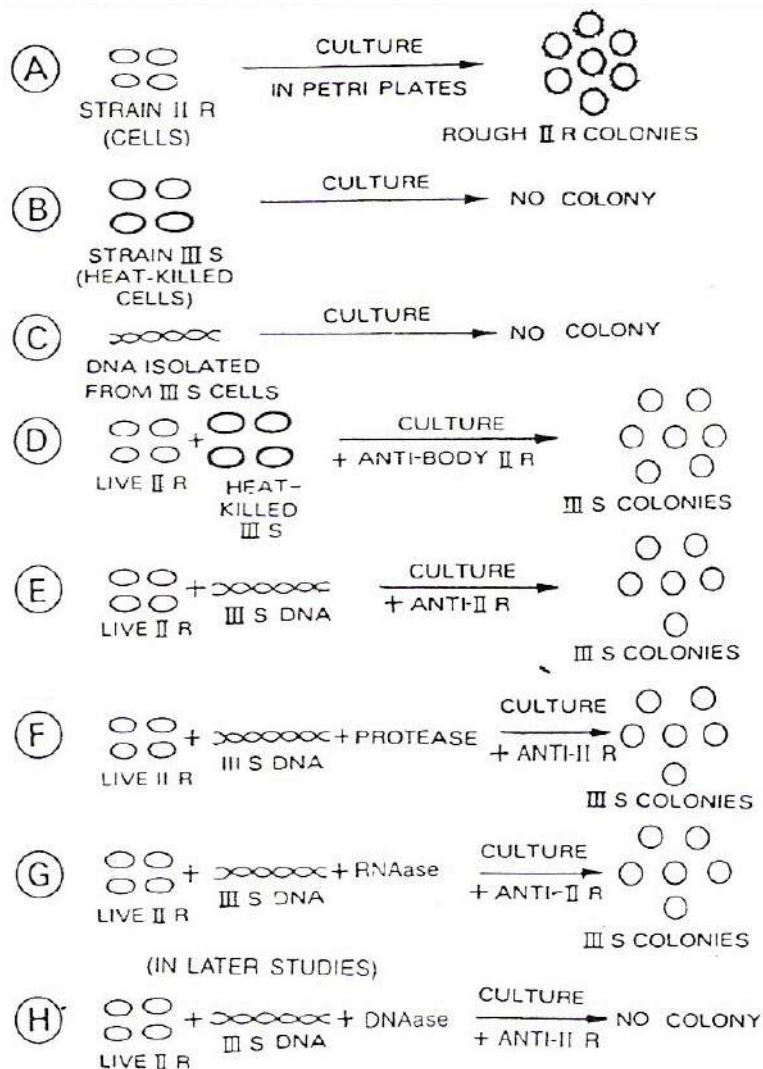


Fig.20.2. Experiments of Avery, Macleod and McCarty with *Diplococcus*

Experiments of Hershey and Chase

Hershey and Chase studied the life cycle of T_2 phase of *E. coli*; they clearly showed that only the DNA component of T_2 particles is transmitted to the progeny phage particles. T_2 phage has a hexagonal head and a contractile tail, a large part of which is enclosed in a tail sheath. The tail ends in a tail plate which has 6 spikes and 6 tail fibers. T_2 and most other bacteriophages are composed of protein and DNA; head coat and tail are made up of protein, while the DNA is packed inside the head coat.

Viruses are obligate parasites since they can reproduce only within host cells. Infection beings when the tail plate of a T_2 particle comes in contact with the cell wall of an *E. coli* cell and attaches to it Now the tail sheath contracts pushing part of the tail core through the bacterial cell wall which is already weakened by certain hydrolytic enzymes present in the phage tail core.

Hershey and Chase labelled T_2 DNA and protein in two separate experiments (Fig. 20.3). DNA contains phosphorus (P) but no sulphur (S), while proteins contain 5 but ordinarily they have no P. Therefore, they labelled T_2 DNA with ^{32}P , while the proteins were labelled with ^{35}S . In order to obtained ^{32}P (or ^{35}S) labelled phage particles, *E. coli* cells were' grown for several generations on a culture medium containing ^{32}P (or ^{35}S). These cells were then infected with T_2 particles; the progeny phage particles thus obtained were appropriately labelled with either ^{32}P or ^{35}S .

In one experiment, they mixed ^{32}P -labelled T_2 particles with *E. coli* cells (grown on a normal culture medium). Infection was allowed for 10 min; the cells were then agitated in a blender to separate the empty phage particles (called ghosts) remaining outside the bacterial cells after infection. The bacterial cells were pelleted out

through centrifugation, thus separating them from the ghost and active phage particles. Most of the ^{32}P label was present in the infected *E. coli* cells, while the ghosts and the phage particles contained only a small fraction of the total label. The progeny phage particles obtained after lysis of these *E. coli* cells also contained the ^{32}P label. This clearly shows that the ^{32}P present in phage DNA is injected into *E. coli* cells and is finally transmitted to progeny phages, and that DNA is transmitted from one generation to the next.

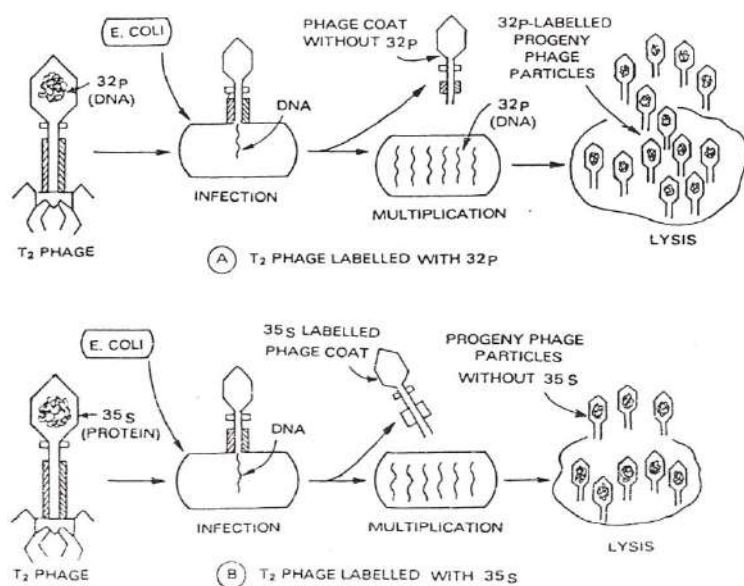


Fig.20.3. Experiments of Hershey and Chase

In the other experiment, they used ^{35}S -labelled T₂ particles for the infection of *E. coli* cells, the experimental procedure being the same as that for the ^{32}P labelled phage particles. In this case, almost all the ^{35}S label was present in the ghosts and the active phage particles, and only a very small fraction of it was found in the infected.

E. coli cells. Further, the amount of ^{35}S present in the progeny phage particles obtained through lysis of the infected *E. coli* cells was almost negligible. Clearly, almost all of the proteins are left outside the *E. coli* cells during infection and that proteins are not transmitted from one phage generation to the next.

Thus the studies of Hershey and Chase clearly showed that DNA of T₂ is transmitted from one generation to the next, while proteins are not transmitted. Therefore, at least in T₂ phage, DNA is the genetic material. It may be pointed out that the results from these studies were not as unambiguous as those from the studies of Avery and associates (the presence of small amounts of ^{35}S label in the infected *E. coli* cells and progeny phages). But the role of DNA in these studies (transmission from one generation to the next) was clear and easily comprehensible, while its role in the experiments of Avery and coworkers (transformation through recombination) was yet unknown and very difficult to comprehend. As a result, the findings of Hershey and Chase led to the universal acceptance of DNA as the genetic material.



GENETIC MATERIAL II. PRIMARY AND SECONDARY STRUCTURES

Chemical analyses have shown that nucleic acids (DNA and RNA) are composed of the following three types of molecules: (1) phosphoric acid, (2) pentose sugar and (3) organic bases.

PHOSPHORIC ACID

Phosphoric acid (H_3PO_4) has three reactive (-OH) groups of which two are involved in forming the sugar phosphate backbone of DNA. A phosphate moiety binds to the 5'C of one and the 3'C of the other neighbouring pentose molecule of DNA to produce the phosphodiester (5'C-0-P-0-C3') linkage.

PENTOSE SUGAR

The pentose present in RNA is called D-ribose from which this nucleic acid gets its name. But DNA contains 2'-deoxy-D-ribose (or simply, deoxyribose) which is the reason for the name deoxyribose nucleic acid. The oxygen atom present at the second carbon of ribose is missing in deoxyribose hence its name 2' -deoxyribose. The position of carbon atoms of the two pentose sugars are denoted as 1', 2', 3', 4' and 5' in order to differentiate them from the corresponding positions in the DNA and RNA bases which are not marked by a prime ('). 5' and 3' carbons of the pentoses participate in the phosphodiester linkage, while the 1' carbon is always occupied by an organic base.

ORGANIC BASES

The organic bases found in nucleic acids are heterocyclic compounds containing nitrogen in their rings; hence they are also called nitrogenous bases. DNA ordinarily contains four different bases called adenine (A), guanine (G), thymine (T) and cytosine (C); RNA contains

the same bases, except for thymine in whose place uracil (U) is found. These five bases (A, G, T, C, U) are grouped into two classes on the basis of their chemical structure: (1) pyrimidine (T, C, U,) and (2) purine (A, G) (Fig. 21.1).

Pyrimidines

Pyrimidine bases consist of a six-membered pyrimidine ring which is similar to the benzene ring except that it contains nitrogen (N) in the place of carbon (C) at positions 1 and 3. The three pyrimidine bases (T, C, U) contain a keto oxygen (=O) at position 2. In cytosine, an amino (-NH₂) group is also present at position 6; 5-methyl cytosine (produced during DNA methylation; see, Chapter 35) has a methyl (-CH₃) group attached to the fifth carbon of cytosine. In the case of uracil, a keto (=O) group is present at the sixth carbon, while thymine is essentially 5-methyl uracil (i.e., a keto oxygen at position 6, and a -CH₃ group at carbon 5). All the pyrimidines, therefore, contain an atom at position 3 which is involved in their linkage with the 1'C of pentoses.

Purines

The two purines (A, G) consist of a purine ring, which has a six-membered and a five-membered ring. The six-membered ring of purines is the same as pyrimidine ring; carbons 4 and 5 of this pyrimidine ring are common to the five-membered ring also. In addition, the five-membered ring has N in 7th and 9th positions. In adenine, an amino (-NH₂) group is present at position 6. But

in guanine a keto (=O) group is found at position 6 and an -NH₂ group is attached with the second carbon. The N present at the 9th position of purines participates in a covalent linkage with the 1'C of the pentoses.

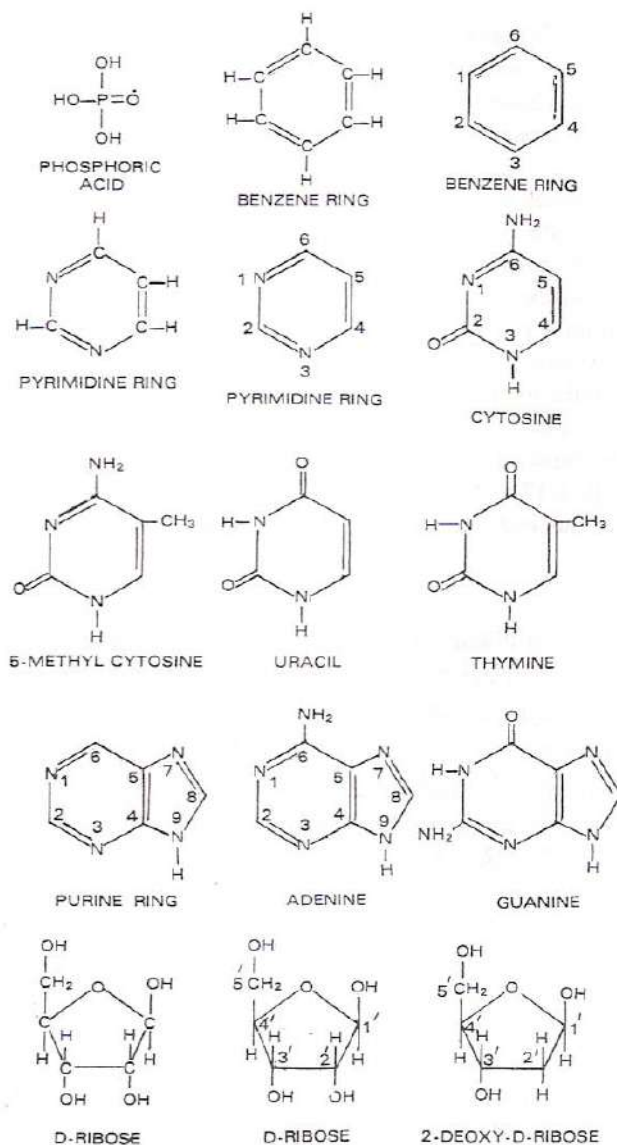


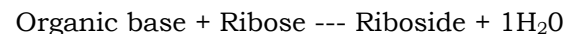
Fig.21.1. Chemical structure of different structural components of DNA

It may be pointed out that both the purines (A and G) are normally found in both RNA and DNA, while only one of the pyrimidines (C) is normally present in both the

nucleic acids. Of the remaining two pyrimidines, T is normally found only in DNA, while U is ordinarily present in RNA only.

NUCLEOSIDES

In nucleic acids, organic bases are linked with the pentose molecules. In this linkage, the - H attached to the - N at position 3 of pyrimidines or that attached to the N at position 9 of purines interacts with the -OH at 1'e of the pentoses and forms a C-N covalent bond between the pentose and the organic base. This reaction releases one molecule of H₂O. Linkage between ribose and the different organic bases yield ribonucleosides, while those between deoxyribose and the organic bases produce deoxyribosides: both ribosides and deoxyribosides together are called nucleosides. The four common ribosides are called adenosine, guanosine, uridine and cytidine. Similarly, the four common deoxyribosides are deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine; since normally only deoxythymidine is present in cells, it is simply referred to as thymidine. Thus,



NUCLEOTIDE

A Nucleotide is formed when a phosphate group is attached to either the 3' C or the 5' C of the pentose molecule of a nucleoside. In the nucleotides participating in DNA replication, the phosphate groups are ordinarily attached to the 5' C. The nucleotides produced by the different ribosides are, 5'-adenylic acid, 5'-guanylic acid, 5'-cytidylic acid and 5'-uridylic acid; together they are known as ribonucleotides or simply ribotides. Similarly, the nucleotides formed by the four deoxyribosides are, deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic

acid and thymidylic acid; together they are called deoxyribonucleotides or simply deoxyribotides. Thus,

Organic base + Ribose + 5' (or 3') phosphate ---

Ribotide + 2H₂O

Organic base + Deoxyribose + 5' (or 3') phosphate ---

Deoxyribotide + 2H₂O

Ribotides and Deoxyribotides = Nucleotides

A nucleotide having phosphate at the 5'C will have a free -OH at the 3'C; such a nucleotide is called 5'-nucleotide or 5'p3' OH nucleotide. Similarly, nucleotides having phosphate at the 3'C have a free -OH at the 5'C; they are called 3'-nucleotides or 3'p S'OH nucleotide. Thus each nucleotides has a phosphate group at its 3'C or, in naturally occurring nucleotides at S'C, and an - OH group at its 5'C or 3'C, respectively.

DNA STRUCTURE

A native DNA molecule is double-stranded. Each of the two strands of a DNA molecule is a multimer of deoxyribonucleotides, and is known as a polynucleotide. The neighbouring nucleotides in a polynucleotide are joined with each other by phosphodiester linkage. In a phosphodiester linkage the 5'C of the pentose of one nucleotide is linked with one -O- of the phosphate, while the 3'C of the pentose of the other nucleotide is linked with another -O- of the phosphate; this generates a 5'C-O-P-O-C3' linkage. This linkage results when one -OH of the phosphate of a nucleotide, e.g., 5' dAMP, reacts with the -OH of the 3'C of the pentose of another nucleotide, e.g., dCMP; one molecule of H₂O is liberated in this reaction. In this dinucleotide, the 5'C of one nucleotide (linked with phosphate) and the 3'C of the other nucleotide have free -OH groups with which other nucleotides can join to produce a polynucleotide; this generate the primary structure of DNA.

Thus a polynucleotide chain consists of several alternating pentose and phosphate molecules linked with each other by phosphodiester linkages. Each pentose has anyone of the four organic bases attached to its 1'C; the base is not directly involved in the formation of sugar-phosphate backbone of a polynucleotide. At one end of the polynucleotide chain, the 5'C of pentose has a free -OH (-OH of the phosphate group attached to the 5'C), while the 3'C of the pentose of the opposite end has a free -DH; these ends are known as 5'- and 3'-ends, respectively. Clearly, a polynucleotide is polar. At the same time, the primary structure of DNA does not impose any restriction on the sequence in which the bases occur in a polynucleotide.

Genetic material

Watson and Crick in the year 1953, proposed double – helix model of DNA, which become universally accepted.

The main features of the model are summarised below :

1. A DNA molecule is made up of two polydeoxy ribo nucleotide (or simply polynucleotide) strands or chains.
2. The two strands of a DNA molecule are oriented anti-parallel to each other i.e., the 5'-end of one strand is located with the 3'-end of the other strand at the same end of a DNA molecule. This anti-parallel orientation of the two strands is essential for the formation of hydrogen bonds between the pairs of DNA bases.
3. Each polydeoxy ribo-nucleotide strand is composed of many deoxy ribo- nucleotides joined by phosphodiester linkage between their sugar and phosphate residues.
4. The base sequences of the two strands of a DNA molecule show the following universal relationship.

- i) Wherever adenine occurs in one strand, thymine is present in the corresponding positions of the other strand and vice-versa.
 - ii) Similarly the sites at which guanine is present in one strand are occupied by cytosine in the second strand and vice-versa. Due to this relationship, the two strands of a DNA molecule are called complementary strands.
5. The adenine present in one strand of a DNA molecule is linked by two hydrogen bonds, with the thymine located opposite to it in the second strand and vice-versa. Similarly G located in one-strand forms three hydrogen bonds with the C present opposite to it in the second and vice-versa. The formation of hydrogen bonds between A and T and between G and C is known as complementary base pairing. These base pairs are called complementary bases. Hydrogen bonds are readily disrupted when DNA is either heated or when the pH of a DNA suspension is raised in to the alkaline range; this produces single stranded DNA called denaturation.
 6. During replication the two strands of a DNA molecule uncoil, and the unpaired bases in the single stranded region of the two strands bind with their complementary bases, present in the cytoplasm, as nucleotides. These nucleotides become joined by phosphodiester linkage generating complementary strands of the old ones. This provides for an almost 'error-free' high fidelity replication of the genetic material.

Modern concept of gene:

Benzer in the year 1956 recognised certain units of the gene based on their function. The classified definition that gene is a unit of physiological function, a unit of transmission and a unit of mutation was revised and a modern definition for the gene was proposed.

Gene is considered to have three distinct units, cistron, recon and muton. Cistron is synonymous to the gene of physiological function. It is the portion of the DNA specifying a single polypeptide chain. Recon is the smallest unit of the DNA capable of recombination in a gene. The recombinationally separable form of a cistron is called the recon. Muton is the smallest unit of the DNA capable of mutational changes. This modern concept of the gene gave a better understanding of the structure and function of the gene.

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 ($G = C$) was always not far from one, $(A + G) : (T + C) = 1$.

The double helical model of DNA was constructed by Watson and Crick (1953) (Fig. 21.2).

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 stair case, 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 $20\text{\AA}$ and makes a complete turn at every $34\text{\AA}$ along its length. The distance between nucleotides is $3.4\text{\AA}$. Each complete turn has a stack of 10 nucleotides. The helix contains two polynucleotide chains or two stacks of 10 nucleotides each per turn.

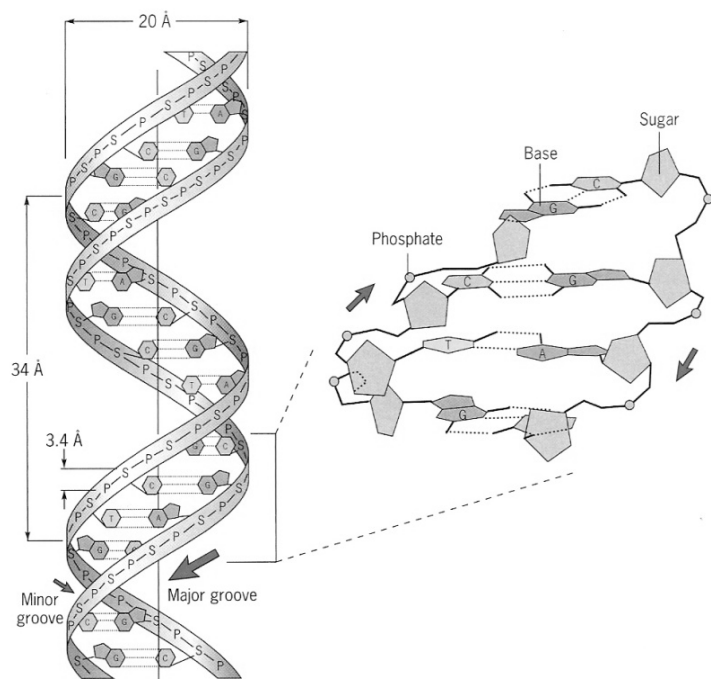


Fig. 21.2. Watson and Crick's Double Helical Model of DNA

The railing consists of phosphate – sugar linkages, which are repeated without change. The half step of one strand extent to meet the half step of other 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 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 charge (N^-) from opposite strands. Adenine pairs with thymine with two H bonds ($A = T$) and guanine with cytosine with three H bonds ($G \equiv C$). These N bases are connected to each other by deoxy ribose sugar 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 ($G \equiv C$) that give rigidity and stability to the molecules.

THE A, B AND Z FORMS OF DNA

The above description pertains to the E-form of DNA. This form shows right-handed coiling and contains 10 A° base-pairs per pitch (instead of the 10 mentioned above). Dehydrated DNA occurs in the A-form which is also a right-handed helix, but it has 11 base pairs per pitch. It is unlikely that native DNA occurs in the A form, but DNA: RNA heteroduplexes (double helices having one DNA and one RNA strand) and RNA double helices occur in this form in vivo.

Certain DNA sequences occur in Z-form, which shows left-handed coiling, contains 12 base pairs/pitch and has a pitch of 45 Å. In Z-DNA, the sugar-phosphate backbone follows a zigzagged path giving it the name Z-DNA or Z-form. Specific segments of a DNA molecule can undergo conformational changes from B -form to Z-form and vice-versa; these changes may be brought about by some specific regulatory proteins. The Z-form DNA is postulated to play a role in gene regulation. The chief differences between the B-form and Z-form DNAs are summarized below.

Characteristic	B-DNA	Z-DNA
Coiling	Right-handed	Left-handed
Pitch	34 Å	45 Å
Base pairs/pitch	10.4	12
Diameter	Ca. 20 Å	Ca. 18 Å
Rise per base pair	3.4 Å	3.7 Å
Sugar-phosphate backbone	Regular	Zig-zag

GENETIC MATERIAL III. REPLICATION

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 determine the base sequence along the other. Hence, each chain of a 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 parental template chain. The free nucleotides are seen together by formation of phosphodiester bonds for linking adjacent deoxyribose, thus forming a new polynucleotide molecule. Three theories were proposed for DNA replication. They are dispersive theory, conservative theory and semiconservative theory.

In dispersive replication, the old DNA molecule would break in to several pieces, each fragment would replicate, and the old and new segment would recombine randomly to yield progeny DNA molecules; each progeny molecule would have both old and new segments along its length.

In conservative scheme, the two newly synthesized strands would associate to form one double helix, while the two old strands would remain together as one double helix.

In contrast, in semiconservative model (Fig. 22.1), each newly synthesized strand of DNA would remain

associated with the old strand against which it was synthesized. Thus each progeny DNA molecule would consist of one old and one newly synthesized strand.

Semi conservative model

Replication is necessary so that the genetic information present in cells can be passed on to daughter cells following cell division. The DNA is copied by enzymes called DNA polymerases. These act on Single-stranded DNA and synthesized a new strand, complimentary to the original strand. DNA synthesis always occurs in the 5'→3' direction. DNA would replicate simultaneously and continuously on both strands in semi discontinuous manner (one strand continuous; the other discontinuous). Replication would occur in both means namely, unidirectional (*E. coli*) and bidirectional (*Bacillus subtilis*). However, bidirectional replication is for more common In prokaryotes such as *E. coli*, two enzymes, DNA polymerases I and III, are responsible for DNA synthesis. In eukaryotes, DNA is replicated by four DNA polymerases (α , β , γ , and δ).

DNA replication begins at certain unique point called replication origin and gradually progresses in both directions (Fig. 22.2). Two enzymes, DNA gyrase and DNA helicases, bind to the origin and unwind the double helix; single-strand binding (SSB) protein keeps the strand separate. Replication origin usually contain sequences rich in weak A–T base pairs. Since, DNA replication never starts at the end of a DNA, the separation of parental strands create a bulge or bubble at the point of origin. The region where the helix unwinds and new DNA is synthesized is called the replication fork.

As the two strands of the double helix run in opposite directions, one strand called the leading strand is copied in the same direction as the unwinding helix and so can be synthesized continuously.

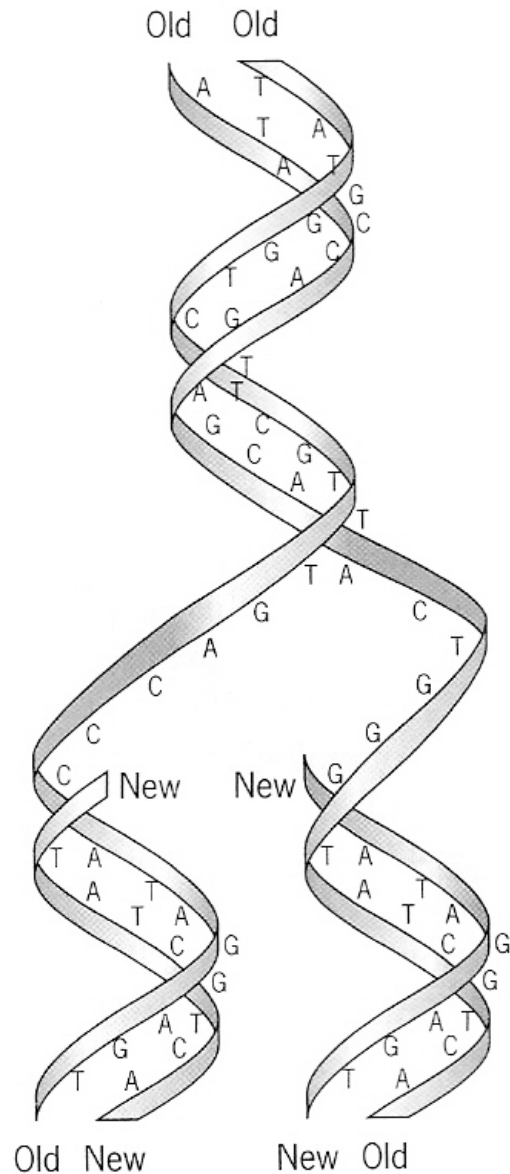


Fig. 22.1. Semiconservative model of replication

The other strand, known as the lagging strand, is synthesized in the opposite direction and must be copied discontinuously as a series of segments known as Okazaki fragments.

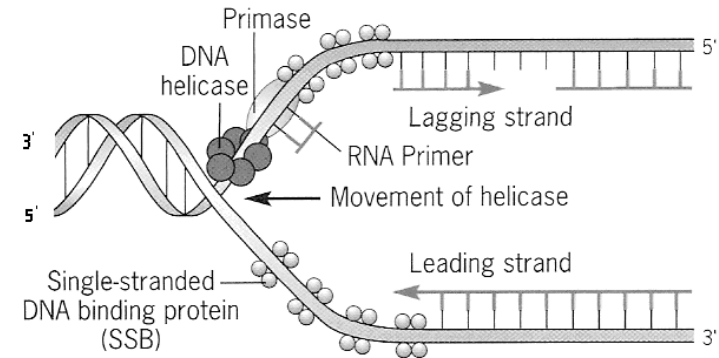


Fig. 22.2. Replication of DNA

DNA polymerases require a short double-stranded region to initiate DNA synthesis. This is produced by an RNA polymerase, called primase which is able to initiate synthesis on single-stranded DNA. The primase synthesizes a short RNA primer sequence on the DNA template creating a short double-stranded region. The DNA polymerase removes the RNA primer and replaces it with DNA. On the leading strand, DNA polymerase III synthesizes DNA beginning at the RNA primer. On the lagging strand, synthesis ends when the next RNA primer is encountered. At this point DNA polymerase I take over DNA synthesis. The final step required to complete synthesis of the lagging strand is for the Okazaki fragments to be joined together by phosphodiester bonds by an enzyme called DNA ligase. Replication needs to be very accurate because even a small error would result in the loss of important genetic information after just a few cell divisions. Accuracy is ensured by the ability of the DNA polymerases to check that the correct bases have been inserted in the newly synthesized strand. This is achieved through the reverse (3' 5') exonuclease activity

GENETIC MATERIAL IV. PROTECTION AND REPAIR SYSTEMS

The genetic material (DNA) must retain its primary structure with a very high fidelity during replication and transcription and protects itself from damages due to the various cellular and extra-cellular factors.

Bacteria and other organisms protect their genetic material from contamination by foreign DNA through the action of restriction enzymes, which clean DNA molecules possessing specific recognition sites, e.g., short specific sequences.

However, the restriction enzymes of an organism do not attack its own DNA; in Bacteria this is achieved through methylation of the restriction sites (DNA modification).

In addition, damage of DNA is handled by a variety of mechanisms which may involve excision of the damaged region and its replacement by a newly synthesized sequence (DNA repair) or even the process of genetic recombination may be involved in the repair of replicating damaged DNA (retrieval system).

DNA modification

DNA modification generally occurs through methylation (addition of $-CH_3$) of DNA bases; usually adenine and cytosine are methylated to yield 6-methyladenine and 5-methylcytosine, respectively. DNA methylation is mediated by enzymes called methylases. *E.coli* has three distinct methylation systems.

- i) The hsd system: This system methylates adenine; similar systems occur in many bacteria.
- ii) The dam system: This system also methylates adenine, it can distinguish the strands of newly

of the enzymes which allows them to remove incorrectly inserted bases from newly synthesized DNA and replace them with the correct base. This is referred to as proofreading ability. It is estimated that just one base in five billion is inserted incorrectly.

Replication of Bacterial DNA (Circular DNA)

The replication of DNA molecules requires unwinding of the DNA double helix (Fig. 22.3). For circular DNA molecules that do not have free ends, this produces super coiling of the DNA preventing the replication fork from progressing. This problem is overcome by the action of enzymes called topoisomerases of which there are two types. DNA topoisomerase I produces a transient break in the polynucleotide backbone of one of the DNA strands a short distance ahead of the replication fork allowing the DNA to rotate freely around the other intact strand removing the super coiling. The enzyme then rejoins the ends of the broken strand. When replication of a bacterial chromosome is completed, two circular daughter molecules that are interlocked are produced. These are separated by the action of DNA topoisomerase II that acts by causing transient breaks in both strands of one of the DNA molecules allowing the other DNA molecule to pass through, thus separating the two daughter molecules. The topoisomerase II enzyme then rejoins the broken strands.

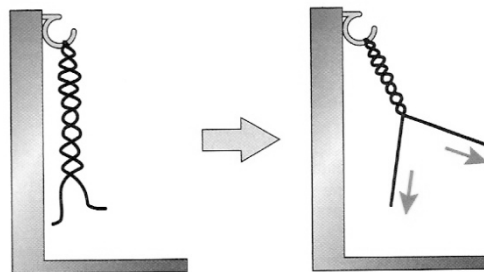


Fig.22.3. Unwinding of a two stranded rope; similar to unwinding of a double stranded circular DNA molecule

replicated DNA. The dam system is involved in the control of DNA replication and in marking DNA strands for repair.

- iii. The dm system: This system methylates cytosine, but its function is still unknown.

In prokaryotes, methylation is involved in the modification of restriction sites (DNA safeguard), DNA repair and control of replication.

In Eukaryotes it is concerned with the regulation of gene action.

DNA molecules having the same methylation pattern as that of the bacterial cell itself will be recognised as own DNA while those lacking this will be regarded as foreign DNA. The restriction enzymes are a type of endo-nucleases i.e., enzymes, which produce cuts within DNA molecules. The restriction enzyme as a rule recognize highly specific, short base sequences for binding to DNA molecules. They may, however, produce the cut either within the recognition site (Type II restriction enzymes) or some ways removed from this site (Type I and III restriction enzymes).

Each type of restriction enzymes is represented by a large number of different enzymes; they are also associated with methylation systems of these, Type II systems consist of separate enzymes for methylation and restriction activity while in type I and III systems the same enzyme carries out both the activities.

The Type^{II} Enzyme system:

The type^{II} system is widespread in bacteria' about 1/3 of the bacterial strains have them. In this system a type^{II} restriction enzyme is responsible for clearing the DNA, while a separate enzyme methylates the same site which is recognized by the restriction enzyme.

Eg : Eco R1 system in which the restriction enzyme is a dimer of identical polypeptides, while the methylase is a monomer (Fig. 23.1).

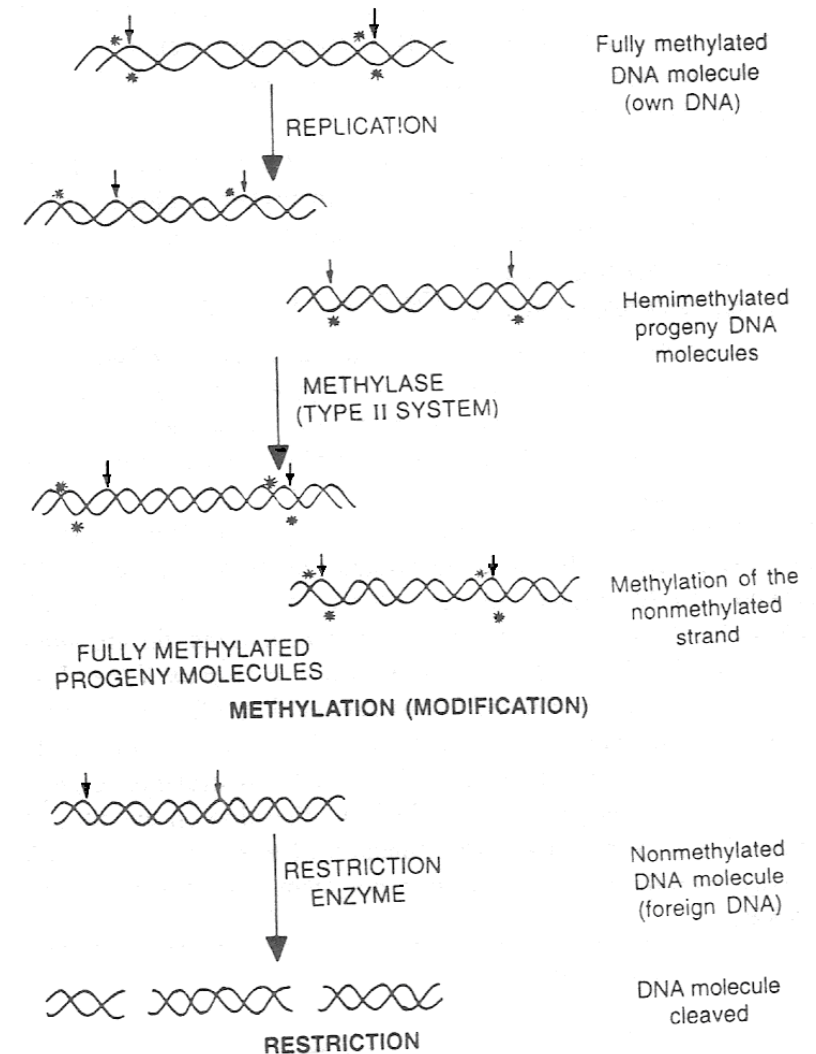


Fig. 23.1. The Type^{II} Enzyme System

The recognition sites for type II enzymes are generally palindromes of 4-6 bp so that the bases to be methylated occur on both the strands. The replication of a fully methylated target site yields target sites that are hemimethylated. “The restriction enzymes do not recognize either fully methylated or hemimethylated generally recognize the hemimethylated DNA molecules and brings about the methylation of their non-methylated strands to make them fully methylated”.

This appears to be the general mode of methylase action *in vivo* so that the methylation pattern, once generated in the specific target sites is perpetuated indefinitely. The methylase adds a single methyl group at a time. A non-methylated (foreign) DNA is rarely methylated since the methylase dissociates from the DNA after adding only one methyl group; a separate binding is needed for the addition of a second methyl group. Generally, a DNA molecule with non-methylated target site is recognized by the restriction enzyme is degraded.

DNA Repair systems

Damages to the genetic material i.e., DNA are taken care of by the DNA repair systems. The various damages to DNA may be grouped into the following two types:

(1) Single Base Changes

Such changes affect a single base of a DNA molecule they do not produce structural disturbance and do not affect either replication or transcription of the affected molecules. These changes are represented by:

- a. The conversion of one base into another.
e.g., Deamination of 5 methylcytosine results in thymine.
- b. By the covalent addition of a small group to a base which affects its pairing behavior. As a result the affected base does not pair properly with its partner base.

(2) Structural Distortions

These changes generally adversely affect the replication and transcription of the affected DNA molecule. They are represented by a single strand nick, removal of a base etc.

The various types of DNA damage systems are (1) Direct repair (2) Excision repair (3) Mismatch repair (4) Tolerance system and (5) Retrieval system.



RIBONUCLEIC ACID (RNA)

Chemical composition of RNA

RNA is formed of a single strand. It consists of several units called nucleotides. Hence each RNA molecule is formed of several nucleotides. Each nucleotide is formed of three different molecules, namely phosphate, ribose sugar and nitrogen base. The nitrogen bases are of two types, namely *purines* and *pyrimidines*. The purines present in the RNA are *adenine* and *guanine*. The pyrimidines present in RNA are *cytosine* and *uracil*.

The RNA molecule is normally single stranded. Sometimes the strand may be folded back upon itself and this double strand may be coiled to form a helical structure like that of DNA.

In RNA the purines and pyrimidines are not present in equal amounts.

Types of RNA

There are three types of RNA. They are the following:

1. Messenger RNA (mRNA)
2. Transfer RNA (tRNA)
3. Ribosomal RNA (rRNA)

1. Messenger RNA (mRNA)

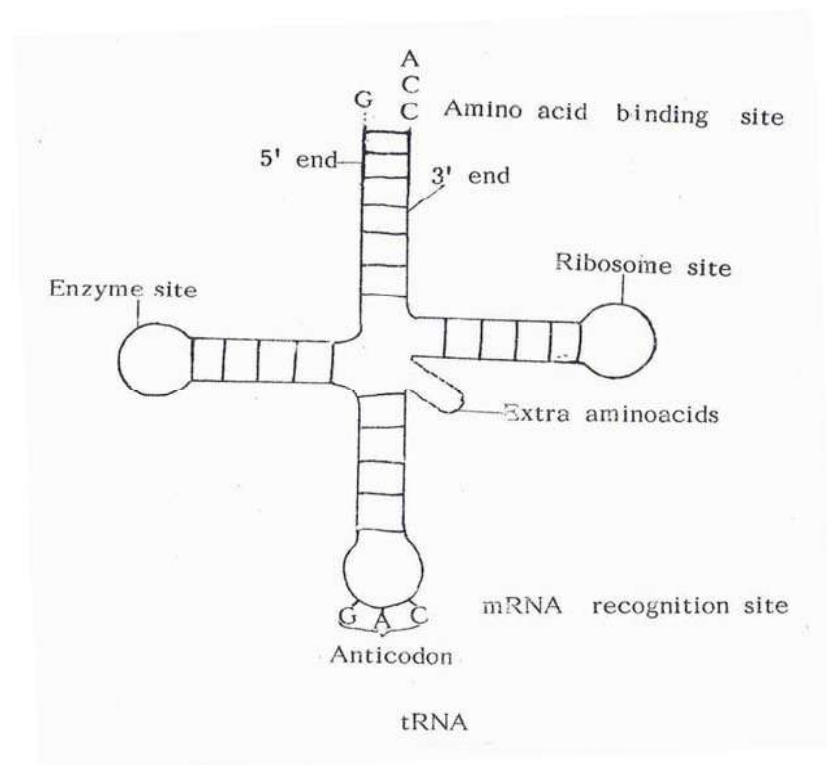
Messenger RNA is a nucleic acid which carries genetic information about protein to be synthesized from the DNA to the cytoplasm. The term mRNA was coined by JACOB and MONOD in 1961. It has the following salient features.

1. It is a single stranded RNA
2. It contains several units called nucleotides.
3. The mRNA contains phosphoric acids, ribose sugars, purines, namely adenine and guanine and pyrimidines, namely cytosine and uracil

4. Messenger RNA is synthesized from DNA. The synthesis of mRNA from DNA is called transcription.
5. For the synthesis of mRNA, one strand of the DNA acts as a template. Hence the sequence of bases in the mRNA is complementary to that of DNA. For example, if the sequence of bases in the DNA is CAATGA, the sequence of bases in the mRNA will be GUUACU.
6. The synthesis of mRNA from DNA is catalysed by an enzyme called RNA polymerase.
7. The mRNA carries genetic information from DNA. The genetic information carried by the mRNA is called **genetic code**. The genetic code is the sequence of nitrogen bases in mRNA. The genetic code is formed of several codons. Each codon is a sequence of three nitrogen bases which codes for one amino acid. As each codon is formed of three nitrogen bases, it is called a triplet code.
8. Among RNAs, mRNA is the longest one. Most of the mRNAs contain 900 to 1500 nucleotides.
9. Each mRNA contains the codons for one polypeptide chain. If the mRNA contains 900 nucleotides, the polypeptide chain synthesized by this mRNA will contain 300 amino acids.
10. The mRNA synthesized in the nucleus, immediately diffuses through the nuclear membrane into the cytoplasm. In the cytoplasm it is deposited on the ribosomes.
11. In some cells the mRNA is linked with proteins to form nucleoprotein complexes called informosomes. They are not deposited on the ribosomes; but they remain free in the cytoplasm. They are stable and are resistant to ribonuclease. They are found in cleavage cells. They take part in protein synthesis in embryonic cells.

12. The life span of mRNAs is short. The average life span of mRNA in *E.coli* is 2 minutes and in eukaryotes it is 4 hours.
13. There are two types of mRNAs, namely monocistronic mRNAs and polycistronic mRNAs. Monocistronic mRNA is formed from a single cistron (gene) and so it has message for a single polypeptide chain. The polycistronic mRNA is formed from many cistrons and so it has message for many polypeptides.

Transfer RNA (tRNA)



Transfer RNA is a nucleic acid which transports amino acids to the site of protein synthesis. Hence it is called transfer RNA. It is also called soluble RNA. The tRNA has the following salient features.

1. It is a single stranded RNA
2. It is smaller than mRNA,
3. It transports amino acids to the site of protein synthesis
4. Each tRNA transports only one variety of amino acid.
5. There are about 60 types of tRNA as there are only 20 types of amino acids, certain amino acids are carried by more than one tRNA.
6. The tRNA is formed of small units called nucleotides. There are about 75 to 80 nucleotides in the tRNA
7. The tRNA contains phosphoric acid, ribose sugar, purines (adenine and guanine) and pyrimidines (cytosine and uracil)
8. The tRNA is folded on itself to form a clover leaf-like structure. It has two terminal ends, three main loops and one mini loop.
9. A tRNA has four main regions, namely
 - Enzyme site
 - Carrier site
 - Anticodon site and
 - Ribosome site.
10. Each amino acid is activated by a specific enzyme. This activating enzyme is attached to main loop of tRNA hence this site is called enzyme site. It recognizes, catalyzes and picks up the appropriate amino acid from the cytoplasm.
11. The activated amino acid is attached to the carrier site. The carrier site is located at one terminal end. It is the 3' end and has a free-OH group of ribose sugar. It is common to all tRNA molecules.
12. The terminal loops of tRNA contains a sequence of three nitrogen bases. It is called **anticodon**. The

anticodon is complementary to a codon in the mRNA. The tRNA is temporarily attached to the mRNA at this site and it reads the sequence of bases of mRNA hence, mRNA is described as the reader of the message written in the mRNAs.

13. The tRNA is attached to the ribosome through one main loop. Hence, this region of tRNA is called ribosome site.
14. One terminal end of the tRNA is the 5' end. It carries a guanine carrying nucleotide.
15. The tRNA contains certain rare and unusual nitrogen bases, namely, pseudouridine, methylguanine, methylaminopurine, inosinic acid etc.,
16. Transfer RNAs are more stable.
17. They are synthesized from the DNA of chromosomes.
18. They contain unusually high C-G base pairing.

Ribosomal RNA or Insoluble RNA

Ribosomal RNA constitutes the largest part of the total cellular RNA. It is found primarily in the ribosomes. But it is synthesized in the nucleus. It is single stranded like mRNA. The prokaryotic cells contain three types of rRNA molecules (based upon the sedimentation constant) namely 23 S rRNA,

Genetic and non-genetic RNA

In all organisms except virus, the RNA is doing non-genetic functions. Such RNAs are called non-genetic RNAs.

In some virus the RNA is doing genetic functions. Such RNAs are called genetic RNAs.

Functions of RNA

RNA has the following functions

1. RNAs are the main components of protein synthesis.

2. In many viruses the RNA functions as the genetic material
3. The rRNA present in the chromatin fibres initiates the replication of DNA.

RNA AS GENETIC MATERIAL

In several viruses, e.g., TMV (tobacco mosaic virus) DNA is absent; these viruses are composed of RNA and protein. TMV particles are like hollow cylinders; their RNA is coiled like a spring which is stabilized by the protein molecules that are arranged on the outside of the coil. Frankel-Conrat and Singer demonstrated in 1957 that RNA functions as the genetic material in TMV. The proteins and RNA of TMV can be separated chemically; when they are remixed under appropriate conditions they reassociate to produce active TMV particles. In one experiment, either RNA or the proteins isolated from TMV were used for the infection of tobacco leaves. Mosaic symptoms developed only when the RNA was used for infection (and not when the proteins were used). Clearly, only RNA fraction of TMV is capable of producing the disease, and hence it appears to be the genetic material.

In another experiment, they produced hybrid TMV particles using proteins and RNA from two different strains of the virus. Strain A of TMV was isolated from tobacco, while strain B was isolated from *Plantago lanceolata* (hence called *Plantago* strain; it is also called Holmes rib-grass virus or HRV). Strains A and B produce distinctive symptoms on a certain tobacco variety. Frankel-Conrat and Singer constructed two types of hybrid virus particles by mixing: (1) RNA from strains A and proteins from strain B, and (2) proteins from strain A and RNA from strain B (Fig. 24.1). When tobacco leaves were infected with hybrid TMV particles of the first type, the disease symptoms of strain A developed; the virus particles isolated from the diseased leaves had proteins identical with those of strain A. Similarly, when hybrid particles of the second type were used for infection of

GENE ACTION

THE 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.

The number and sequence of bases in mRNA specifying an amino acid is known as codon, while the set of bases in a tRNA that base-pair with a codon of mRNA is known as anti-codon. The sequence of bases in an anti-codon is exactly the opposite of that present in the codon, since the codon and anti-codon segments run anti-parallel to each other when they base-pair. For eg: the codon (5') AUG (3') has the anti-codon (3') UAC (5') which will read CAU if read from the 5' end as is the case for the codon (AUG). The set of all the codons that specify the 20 amino acids is termed as the genetic code, genetic language or coding dictionary.

The genetic language has only four alphabet, A,T,G, and C, the four bases making up polynucleotides. Each word of this language i.e., codon may consist of 1,2,3,4,5 or more letters. Codons of 1 and 2 letters will be inadequate since there can be only $4 = (4^1)$ and $16 = (4^2)$ different codons, respectively to code for 20 different amino acids. This would require some ambiguity in the genetic code, That is a codon would be required to code for more than one amino acid. Ambiguity is highly

tobacco leaves, symptoms identical with those of strain B were observed. Further, the virus particles isolated from such diseased leaves had proteins identical to those of strain B.

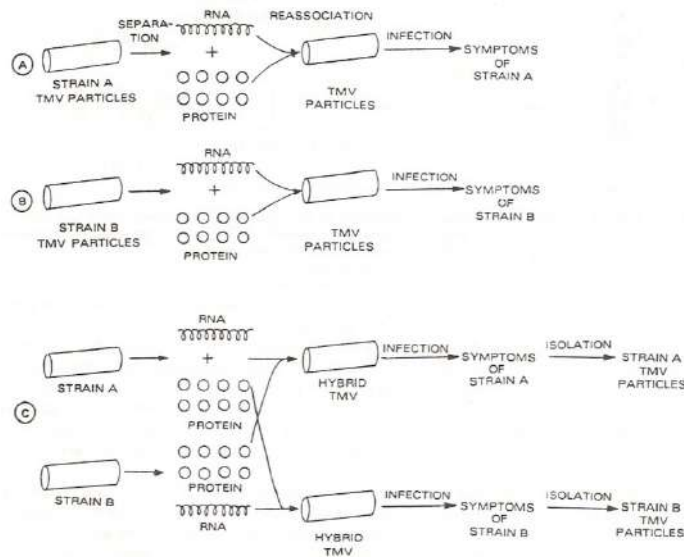


Fig. 24.1. Experiments Frankle-Conrat and Singer on TMV

It is evident from these findings that only RNA (and not the protein) of TMV has the capacity to produce the disease, and that the type of proteins present in the virus particles is determined by the RNA. Clearly, RNA is the genetic material in TMV. It is now known that in many other viruses, e.g., animal viruses causing poliomyelitis, influenza, encephalitis etc., most of the plant viruses, and some small bacteriophages (such as, f2, MS2, R17 etc.), RNA functions as the genetic material.



undesirable since it would lead to a certain degree of random variation in the amino acid sequence of the protein produced by a single gene, while the cellular function requires this sequence to be highly precise.

A three letter code, triplet code, would produce $64 = (4^3)$ different codons (Fig. 25.1). This is as follows:

		Second position				
		U	C	A	G	
First position	U	UUU } Phe	UCU } Ser	UAU } Tyr	UGU } Cys	U
		UUC } Phe	UCC } Ser	UAC } Tyr	UGC } Cys	C
		UUA } Leu	UCA } Ser	UAA Stop	UGA Stop	A
		UUG } Leu	UCG } Ser	UAG Stop	UGG Trp	G
	C	CUU } Leu	CCU } Pro	CAU } His	CGU } Arg	U
		CUC } Leu	CCC } Pro	CAC } His	CGC } Arg	C
		CUA } Leu	CCA } Pro	CAA } Gln	CGA } Arg	A
		CUG } Leu	CCG } Pro	CAG } Gln	CGG } Arg	G
	A	AUU } Ile	ACU } Thr	AAU } Asn	AGU } Ser	U
		AUC } Ile	ACC } Thr	AAC } Asn	AGC } Ser	C
		AUA } Ile	ACA } Thr	AAA } Lys	AGA } Arg	A
		AUG Met/start	ACG } Thr	AAG } Lys	AGG } Arg	G
	G	GUU } Val	GCU } Ala	GAU } Asp	GGU } Gly	U
		GUC } Val	GCC } Ala	GAC } Asp	GGC } Gly	C
		GUA } Val	GCA } Ala	GAA } Glu	GGA } Gly	A
		GUG } Val	GCG } Ala	GAG } Glu	GGG } Gly	G

Fig. 25.1. Genetic Code

As a result either a large number (44) of codons will not code for any amino acid i.e., will be nonsense codons, or else more than one codon will code for a single amino acid, such a codon being known as redundant or degenerate.

Based on such considerations physicist George Gamow proposed a coding scheme in 1954; he suggested that each codon contained three bases and that the adjacent codons overlapped each other. In an overlapping system the 3 letters of a codon are shared by the neighbouring codon, whereas in a non-overlapping system the 3 bases of a codon are separate from those of the neighbouring codons.

The Coding Dictionary:

It has become clear from various experiments that a sequence of three nucleotides in the mRNA codes for an amino acid (a triplet code or codon), and that the code is non overlapping and commaless (Fig. 25.2). A commaless code means that all the bases in a polynucleotide are parts of codons and that no base serves as a punctuation mark. The meaning of all the possible 64 triplet codons has been determined beyond doubt. Three codons, UAA, UAG and UGA are nonsense codons as they do not code for any amino acid; those codons act as polypeptide termination codons. There is a considerable degeneracy in the code.

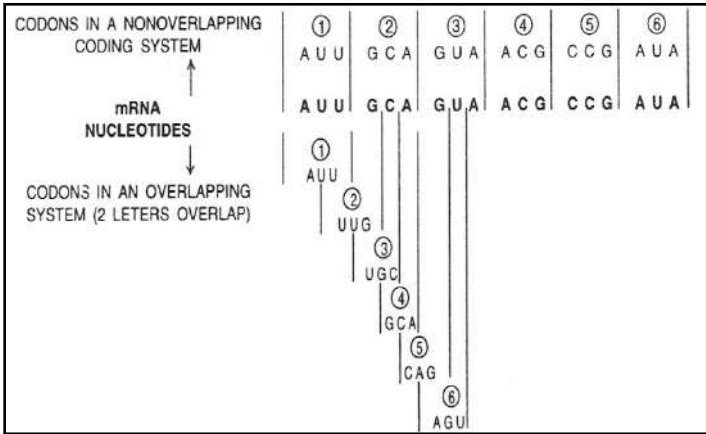


Fig. 25.2. Coding Dictionary

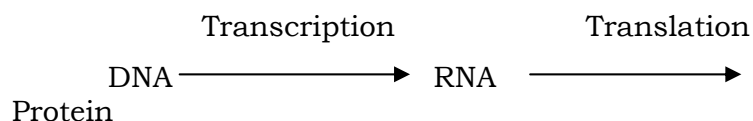
GENE EXPRESSION / PROTEIN SYNTHESIS

Central dogma

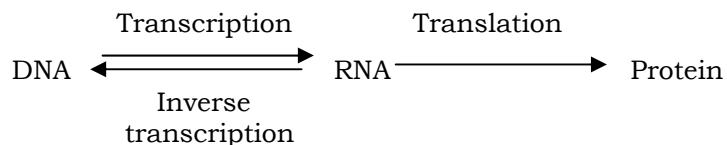
The process of protein synthesis involves one of the central dogma of molecular biology postulated by Crick (1958) according to which genetic information flows from nucleic acid to protein.

Protein synthesis involves two steps viz., transcription and translation. Transcription involves a sequential flow of information from DNA to mRNA. This does not involve a change of code since DNA and mRNA are complementary. Translation involves a change of code from nucleotide sequences to amino acid.

Generally, the flow of information is one way, from DNA to RNA and from RNA to protein.



In certain viruses, the existence of an enzyme 'RNA dependent DNA polymerase' (also called inverse transcriptase) was reported and this enzyme could synthesize DNA from a single stranded RNA template. These findings of Baltimore (1970) and others gave rise to the concept of 'central dogma reverse'. According to this, the sequence of information flow is not necessary from DNA to RNA to protein but can also take place from RNA to DNA.



Protein Synthesis

Proteins form the structural constituents of a cell. They also form enzymes that catalyze all the chemical reactions in a cell. Therefore, the structure and function of the cell is maintained by its proteins.

Proteins are made up of amino acids. Many amino acids link together to form a polypeptide chain. Each protein is formed of one or more polypeptide chains.

The synthesis of protein is under the control of DNA.

The information for the synthesis of protein remains in DNA in the form of genetic code.

Requirements of Protein Synthesis

The synthesis of protein requires the following components:

1. DNA
2. Messenger RNA - Codon
3. Ribosomes - Poly ribosomes
4. Magnesium ions (Mg^{H})
5. Amino acids
6. ATP
7. Activating enzyme
8. Transfer RNA
9. GTP (Guanosine triphosphate)
10. Condensing enzyme
11. Releasing enzyme etc.

Site of Protein Synthesis

A small amount of protein is synthesized in the nucleus, but the greater proportion is synthesized in the

cytoplasm. In the cytoplasm the ribosomes are the sites of protein synthesis.

Mechanism of Protein Synthesis

Protein synthesis involves the following steps :

1. Transcription of mRNA
2. Activation of amino acids
3. Attachment of amino acids with tRNA
4. Translation
5. Elongation of polypeptide chain
6. Termination of polypeptide chain and
7. Processing of polypeptide chain

1. Transcription of mRNA

Synthesis of mRNA from DNA is called transcription. It involves the following steps (Fig. 26.1).

1. The DNA strands unwind and separate.
2. One strand of DNA functions as a template.
3. Synthesis proceeds from 5' end towards 3' end.
4. Synthesis starts from a specific point in the DNA strand called initiator site.
5. It is catalyzed by an enzyme called RNA polymerase.
6. The newly synthesized mRNA remaining inside the nucleus is called Hn-RNA (heterogeneous nuclear RNA). It is unstable.
7. The Hn-RNA is stabilized by the addition of a sequence of about 200 nucleotides of adenylic acid called poly- A to the 3' end.
8. At the 5' end a cap-like structure is formed by the addition of 7-methyl guanosine. This is the initiation site of translation.

9. Now the mRNA diffuses through the nuclear membrane and enters the cytoplasm for protein synthesis.

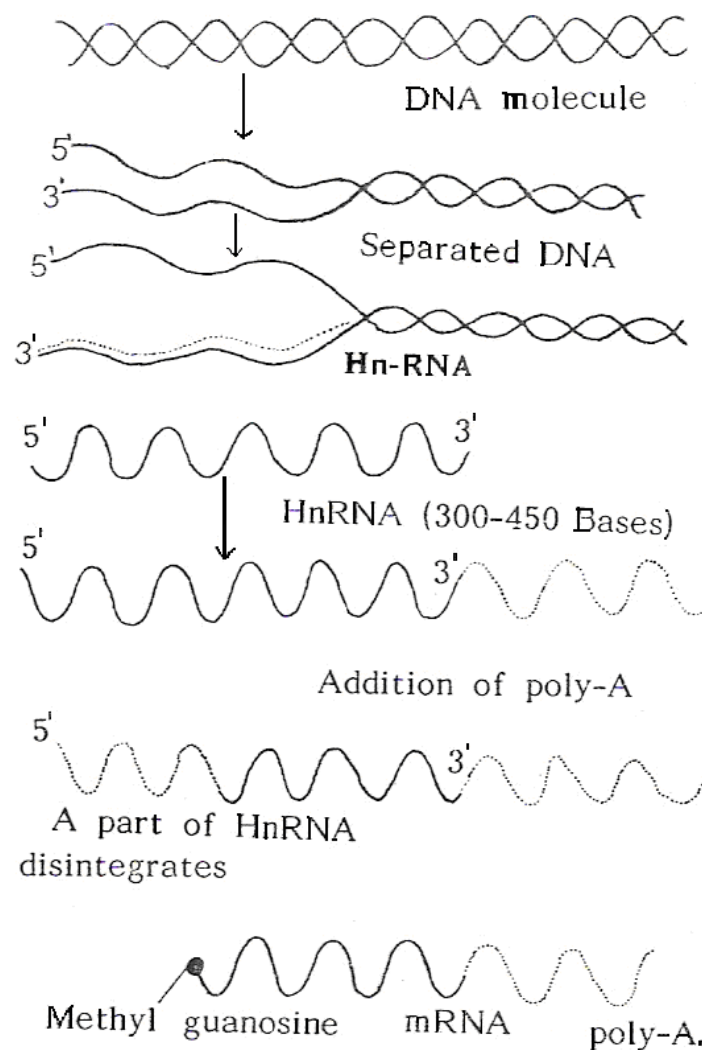
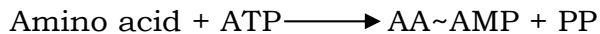
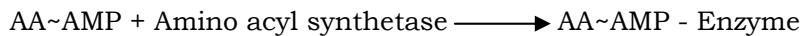


Fig. 26.1. mRNA transcription

2. Activation of aminoacids

Amino acids are the building blocks of proteins. In protein synthesis, the various aminoacids are linked in a specific sequence as per the information carried in the mRNA. Cytoplasm provides all the aminoacids necessary for protein synthesis. The amino acid present in the cytoplasm is activated during protein synthesis. This activation is brought about by ATP and an activating enzyme called amino acyl synthetase.

First of all amino acid reacts with ATP to form amino acid ~ AMP or amino acyl adenylate (AA~AMP) and pyrophosphate (PP). Then the enzyme amino acyl synthetase links with amino acid ~ AMP to form amino acyl adenylate enzyme complex. This complex is called activated amino acid. It remains in the cytoplasm.



Each amino acid has a specific amino acyl synthetase enzyme and it binds to its specific enzyme without any error.

3. Attachment of activated aminoacid to tRNA

When a specific tRNA collides with the activated amino acid, the enzyme of activated amino acid binds with the enzyme - site of tRNA. At the same time, the activated amino acid binds with the amino acid attachment site of tRNA. As a result the AMP and the enzyme are free from the complex and an aminoacyl tRNA is formed. The aminoacyl tRNA moves towards the ribosome which is involved in protein synthesis (Fig. 26.2).

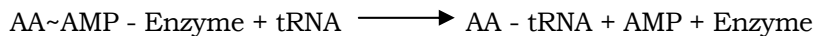
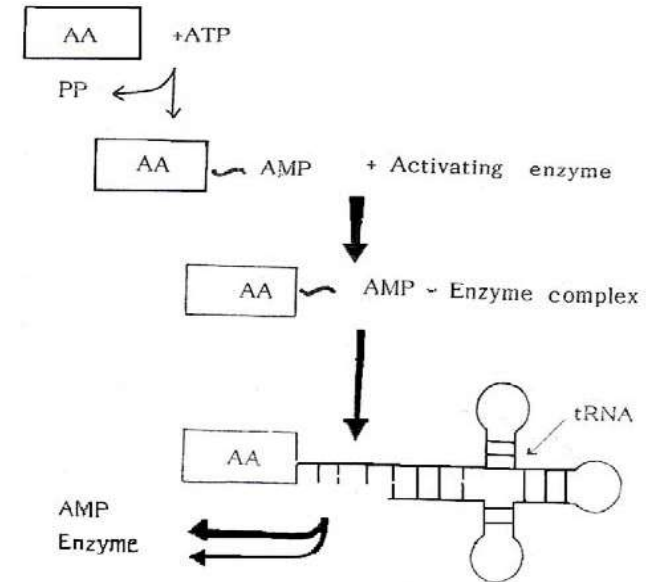


Fig. 26.2. Activation and attachment of aminoacids

4. Translation

Translation is a process by which the sequence of nucleotides present in the mRNA is translated into the sequence of aminoacids of a polypeptide chain. Translation is initiated by the initiation codon of mRNA. The initiation codon is always AUG (Fig. 26.3). It codes for the aminoacid methionine. This aminoacid is picked up by the tRNA. The 5' end of mRNA is attached with the small subunit of ribosome. Here the initiation codon and the small sub unit forms the initiation complex. The tRNA with the activated amino acid is attached to the initiation complex. This combines with large subunit of ribosome. This forms a complex called ribosome - mRNA - aminoacid tRNA complex.

The amino acid - tRNA complex is now attached to the P-site of large sub unit and its anticodon binds to the first codon of mRNA. The second aminoacid - tRNA is attached to the A-site and its anticodon binds to the

second codon. Now a peptide bond is formed between the aminoacids of site-P and site-A. Hence a dipeptide is formed. After the formation of peptide bond, the tRNA from P-site is released and the dipeptide is attached to A-site only. As soon as the tRNA from P-site is released, the tRNA from A site is transferred to site-P from site-A.

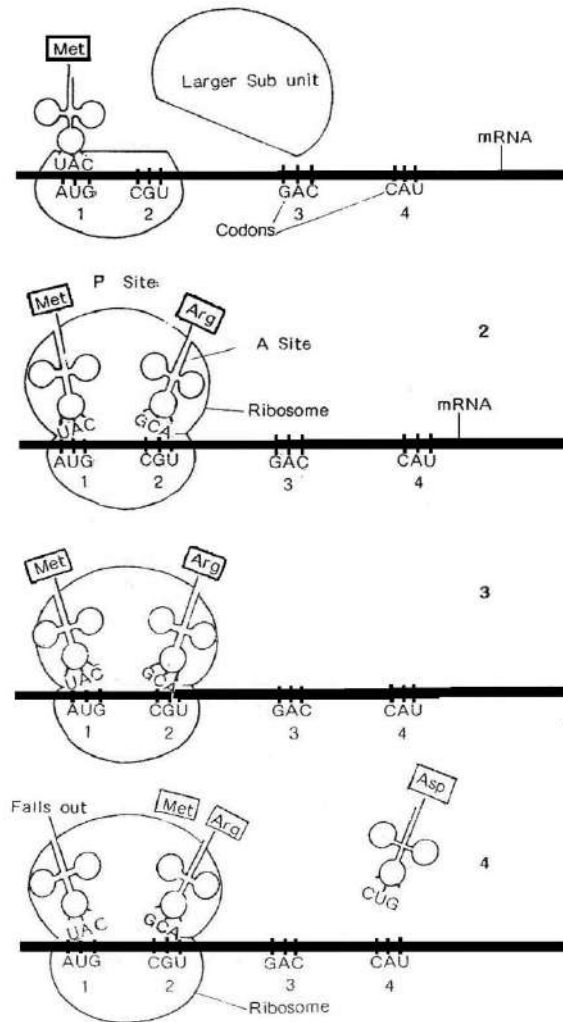
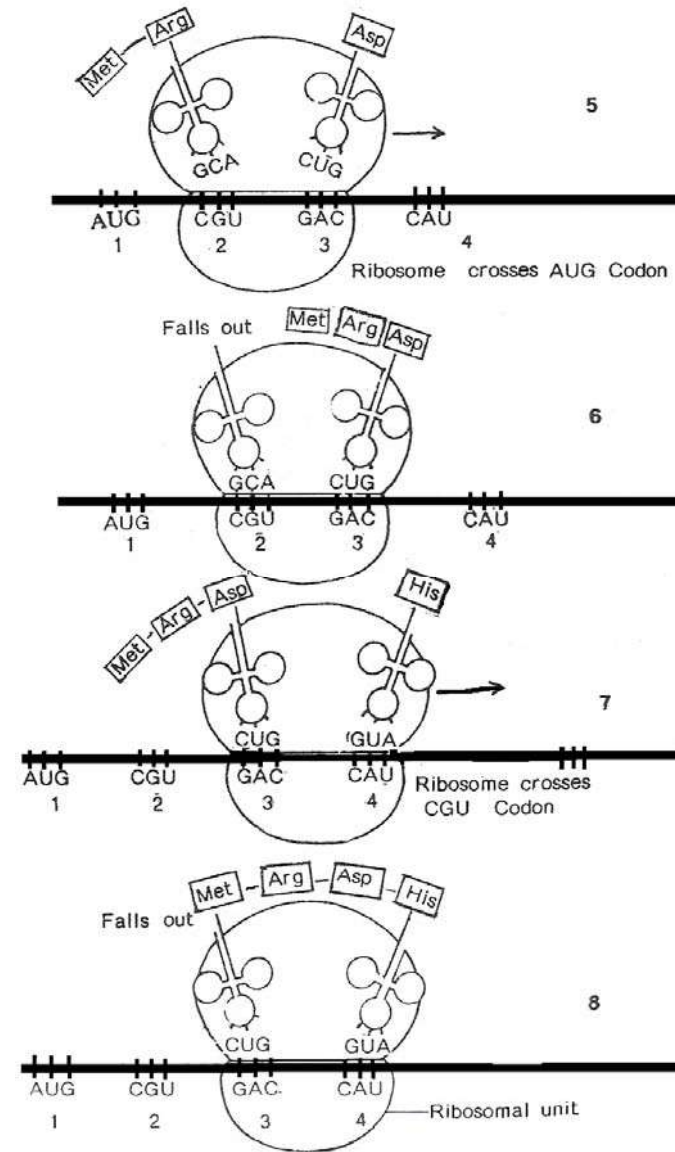


Fig. 26.3. Stages involved in protein synthesis

5. Elongation of polypeptide chain

During this process ribosome moves along mRNA in 5' to 3' direction. This brings the third codon to A-site. Now the third aminoacid - tRNA is attached to A-site with its anticodon binding to the third codon. In this way, more aminoacids are linked to the dipeptide to make it a polypeptide chain. The chain gradually elongates as it moves along the ribosome.

6. Termination of polypeptide chain

At the end of each cistron of mRNA, there is a termination codon. This codon may be UAA or UAG or UGA. It does not code for any aminoacid. So when the ribosome reaches the termination codon, no aminoacid is added to the polypeptide chain. Thus one polypeptide chain is completed. The polypeptide chain is released by an enzyme called peptidyl transferase. The ribosome now dissociates into small and large subunits.

7. Processing of polypeptide chain

On release, the first amino acid methionine is removed by the enzyme deformylase. Some more aminoacids from the ends are removed by the enzyme exopeptidase. Then the polypeptide chain gets folded up to form functional protein.

Polyribosome

A single mRNA can be used repeatedly for the synthesis of hundreds of the same type of polypeptide chains (Fig. 24.4). When the first ribosome moves away from the initiation codon, a second ribosome gets attached with the initiation codon and starts synthesizing a second polypeptide chain. Thus during protein synthesis, a number of ribosomes can be seen attached to a single mRNA molecule at one time, each with a polypeptide chain under formation.

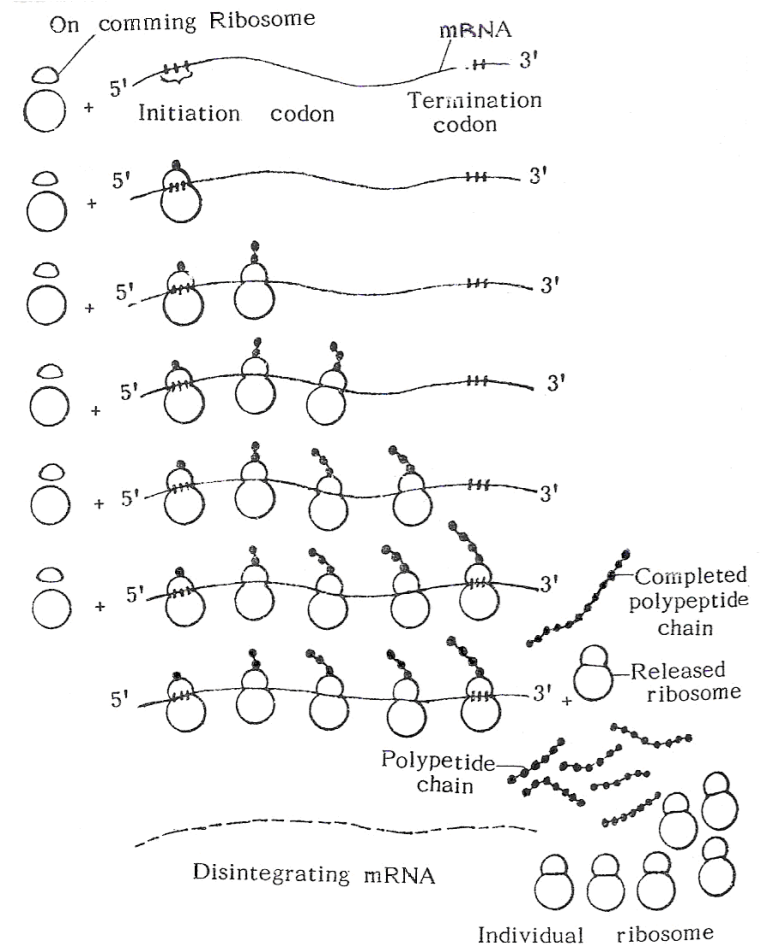


Fig. 26.4. Polyribosome and protein synthesis

Rate of Synthesis

The synthesis of protein on a messenger RNA template progresses at the rate of about one aminoacid per half second. A haemoglobin molecule of 150 aminoacids is completed in 115 minutes. The mRNA molecule may act as a template repeatedly. In some cases a single mRNA molecule is used repeatedly for the synthesis of 20 molecules of protein.

Protein Synthesis and House Construction

The process of protein synthesis can be compared with the construction of a house. The brain of an engineer (DNA) is filled with informations to construct various types of buildings. DNA is filled with informations to synthesize various types of proteins. As a first step in the construction of a house, the engineer decides the nature and the type of house, or he produces a plan or blue print (mRNA) for constructing the house. The blue print reflects the informations present in the brain and it contains all the informations about the nature and the type of the house (mRNA reflects the genetic information present in DNA). It is possible to see the structure of a house from its blue print (It is possible to study the structure of the protein from its mRNA). These blue prints are sent out to the workmen (ribosomes). The workmen, however must have materials with which to build the house. The bricks, nails, cement and other materials (various types of amino acids) are brought to the workmen, by delivery trucks. Normally we use a specific truck to carry a specific material (specific tRNA to carry a specific aminoacid). Money plays a very important role to carry out all these works (The cells require energy to synthesize the proteins). The money may be in the form of currency or coins (The energy may be in the form of ATP or GTP). The adjacent bricks are pasted with one another by some pasting materials (the adjacent aminoacids are linked with one another by a covalent bond). Thus the house is constructed according to the ideas of the engineer (protein is synthesized according to the information of the DNA).

Inhibitor of Protein Synthesis

Antibiotic and chloramphenical are the main inhibitors of protein synthesis. These inhibitors affect the formation of peptide bonds between the amino acids. The inhibitor binds to a specific site in the ribosome and does its function.

Protein Synthesis in non-nucleated cells

The human red blood cells (RBC) are non-nucleated. The red blood cells are produced in the red bone marrow and they contain nuclei in their premature stage. At that period the nucleus (DNA) produces sufficient amount of mRNA to synthesize sufficient amount of proteins to keep the cell alive and active even after the nucleus has been destroyed. The red blood cells do not continue to grow and synthesize new protein, and their life span is limited to about 120 days. It shows that the ribosomes can continue to synthesize proteins for a short time with the existing supply of mRNA which is already in the cytoplasm.



REGULATION OF GENE EXPRESSION IN PROKARYOTES

The Operon Concept

It was proposed by F. Jacob and L. Monod in 1961. The operon concept is outstanding for its simplicity, specificity and precision in the regulation of transcription according to the needs of a cell.

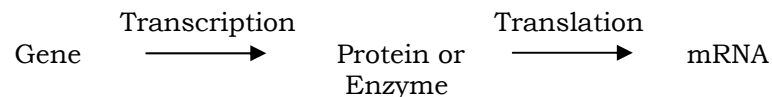
An Operon is a group of structural genes whose transcription is regulated by the coordinated action of a [1] regulator 'r' [2] a promotor 'p' and [3] an operator 'o' gene.

“Gene expression refers to the molecular mechanism by which a gene expresses a phenotype. The gene expresses the phenotype by synthesizing a protein or an enzyme. The protein or the enzyme determines the character”.

The gene contains the information for the protein or enzyme. The information is transferred to mRNA by the synthesis of mRNA. The synthesis of mRNA is called transcription. The mRNA moves into the cytoplasm from the nucleus. In the cytoplasm, it is attached to the ribosomes. The rRNA brings amino acids to the ribosomes.

The amino acids are linked in a particular sequence as per the sequence of nucleotides in the mRNA is called translation. By translation a polypeptide chain is produced. This polypeptide chain may be a protein or an enzyme. This protein may form a structural component of the cell or the enzyme may catalyze a metabolic reaction. So the gene expresses the character.

The gene expression involves the following steps.



The Operon Hypothesis

Jacob and Monod in 1961 proposed a mechanism for the expression of genes in *E. coli*, an intestinal bacterium. As *E. coli* is a prokaryote the operon hypothesis explains the mechanism of gene expression in prokaryotes.

“The operon hypothesis states that each metabolic reaction is controlled by a set of genes. All the genes controlling a metabolic reaction constitute an operon”.

Jacob and Monod studied the catabolism of lactose in *E. coli*. It involves a set of enzymes produced by a set of genes. All these genes involved in the catabolism of lactose are called lac operon.

Structure of operon model

The operon model is formed by two types of genes (Fig. 27.1), namely

1. Structural genes and
 2. Control genes
1. Structural genes: The structural genes have the codes for the synthesis of enzymes (proteins) necessary for metabolic reactions.
 2. Control genes: The control genes control the activity of structural genes. The control genes are of three types, namely regulator gene, promotor gene and operator gene.

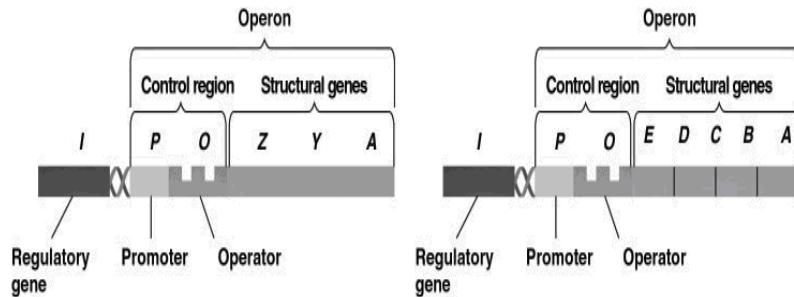


Fig. 27.1. Structure of the operon. The operon consists of the promoter (P), and operator (O) sites, and structural genes which code for the protein. The operon is regulated by the product of the regulatory gene (I).

- Regulator gene: The regulator gene suppresses the activity of the structural genes. It brings about its action by producing a protein called REPRESSOR. The repressor binds with the operator gene and suppresses their activity.
- Promotor gene: This gene marks the site at which transcription of m RNA starts. The RNA polymerase binds with this gene.
- Operator gene: The operator gene directly controls the structural genes. The repressor binds with the operator gene. It lies close to the structural genes.

Mechanism of operon model

When *E. coli* is cultured in a medium devoid of lactose, the enzymes for lactose catabolism are not required. In such cases the lac-operon remains switched off or repressed (Fig. 27.2). Under such conditions, the regulator

Operon model in *E.coli* : Repressed stage (Switched off)

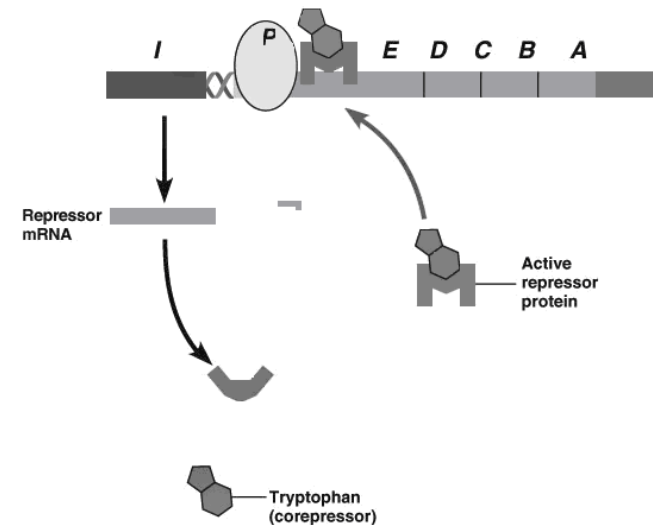


Fig.27.2. Repressor active, operon off. When the corepressor typtophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

Depressed stage (Switched on)

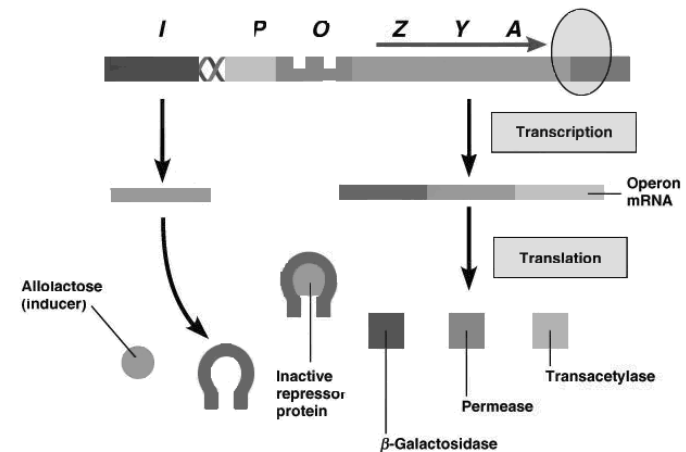


Fig. 27.3. Reperssor inactive, operon on. When the inducer allolactose binds to the repressor protein, the inactivated repressor can no longer block transcription. The structural genes

are transcribed, ultimately resulting in the production of the enzymes needed for lactose catabolism.

gene produces a repressor protein. This repressor protein binds with the operator gene and prevents its transcription. As a result the structural genes do not synthesize mRNA and enzymes are not produced. This phenomenon is called repression.

When *E.coli* is cultured in a medium containing lactose, the *E.coli* needs enzymes for lactose – catabolism. So the lac-operon is switched on or depressed (Fig. 25.3). In such cases the lactase enters *E. coli* and it gets modified into an inducer substance. It binds with the repressor. The repressor is unable to bind with the operator. The operator is therefore free. It induces the RNA polymerase to bind with the promoter gene. The promoter initiates transcription and the structural genes synthesize mRNA to produce enzymes.



REGULATION OF GENE EXPRESSION IN EUKARYOTES

Gene and Enzyme sequences

In bacteria and viruses, genes governing enzymes of a metabolic pathway are, in many cases clustered together. Often the sequence of these genes closely corresponds the sequence of involvement of the enzymes produced by them in the metabolic pathway. For eg., in *Salmonella*, histidine biosynthesis involves nine clustered genes occurring in the order E-I-F-A-H-B-C-D-G. This is precisely the sequence in which the enzymes produced by them participate in histidine biosynthesis, except that the last gene, G controls the first enzyme pyrophosphorylase. However, in eukaryotes such a correlation is generally lacking. For eg., in *Neurospora* genes concerned with the biosynthesis of the same amino acid are located in different chromosomes. Thus the situation found in *salmonella*, *E.coli* etc. appears to be a primitive genetic organization.

One – Gene – One Enzyme Hypothesis

This was originally postulated by Beadle and Tatum in 1941. From their work on *Neurospora* biochemical mutants, they concluded that each gene controlled a single specific chemical reaction. Genes control chemical reactions by controlling the presence of specific enzymes catalysing these reactions. Thus one – gene – one enzyme hypothesis simply states that each gene controls a single specific enzyme. One of the first clear-cut proofs for this hypothesis came from arginine biosynthesis pathway in *E. coli*. The pathway consists of eight chemical reactions each catalysed by a specific enzyme. The presence of each gene as specific enzymes are absent in mutants for specific genes.

One - Gene – One Polypeptide Hypothesis

Many enzymes (and protein molecules) are composed of a single polypeptide and are termed as monomers, while others are made up of two or more polypeptides and are known as dimers, tetramers etc., depending on the number of polypeptides in the molecule. A polypeptide consists of several amino acids joined by peptide bonds and generally lacks full protein activity. It is now accepted that each polypeptide is controlled by a single specific gene. For eg., tryptophan synthetase in *E.coli* consists of four polypeptide chains, two α and two β chains, which are governed by two different genes located adjacent to each other. Similarly, a haemoglobin molecule has four polypeptide chains of two distinct types therefore two α and two β polypeptides. Polypeptides α and β are specified by two different genes located in two different chromosomes.

Thus the one – gene – one enzyme hypothesis is valid for only those enzymes that are composed of a single polypeptide. In addition, many genes specify non-enzyme protein molecules, eg., haemoglobin, myoglobin structural proteins etc. Therefore the one - gene – one polypeptide hypothesis proposed by Ingram is more appropriate and accounts for monomeric as well as dimeric etc., enzymes and for non-enzyme proteins.

“According to this hypothesis, a gene specifies a single polypeptide chain”. However, many genes do not produce any polypeptides, eg., operator and promoter genes, and the genes specify tRNA (transfer RNA) and rRNA (ribosomal RNA). Therefore one – gene – one polypeptide hypothesis may be regarded as applicable to those genes which specify polypeptide sequences, that is structural and regulator genes.

Genetic control of protein (or) polypeptide structure

Proteins (or polypeptides) are composed of several smaller sub units, amino acids. There are 20 different amino acids that occur in most of the proteins. Each

amino acid has an alpha carbon atom to which four groups are attached: a carboxyl group ($-\text{COOH}$) with a potential negative charge an amino group ($-\text{NH}_2$) with a potential positive charge (NH_3^+), an H atom and a polar or non polar side chain (R). The side chain (R) may consist of a single H atom (alanine), several carbon atoms (lysine, arginine) or even a benzene ring (phenylalanine). The carboxyl group of one and the amino group of the other of two neighbouring amino acids react to produce a peptide linkage and one molecule of water is released. The peptide linkages, elucidated by Emil Fischer, link together sequence yielding a polypeptide chain. The polypeptide chain, therefore has a back bone of alpha carbon alternated with linkage i.e., repeats, with the side chains (denoted by R) dangling away from the alpha-carbon atoms; this may be represented as polypeptide structure.

The Translation Process

Translation of mRNA message, i.e., synthesis of a polypeptide with the amino acid sequence coded by the mRNA molecule, begins by the formation of an initiation complex.

In prokaryotes, the initiation complex is formed when the initiation codon, AUG of mRNA binds to the A site of 30s sub unit of a ribosome and a tRNA, f_{met} molecule, carrying formylemethionine (Formylmethionyl tRNA f_{met}) base-pairs with the codon AUG. A formylemethionine molecule has CHO (formyl) group attached to the amino group of methionine.

In Eukaryotes, a methionine (not formylemethionine) tRNA f_{met} (methionyl tRNA f_{met}) first binds to the 40s sub unit at the A site, and then an mRNA molecule attaches to this complex requires a number of initiation factors *viz.*, GTP, f_1 , f_2 and f_3 . However, GTP is not hydrolyzed during this process.

MUTATION

In genetic material, the sudden heritable changes in the characters are called mutation. The individuals showing these changes are known as mutants. The allele producing the changed phenotype is called mutant allele. A mutation may arise due to a change in the base sequence of a gene; such mutations are called gene mutations or point mutations. Changes in chromosome number and structure also produce heritable changes in phenotype; these are termed as chromosomal mutations.

The term mutation was introduced by Hugo de Vries in 1900 in connection with sudden heritable changes observed by him in *Oenothera*. Systematic studies on mutations began in 1910 with the discovery and genetic analysis of the white eye mutant of *Drosophila* by Morgan. The utility of spontaneous mutations in genetic material was prompted by H.J. Muller in 1927 using the mutagenic action of X-rays in *Drosophila*; he was awarded the Nobel Prize for Physiology and Medicine in 1946. Stadler in 1929, described the mutagenic effects of X-rays in barley. The first plant breeding programme designed to exploit induced mutations (mutation breeding) was initiated in 1929 in Sweden, Germany and U.S.S.R.; in India, the first mutation breeding programme began in the early 1930's.

Characteristics Of Mutations

The main characteristics of mutations may be summarized as follows.

1. Generally, mutant alleles are recessive to their wild type or normal alleles.
2. Mutations are random events in terms of the time of their occurrence and the gene in which they occur.

3. Most mutations have harmful effect. The frequency of beneficial mutation is about 0.1 % of all the induced mutations; only these mutations are useful in crop improvement
4. Mutations are recurrent, that is, the same mutation may be expected to occur in different individuals of a given generation or those belonging to different generations.
5. Mutations occur at very low frequencies in nature without any known cause; such mutations are called spontaneous mutations.
6. The rate of spontaneous mutations for most of the genes is very low, ranging between 10^{-7} and 10^{-4} .
7. The rate of spontaneous mutations varies considerably from one gene to another. Some genes, e.g., waxy locus of maize, are highly stable, while some others, e.g., yellow body locus of *Drosophila*, show a relatively high mutation rate.
8. Some genes show exceptionally high rates of spontaneous mutations: they are known as mutable genes.
9. Some genes increase the spontaneous mutation rates of some other genes of the genome; such genes are called mutator genes.
10. Some genes suppress the mutation of some other genes of the genome; they are termed as antimutator genes.
11. Some sites within genes show very high rates of mutation, while some other sites show little or no mutation. The highly mutable sites within a gene are commonly known as hot spots.
12. Many agents, both physical and chemical, increase the frequency of mutations; they are known as mutagens.

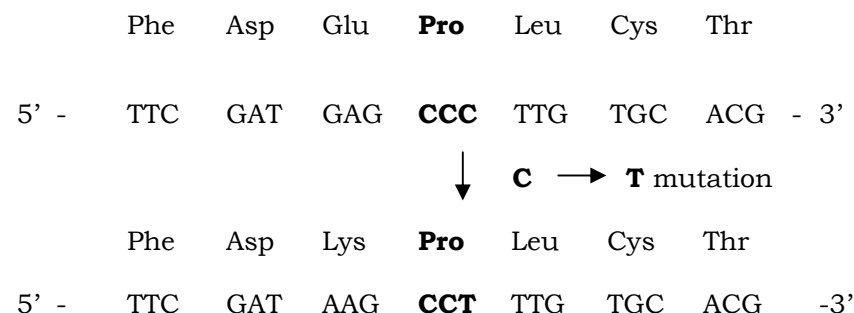
13. Mutations can occur in any tissue/cell (either somatic or germinal) of an organism.
14. Mutational events can occur during any developmental stage of organisms, but treatments with mutagens during certain stages of cell division (e.g., S phase) may yield mutations at a higher rate than those during other phases of the cell cycle.
15. The frequency of induced mutation is markedly affected by the environmental conditions prevailing just prior to, during and even after the mutagen treatment. For example, irradiation with X-rays or gamma-rays of seeds having low moisture content (about 4-5% in wheat, barley and oats) produces a markedly higher mutation rate than that of seeds having normal moisture content (e.g., 10-12%).
16. Mutations occur in both forward (from wild type allele to mutant allele) and reverse (from mutant allele to wild type allele) directions. Generally, the rates of forward mutations are much higher than those for reverse mutations.

Classification of Mutations

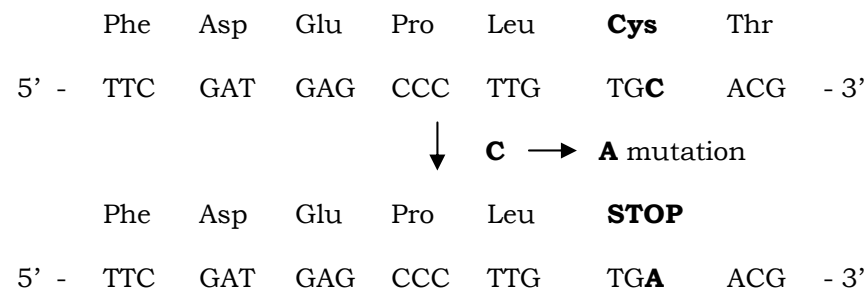
I. Based on Size

(A) Point mutation: A change in a very small segment of DNA; usually considered to involve a single nucleotide or nucleotide pair.

1. Samesense / silent mutation: Mutations may occur at the third base of a codon and the amino acid will not be altered. Silent mutations have no effect on the encoded protein and do not result in a mutant phenotype. They tend to accumulate in the DNA of organisms where they are known as polymorphisms. They contribute to variability in the DNA sequence of individuals of a species.



2. Nonsense mutation: Nonsense mutation change a codon for an aminoacid into a termination codon. The mutation causes translation of the messenger RNA to end prematurely resulting in a shortened protein which lacks part of its carboxyl-terminal region. Nonsense mutation have a serious effect on the activity of encoded protein and often produces a mutant phenotype.



3. Missense mutation: Missense mutation alters a single base which changes a codon such that the encoded aminoacid is altered. Missense mutation occurs in one of the first two bases of a codon. Most proteins will tolerate some change in their aminoacid sequence. Alterations of aminoacid in parts of the protein that are important for structure or function are more likely to have a deleterious effect and produce a mutant phenotype. Missense mutation have serious effect on the encoded protein and produce mutant phenotypes.

	Phe	Asp	Glu	Pro	Leu	Cys	Thr	
5' -	TTC	GAT	GAG	CCC	TTG	TGC	ACG	- 3'
			↓		G → A	mutation		
	Phe	Asp	Lys	Pro	Leu	Cys	Thr	
5' -	TTC	GAT	AAG	CCC	TTG	TGC	ACG	- 3'

4. Frameshift mutation: Insertion of extra bases or the deletion of existing bases from the DNA sequence of a gene. If the number of bases inserted or deleted is not a multiple of three the reading frame will be altered and the ribosome will read a different set of codons downstream of the mutation substantially altering the AA sequence of the encoded protein.

	Phe	Asp	Glu	Pro	Leu	Cys	Thr	
5' -	TTC	GAT	GAG	CCC	TTG	TGC	ACG	-3'
			↓	Insertion of A				
	Phe	Asp	Lys	Thr	Leu	Val	His	
5' -	TTC	GAT	AAG	ACC	CTT	GTG	CAC	-3'

(B) Gross mutation: Changes involving more than one nucleotide pair, may involve the entire gene, the entire chromosome, or sets of chromosomes (polyploidy).

II. Based on Cell Type

(A) Somatic mutation: Occurs in nonreproductive cells of the body, often producing a mutant phenotype in only a sector of the organism (mosaic or chimera).

(B) Gametic mutation: Occurs in the sex cell, producing a heritable change.

III. Based on Quality(Molecular basis)

(A) Structural mutation: Changes in the nucleotide content of the gene.

1. Substitution mutation – Substitution of one nucleotide for another.

a. Transition mutation substitute one purine for another or one pyrimidine for another.

b. Transversion mutation substitute a purine for a pyrimidine or vice versa.

2. Deletion mutation: It involves the loss of a portion of the DNA sequence. The amount lost varies greatly. Deletion mutation can be as small as a single base or much larger in some cases corresponding to the entire gene sequence.

3. Insertion mutation: Insertion or addition of extra bases, usually from another part of a chromosome causes insertion mutation. The amount inserted may be one or two bases or may be much larger.

(B) Rearrangement mutation: Changing the location of a gene within the genome often leads to “position effects”.

1. Within a gene – two mutations within the same functional gene can produce different effects, depending on whether they occur in the cis or trans position.

2. Number of genes per chromosome – different phenotype efforts can be produced if the number of gene replicas are nonequivalent on the homologous chromosomes.

3. Moving the gene locus may create new phenotypes, especially when the gene is relocated near heterochromatin.

a. Translocation – movement to a non-homologous chromosome.

b. Inversion – a portion of the DNA sequence is excised then reinserted at the same position but in the opposite orientation.

IV. Based on Origin

(A) Spontaneous mutation: Origin is unknown, also known as “background mutation”. They occur in natural populations (without treatment by man) with a frequency of 10^{-6} . e.g. R locus in maize mutates at a frequency of 4.92×10^{-4} .

(B) Genetic control: The mutability of some genes is known to be influenced by other ‘mutator genes’.

1. Specific mutator – effects limited to one locus.
2. Nonspecific mutator – simultaneously affecting many loci.

(C) Induced mutation: Mutations produced due to the treatment with either a chemical or a physical agent are called as induced mutation. The agents capable of inducing mutations are known as mutagens and their capacity for inducing mutations, is termed as mutagenic property. Induced mutations are useful in 2 ways (1) genetic and biochemical studies (2) in crop improvement. The process of inducing mutations through treatment with a mutagen is known as mutagenesis, while the exploitation of induced mutation for crop improvement is called mutation breeding.

1. Ionizing radiation : Changes in chemical valence through the ejection of e⁻.

- a. Particulate radiation α -rays, β -rays, fast neutrons, thermal neutrons.
- b. Nonparticulate / electromagnetic radiation – X rays, γ -rays.

2. Non-ionising radiation: It raises the energy levels of atoms (excitation), rendering them less stable. eg.

UV-rays and heat. UV produces thymine dimers i.e., bonding between thymines on the same strand.

3. Chemical mutagens: These are chemical substances that increase the mutability of genes. They are as follows;

1. Alkylating agents – sulphur mustards, nitrogen mustards, epoxides, ethylene imine (EI), sulphate and sulphonates (eg. Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS), diazoalkanes, nitroso compounds.

2. Acridine dyes – acriflavine, proflavine, acridine orange, acridine yellow, ethidium bromide (EB).

3. Base analogues – 5- bromouracil, 5-chlorouracil.
4. Others – nitrous acid, hydroxyl amine, sodium azide.

V. Based on Direction

(A) Forward mutation : Creates a change from wild type to abnormal phenotype.

(B) Reverse or back mutation: Produces a change from abnormal phenotype to wild type.



1. Single site mutation – changes only one nucleotide in a gene.

2. Mutation suppressor – a gene change which occurs at a different site from the primary mutation, yet reverses its effect.

a. Extragenic (intergenic) suppressor – occurs in a different gene from that of the mutant.

b. Intragenic suppressor – occurs at a different nucleotide within the same gene; shifts the reading frame back into register.

VI. Based on Magnitude of Phenotypic Effect

(A) Change in mutation rate: Some alleles can be distinguished only by the frequency with which they mutate.

(B) Isoalleles: Produce identical phenotypes in homozygous or heterozygous combinations with each other, but prove to be distinguishable when in combination with other alleles.

(C) Mutations affecting viability:

- i) Subvitals – relative viability is greater than 10% but less than 100% compared to wild type.
- ii) Semilethals – cause more than 90% but < 100% mortality.
- iii) Lethals – kill all individuals before adult stage.

Mutagens

Mutations can be induced by a number of agents; the agents capable of inducing mutations are called mutagens. The different mutagenic agents may be classified into the following two broad groups: (1) physical mutagens (different types of radiations) and (2) chemical mutagens (a variety of chemical compounds).

Physical Mutagens

The different types of radiations having mutagenic properties are known as physical mutagens. These radiations may be a part of the electromagnetic spectrum having shorter wavelength and higher energy than visible light (e.g., UV rays, X rays, gamma-rays and cosmic rays), or may be particulate radiations produced by the decay of radioisotopes (e.g., ^{14}C , ^3H , ^{35}S , ^{60}Co etc.). Radiations are grouped into two classes depending on the kind of effects they have on the atoms in their path: (1) ionizing and (2) nonionizing radiations.

Nonionizing radiations

Ultraviolet rays (present in solar radiation or produced by mercury-vapour lamp or tubes) is the only nonionizing radiation with mutagenic properties. Their wavelength ranges from 100-3900 Å. (10-390 nm) and they are specifically absorbed by purines and pyrimidines present in DNA. UV is relatively low energy radiation, has a very poor penetrating power. UV rays are used to irradiate pollen grains.

Some of the commonly used physical and chemical mutagenic agents

Class	Mutagen
Ionizing Radiations	Physical Mutagens
1. Particulate radiations	Alpha-rays (α -rays) Beta-rays (β -rays) Fast neutrons * Thermal neutrons *
2. Nonparticulate radiations	X-rays * Gamma-rays (γ -rays)
Nonionizing radiations	Ultraviolet rays (UV rays)
Alkylating Agents	Chemical Mutagens Mustard gas or sulphur mustard Nitrogen mustard Ethylmethane sulphonate(EMS)* Methylmethane sulphonate(MMS) Ethylethane sulphonate (EES) N-Methyl-N' -nitro-N' nitrosoguanidine (NTG)
Base Analogues	5-Bromouracil (5-B U) 2-Aminopurine (2-AP)

Acridine Dyes	Acriflavin
	Proflavin
	Acridine orange
	Ethidium bromide
Deamination agents	Nitrous acid (NH ₂ O)
Other chemical mutagens	Hydroxylamine(HA)
	Sodium azide*
	DNA sequences

* Mutagens commonly used in plants

Ionizing Radiations

An ion is an atom carrying either a positive or a negative charge; it is produced when an atom either gains or loses an electron. The process of ion production is known as ionization. There are two types of ionizing radiations, particulate and non-particulate radiations. Particulate radiations consist of high energy atomic particles generated due to radioactive decay. Beta-rays are high energy electrons produced by radioactive decay of ³R, ³²p, ³⁵S etc. and are negatively charged. Alpha-rays are composed of alpha-particles having two protons and two neutrons each; thus alpha-particles have double positive charge. Fast neutrons are produced in cyclotrons or atomic reactors due to radioactive decay of heavier elements. The velocity of fast neutrons is reduced by graphite or heavy water to generate thermal or slow neutrons. Both the types of neutrons are uncharged particles, and are highly penetrating in biological tissues. Alpha-particles and the two types of neutrons are densely ionizing radiations.

The non particulate ionizing radiations are represented by X -rays and gamma rays, which are high energy radiations composed of photons. The physical properties and biological effects of X-rays and gamma-rays are similar, but they differ in the source of their origin: X-rays are produced by X-ray tubes, while

gamma-rays are produced by the decay of some radioactive isotopes, e.g., ¹⁴C, ⁶⁰CO etc. X-rays are often referred to as hard (0.1-0.001 Å) or soft (1-10 Å) depending on their wavelength. Both X-rays and gamma-rays are highly penetrating in biological tissues and are sparsely ionizing. The dose of X -rays and most other ionizing radiations is measured as roentgen units (r units). .

According to the target theory, gene is a target within which a single hit produces a mutation. A 'hit' may be represented by an ionisation or by the production of a free radical/peroxide molecule capable of causing a mutational change in DNA. One such event (hit) within the gene is capable of producing one mutation. But it does not mean that every hit would produce a mutation; it simply means that every hit has some fixed probability of inducing a mutation depending on the conditions of irradiation.

The efficiency of radiations in inducing mutations is affected by a number of factors,

- (1) The stage of cell division, S phase
- (2) O₂ concentration, Presence of O₂ during irradiation increases its mutagenic effects while removal of oxygen reduces irradiation damage
- (3) Temperature
- (4) The plant or animal species
- (5) Moisture content of seeds, reduced moisture content (ca. 4-5%) in plant seeds yields a higher frequency.

Chemical Mutagens

A large number of chemicals is reported to be mutagenic. Some of the important chemical mutagens are listed in Table.

These chemicals can be divided into five main classes: (1) base analogues, (2) alkylating agents, (3)

acridine dyes, (4) deamination agents, (5) other mutagenic chemicals.

Base Analogues

Base analogues are very similar to the bases normally found in DNA and, as a result, are incorporated in DNA during replication. Some of the base analogues are

5-bromouracil (5-BU), 5-chlorouracil (5-CU), 2-aminopurine (2-AP) and 2, 6-diaminopurine; of these 5-BU and 2-AP are the most commonly used base analogues. The mechanism of mutagenic action of 5-BU is more clearly known than those of the other bases. The 5-BU behaves like thymine and usually pairs with adenine.

Alkylating Agents

Several chemicals have reactive alkyl (e.g., methyl, -CH_3 ; and ethyl, $\text{CH}_3\text{-CH}_2$) groups which they transfer to DNA bases and to phosphate groups of DNA; they are called alkylating agents, and this process is known as alkylation.

1. It interfere with DNA replication and cause breakage in the sugar-phosphate backbone.
2. During the repair of alkylated bases, errors may occasionally occur producing transitions, transversions or even frame-shift mutations.
3. Some alkylation agents, especially those having two (difunctional) or more (polyfunctional) active alkyl groups, prevent DNA replication and induce chromosome breakage resulting in structural aberrations.

Deamination Agents

Nitrous acid is a potent mutagen which acts both on replicating and nonreplicating DNA. It leads to oxidative deamination of cytosine (removal of -NH_2) in which the

amino groups (-NH_2) are replaced by keto ($=\text{O}$) groups yielding uracil. Uracil base-pairs with adenine leading to G:C $\rightarrow$ A:T transitions.

Acridine Dyes

Acridine dyes, e.g., acriflavin, proflavin are very powerful mutagens particularly in prokaryotes. Since acridines are positively charged, they insert themselves between two base-pairs of DNA; this is known as intercalation. Intercalation increases the rigidity of DNA double helix as well as disturbs its configuration. Replication of intercalated DNA molecules often results in addition or deletion of one to few (usually one) base pairs, which produces frame-shift mutations.

Other Chemical Mutagens

In addition to the above four groups of mutagens, a number of other chemicals possess mutagenic properties, but the mechanisms of their mutagenic actions are not known. The mechanism of action of hydroxylamine (CNH_2OH) is known to some extent. Hydroxylamine induces G:C $\rightarrow$ A:T transitions most likely by inducing hydroxylation of the amino group of cytosine.

Chemical mutagens may be classified into two broad groups depending on the state of DNA they affect. The first group consists of those mutagens which affect both replicating as well as nonreplicating DNA, e.g., alkylating agents, nitrous acid, hydroxylamine and acridine dyes. The second group includes those chemicals which affect only replicating DNA, e.g., base analogues.

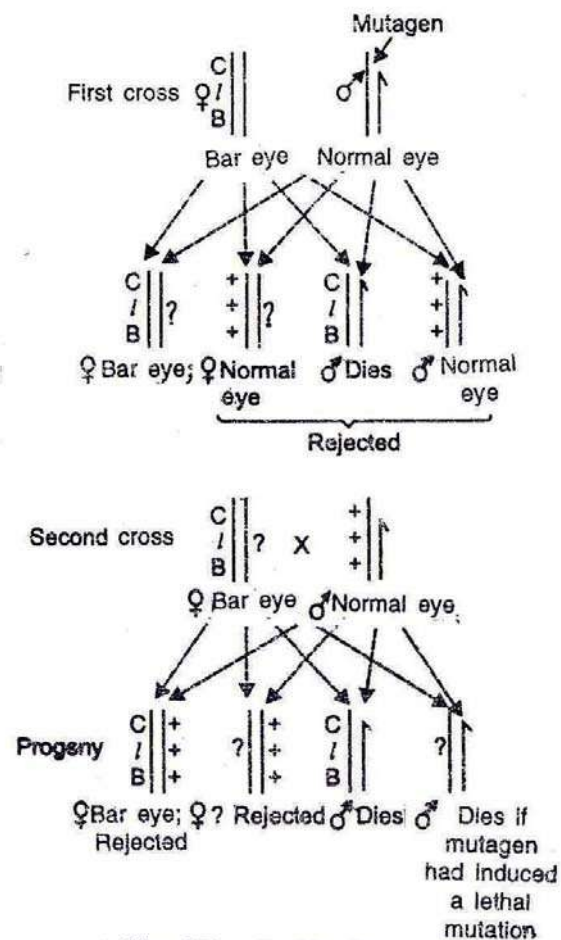
CIB technique

CIB Stock of *Drosophila*

This technique was developed by Muller and used for the unequivocal demonstration of mutagenic action of X-rays. The CIB stock of *Drosophila* has a special X-chromosome a large part of which is inverted (paracentric inversion); this acts as a crossover suppressor in the inverted region and is designated by C. A recessive lethal (l) gene and the

dominant gene for bar eye (B) are located within the inverted segment; as a result, the l and B are always inherited together in the same chromosome. Consequently all CIB females, identified by the bar eye shape, are heterozygous for this chromosome, while males having the CIB chromosome do not survive (because of the l gene).

The CIB technique is used for the detection of sex-linked recessive lethal mutations induced by treatment of *Drosophila* males with a mutagen. Such males are crossed with CIB females. In the F₁, half of the females will have the CIB chromosome (easily identified by the bar-shaped eyes); the remaining half of the females will not have the CIB chromosome and are rejected. All the surviving F₁ males will have the normal chromosome will die due to the gene l. Each F₁ CIB female is mated to a normal male; progeny from each such mating are kept in separate culture bottles. Each F₁ CIB female will have one CIB chromosome and one X chromosome from the mutagen-treated male parent. Half of the male progeny from the F₁ CIB females will receive the CIB chromosome, and still die (due to l). The remaining half of the male progeny will get their X chromosome from their mutagen-treated grandfather. If a lethal mutation was induced by the mutagen in this X chromosome these males will also die; (fig--). Thus if the normal X chromosome of an F₁ CIB female had a lethal mutation, there would be no male in the progeny of this female. The detection of sex-linked recessive lethals in this method is based only on the presence or absence of male progeny in the crosses between the F₁ CIB females and normal males. The frequency of recessive lethal mutations in the X chromosome is obtained as the ratio between the number of F₁ CIB females producing no male progeny and the total number of the females tested. This technique is simple, rapid and there is little chance of an error in scoring. However, it is suitable for the scoring of sex-linked recessive lethals only.



The CIB method for the detection of sex-linked recessive lethals in *Drosophila*. If a recessive lethal mutation is induced in a X chromosome of the mutagen-treated male (used in the first cross), no male progeny will be recovered from the F₁ CIB females carrying such an X chromosome.

CYTOPLASMIC INHERITANCE

In the case of cytoplasmic inheritance, generally the character of only one of the two parents (usually the female parent) is transmitted to the progeny. As a result, reciprocal crosses exhibit consistent differences for such characters, and there is lack of segregation in F_2 and subsequent generations. Such inheritance is also referred to as extra nuclear inheritance, extra chromosomal inheritance and maternal inheritance.

Since the genes governing traits showing cytoplasmic inheritance are located outside the nucleus and in the cytoplasm, they are referred to as plasmagenes, cytoplasmic genes, cytogenes, extra nuclear genes or extra chromosomal genes. Out of these terms, plasmagene has been preferably used.

Plasmagenes are located in the DNA present in mitochondria (abbreviated as mt -DNA) and in chloroplasts (shortened as cp - DNA); together these DNA's are termed as organelle DNA. The sum total of the genes present in cytoplasm of a cell is known as plasmon, while all the genes present in a plastid constitute a plastom.

Characteristics Of Cytoplasmic Inheritance

Reciprocal Differences

Reciprocal crosses show marked differences for the characters governed by plasmagenes. In most cases, plasmagenes from only one parent, generally the female parent, are transmitted; this phenomenon is known as uniparental inheritance. As a consequence, F_{1s} and the subsequent generations from reciprocal crosses show marked and consistent differences for such traits; this is a characteristic feature of cytoplasmic Inheritance.

Lack of Segregation

In general, F_2 , F_3 and the subsequent generations do not show segregation for a cytoplasmically inherited trait. This is because the F_1 individuals generally receive plasmagenes from one parent only.

Irregular Segregation in Biparental Inheritance

In some cases, plasmagenes from both the parents are transmitted to the progeny; this is known as biparental inheritance. Biparental inheritance gives rise to irregular segregation ratios in the F_1 generation of higher plants. This shows a lack of association between the segregating character and the process of meiosis. But in *Chlamydomonas* often zoospores show 1 : 1 ratio for the alleles of a plasmagene, but only after they have undergone several mitotic divisions.

Somatic Segregation

Plasmagenes generally show somatic segregation during mitosis, a feature of rare occurrence in the case of nuclear genes. This indicates a lack of association between the segregating trait and the mitotic spindle apparatus.

Association with Organellar DNA

Several plasmagenes have been shown to be associated with cpDNA or mtDNA, e.g., streptomycin resistance with cpDNA of *Chlamydomonas*, male sterility with mtDNA in maize and jowar etc. In fact, many genes have been provisionally mapped in cpDNA and in mtDNA. The demonstration of an association of a plasmagene with these known markers is a definite evidence for the trait produced by the former to be cytoplasmically inherited.

Nuclear Transplantation

If nuclear transplantation reveals a trait to be governed by the genotype of cytoplasm and not by that of nucleus, cytoplasmic inheritance of the trait is strongly indicated. In *nuclear transplantation*, nucleus of a cell is removed and replaced by a nucleus of another genotype taken from a different cell. Generally in such studies, nuclei of somatic cells are transplanted into zygotes before the first mitotic division is initiated.

Transfer of Nuclear Genome Through Backcross

The nucleus of a variety or species may be transferred into the cytoplasm of another species or variety through repeated backcrossing with the former; the donor of nucleus is used as the recurrent male parent. Lines produced in this way are known as alloplasmic lines since they have nuclei and cytoplasms from two different species. A comparison of the various characters of alloplasmic lines with those of the corresponding euplasmic lines (lines having nuclei and cytoplasms from the same species) demonstrates cytoplasmic effects, if any, on these traits. This technique is time consuming, but extremely powerful; it has been extensively used to study cytoplasmic differentiation during evolution, particularly in Triticale (wheat and its relatives).

Mutagenesis

Some mutagens, e.g., ethidium bromide, are highly specific mutagens for plasmagenes, while nuclear genes are not affected by them. Induction of mutation by such agents in a gene indicates to be a plasmagene.

Lack of Chromosomal Location

In many organisms, extensive linkage maps of nuclear genes are available. If a gene is shown to be located in one of these linkage groups it cannot be a

plasmagene. Failure to demonstrate the location of a gene in one of the linkage groups of an organism is indicative of its cytoplasmic location, but this is highly tentative.

Lack of Association with a Parasite, Symbiont or Virus

In many cases, a cytoplasmically inherited character is associated with a parasite, symbiont or virus present in the cytoplasm of the organism. Such cases cannot be regarded as cases of cytoplasmic inheritance. Only those cytoplasmically inherited characters, which are not associated with parasites, symbionts or viruses can be regarded as governed by plasmagenes. The known cases of true cytoplasmic inheritance are concerned with either chloroplast or mitochondrial traits and are usually associated with their DNA. Such cases are, therefore, often referred to as organellar inheritance, plastid inheritance and mitochondrial inheritance.

Maternal Effect

Development of some characters in several organisms is either governed or markedly influenced and affected by the genotype of the female parent. This is known as maternal effect.

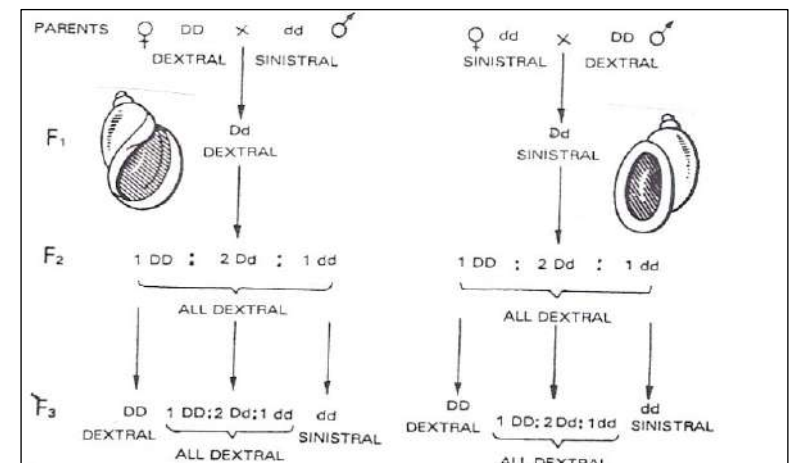


Fig. 30.1. Shell Coiling in *Limnaea* (a snail)

An extreme example of maternal effect is known in the snail *Limnaea* (Fig 30.1). In the snail, the direction of coiling of its shell is controlled by a single nuclear gene D/d; the dominant allele D produces right-handed coiling, while the recessive allele d produces left-handed coiling. The direction of shell coiling in an individual is governed by the genotype of its female parent and not by its own genotype. As a result, reciprocal crosses show difference in coiling in F₁ and there is no phenotypic segregation in F₂. The phenotypic effect of segregation is observable in F₃ crosses between female with left handed coil (dd) and males having right handed coil (DD) produce F₁ progeny (Dd) with left handed coil since the genotype of the female parent is dd. In F₂, segregation of Dd produces three genotypes. DD, Dd, dd, in the ratio 1:2:1. But snails with DD, Dd as well as dd genotype exhibit right handed coiling since their female parent has the genotype Dd producing right handed coiling in all its progeny (irrespective of the genotype of the progeny). The F₃ progeny from F₂ individuals with the genotype DD and Dd will show right handed coiling while those from dd F₂ individuals will exhibit left handed coiling of their shells this produces the typical 3:1 ratio (right handed : left handed) in F₃.

The reciprocal crosses (female right handed DD x male left handed dd) on the other hand, yields right handed coiling in F₁ (Dd) as well in the three genotypes, 1 DD : 2Dd : 1dd, obtained in F₂. But in F₃ 2/3 of the progenies show right handed coiling since they are derived from F₂ individuals with the genotypes DD and Dd. The remaining 1/3 of the F₃ progenies exhibit left handed coiling since the female parents had the genotype dd; this yields the typical 3:1 ratio in F₃.

Plastid Inheritance

Inheritance of plastid characters due to plasmagenes located in plastids is known as plastid

inheritance. This was the first case of cytoplasmic inheritance to be discovered by Correns and Baur in 1908.

Leaves of *Mirabilis jalapa*, the four o'clock plant, may be green, white or variegated and some branches may have only green, only white or only variegated leaves (Fig. 30.2). Correns made reciprocal crosses in all combinations among the flowers produced on these three types of branches. When flowers from a green branch are used as female, all the progeny are green irrespective of the phenotype (green, white or variegated) of the male parent. Similarly, progeny from the crosses involving flowers from white branches as the female parent were all white irrespective of the male flowers being from green, white or variegated branches. But in the progeny from all the crosses involving flowers from variegated branches as the female parent, green, white and variegated plants were recovered in variable proportions.

The maternal transmission of plasmagenes in higher plants and animals is explained on the basis of unequal contribution by male and female gametes to the cytoplasm of the zygote. During fertilization, ordinarily only the nucleus enters the egg cell and its cytoplasm is left outside the egg cell. As a result, the mitochondria and proplastids present in the zygote are derived from the female parent only. Therefore, plasmagenes located in these organelles will also be derived from the female parent only.

Nuclear Cytoplasmic Interaction

An unusual case of interaction between nuclear and cytoplasmic genomes is reported in maize. A type of variegation, called *iojap*, is produced by a recessive gene *ij*; plants homozygous (*ij ij*) for this gene develop the typical *iojap* variegation. But once this variegation is produced by the nuclear gene *ij*, it shows a typical cytoplasmic inheritance. Clearly, the nuclear genotype *ij ij*

has a mutagenic effect on the plastid genome. Once, this mutation is induced in some cp-DNA molecules the variegation is inherited cytoplasmically.

A cross between normal (Ij Ij) plants as female and iojap (ij ij) plants as males produces all green plants in F₁ with the nuclear genotype Ij ij. In F₂ generation of this cross ¼ progeny are ij ij and develop the iojap variegation, ¾ of the progeny are normal green. When the iojap F₂ plants are mated with normal green plants, a marked-reciprocal difference is observed in the progeny. When iojap plants are used as males and green plants as females, all the progeny are normal green. But in the reciprocal cross green, white and iojap progeny are recovered, the ratio between the three types of progeny is quite variable.(Fig.30.3.)

Findings of Correns from various types of crosses in *Mirabilis jalapa*

Leaf phenotype of the branch used as the female parent	Leaf phenotype of the branch used as the male parent	Leaf phenotype of the progeny (F ₁)
Green	1. Green 2. White 3. Variegated	Green Green Green
White	1. Green 2. White 3. Variegated	White White White
Variegated	1. Green 2. White 3. Variegated	Green, White and Variegated in variable ratios in each of the cases.

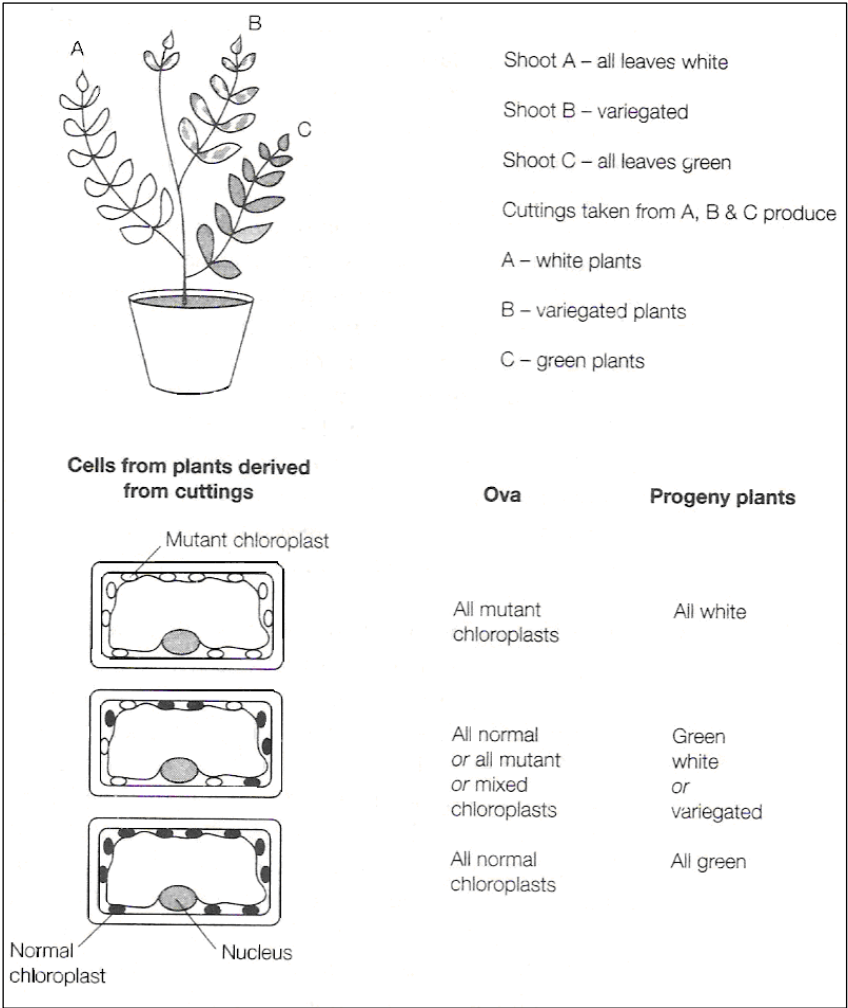


Fig. 30.2. Inheritance of chloroplast phenotypes in variegated plants

SEXUAL REPRODUCTION

It involves fusion of male and female gametes to form a zygote, which develops into an embryo. In crop plants, male and female gametes are produced in specialized structures known as flowers.

Flower

A flower usually consists of sepals, petals (or their modifications), stamens and/or pistil. A flower containing both stamens and pistil is a perfect or hermaphrodite flower. If it contains stamens but not pistil, it is known as staminate, while a pistillate flower contains pistil but not stamens. Staminate and pistillate flowers occur on the same plant in monoecious species, such as maize, colocasia, castor (*Ricinus communis*), coconut etc. But in dioecious species, staminate and pistillate flowers occur on different plants, e.g., papaya, date palm (*Phoenix dactyifera*), pistachia (*Pistacia vera*), hemp (*Cannabis indica*) etc. In crop plants, meiotic division of specific cells in stamen and pistil yields microspores and megaspores, respectively. This is followed by mitotic division of the spore nuclei to produce gametes; the male and female gametes are produced in microspores and megaspores respectively.

Sporogenesis

Production of microspores and megaspores is known as sporogenesis. Microspores are produced in anthers (microsporogenesis), while megaspores are produced in ovules (megasporogenesis).

1. Microsporogenesis

Each anther has four pollen sacs, which contain numerous pollen mother cells (PMCs). Each PMC

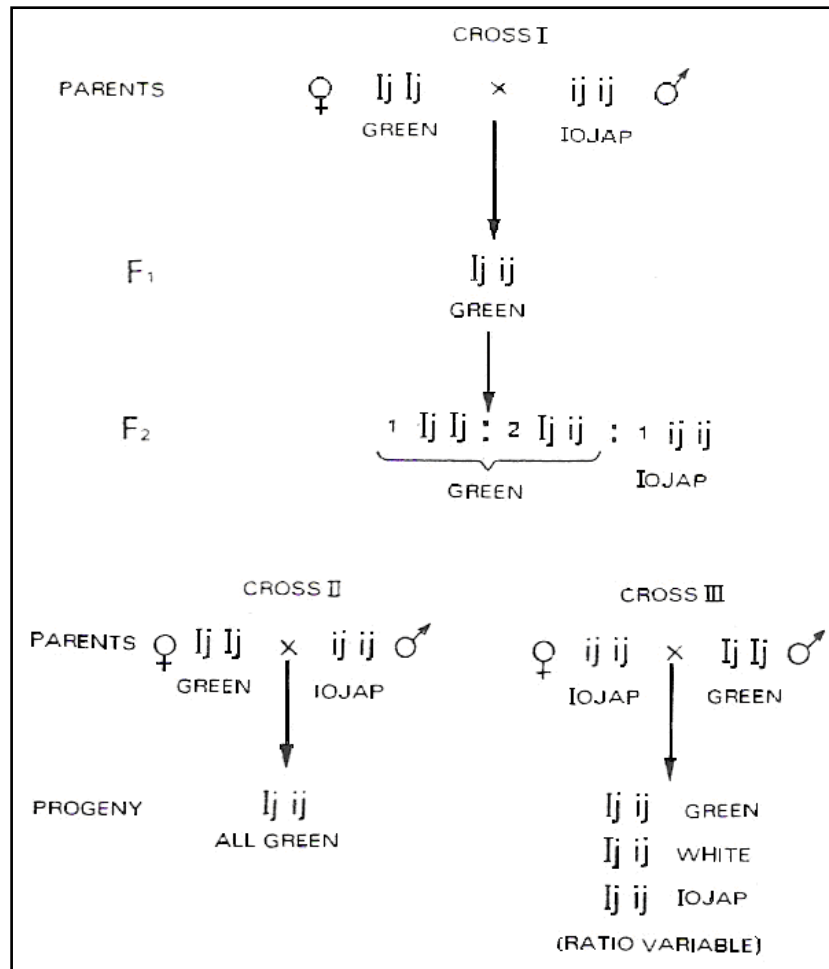


Fig.30.3. The produce of *iojap* variegation in maize by a recessive nuclear gene *ij*. But once the variegation is produced, it shows cytoplasmic inheritance

undergoes meiosis to produce four haploid cells or microspores. This process is known as microsporogenesis. The microspores mature into pollen grains mainly by a thickening of their walls.

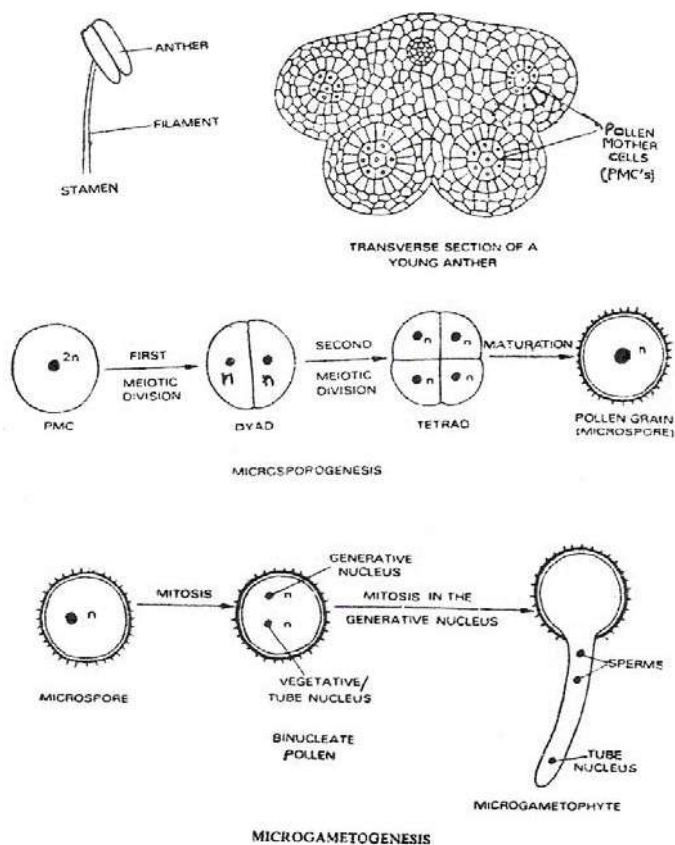


Fig.31.1 Microsporogenesis and Microgametogenesis

2. Megasporogenesis

Megasporogenesis occurs in ovules, which are present inside the ovary. A single cell in each ovule differentiates into a megaspore mother cell. The

megaspore mother cell undergoes meiosis to produce four haploid megaspores. Three of the megaspores degenerate leaving one functional megaspore per ovule. This completes megasporogenesis.

Gametogenesis

The production of male and female gametes in the microspores and the megaspores, respectively, is known as gametogenesis.

1. Microgametogenesis

This refers to the production of male gamete or sperm. During the maturation of pollen, the microspore nucleus divides mitotically to produce a generative and a vegetative or tube nucleus. The pollen is generally released in this binucleate stage. When the pollen lands onto the stigma of a flower, it is known as pollination. Shortly after pollination, the pollen germinates, the pollen tube enters the stigma and grows through the style. The generative nucleus now undergoes a mitotic division to produce two male gametes or sperms. The pollen, along with the pollen tube, is known as microgametophyte. The pollen tube finally enters the ovule through a small pore, micropyle, and discharges the two sperms into the embryo sac.

2. Megagametogenesis

The nucleus of a functional megaspore divides mitotically to produce four or more nuclei. The exact number of nuclei and their arrangements varies considerably from one species to another. In most of the crop plants, megaspore nucleus undergoes three mitotic divisions to produce eight nuclei. Three of these nuclei move to one pole and produce a central egg cell and two synergid cells on either side of the egg cell. Another three moves to the opposite pole to give rise to antipode cells. The two remaining in the centre is the polar nuclei and fuse to form a secondary nucleus. The megaspore thus develops into a mature megagametophyte or embryo sac.

The development of embryo sac from a megaspore is known as megagametogenesis. The embryo sac generally contains one egg cell, two synergids, three antipodal cells (all haploid), and one diploid secondary nucleus.

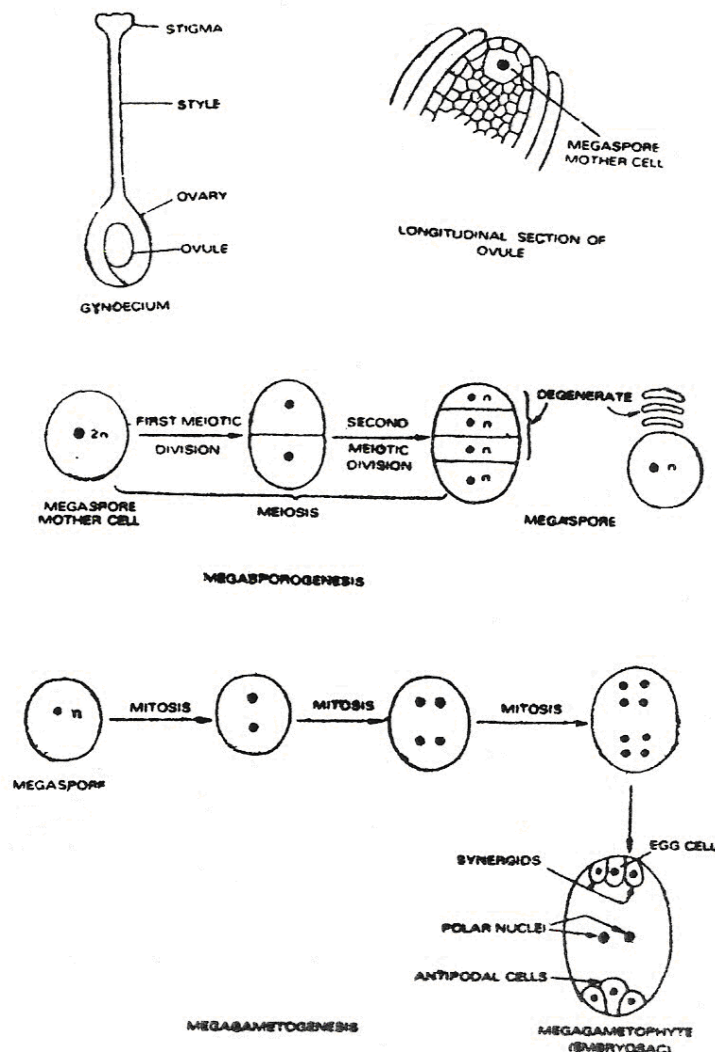


Fig.31.2 Megasporogenesis and Megagametogenesis

Fertilization

The fusion of one of the two sperms with the egg cell, producing a diploid zygote, is known as fertilization. The fusion of the remaining sperm with the secondary nucleus, leading to the formation of a triploid primary endosperm nucleus is termed as triple fusion. The zygote divides mitotically to produce a diploid embryo. The primary endosperm nucleus produces endosperm through repeated mitotic divisions. During seed development, endosperm provides nutrition to the developing embryo. It may be absorbed completely, e.g. in legumes or may form the major portion of seeds, e.g., in monocots like maize (*Z. mays*), wheat (*Triticum sp.*) etc., and some dicots, e.g., castor (*R. communis*), *Brassica sp.* etc.

Significance of sexual reproduction

Sexual reproduction makes it possible to combine genes from two parents into a single hybrid plant. Recombination among these genes produces a large number of genotypes. This is an essential step in creating genetic variation through hybridization. Almost the entire plant breeding is based on sexual reproduction. Even in asexually reproducing species, sexual reproduction, if it occurs, is used to advantage, e.g., in sugarcane, potato, sweet potato etc.



Effects of the Environment on the Development of Human Characters

A reliable investigation of the effects of environmental factors on the development of different characters of an organism requires that individuals having the same genotype be grown under different environments and observations on them be recorded at identical stages of development. This is achieved with relative ease in almost all the plants and many animals, but is ordinarily not possible in human beings. This is because every human being, except identical twins, has a different genotype from the rest and experimental populations of human beings can not be created as in the case with other organisms. Therefore, a study of environmental effects on the development of human characters is based on twins, the chance birth of two babies to a mother from a single pregnancy. The twins are of two types: (1) fraternal twins and (2) identical twins. Fraternal twins are derived from two different zygotes resulting from fertilization of two different eggs by separate sperms. As a result, such twins have different genotypes, and may be of different sexes. In contrast, identical twins are derived from a single zygote; an abnormal division of the embryo very early in development divides a single embryo into two parts each of which develops into a complete embryo. Identical twins, therefore, are always of the same sex and have identical genotypes.

There are two approaches for estimating the effects of environments on various human traits, each suited for a different situation. These approaches are: (1) comparison of concordance between fraternal and identical twins and (2) comparison between identical twins reared apart.

Concordance between Fraternal and Identical Twins

When a character is present in both the individuals of a twin pair, the pair is classified as concordant and the condition is known as concordance. On the other hand, when the two individuals of a twin pair show different forms of the character, the pair is classified as discordant, and the situation is referred to as discordance. The total number of twin pairs showing concordance and discordance may be recorded and expressed as per cent of the total number of pairs showing concordance and discordance; this is known as the frequency of concordance and discordance. A comparison of the frequencies of concordance for a character in identical and fraternal twins provides some information on the effect of environment on the development of the development of that character. It may be noted that in such studies, only those fraternal twins in which the two individuals in a twin pair are of the same sex are included.

The two individuals of each identical twin pair have identical genotypes. Therefore, discordance for a character in identical twins will be entirely due to the effect of environment on the development of the concerned character. However, concordance for a trait in identical twins will be entirely due to both heredity and environment. In contrast, the two individuals of each fraternal twin are expected to have different genotypes. As a result, discordance in fraternal twins will be due to both heredity and environment, but concordance will be mainly due to environment. Thus, concordance for a trait will be due to both heredity and environment, while that in identical twins is due to both heredity and environment. Thus a comparison of concordance for a trait in identical twins with that in fraternal twins

provides an idea of the effect of environment on the development of the character.

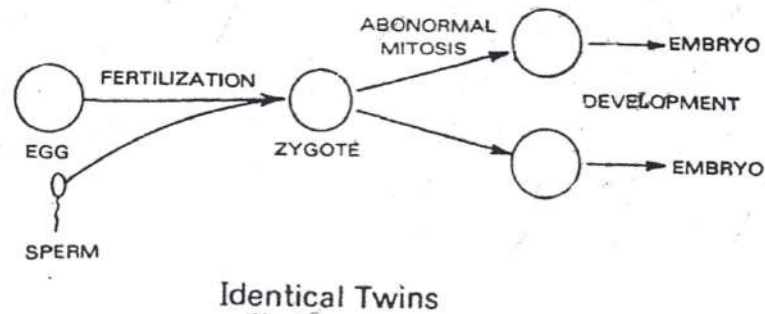
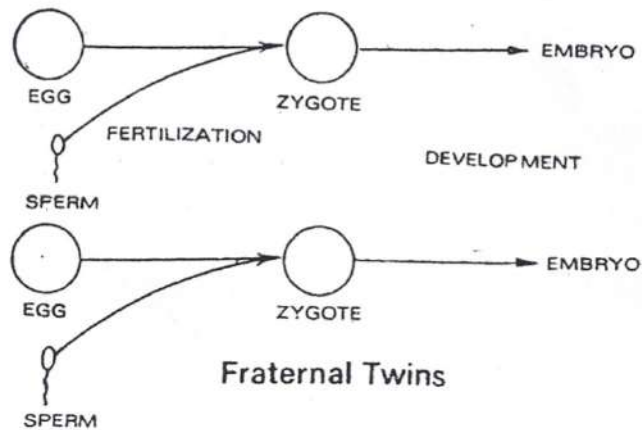


Fig.32.1 Identical and Fraternal Twins

A comparison of this type may reveal one of the following three situations. First, the concordance in identical twins may be very high as compared to that in fraternal twins, eg. Hair colour, eye colour and diabetes. Obviously, the expression of such characters is mainly under genetic control, and the environment plays a relatively smaller role. Second, the concordance in the identical twins may be comparable (e.g. right-or left

handedness) to or slightly higher than (e.g alcohol drinking) than that in fraternal twins. Clearly the development and the genetic component appears to be quite small. In no condition, fraternal twins are likely to show a greater concordance than identical twins. The third situation represents intermediate conditions between the first two situations. The expression of such characters is obviously equally affects by both genotype and environment.



EUGENICS AND EUTHENICS

The gene pool of human population is undergoing a slow but inexorable decline in quality because of **dyogenic** influence (**dyogenic** signifies 'bad born'). Conscious efforts for the improvement of gene pool so as to avoid this senile genetic decay of human population are essentially required. Geneticists are greatly concerned with the science of well being 'well born'. A separate discipline **Eugenics**, well being was established in 1883 by Sir FRANCIS GALTON, for the study of all such problems and the protective measures for the improvement of gene pool of human population .

GALTON has defined eugenics as "*the study of agencies under social control which may improve or impair the racial qualities of future generations both physically and mentally with the application of principles of genetics*".

The **eugenics** is the science of bringing into the world only the good germplasm or improving the genic qualities of individuals yet to be born by exercising selective mating.

Euthenics is the science of better living. It involves the improvement of qualities of those individuals, who have already been born, by providing them the best suitable environment, providing them good food, clothing, education and training.

EUGENIC MEASURES

The eugenic measures involve two types of steps

1. **Negative eugenics:** It comprises of all those eugenic measures, which helps in preventing the multiplication of defective germplasm.

2. **Positive eugenics:** It comprises of all those eugenic procedures which help in the multiplication of germplasm.

1. Negative eugenic measures:

1. Regulation of marriages:

Certain countries have marriage laws. According to these laws the feeble minded ,idiots, narcotics and persons with other hereditary diseases do not have the right of marriage with the result they are excluded from reproduction and bearing the offspring and the future progeny is saved from these hereditary defects. Some of the hereditary diseases express themselves in the adults. Therefore, child marriages should be banned .The legislations should be followed strictly and performance of marriages should be completed when each prospective bride and bridegroom is found medically fit.

2. Sexual separation of defectives:

The defective or undesirable persons who possess inherited defects of diseases (hemophilia, colourblindness and diabetics *etc.* or epilepsies, feeble mindedness, idiocity *etc.*) should not be permitted to reproduce so that the unwanted genes are gradually eliminated from the gene pool of human population. There are some possible methods:

- a) Segregation
- b) Sterilization
- c) Birth control and termination of pregnancy
- d) Selective fusion of gametes

3. Consanguineous marriages or marriages in blood relations:

Consanguineous union (union of gametes within same genetic ancestry) increases the threat that the recessive defects will appear more frequently. In such unions a heterozygote finds a first cousin heterozygote for the same recessive allele and encourages the appearance of recessive homozygote.

This can be illustrated by a rare gene, the recessive albinism. The frequency of heterozygote for albinism is around $1/140$. The marriage of heterozygotes by chance would be $1/140 \times 1/140 = 1/19600$ (according to the law of probability). The chances that they have a homozygous recessive child would be $1/19600 \times 1/4 = 1/78400$.

On contrary, if the women in the parental generation are heterozygous for a recessive gene, the probability that her offspring will inherit the recessive allele is $1/2$. The probability that the individuals in the third generation receiving the recessive allele is $1/2 \times 1/2 = 1/4$.

In this pedigree, the chance that both of the first cousins who marry are heterozygotes is $1/4 \times 1/4 = 1/16$. The chance that they will produce homozygous child is $1/4 \times 1/4 \times 1/4 = 1/64$. By comparing the two results it becomes evident that chances of the couple being heterozygous for a particular gene and the appearance of a homozygous recessive increases drastically, if they are related. The degree of closeness of relationship between husband and wife increases the chances of their being heterozygotes. The **coefficient of consanguinity** as defined by Mc KUSICK is the probability that any single locus is homozygous by descent from a common

ancestor. The coefficient of consanguinity for various parental relationships are given below.

For the above reasons, most societies impose some sort of restrictions or have tabbos for marriages between close relatives and first cousins.

4. Controlled immigration

Immigration laws should be framed and followed strictly. The diseased, undesired or feeble minded persons or persons with hereditary defects should not be permitted for immigration.

Coefficient of consanguinity for various parental relationships

Parental relationship	Frequency
Father-daughter	$1/4$
Offspring of identical twins	$1/8$
Uncle-niece	$1/8$
Double first cousin	$1/8$
First cousins	$1/16$
Half first cousins	$1/32$
Second cousins	$1/64$
Third cousins	$1/256$

2. Positive Eugenics

1. Selective mating:

The family histories should be checked thoroughly before settling the marriage and persons with defective pedigrees should be avoided. On the other hand persons

with normal or talented family histories should be preferred for marriage. It is a fact that children from healthy parents are always healthy and intelligent.

2. *Removal of Social Hindrances:*

Social rules and regulations put hindrances in the way of marriages. Religion, castes, racialism and dowry are major hindrances, as a result which the good germplasm from different sources fails to come together. These social hindrances should be removed and inter-caste, inter-racial and inter-religion marriages should be encouraged. These marriages bring together two different lineages and provide an opportunity for mixing of two gene pools. The combinations may prove useful.

3. *Early Marriage and prevention of loss or wastage of good germplasm*

Often marriages are made late in life time. It is especially true of highly placed persons having great ambition for their career. In achieving their ambition they sacrifice best part of their youth remaining unmarried. Majority of scientist, politicians, poets and writers, etc. prefer to remain unmarried.

The biological and psychological investigations have revealed that emotional warmth and sexual activities decrease with age and moreover, the germplasm also loses its vigour. Therefore, good germplasm goes waste. Early marriages of superiors should be encouraged to produce more offsprings. The intellectuals, philosophers, scientists, braves, politicians should be subsidized to produce their kind. During war braves and intellectuals are killed and mutilated. This also leads to wastage of good germplasm. Therefore, war should be avoided as a Eugenic measures.

4. *Preservation of good Germplasm*

H.S.MULLER has suggested that the germplasm(semen and ova) of outstanding persons may be preserved and may be used for artificial insemination. Thus these persons could serve to be the father of many more children than would be otherwise.very recently scientists felt the urgent need of establishing sperm and egg banks to protect the precious germ cells from radiation and age effect. The germ cells could be collected in adulthood and stored in lead-lined containers in deep freeze.

5. *Eugenic consultation or genetic counselling*

Eugenics may be applied most efficiently in the form of consultation before marriage. Doctors with good knowledge of genetics could be made available at definite centres, who could provide the proper guidance.

6. *Consanguineous Marriage*

Consanguineous marriage between intellectuals or among persons with talents could help in bringing together the good germplasm. The eugenic consultant can help in such matters.

EUTHENIC MEASURES

It is not easy to differentiate the hereditary and environmentally induced traits. In such circumstances the efficacy of eugenic measures is doubted. Therefore the euthenic measures to develop best from the available genotype provide more appropriate and as an immediately rewarding alternative .These are

1. *Good nature and nurture*

The people should be provided the best possible environment for the development of their caliber. They should get good food, better facilities for living, schooling and entertainment. They must be provided with suitable jobs of their interest.

2. *Education*

Persons should be taught about basic principles of human biology, human genetics and eugenics. They should be instructed for basic laws of hygiene and importance of good health.

Neither eugenics nor euthenics alone could bring about the betterment of human race. The collective efforts of both can provide some success in future generations. The eugenic programme is a long term method but will help in permanent changes in the gene pool.

EUPHENICS

Euphenics is the symptomatic treatment of genetic diseases. It deals with the control of inherited diseases, especially the inborn errors of metabolism. Such metabolic disorders are caused due to some defect in the genotype which fails to produce one of the specific enzyme required in the metabolism of a specific substance. For example, a disease **Phenylketonuria** (Pku) is caused by the absence of the enzyme which catalyses the first step in the metabolism of phenylalanine. Such persons are unable to utilize amino acid phenylalanine which accumulates in the blood and causes severe mental retardations and epilepsy.

Hence, to introduce good genotype in human gene pool the following methods could be practiced.

1. Genetic Cloning
2. Over-riding gene expression
3. Genetic Surgery or Genetic Engineering
4. Gene insertion of Mammalian Cells and
5. Protoplast fusion and Parasexuality

Mobile genetic elements

Mobile genetic elements (MGE) are a type of DNA that can move around within the genome. They include:

1. Transposons (also called transposable elements)
2. Retrotransposons
3. DNA transposons
4. Insertion sequences
5. Plasmids
6. Bacteriophage elements, like Mu, which integrates randomly into the genome
7. Group II introns

The total of all mobile genetic elements in a genome may be referred to as the **mobilome**. **Barbara McClintock** was awarded the 1983 Nobel Prize "for her discovery of mobile genetic elements". Mobile genetic elements play a critical role in the spread of virulence factors, such as exotoxins and exoenzymes amongst bacteria.

siRNA and RNAi

Small interfering RNA (siRNA), sometimes known as **short interfering RNA** or **silencing RNA**, is a class of double-stranded RNA molecules, 20-25 base pairs in length. siRNA plays many roles, but its most notable is in the **RNA interference (RNAi)** pathway, where it interferes with the expression of specific genes with complementary nucleotide sequence.

siRNA also acts in RNAi-related pathways, *e.g.*, as an antiviral mechanism or in shaping the chromatin structure of a genome.

siRNAs and their role in post-transcriptional gene silencing (PTGS) in plants were first discovered **by David Baulcombe's group** at the Sainsbury Laboratory in Norwich, England.

siRNAs have a well-defined structure: a short (usually 21-bp) double-stranded RNA (dsRNA) with phosphorylated 5' ends and hydroxylated 3' ends with two overhanging nucleotides:

siRNAs can also be introduced by transfection. Since in principle any gene can be knocked down by a synthetic siRNA with a complementary sequence, siRNAs are an important tool for validating gene function and drug targeting in the post-genomic era.

Metagenomics

Metagenomics is the study of **metagenomes**, genetic material recovered directly from environmental samples. The broad field may also be referred to as **environmental genomics**, **ecogenomics** or **community genomics**.

While traditional microbiology and microbial genome sequencing and genomics rely upon cultivated clonal cultures, early environmental gene sequencing cloned specific genes (often the 16S rRNA gene) to produce a profile of diversity in a natural sample. Because of its ability to reveal the previously hidden diversity of microscopic life, metagenomics offers a powerful lens for viewing the microbial world that has the potential to revolutionize understanding of the entire living world.

Transcriptomics

The **transcriptome** is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells.

The term can be applied to the total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome can vary with external environmental conditions. Because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as **transcriptional attenuation**. The study of *transcriptomics*, also referred to as **expression profiling**, examines the expression level of mRNAs in a given cell population, often using high-throughput techniques based on DNA microarray technology. The use of next-generation sequencing technology to study the transcriptome at the nucleotide level is known as RNA-Seq.

Metabolomics

Metabolomics is the scientific study of chemical processes involving metabolites. Specifically, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles.

The metabolome represents the collection of all metabolites in a biological cell, tissue, organ or organism, which are the end products of cellular processes. Thus, while mRNA gene expression data and proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that cell.

One of the challenges of systems biology and functional genomics is to integrate proteomic, transcriptomic, and metabolomic information to give a more complete picture of living organisms.

Phenomics

Phenomics is an area of biology concerned with the measurement of phenomes — the physical and biochemical traits of organisms — as they change in response to genetic mutation and environmental influences. It is used in functional genomics, pharmaceutical research, and metabolic engineering.

An important field of research today is trying to improve, both qualitatively and quantitatively, the capacity to measure phenomes. This includes developing high-throughput measurement systems. For example, the Australian Plant Phenomics Facility, an initiative of the Australian government, has developed a number of new instruments for comprehensive and fast measurements of phenotypes in both the lab and the field.

Plant genome structure

Eukaryotic genomes are much more complex than prokaryotic genomes. Further, plant genomes are more complex than other eukaryotic genomes. Prior to the development of recombinant DNA technology, genomes were analyzed by reassociation kinetics techniques. Reassociation kinetic experiments are performed by melting DNA and allowing it to reanneal upon itself or with another population of either DNA or RNA molecules. The kinetics of the reassociation provide data that can be used to analyze the overall structure, evolution and expression of genomes.

The most common method of denaturing duplex DNA is by heating to 100°C. But how can we monitor this denaturation and subsequent renaturation? The most common method is by measuring the absorbance change in the ultraviolet region at 260 nm. The important property is that melted, single-stranded DNA absorbs about 40% more at 260 nm than duplex DNA. If we slowly heat DNA the absorbance will increase dramatically over

a short range of temperatures. The mid-point of this transition is called the **melting temperature** or **T_m**. Under physiological conditions, the T_m usually lies in the range of 85-95°C. Thus, without altering the cellular conditions, the duplex DNA is stable in the cell. The exact temperature that a particular DNA melts depends on several parameters. The **GC content** is important because GC base pairs have three hydrogen bonds compared to the two for AT base pairs. Thus, the higher the GC content the higher the T_m.

Effect of GC content on T_m

%GC	Melting Temperature
40	87°C
60	95°C

DNA denaturation is reversible, and the reversible process is called **renaturation**. This process requires that the temperature be lowered gradually. As this lowering of temperature proceeds the following occurs:

- 1. Single-stranded molecules randomly encounter each other.
- 2. Short complementary stretches of duplex DNA form.
- 3. The DNA then zips back together to form the original structure.

Obviously if a single molecule is denatured it should be able to reform completely. But if two separate molecules are denatured together, for example wheat and barley DNA, then complementary regions between the two DNAs will be able to form duplex DNA. The ability of two molecules to renature is called hybridization.

Hybridization

It is pairing of complementary nucleic acids. Performing hybridization experiments in a solution is called liquid hybridization.

Analysis of Genomes by Reassociation Experiments

If a DNA molecule is melted and allowed to reassociate, the complexity of the genome dictates the rate in which duplex DNA will form. If we consider a simple molecule that consists of alternating GCs, this molecule will be able to form a duplex quicker than a molecule that consists of repeating blocks of AGCT. As the number of different combinations of bases increases, the time required for complete duplex formation to occur will increase. Renaturation, or duplex formation requires random collisions between two single-stranded molecules. This process follows second-order kinetics and is concentration dependent. We will not go through the derivation of the formula but the important parameter used to define a certain DNA is: $C_0t_{1/2}$.

This value is defined as the amount of time required for one-half of the DNA to reanneal or form duplex DNA. The units for this parameter is moles of nucleotides per liter per second. The more complex the genome of interest, the longer it will take for like sequences to reanneal. Consequently, the $C_0t_{1/2}$ will be larger. Thus in terms of reassociation kinetics complexity has a specific definition.

Complexity - the total length of different sequences

For example, *E. coli* is considered to have a complexity of 4.2×10^6 base pairs. What is the experimental procedure used to derive these values? In general the procedure is:

1. Shear the DNA to be analyzed to a length of about 300 bp.

2. Melt the DNA (usually in 0.12 M phosphate buffer) by boiling for 5 min.
3. Quickly place at 60°C.
4. Take aliquots at different time points. Separate single-stranded DNA from double-stranded DNA by hydroxyapatite. Measure the amount of DNA that is double-stranded by absorbance at 260 nm.
5. Plot the amount that is single-stranded versus the C_0t value. The C_0t value is expressed in log equivalent. This plot depicts the C_0t curve.

When this type of experiment is performed with eukaryotic DNA three components are usually seen. These components each reanneal with their own unique $C_0t_{1/2}$ value. The three components are termed the **fast, intermediate, and slow components**. Why do we see these three components? Eukaryotic genomes are characterized by sequences that are represented by different copy numbers. If a sequence is found many times in the genome, it will reanneal much quicker than those sequences that are found only once in the same genome. Thus the equivalent C_0t curve for a eukaryotic genome will be different than a genome, such as *E. coli*, which only contains single copy sequences.

A comparison of the C_0t value of each of these components with an *E. coli* standard allows us to derive the complexity of each component. The complexity of the slow component of the genome is greater than that for the other two components and is considered to represent the single copy portion of the genome. The complexity of the slow component can be used as a good estimate of the **genome size**. The genome size will be the sum of the lengths of all the unique sequences. Using the example from Genes V - Lewin, p.664, the complexity of the slow component is 3×10^8 bp and the complexity of the intermediate component is 6×10^5 . If we divide the complexity of the slow component into the intermediate

component we get 2×10^{-3} . This demonstrates that the intermediate component contributes very little to the complexity of the genome. Therefore, the complexity of the slow or single copy portion of the genome can be considered equal to the genome size.

To derive the complexity of each component, it is necessary to run a standard, such as *E. coli* DNA, with each experiment. As stated above, *E. coli* is considered to consist of only single-copy sequences. Let's say that in the experiment from which the C_0t for each component was derived, the $C_0t_{1/2}$ value for *E. coli* was 4. Experimentally it was determined that the slow component comprised 45% of the total DNA. Therefore, if only that component was annealed, the $C_0t_{1/2}$ value would be 283 (630×0.45). That value is 71 ($283/4$) times slower than for *E. coli*. Therefore the complexity of the slow component is 71 times that of *E. coli* or 3.0×10^8 ($71 \times 4.2 \times 10^6$). The complexity of the other components is derived similarly.

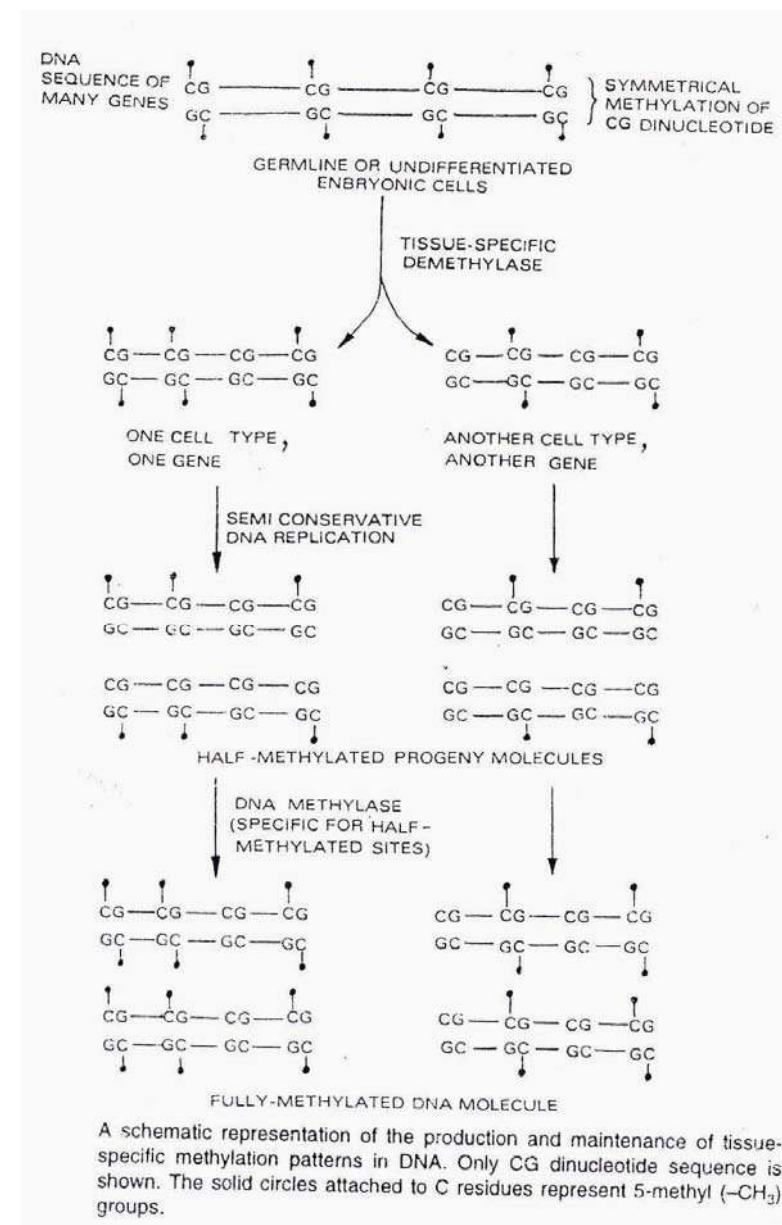
Genome size is quite variable throughout the biological world and the genome size in plants shows the greatest variation of any kingdom in the biological world.

Variation in Genome Size among Plants

Species	kb/haploid	pg/haploid ¹
Arabidopsis	7×10^4	0.15
Lily	1×10^8	100.00

Conversion factor: $1 \text{ pg} = 0.965 \times 10^9 \text{ bp} = 6.1 \times 10^{11} \text{ daltons}$

DNA Methylation



About 2-7% of the cytosine residues in animal DNA are methylated, the value depending on the species. Most of the methyl groups are found in C-G 'doublets', and the majority of C residues in them may be methylated. The C-G doublet generates a C residue on both the strands and the DNA is known as **fully methylated** if C in both the strands in this doublet sequences is methylated; if the C in only one of the strands is methylated, the DNA is called **hemimethylated**. Replication of fully methylated DNA regularly yields hemimethylated DNA, which is then methylated by methylases. The methylases that act constitutively on hemimethylated sites to make them fully methylated are called **maintenance methylase**. Maintenance methylases are the only known methylases in mammalian cells. These methylases are universally present and are essential in that mouse embryos in which the methylase gene is disrupted do not survive beyond early embryogenesis. Methylation at new sites is established by **de novo methylases**, but this type of enzyme has not been characterized in eukaryotes. Methyl groups from DNA are removed by **demethylases**.

In case of many genes, most of the C-G 'doublet' sites are methylated in all the tissues. But a minority of such sites are methylated in a tissue-specific manner, viz, they are methylated in the tissues in which the gene is not expressed, but are nonmethylated where the genes are being expressed. In general, the methylation at the 5'-end of a gene seems to suppress gene expression, while the 3'-end could remain methylated. The promoter activity is suppressed by methylation of promoter DNA, while demethylation restores this function. For example in case of globin gene, presence of methyl groups in the region from 200 bp upstream of the startpoint suppresses transcription. The removal of either the 3 methyl-groups located upstream or that of the three groups located downstream of the startpoint is unable to relieve this repression; however, the removal of all the methyl groups in this region allows promoter function. Thus

transcription appears to require a methylation-free region at the promoter. But some genes can be expressed when they are extensively methylated.

Functional genomics

By February 2001, when conclusions from the human draft sequence were published, complete sequences of genomes of 599 viruses and viroids, 205 naturally occurring plasmids, 185 organelles, 31 eubacteria, 7 archaea, one fungus, two animals and one plant had become available. Once the complete base sequence of a genome becomes available, scientists can begin to unravel the total information contained in the genome; this is termed as **functional genomics**. Models then can be developed that would explain how biological systems develop and respond using this information. The following activities therefore, may be expected to predominate the genomics science after the genome sequence becomes available.

1. The list of all the proteins encoded by genomes, is known as a **proteome**. *Determination of the proteome is called **proteomics***, and this will be the next step in genomics. This is going to be a demanding and time consuming task since it is not easy to identify new/unknown genes from a DNA sequence. For example, human chromosome 22 is expected to contain approximately 1000 genes, but only 545 are easily identified because they encode proteins that are similar to those studied previously in various organisms. Putative genes are predicted by complex computer modeling that is only partly accurate. Therefore, **demonstration that the putative genes are real genes will be a continuing challenge**.
2. The next step will be to determine the functions of each of the proteins encoded by the genome; this is known as **functional genomics**. The amino acid

sequence of a protein may give a clue to its putative function based on its similarity to already known proteins. In any case, this step is going to be quite challenging and would require prolonged experimentation.

3. Biologists will need to determine the set of genes active in various tissues, developmental stages etc, and the relative levels of activities of these genes. This would be greatly facilitated by the use of 'micro-arrays' and 'micro-chips' that allow a comprehensive assay of the variation in levels of different RNA species.
4. Determination of the complete set of functional sequences, other than genes, e.g., regulatory sequences, present in a genome will be much more difficult than that of genes themselves. These sequence will have to be identified and their functions elucidated in order to be able to appreciate the functional aspects of the genes contained in a genome.
5. The most challenging task will be to develop models, based on the proteins encoded by specific genomes that account for the development and responses of biological systems and phenomenon and to evaluate the validity of these models.
6. The field of genetic epidemiology is expected to make rapid progress after genomic sequencing. **Genetic epidemiology** is the analysis in large populations of the interactions of gene, genetic variation and environment; this allows the discovery of new drug treatments, and to tailor them to individual genetic and life profiles. Any two unrelated human beings differ from each other by one base pair in every 1000 bp (approx) of their genomes; these differences are called **single-nucleotide polymorphisms or SNPs (often called**

'snips') since the differences are of one base pair only. About 85 % of all difference in human DNAs are SNPs ; it is generally considered that SNPs are probably the cause of most common genetic disorders. It is proposed to determine the 3,00,000 of the commonest SNPs in human populations, and to use this information to design new treatments for the common disorders.

Meaning of Developmental Genetics

All multicellular organisms begin their life cycle as single cell from which their various tissues and organs develop through differentiation. The development consists of the formation of different types of tissues/organs/cells, while differentiation denotes a permanent or irreversible change in the function of cells(relative to the single -celled zygote),which is often accompanied with changes in their structure. For example, cells derived from the same zygote may develop, among several others, into skin cells, nerve cells or red blood cells; these cell types differ both in their structure as well as function (the type of gene products to be synthesized). Differentiation is more apparent in multicellular organisms, but it also occurs in unicellular organisms. *e.g.*, bacteria and viruses. Development in both prokaryotes and eukaryotes involves the production of proteins that generate in them specific structural and functional phenotypes.



GLOSSARY

Aberration (Chromosomal aberration): Structural changes in chromosomes; chromosome mutation.

Accessory chromosome : Supernumerary heterochromatic chromosomes found in some plant and animal species, in addition to normal or A chromosomes ; they are designated as B chromosomes.

Acentric : Chromosome or chromosome fragment without a centromere.

Acentric chromosome: A chromosome fragment without centromere.

Acrocentric : A chromosome or chromatid where the centromere is very close to one end.

Acrocentric chromosome: A chromosome with its centromere near one end.

Additive gene effect: In case of polygenes; the effects of different genes having cumulative and equal effect

Adenine: A purine base designated as "A", found in nucleic acids.

Adenosine: Adenine attached to ribose.

Allele (Allelomorph) : One of a pair or more alternative forms of a gene situated at the same locus in homologous chromosomes. More than two alternative forms of a gene are called multiple alleles.

Allopolyploid: A polyploid having two or more distinct genomes; usually produced by chromosome doubling of interspecific hybrids (F₁s).

Allotetraploid: An allopolyploid having four genomes; usually two copies each of two genomes; may be four different genomes.

Amphidiploid: An allopolyploid having two copies each of two or more distinct genomes; natural amphidiploids behave as diploids during meiosis, *i.e.*, they show normal bivalent formation, *e.g.*, wheat, tobacco, oats, cotton etc.

Anaphase: The stage in which sister chromatids of each chromosome (in mitosis and AII of meiosis) or the homologous chromosomes (at AI of meiosis) separate by their movement toward opposite poles of the spindle.

Aneuploid: An organism or a cell having one or few chromosomes more or less than the normal disomic number of the species; the chromosome number is not a multiple of the basic number (*x*).

Antibody: A protein molecule that interacts specifically with an antigen molecule; usually produced by an animal in response to the antigen in question.

Anticodon: A sequence of three bases (triplets) in tRNA, complementary to the three bases of mRNA coding for an amino acid.

Antigen: A substance, usually a protein, which induces specific antibody production when introduced into a living system, usually an animal, in which it does not occur naturally.

Apomixis: The development of an embryo (and seed) without fertilization.

Apospory: Formation of a diploid embryo sac directly from a cell of the nucellus or integument.

Asexual reproduction: The progeny are produced through a process which does not involve the fusion between male and female gametes.

Asynapsis: Failure of pairing (synapsis) of homologous chromosomes during meiosis (zygotene of prophase I).

ATP: Adenosine triphosphate; consists of adenine, ribose (5-carbon sugar) and three phosphate groups ; it is a major carrier of energy and phosphate in biological system.

Autoallopolyploid : Cell or individual whose chromosome complement shows the characteristic of auto-and allo-polyploidy. If A and B represent different genomes, the autoallopolyploid may be AAAABB, AABBBB or AAAABBBB.

Autogamy: Self-fertilization; union between male and female gametes produced by the same individual; in *Paramecium*, sexual reproduction within a single animal without conjugation.

Autopolyploid: An individual having more than two copies of a single genome.

Autosome: Chromosomes other than sex chromosomes; their kind and number is the same in males and females of a species.

Autotetraploid: A cell or individual possessing 4 sets of the basic chromosome number.

Balanced lethals: Lethal genes linked in repulsion phase; they are maintained in this phase either due to tight linkage or crossover suppression; only their heterozygotes survive.

Base pair (bp) : Two nitrogenous bases (one purine and one pyrimidine) which pair in double-stranded DNA or RNA molecules. Adenine pairs with thymine

Base pair ratio : The ratio $A + T / G + C$.

Basic number: The haploid or gametic chromosome number of a diploid species (*genomic number*); in polyploid species, haploid number of the parental diploid species; represented by *x*.

Bisexual : In plants, the flower having both androecium and gynaecium ; in animals, the individual having both testes and ovaries.

Bivalent: The structure formed by pairing of two homologous chromosomes during meiosis; seen during pachytene onward till the beginning of anaphase I.

C value: Total amount of DNA in a haploid genome.

Cell: The smallest membrane-bound structural; the functional unit of all living matter, capable of independent reproduction.

Cell cycle: The life cycle of the individual cell, the period from one division to the next. In somatic cells, it consists of interphase (G-phase, S-phase)

Cell differentiation: The process by which the descendants of a single cell achieve and maintain specialization of structure and function during the development of the individual.

Central dogma: The functional relationship among DNA, RNA and protein regarding the direction of information flow. DNA serves as a template for its own replication and for RNA synthesis (transcription) and RNA is template for protein synthesis (translation), thus the information flows as : DNA- RNA-Protein. However, in certain RNA viruses, DNA is synthesized on RNA template.

Centromere: In a chromosome, the specialized region to which the spindle fibre is attached during cell division; it is involved in chromosome movement at anaphase.

Character: Derived from characteristic; a morphological, anatomical, physiological or behavioural feature of an organism; usually, the end-product of development; a result of the genotype, environment and genotype x environment interaction effects.

Chiasma: The cross-shaped points of contact between nonsister chromatids of homologous chromosomes during diplotene-MI, formed as a result of crossing over.

Chimera (chimaera) : A plant (rarely an animal) composed of tissues of two or more genotypes or ideotypes ; as a consequence of mutation, fusion of different zygotes or grafting.

Chi-Square (χ^2) Test: A statistical test for determining whether observed data agree with an expected ratio.

Chloroplast: A highly differentiated plastid with typical granna; contains all the chlorophyll in higher plants; has DNA and participates in cytoplasmic inheritance.

Chromatid: Of a metaphase chromosome, one of the two identical longitudinal strands produced by replication of the single chromatid of a telophase chromosome; the two chromatids of a chromosome are called *sister chromatids*.

Chromatin: A complex between DNA, histones and other proteins, and chromosomal RNA, making up the chromosomes; in form of 300 A⁰ *chromatin fibers* (derived from, chromaticity = property of taking up stain).

Chromocentre: In salivary gland chromosomes of certain Diptera; the body produced by a fusion of the heterochromatic regions of the autosomes and X chromosome, and the entire Y chromosome.

Chromomeres: Bead-like condensed chromosome segments linearly arranged in prophase of mitosis and meiosis; they are called bands in polytene chromosomes.

Chromosomal aberration: A change in chromosome number from the diploid state, or a change in chromosome structure from the normal chromosome complement.

Chromosome: Nucleoprotein bodies composed of 300 A chromatin fibers; clearly observable during cell division; dark-staining with basic dyes; bearers of the genetic material and the genes.

Chromosome banding: The staining of chromosomes following specific procedures which produce many dark and light zones along their lengths; the pattern of banding is highly specific, and all the 23 human chromosomes can be identified.

Chromosome theory: The chromosome theory of inheritance states that the chromosomes are the carriers of genes.

Cistron: Functional unit of gene; a section of DNA or RNA which codes for a specific gene product.

Clone: Group of cells or organisms which are genetically identical and are derived from a single cell or common ancestor by mitosis. Also, the molecules identical with a single ancestral molecule.

Codominance: Both the alleles of a gene express themselves in the heterozygotes.

Codon: A group of three adjacent nucleotides of mRNA which code for one amino acid; *nonsense codons* specify no amino acid and function in polypeptide chain termination.

Colchicine: An alkaloid derived from autumn crocus; it prevents the organisation of spindle apparatus and, thereby, induces chromosome doubling.

Colour blindness: Inability of an individual to distinguish between two colours, e.g., red-green colour blindness; usually, sex-linked.

Complementary genes: Two (or more) dominant genes capable of producing a character only when both (or all) of them are present together in the dominant state.

Continuous variation: Variation not separable into distinct classes; governed by polygenes; markedly affected by the environment; in quantitative traits; analysis based on measurement data.

Coupling phase: In linkage; the dominant alleles of two or more genes present in the same chromosome and, hence, linked together; contributed by the same parent

Crossing over: The exchange of segments between nonsister chromatids of homologous chromosomes leading to recombination.

Crossover unit: The distance between two linked genes which permits 1 % recombination between them (Syn., map unit, Morgan).

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

Cytokinesis: The process of the division of cytoplasm of a cell into two or more parts ; usually it occurs after nuclear division (karyokinesis).

Cytology: A study of the structural and functional organisation of the cell.

Cytoplasm: The entire content of a cell other than the nucleus; includes ER, plastids, mitochondria, microtubules etc., and the hyaloplasm.

Cytoplasmic inheritance: Inheritance due to the genes located in the cytoplasm (Plasmagenes), *e.g.*, mitochondria and plastids; typical uniparental transmission and the lack of segregation; in higher plants and animals, transmission through the female parent. (Syn., extranuclear, extrachromosomal and maternal inheritance).

Cytoskeleton: The networks of fibres in the eukaryotic cytoplasm.

Deoxyribonucleic acid (DNA) : The genetic material of all cells; a polymer of deoxyribonucleotides which are joined by phosphodiester bonds.

Deficiency: The loss of a segment from a chromosome; deficiency heterozygotes are hemizygous for the genes located in the deleted segment; many deficiencies produce genetic effects similar to gene mutations (Syn., deletion).

Denaturation: The loss of natural or native configuration of a macromolecule; generally, accompanied with a loss in the biological activity of the molecule; a separation of the two strands of DNA double-helix producing single-stranded DNA molecules.

Diakinesis: A substage of prophase I of meiosis; the chiasma terminalization is complete; the bivalents are short and thick, and well-spread in the cell.

Dicentric chromosome: A chromosome having two centromeres.

Differentiation: The modification of identical cells derived from a zygote into the different cell types; usually associated with organ formation, and characterised by differences in the kinds of genes being transcribed.

Dihybrid: The progeny from a cross between two homozygous parents differing for two genes; A cross involving two gene pairs (character pairs).

Diploid: A cell, organism or species having two copies of a single genome; specified by $2x$ (not $2n$).

Diplospory: A type of apomixis in which the embryo sac (gametophyte) is formed after mitotic divisions of the archesporial cell ; meiosis either does not occur or does not result in reduction of chromosome number.

Diplotene: The state after pachytene and before diakinesis during prophase I of meiosis; characterised by the movement of homologous chromosomes, paired to form bivalents, away from each other and the appearance of chiasmata.

Discontinuous variation: Variation which can be divided into distinct classes; in case of qualitative characters; usually little affected by the environment; yields typical F_2 ratios.

Disomic: Cell or individual having two homologous sets of chromosomes ($2n$), *i.e.*, each chromosome is represented by a pair of homologues.

DNAase: An enzyme that specifically digests or hydrolyses DNA.

Dominance: In the heterozygote, one allele expresses itself at the expense of the other.

Duplex: The condition where two dominant alleles of a given locus are present in an autopolyploid, *e.g.*, AAa in triploid, $AAaa$ in autotetraploid.

Duplicate genes: Two dominant genes produce the same phenotypic effect whether they are alone or together, the alternative phenotype being produced only when both are in the recessive state.

Duplication: The occurrence of a chromosome segment more than once in the same chromosome

Dyad : A pair of daughter cells produced from the first meiotic division.

Egg (Ovum) : The gamete produced by a female organism.

Embryo sac: In plants; the structure derived from a megaspore through mitosis; generally, contains one egg cell, two synergids, three antipodal cells and two polar nuclei.

Embryo: The early stages of development of an organism from the zygote.

Endomitosis: Replication of chromosomes without cell or nuclear divisions, leading to polyploidy.

Endonuclease: An enzyme which induces internal cuts or cleavages in a DNA molecule.

Endoplasmic reticulum: A network of membranes in the cytoplasm; consists of tubules, vesicles and cisterns; provides the attachment site for ribosomes; may be rough or smooth.

Endosperm: The nutritive tissue of developing seeds derived from repeated mitotic divisions of the fusion product of the two polar nuclei of all embryo sac with a sperm; usually it is $3n$, but in some cases it is $5n$.

Enzyme: A protein which catalyses or speeds up a specific chemical reaction; usually in a living system.

Epistasis: The expression of a gene is modified by another gene; in quantitative genetics, nonallelic interaction or simply interaction.

Equational division: The second division of meiosis when the sister chromatids separate; when crossing over occurs between a gene and its centromere, segregation for the gene takes place during this division.

Equatorial plate: It is the arbitrary plane at which the chromosomes are arranged during metaphase.

Euchromatin: The chromosome or chromosome regions which show the normal coiling-decoiling cycle and normal staining properties.

Eukaryote: An organism whose cells have true nuclei, *i.e.*, nuclei enclosed by typical nuclear envelope.

Euploid: A cell or an organism in which the chromosome number is an exact multiple of the basic number (x) for the concerned species.

Evolution: The development of new varieties, races, species, genera etc. through the origin of variation and natural selection.

Exon: The part of an eukaryotic gene which is represented in the mature mRNA and in the polypeptide specified by the gene.

Exonuclease: An enzyme which degrades DNA or RNA from the end of the strand. Exonuclease may be specific for either the 5' end or 3' end of the polynucleotide chain.

Expected ratio: In chi-square test; the ratio in which the data are expected to occur in a given experiment; based on known facts and theoretical considerations.

Expressivity: The degree of phenotypic expression of a gene in the different individuals; it may be uniform or variable.

Extranuclear gene: Genes residing outside the nucleus, in organelles such as mitochondria and chloroplasts.

F₁ : The progeny obtained by crossing or mating two strains or individuals; first filial generation.

F₂ : The second filial generation; obtained by self-fertilization of or intermating among the individuals of F₁ generation.

Fertilization: The fusion of male and female gametes to produce a zygote.

Frameshift mutation (reading frame mutation) : Mutations that arise by insertion or deletion of base pairs which are not a multiple of 3. They change the reading frame due to displacing the starting point of transcription, thus the mRNA is misread.

G₁ (gap-1 period) : In eukaryotic cell cycle, the first gap period of interphase ; the period (stage) between the end of mitosis and beginning of DNA synthesis.

G₂ (gap-2 period): In eukaryotic cell cycle, the second gap period of interphase; the period between the end of DNA synthesis and start of next mitosis.

Gamete: A specialized haploid (usually) cell which participates in sexual reproduction; one male and one female gametes fuse to produce a zygote.

Gametic chromosome number: The chromosome number normally present in the gametes of a species; ordinarily, it is the haploid number (n).

Gametogenesis: The production of male and female gametes.

Gene (cistron): Segment of DNA (or RNA in certain viruses) which represent a functional unit of inheritance, defined by the cis-trans test or a particular sequence of nucleotides along DNA molecule (or RNA in certain viruses) involved in producing a polypeptide chain.

Gene mutation: A change in the base sequence of a gene, usually producing a mutant phenotype.

Gene pool: The sum total of all the alleles present in the breeding or reproductive members of a random mating population.

Gene symbol: A letter or a group of letters used to denote a gene; usually derived from the name of the mutant phenotype; the first letter of the symbol for the dominant allele is capital, while all the letters in the symbols for recessive alleles are in lower case.

Gene: A segment of DNA which specifies a single polypeptide (structural gene); also, a segment of DNA which codes for an RNA molecule (e.g., rRNA and tRNA genes), or may serve as a binding site for regulatory proteins (e.g., operator and promoter genes).

Genetic code: In DNA and RNA, the base triplets that carry genetic information for protein synthesis.

Genetic map: The linear arrangement of mutational sites belonging to the gene concerned (cistron) in a chromosome plotted graphically to correspond with their relative distances from one another, as deduced from recombination experiments.

Genetics: The branch of biology concerned with the study of heredity and variation.

Genome: A complete set of chromosomes of a diploid species; all the members of a genome are distinct from each other in gene content and, often, in morphology; members of a genome do not pair.

Genotype: The genetic constitution of an organism.

Germ cell: A cell 'destined to produce gametes for sexual reproduction.

Germplasm: The sum total of hereditary material or genes present in a species.

Haploid: The cell or individual with gametic chromosome number, symbolised by "n".

Heredity: The transmission of traits (more precisely, genes) from parents to offspring.

Heritability: The ratio of genotypic variance (broad sense heritability) or additive genetic variance (narrow sense heritability) to the total phenotypic variance.

Heterochromatin: The parts of chromosomes (or chromatin) which take up dark stain during interphase; genetically inactive; usually, contains repetitive DNA sequences which are rarely transcribed; may be constitutive or facultative.

Heteromorphic chromosomes: Homologous chromosomes differing in morphology.

Heterosis (Hybrid vigour): Superiority of the heterozygote over the corresponding homozygotes with respect to one or more characters (or superiority of the F_1 over both the parents.).

Heterosis: The superiority of a heterozygote over both the concerned homozygotes in respect of one or more characters; in practice, the superiority of an F_1 over both its parents.

Heterozygote: An individual having two different alleles for one or more genes.

Hexaploid: An individual having six genomes of one (auto hexaploid) or more (allohexaploid) kind.

Histones: The conserved basic proteins complexed with DNA in eukaryotes, forming nucleosome.

Hollandic gene: A gene carried in the Y chromosome and, thereby, showing only male-to-male transmission; confined to the male sex only (in mammals).

Homoeologous chromosomes: Partially homologous chromosomes; they are derived from ancestral chromosomes which were homologous.

Homogametic sex: The sex which produces only one type of gametes with regard to the sex chromosome.

Homologous chromosomes: The chromosomes which pair regularly and freely with each other during meiosis; they are identical in gene content and, usually, in morphology.

Homozygote: A cell or individual having identical alleles at one or more loci.

Hormone: A compound produced by the cell of one part of the body which acts in the other parts of the organism.

Hybrid: The progeny obtained by crossing or mating two individuals or strains having different genotypes.

Hybridization: The process of obtaining progeny through the union between male gametes of one strain with the female gametes of another strain. (Syn., crossing, mating).

Idiogram (Karyogram) : A diagrammatic representation of the chromosome morphology (karyotype) of an organism.

Idiotype: Refers to the sum total of hereditary determinants of an organism, comprising its chromosomal genes (genotype) and extrachromosomal genes (plasmotype). in translation.

In vitro : When biological processes are made to occur outside the organism in a vessel or test tube.

In vivo: Within the living organism.

Inbreeding: Mating between two individuals related by descent, i.e., having a common ancestor.

Incomplete dominance: The phenotypic expression of heterozygotes for a gene being intermediate between those of the two concerned homozygotes.

Independent assortment: The segregation of one pair of alleles being independent of that of another pair of alleles, that is, an allele of one gene passes with either one of the two alleles of the other gene with equal frequency.

Inhibitory gene: A dominant gene which prevents the expression of another dominant gene; generally, denoted by I.

Interference: Effect of one crossing over event on the occurrence of a second crossing over (double crossing over) in its vicinity (adjacent region). It may be positive or negative interference (see, chromatid interference chiasma interference).

Interphase: The stage in cell cycle when the nucleus of a cell is not undergoing division; a period of active protein (G1 and G2 phases) and DNA (S phase) synthesis.

Intron: The part of an eukaryotic gene which are not represented in the mature mRNA molecules; derived from 'intervening sequences'.

Inversion: Rotation of a segment of a chromosome by 180° so the genes in this segment are present in the reverse order; characteristic inversion loops are produced during meiosis in the inversion heterozygotes.

Iojap : In maize, a mutant nuclear gene changing the chloroplast characters; the mutant chloroplasts behave autonomously after it.

Isochromosome: A chromosome whose arms are equal and genetically identical homologous.

Isogenic lines: Lines having identical genotype, except for one gene at which they differ e.g. A and B lines is male sterility.

Karyokinesis: The division of a nucleus into two daughter nuclei; through mitosis or meiosis.

Karyotype: Chromosome complement of an individual defined by the number and morphology of the chromosomes usually at mitotic metaphase. It is often used for photomicrographs of metaphase chromosomes, arranged in a standard sequence.

Lagging chromosome (s) (laggards) : At anaphase of mitosis or meiosis, the chromosomes which either do not move or move much slowly towards poles are called laggards and so they are not included in the daughter nuclei.

Lagging strand (of DNA): The DNA strand synthesized discontinuously in the form of short fragments (5' to 3' direction) which are later connected covalently by the enzyme ligase, thus the overall growth being in 3' to 5' direction.

Lampbrush chromosome: In diplotene stage of vertebrate oocytes, particularly, in amphibia; the two chromosomes of each bivalent have numerous paired loop like lateral extensions; loops are the sites of active transcription.

Leading strand (of DNA) : The DNA strand synthesized continuously in the 5' to 3' direction.

Lethal gene: An allele of a gene which causes the death of all those individuals which carry it in the appropriate genotype; death occurs any time before the individuals reach adulthood.

Ligase: An enzyme which seals the nicks or the cuts in a DNA strand.

Linkage map: A linear map of the genes showing linkage with each other; it depicts the sequences in which genes are located in the chromosome as well as the frequency of recombination between the adjacent genes.

Linkage: The tendency of genes to stay together during inheritance; due to the genes being located relatively close to each other in the same chromosome.

Locus: The fixed position in a chromosome occupied by a gene; often used as a synonym of gene since, ordinarily, genes occupy a fixed position in the concerned chromosome.

Malesterility: The male gametes produced by an individual being nonfunctional.

Maternal effect: The phenotypes of progeny being affected by the genotypes of their mothers.

Maternal inheritance: The transmission to progeny of a character from the female parent only.

Megaspore: Haploid spores produced by meiosis in megasporocytes in angiosperms. One of the haploid megaspores divides to produce the embryo sac (female gametophyte).

Megasporogenesis: The production of female spore through meiosis in the ovules of plants.

Meiosis: The eukaryotic cell division that produces haploid spores. It completes in two successive cell divisions following one replication of chromosomes (DNA replication). In the I division, homologous chromosomes pair and separate and are reduced to half in number (n); in the II division chromatid separation occurs to produce four haploid daughter cells - the spores (plants) or gametes (animals).

Merozygote: In bacteria, a partially diploid cell (zygote) containing complete genome of the recipient and a chromosome fragment (merogenote) derived from the donor cell.

Messenger RNA (mRNA) : The RNA molecules formed by transcription of DNA and carrying information for amino acid sequences of specific polypeptides and serve as template for protein synthesis ..

Messenger RNA (mRNA): The RNA molecules which carry the information for amino acid sequences of specific proteins which is deciphered with the help of ribosomes and specific tRNAs.

Metacentric chromosome: A chromosome with the centromere in its centre or close to the centre; the two arms of such a chromosome are nearly equal.

Metaphase: The stage during cell division after prophase and before anaphase; the chromosomes are short, thick distinct from each other and arranged on the equatorial plate.

Microsporogenesis: The production of haploid male spores (pollen grains) through meiosis in the anthers of plants.

Mitochondria: Cytoplasmic organelles enclosed by two concentric membranes, the inner membrane having characteristic infolds called cristae; concerned with oxidative phosphorylation and production of ATP; contains DNA and participates in cytoplasmic inheritance.

Mitosis: The cell division in which each nuclear division is preceded by DNA replication; the daughter cells are identical in chromosome number and genetic content to one another and to the parent cell.

Mitotic apparatus: The organelle consisting of asters which surround the centriole, mitotic spindle which connect the centromeres of the chromosomes to centrosome and the matrix in which the spindle fibres are embedded,

Mitotic crossing over: (somatic crossing over): Crossing over during mitosis of somatic cells resulting in the segregation of heterozygous alleles.

Modifying gene: A gene that increases the phenotypic expression of a major gene; usually, several modifying genes act in an additive manner and generate a continuous variation in an otherwise qualitative trait.

Monogenic: Governed by or involving a single gene.

Monohybrid cross: Hybridization between two individuals or strains which differ for a single character, or which are taken to differ for a single character.

Monohybrid: The progeny derived by mating or crossing two individuals or strains which differ for one gene (in practice, one character).

Monoploid: A cell or individual having the basic chromosome number (x) ; haploid from a diploid (2x) species.

Monosomic: An individual having one chromosome less than the normal somatic complement of the species.

Mosaic: An individual or tissue having cells of two or more different genotypes.

Multiple alleles: More than two alleles of a single gene (more precisely, of a single cistron).

Mutable gene: A gene having an unusually high spontaneous mutation rate.

Mutagen: A chemical or physical agent which induces mutation.

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

Mutation: A sudden and heritable change in the character of an organism which is not due to segregation or recombination.

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

Muton: The unit of mutation; the smallest unit of a gene capable of undergoing mutation; represented by one nucleotide.

Natural Selection: Differential rate of reproduction of different genotypes of an organism in response to the environmental factors; the basis of organic evolution.

Nondisjunction: The failure of a chromosome pair (during A I of meiosis) or of sister chromatids of a chromosome (during A II of meiosis and anaphase of mitosis) to separate during anaphase; as a result, they pass to the same pole leading to the production of $n + 1$ and $n - 1$ gametes (meiosis) or $2n + 1$ and $2n - 1$ daughter cells (mitosis).

Nonsense codon: The codons that do not specify an amino acid, and used for protein chain termination; they are UAA (called ochre), UAG (called amber) and UGA.

Nuclear envelope: The double-membraned structure surrounding the nucleus (in eukaryotes).

Nuclear lamina: A proteinaceous layer consisting of 3 lamin proteins on the inside of the nuclear envelope.

Nuclear pores: Pores in the nuclear envelope, used for transport of macromolecules.

Nucleic acid: The macromolecules composed of phosphate, pentose and organic bases, viz., DNA and RNA.

Nucleoid: In bacterial cell, the DNA containing region which is functionally equivalent to the nucleus of eukaryotes.

Nucleolus Organizer Region (NOR): The segment of a chromosome containing the genes concerned with the synthesis of ribosomes; usually, seen as a constriction in metaphase chromosomes and associated with the nucleolus during the prophase I of meiosis.

Nucleolus: A relatively dense, spherical structure rich in RNA and protein, and associated with the NOR; the site of ribosome assembly and synthesis of ribosomal components.

Nucleoside: A nitrogenous base (purine or pyrimidine) attached to ribose or deoxyribose.

Nucleosome: The bead like structure of chromatin fibers; each composed of a histone octamer and 146 base pairs of DNA.

Nucleotide: The unit of DNA and RNA organisation; each nucleotide has one molecule each of phosphate, pentose (ribose in RNA, deoxyribose in DNA) and one of the five organic bases [A, G, C, T in DNA) and U (in the place of T in RNA)].

Nucleus: In eukaryotes; the organelle containing chromosomes and nucleolus, and surrounded by a typical nuclear envelope composed of two concentric membranes.

Nullisomic: An individual having one chromosome pair less than the normal somatic complement of the species.

Octaploid: A cell or an organism having eight identical (autooctaploid) or different (allooctaploid) genomes.

Okazaki fragments: Short segments of DNA (about 1000 to 2000 bases) synthesized during discontinuous replication; they are later joined by ligase into a covalently intact strand.

Oligogene: A gene producing a marked effect on the expression of a character so that the individuals can be easily grouped into separate classes on the basis of this trait; its expression is relatively little affected by the environment.

Operator gene: In operons; the gene to which the repressor molecule binds; it is the gene adjacent to the first structural gene of an operon.

Operon: A group of structural genes whose transcription is regulated by act of regulator, promoter and operator genes, which are often located next to (upstream or onto the left of) the structural genes.

Organelle: A specialized structure located in the eukaryotic cytoplasm surrounded by a membrane and performing specialized functions.

Ovary: The gonad of a female animal; in plants, the basal part of pistil containing ovules.

Ovule: In plants, the megasporangium which develops into seed (embryo).

Pachytene (pachynema): A substage of meiotic prophase I, between zygotene and diplotene, the homologous chromosomes have paired to become bivalents, each bivalent contains 4 strands (tetrad) made up of 2 chromatids of each chromosome. Crossing over occurs at this stage.

Parthenogenesis: The development of an individual from an unfertilized egg.

Penetrance: The ability of a gene to express itself in all the individuals which carry it in the appropriate genotype (complete penetrance); many genes fail to do so (incomplete penetrance).

Phenotype: The observable characteristics (of appearance, anatomy etc.) of an organism.

Pleiotropy: A single gene affecting more than one character.

Polygenes: Genes having individually small but cumulative effect on a character; they govern quantitative characters.

Polymorphism: Two or (usually) more phenotypes maintained in a random mating population; due to the effects of environment.

Polynucleotide: A macromolecule in which several nucleotides are joined by the phosphodiester linkage, e.g., DNA and RNA.

Polypeptide: A macromolecule in which several amino acids are joined by the peptide bond.

Polyploid: A cell or an organism having more than two genomes; they may be copies of a single genome (autopolyploidy) or of different genomes (allopolyploidy).

Polysome (polyribosome): A series of ribosomes bound to an mRNA.

Polytene chromosome: A giant chromosome consisting of several fibrils.

Prokaryote (procaryote): The organism lacking a true nucleus and meiosis, i.e., chromosomes are not enclosed by a nuclear envelope, e.g., bacteria and viruses.

Promoter gene: The gene or DNA segment of an operon to which RNA polymerase binds before it initiates the transcription of an operon.

Proof reading: Correction of errors in nucleic acid or protein at the time of their synthesis.

Prophase: The first stage of cell division after interphase and before metaphase; the chromosomes become shorter and thicker, and are visibly double-stranded toward the end (mitosis, prophase II of meiosis); chromosomes pair to form typical bivalents (prophase I of meiosis).

Puff: Expansion of a band of a polytene chromosome involved in RNA synthesis (RNA puff). A DNA puff synthesizes excessive amounts of DNA.

Recessive: An allele which is incapable of expressing itself phenotypically in the heterozygous state.

Reciprocal Crosses: The two crosses in which the same two parents are involved, but the strain which serves as the male parent in one cross is used as the female parent in the other, and *vice-versa*.

Recombination: Usually, in the case of linked genes or characters; the production of new combinations of genes or characters not present in the parents of a cross or generation; involves crossing over.

Recon: The unit of recombination; is represented by one nucleotide; the part of gene within which recombination cannot take place.

Repetitive (repetitious) DNA: Repeated nucleotide sequences, a type of DNA making up significant fraction of the total DNA of most eukaryotes. Replication and transcription; may be unique or repetitive.

Replication fork: The point where strands of double-stranded DNA are separated so that replication can proceed.

Replication: The process of production of new copies of a macromolecule by exactly copying the preexisting macromolecule.

Replicon: A segment of DNA capable of undergoing replication; it has a unique 'origin' site at which the replication begins.

Repulsion Phase: A linkage between the dominant allele(s) of one (or more) gene(s) and the recessive allele(s) of another (several other) gene(s); in such a case one parent involved in a cross contributes the dominant allele of one (or more) gene(s), while the second parent provides the dominant allele(s) of the other gene(s). Respect to a single specialized pair of allelic genes.

Restriction endonuclease: An enzyme which cleaves a DNA molecule either within or close to a site having specific sequence of nucleotides.

Retrovirus: An RNA virus which propagates via reverse transcription into duplex DNA.

Reverse mutation: The mutation of a mutant allele back to the wild type allele.

Reverse transcriptase: RNA dependent DNA polymerase enzyme that synthesizes DNA on a template of RNA.

Ribonucleic acid: A polynucleotide having ribose as the pentose sugar and ordinarily uracil in place of thymine; genetic material in many viruses and may undergo replication; generally, single-stranded; is of five types: mRNA, tRNA, rRNA, chromosomal RNA, and genetic RNA ; produced by the transcription of DNA.

Ring chromosome: Circular chromosomes. In prokaryotes, they are normally found in *E.coli* and some viruses. In eukaryotes, they arise as a result of chromosome structural change.

SAT-chromosome: A chromosome containing a satellite.

Satellite: A distal segment of a chromosome separated from the rest of the chromosome by a secondary constriction.

Segregation: Separation of the two alleles of a gene during gamete formation and their passage into different gametes; usually due to the separation of homologous chromosomes during AI; each gamete contains only a single copy of only one allele, and leads to the typical 3 : 1 or 1 : 2 : 1 ratios in the F_2 generations of monohybrid crosses.

Self-fertilization: The union between male gametes of an individual with the female gametes produced by the same individual; usually in plants, self-pollination.

Self-incompatibility: The inability of fully functional pollen grains to effect fertilization and seed set on self-pollination; usually, due to multiple alleles of a single gene S; gametophytic or sporophytic in nature.

Semiconservative replication: The mode of DNA replication in which the DNA molecule separates into two strands, each strand is conserved and acts as a template for synthesis of a new strand.

Sex chromosome: A chromosome which differs in number or kind between the male and female individuals of the same species, and is involved in sex determination (Syn., allosome.)

Sex-influenced trait: Alleles of the gene governing such a trait show opposite dominance relationship in the males and females of the species.

Sex-limited character: A character which is expressed in one of the two sexes only.

Sex-linkage: The association between a character and the sex during inheritance; the concerned gene is located in the X chromosome; in human beings several genetic diseases are sex-linked.

Sexual reproduction: The production of new individuals through the fusion of male and female gametes.

Somatic cell: A cell making up the body of an organism, excluding the germ cell; such cells have the full somatic complement of the species.

Somatic chromosome number: The chromosome number normally found in the somatic cells, more specifically in the meristematic cells, of a species; represented by $2n$.

S-phase: The part of interphase of eukaryotic cell cycle during which DNA synthesis occurs.

Spindle: A bipolar collection of fibres (called spindle fibres) observed during cell division in eukaryotes.

Spontaneous mutation: A mutation that arises naturally without the application of any mutagen (by man); the cause of such mutations is not definitely known.

Sporogenesis: The production of haploid micro- and megasporangia from the diploid microspore and megaspore mother cells, respectively, through meiosis; in plants.

Staggered cut: In double-stranded DNA cut made at different points near each other in the two strands.

Sterility: Inability to produce progeny usually due to nonfunctional gametes; male and female sterility.

Sticky ends: Complementary single strands of DNA at opposite ends of a double-stranded DNA or at ends of different duplex molecules.

Sub vital: A gene which kills some of the individuals which carry it in the appropriate genotype.

Sublethal gene: A gene which causes the death of most, but not all, of the individuals that carry it in the appropriate genotype.

Synapsis: During prophase I of meiosis; close, longitudinal, point-to-point precise association between homologous chromosomes. (Syn., Chromosome pairing.)

Synaptenemal complex: A proteinaceous structure about 1000 Å wide formed between homologous chromosomes during synapsis; considered to facilitate recombination or crossing over.

Transcription: RNA synthesis on a DNA template.

Telocentric: A chromosome having centromere at one end (in the place of telomere).

Telomere: The natural unipolar chromosome ends in eukaryotes; the DNA sequence consists of a short repeating unit with a protruding single stranded end.

Telophase: The last stage of cell division following anaphase preceding interphase; the chromosomes gather at the opposite poles and begin to uncoil to finally form loosely packed masses of chromatin fibers.

Template: A mould; a substance used to create identical copies as specified by itself; DNA molecules serve as templates during replication and transcription.

Terminal deletion: Loss of segment at the end of a chromosome.

Testcross: The cross of an F_1 hybrid with an individual or strain having the recessive phenotype for the concerned trait.

Tetrad: The group of four daughter cells produced by meiotic division of a single cell; also, the four-stranded structure (i.e., bivalent) produced due to the synapsis of two homologous chromosomes, each having two chromatids.

Tetraploid: A cell or an organism having four genomes; the genomes may be copies of a single genome (autotetraploid) or of different genomes (allotetraploid).

Tetrasomic: A cell or an organism having one chromosome pair in addition to the normal somatic complement of the species; one chromosome of the genome has four copies or homologues.

Transcription: The production of an RNA molecule complementary to a DNA molecule which serves as the template for RNA polymerase, the enzyme that catalyzes transcription.

Transduction: Transfer of a bacterial gene from one bacterium to the other by a phage particle.

Transduction: Genetic recombination in bacteria following the transfer by a virus of a segment of the bacterial chromosome from one bacterial cell into another.

Transfer RNA (tRNA): RNA molecules that transport specific amino acids to the ribosome; each tRNA species has a specific anticodon which base-pairs with the appropriate mRNA codon.

Transformation: The genetic modification of a cell induced by the incorporation of DNA purified from cells or viruses.

Transgenic: Individual created by introduction of new DNA sequences into the germ line via addition to the egg.

Transition: The substitution, in a DNA or an RNA molecule, of purine by another purine or of a pyrimidine by another pyrimidine; leads to gene mutation.

Translation: The process of protein synthesis ; genetic information present in an mRNA molecule directs the order of the specific amino acids to produce the polypeptide.

Translocation: Integration of a segment of one chromosome into a nonhomologous chromosome; this integration may be reciprocal, i.e., the two nonhomologous chromosomes involved in the translocation may exchange segments.

Transposable element (transposon): A sequence of DNA that has the ability to insert itself at a new location in the genome.

Transposase: Enzyme involved in insertion of a transposable element at a new site.

Transversion: In DNA or RNA; substitution of a purine by a pyrimidine and *vice versa*.

Triplet code: A code consisting of a set of 3 nucleotides to specify an amino acid.

Trisomic: A cell or an organism having one chromosome in excess of the normal somatic complement of the species; one chromosome has three homologous in place of the normal two; denoted by $2n + 1$.

Unidirectional replication: Movement of a single replication fork from a given origin.

Variation: The occurrence of differences among the individuals belonging to a single species for one or more traits.

Watson - Crick model: A model of DNA structure given by Watson and Crick in 1953. According to this model DNA forms double helix which is held together by hydrogen bonds between specific pairs of bases.

W - Chromosome: The sex chromosome limited to the female sex in case of female heterogamety (ZW = female; ZZ = male).

Wild type: Refers to a strain, organism or gene commonly found in nature or arbitrarily designated as "normal".

X - Chromosome: The sex chromosome which occurs in both male and female individuals but in different numbers, e.g., human male has one X chromosome, while females have two; in most animals and many plants, it has the genes for femaleness.

Y - Chromosome: The sex chromosome which occurs either in the males (man, rodents, *Drosophila* etc.) or females (birds, reptiles) of a species, but never in both the sexes; usually the major portion of Y is genetically inactive; may or may not determine maleness depending on the species.

Z - Chromosome: The sex chromosome present in both sexes where female is heterogametic sex (ZZ = male; ZW or ZO = male).

Zygote: The cell derived through fusion of one male and one female gamete; it has 2n chromosome complement as opposed to the n for the gametes; it develops into a new individual through mitosis (higher plants and animals) or may undergo meiosis to yield the haploid phase.

INDEX

'A' form of DNA	196	Cistron	194
Alloplasmic lines	253	Coding dictionary	214
Allopolyploidy	91	Co-dominance	116
Amphidiploids	91	Codon	208
Amino acid	221	Colchicine	89
Anaphase	55	Complete dominance	115
Aneuploidy	79	Conditional lethals	121
nullisomy	81	Cris-cross inheritance	170
monosomy	81	Crossing over	143
double monosomic	80	Cytoplasm	18
trisomy	83	Cytoplasmic inheritance	251
tetrasomy	85	Cytokinesis	56
double trisomy	80	Cytoskeleton	28
Anti-codon	214	Deletion	71
Antimutator gene	237	Diakinesis	63
Autopolyploidy	88	Dimers	234
B & Z forms of DNA	196	Dioecious	259
Balanced lethals	122	Diploid	79
Biparental inheritance	252	Diplotene	62
Bridging species	97	Disomic	79
Cell cycle	52	DNA repair system	203
Cell division	50	Dominance and lethal genes	114
Cell theory	29	Dominant lethals	121
Cell wall	16	Double helical model of DNA	194
Central dogma	217	Duplication	74
Centromere	32	Embryo	259
Centrosome	27	Endoplasmic reticulum	23
Chloroplast	20	Epigenesis	12
Chromosomal aberrations	71	Ergastic substances	29
Chromosomal theory of linkage	154	Eukaryotic cell	30
Chromosomal theory of inheritance	110	Euplasmic lines	253
Chromosome	32	Euploidy	86
		Expressivity	141
		Extranuclear inheritance	251
		Extra chromosomal gene	251

Chromosome pairing	81	Extra chromosomal inheritance	251
Cilia	29		
Circular DNA	202	Fertilization	262
Fine structure of gene	177	Interphase	52
First meiosis	58	Inversion	75
Flower	259	Io jap variegation	257
Folded fiber model	40	Ionizing radiation	245
G1 – Phase	53	Isochromosomes	35
G2 – Phase	53	Karyotype	35
Gamete	57	Lampbrush chromosomes	45
Gametic lethals	123	Leptotene	60
Gametogenesis	261	Lethal genes	118
micro	261	recessive lethals	118
mega	261	dominant lethals	121
Gene expression	217	conditional lethals	121
Gene Interaction	126	balanced lethals	122
Genetic code	214	gametic lethals	123
Genetic language	214	Linkage	143
Genetic organelles	19	Linkage map	154
Genic balance theory	163	Lomasomes	27
Germplasm theory	13	Lysosome	26
Giant chromosomes	47	Maternal effect	254
Glyoxysomes	27	Maternal inheritance	251
Golgi complex	25	Meiosis	57
Gynandromorphs	163	Mendel's laws of heredity	104
Haploidy	86	Mendelian inheritance	99
Hemizygous	163	Metaphase	55
Hermaphrodite	259	Methylation	281
Heterogametic	162	Microbodies	26
Heteroploidy	79	Microtubules	28
Holandric genes	171	Mitochondria	19
Homeologous chromosomes	85	Mitosis	50
Homogametic	162	Modern concept of gene	177
Homologous chromosome	37	Monoecious	259
		Monomers	43
		Monoploidy	86
Hot spots	237	Multiple alleles	172

Idiotype	35	Multiple factor hypothesis	138
Incomplete dominance	115	Mutable gene	237
Incomplete linkage	147	Mutagens	237
Intersex	163	physical	244
chemical	242	Peroxisomes	27
Mutant allele	236	Phenocopy effect	142
Mutants	236	Physical basis of heredity	110
Mutation	236	Pistillate	259
gene	236	Plasmagenes	251
point	236	Plasmon	251
chromosomal	236	Plastid inheritance	254
characteristics	236	Plastids	27
spontaneous	237	Plastom	251
forward	238	Plectonemic	195
reverse	238	Pollination	261
classification	238	Polygenic inheritance	138
induced	242	Polynucleotide	192
breeding	242	Polyploidy	88
Mutator gene	237	Polyribosome	224
Muton	194	Preformation	12
Nonionizing	244	Prokaryotic cell	29
Nuclear cytoplasmic interaction	257	Prophase	54
		Protein	217
Nucleolus organiser regions	35	Protein synthesis	217
		requirements	217
Nucleosome	42	site	217
Nucleosome solenoid model	41	mechanism	217
		rate	226
Nucleolus	18	house construction	226
Nucleus	16	inhibitor	227
Nucleosides	190	non-nucleated cells	227
Numerical changes	79	Raphanobrassica	91
Okazaki fragments	201	Reciprocal differences	251
Operon	229	Recon	194
hypothesis	229	Replication	198
structure	229	dispersive theory	198
mechanism	231	conservative theory	199

lac-operon	231	semi-conservative	199
Over dominance	117	Ribosomes	24
Pachytene	61	S – Phase	53
Pangensis	13	Sat chromosomes	34
Penetrance	141	Second meiosis	65
Semi lethal genes	124	Telophase	56
Sex – influenced inheritance	166	Transcription	208
Sex – limited inheritance	165	Transformation	181
Sex chromosome	159	Translation	217
Sex determination	159	Translocation	77
Sex linkage	167	Triple fusion	262
Sexual reproduction	259	Triplet code	208,214
Shell coiling (Snail)	254	Triticale	92
		Type ^{II} enzyme system	205
Spherosomes	27	Ultimate fine structure of gene	179
Spontaneous generation	11	exons	179
Sporogenesis	259	introns	179
micro	259	split genes	179
mega	260	Uniparental inheritance	251
Staminate	259	Univalent shift	82
Structural aberrations	71	Vacuole	28
deletion	71	Variegated leaves	255
		Virulent	181
duplication	74	Vital genes	125
inversion	75	X-Chromosome	159
paracentric	76	X-linked genes	167
pericentric	76	Y-chromosome	162
translocation	77	Y-linked genes	171
Subvital genes	125	Zygote	102
Supervital genes	125	Zygotene	60
Super females	163		
Super males	163		
Supernumerary chromosomes	49		
Telomere	34		

Probability in Genetics (UG Agriculture Genetics & Plant Breeding Notes)

Definition Probability is the chance or likelihood that a particular event will occur.

Formula:

Probability (P) = Number of favourable outcomes / Total number of possible outcomes.

- Probability ranges from 0 to 1.
- 0 = Impossible event.
- 1 = Certain event.

Why is Probability Important in Genetics?

Probability helps predict segregation of alleles, gamete formation, genotype frequencies, phenotype frequencies, inheritance of traits and expected Mendelian ratios. Example: In Aa x Aa, probability of AA = 1/4.

Basic Terms

Term	Meaning
Experiment	Genetic cross performed
Event	Outcome of the experiment
Outcome	Individual result
Sample space (S)	All possible outcomes

Example: Aa x Aa → Sample space = (AA, Aa, Aa, aa); Total outcomes = 4.

Addition Rule

Used when either one event or another event occurs.

Formula:

$P(A \text{ or } B) = P(A) + P(B)$ (mutually exclusive events).

Example: AA (1/4) or aa (1/4) = 1/2.

Application: Finding probability of either dominant homozygote or recessive homozygote.

Multiplication Rule

Used when two independent events occur together.

Formula: $P(A \text{ and } B) = P(A) \times P(B)$.

Example: Probability of AA and aa in two independent progenies = $1/4 \times 1/4 = 1/16$.

Application: Two or more independent genes.

Product Rule

Another name for multiplication rule. Used when all events occur simultaneously.

Example:

Probability that two children are males = $1/2 \times 1/2 = 1/4$.

Sum Rule

Another name for addition rule. Used when any one of several mutually exclusive events occurs.

Example: AA or aa = $1/2$.

Probability in Monohybrid Cross

Genotype	Probability
AA	$1/4$
Aa	$1/2$
aa	$1/4$

Phenotypic ratio: 3 Dominant: 1 Recessive.
Dominant phenotype = $3/4$; Recessive phenotype = $1/4$.

Probability in Dihybrid Cross

Phenotype	Calculation	Probability
A B	$3/4 \times 3/4$	$9/16$
A bb	$3/4 \times 1/4$	$3/16$
aaB	$1/4 \times 3/4$	$3/16$
aabb	$1/4 \times 1/4$	$1/16$

Phenotypic ratio: 9:3:3:1.

Binomial Expansion

Formula: $(p+q)^n$,

Where, p = probability of one event,

q = probability of another event,

n = number of offspring.

Used to calculate probabilities among many offspring.

Probability and Meiosis: Homologous chromosomes segregate randomly during meiosis.

Probability of receiving allele A = $1/2$ and allele a = $1/2$. This forms the basis of Mendel's Law of Segregation.

Independent Assortment

Gamete	Probability
AB	$1/4$
Ab	$1/4$
aB	$1/4$
ab	$1/4$

Conditional Probability

Conditional probability is the probability of an event given that another event has already occurred.

Example: In Aa x Aa among dominant plants (3), heterozygous plants (2); therefore $P(\text{Aa} | \text{Dominant}) = 2/3$.

Applications

- Predicting genotypes

- Predicting phenotypes
- Mendelian inheritance
- Pedigree analysis
- Plant breeding
- Animal breeding
- Population genetics
- Genetic counselling
- Risk prediction of inherited disorders

Addition vs Multiplication Rule

Feature	Addition Rule	Multiplication Rule
Meaning	Either A or B	A and B
Used for	Mutually exclusive events	Independent events
Operation	+	X
Example	AA or aa	AA and aa in two offspring

One-Line Takeaways

- Probability = Chance that an event will occur.
- Probability ranges from 0 to 1.
- Addition rule → Either/Or events.
- Multiplication rule → Both/And events.
- Product rule = Multiplication rule.
- Sum rule = Addition rule.
- Probability of AA in Aa x Aa = $1/4$.
- Probability of dominant phenotype = $3/4$.
- Dihybrid ratio = 9:3:3:1.
- Random segregation during meiosis forms the basis of probability in genetics.

Frequently Asked UG Examination Questions

1. Define probability and explain its importance in genetics.
2. State and explain the addition rule with an example.
3. State and explain the multiplication (product) rule with an example.

4. Explain the role of probability in monohybrid and dihybrid crosses.
5. What is conditional probability? Give a genetic example.
6. Explain the application of probability in plant breeding.
7. Differentiate between the addition rule and multiplication rule.

Model Organisms in Genetics and Plant Breeding

(UG Agriculture Notes)

Model organisms are species that are extensively studied because they are easy to grow, reproduce quickly, and have well-understood genetics. Knowledge gained from these organisms is applied to other plants and animals.

Model organism	Scientific name	Importance
Fruit fly	<i>Drosophila melanogaster</i>	Classical genetics
Garden pea	<i>Pisum sativum</i>	Mendelian inheritance
Thale cress	<i>Arabidopsis thaliana</i>	Plant molecular genetics
Bacterium	<i>Escherichia coli</i>	Molecular biology and genetic engineering

Mouse	<i>Mus musculus</i>	Mammalian genetics and biomedical research
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1. Drosophila (Fruit Fly)

- **Scientific name:** *Drosophila melanogaster*
- **Why is it a model organism?:** Small body size (2-3 mm)
 - Easy and inexpensive to rear
 - Short life cycle (10-12 days)
 - Produces hundreds of offspring
 - Only 4 pairs of chromosomes
 - Giant polytene chromosomes in salivary glands
 - Complete genome sequenced
- **Major Contributions:** Chromosome theory of inheritance; linkage and crossing over; gene mapping; sex-linked inheritance; mutation studies.
- **Important Scientists:** Thomas Hunt Morgan - White eye mutation & sex linkage;

A.H. Sturtevant - first linkage map; H.J. Muller - X-ray induced mutations.

- **Importance in Agriculture:** Understanding inheritance, mutation breeding, gene mapping, molecular genetics.
- **UG Exam Points:** Life cycle: 10-12 days; Chromosomes: $2n=8$ (4 pairs); Nobel-winning model organism.

2. Garden Pea

- **Scientific name:** *Pisum sativum*
- **Why selected by Mendel?:** Easy cultivation; self-pollinated; easy crossing; short generation; many seeds; true-breeding lines; seven contrasting traits.
- **Major Contributions:** Laws of inheritance, dominance, segregation, independent assortment.
- **Important Scientist:** Gregor Johann Mendel - Father of Genetics.
- **Seven Characters:** Seed shape: Round/Wrinkled; Seed colour: Yellow/Green; Flower colour: Purple/White; Pod shape: Inflated/Constricted; Pod colour:

Green/Yellow; Flower position: Axial/Terminal; Plant height: Tall/Dwarf.

- **Importance:** Foundation of classical genetics, inheritance studies, parent selection, hybridization.
- **UG Exam Points:** Self-pollinated; $2n=14$; basis of Mendelian genetics.

3. Arabidopsis

- **Scientific name:** *Arabidopsis thaliana*
- **Why model plant?:** Small flowering plant; genome ~135 Mb; life cycle ~6 weeks; self-pollinated; many seeds; easy transformation; genome sequenced.
- **Major Contributions:** Plant development, flower development, hormone signaling, stress tolerance, gene function, CRISPR, functional genomics.
- **Importance:** Genes for drought, salinity, disease resistance, flowering, root development.
- **UG Exam Points:** First plant genome sequenced; $2n=10$; genome ~135 Mb; life cycle ~6 weeks.

4. *Escherichia coli* (E. coli)

- **Why model organism?:** Single-celled bacterium; generation time ~20 min; easy culture; simple genome; easy genetic modification.
- **Major Contributions:** DNA replication, gene regulation, lac operon, recombinant DNA, cloning, plasmids, CRISPR studies.
- **Importance:** Insulin, vaccines, enzymes, DNA cloning, protein expression, gene engineering.
- **Scientists:** François Jacob & Jacques Monod (Lac operon).
- **UG Exam Points:** Prokaryotic model organism; circular chromosome; ~20 min generation time.

5. Mouse

- **Scientific name:** *Mus musculus*
- **Why model organism?:** High similarity to humans; short generation interval (~10 weeks); easy breeding; genome sequenced; transgenic technology.

- **Major Contributions:** Mammalian genetics, developmental biology, gene knockout, disease research, cancer genetics, immunology.
- **Importance:** Functional genomics, gene editing, drug development, disease resistance studies, biomedical research.
- **UG Exam Points:** 2n=40; mammalian model; knockout & transgenic studies.

Comparison of Major Model Organisms

Feature	Drosophila	Pea	Arabidopsis	E. coli	Mouse
Type	Animal	Plant	Plant	Bacterium	Mammal
Life cycle	10-12 days	3-4 months	6 weeks	20 min	10 weeks
Main use	Classical genetics	Mendelian gen	Plant genomics	Molecular biology	Mammalian genetics

		etic s			
Chro moso mes	2n= 8	2n= 14	2n=1 0	One circu lar chro moso me	2n=4 0
Major scient ist	T.H. Mor gan	G.J. Men del	Man y resea rcher s	Jaco b & Mono d	Bio medi cal rese arch ers

One-Line Takeaways

- Drosophila → Best model for classical genetics, linkage and sex-linked inheritance.
- Pea plant → Used by Mendel to establish the laws of inheritance.
- Arabidopsis → Model plant for molecular genetics and functional genomics.

- E. coli → Model bacterium for molecular biology, recombinant DNA technology and gene regulation.
- Mouse → Model mammal for human genetics, gene knockout and disease research.

Frequently Asked UG Questions

1. Define a model organism.
2. Why did Mendel choose pea plants?
3. Why is Drosophila ideal for genetic studies?
4. Why is Arabidopsis called the 'plant laboratory rat'?
5. Mention the applications of E. coli in biotechnology.
6. Why is the mouse widely used in biomedical genetics?
7. Compare Drosophila, Arabidopsis and E. coli as model organisms.