

BZYCT-137

GENETICS AND EVOLUTIONARY BIOLOGY

VOL

1

GENETICS

BLOCK 1

HEREDITY AND PHENOTYPE

7

BLOCK 2

THE PHYSICAL BASIS OF HEREDITY

127

GENETICS AND EVOLUTIONARY BIOLOGY

Genetics goes hand in hand with evolution. All traits are inherited whenever we bring up a trait, we should ask when that trait evolved and place it in on the appropriate phylogenetic tree. This tree not only shows when certain traits evolved, but can also be used to infer who has them. This tree can be used to show how our genetic traits go back to the universal ancestor that lived 3-5 billion years ago and that all of today's genomes are product of duplication and divergence. The evidence for the evolution can be read from our genomes.

Understanding the connection between Mendel's principles of heredity and DNA is of paramount importance. There are concrete examples of mutations in DNA that change proteins which in turn, change phenotype is one of the best ways to make these connections we now understand some of the Mendel's traits at molecular level. Genes code for proteins and proteins determine phenotype. The more vividly you can make this connection, the richer will be their understanding of both genetics and evolution.

Looking at the functioning of DNA, RNA and protein in determining phenotype, students can begin to understand that they are guardians of 3.5 billion years of evolution and this provides motivation for taking care of our fragile planet that makes life possible.

We welcome you to the study of the first volume of this course which tells you about Genetics. This volume consists of 2 Blocks – First block entitled “Heredity and Phenotype” and Second block is “The Physical Basis of Heredity”. First Block comprises five units and the second Block consists of four units, making nine units in all in first volume.

Genetics is the science of heredity that relates to the study of the structure and function of genes and the mechanisms of their transmission from one generation to the next. The differences in many a characteristics among organisms are a direct result of differences in the genes they carry. These differences have resulted due to the evolutionary processes. *Mutations* – heritable changes in the genes, and *recombination* – the shuffling of genes and chromosomes at meiosis are the raw materials on which *selection* acts favouring a particular combination of genes for a given environment. The principles of heredity were first recognized by Gregor Mendel in 1860s but the systematic study of this discipline began in about 1900. And ever since the science of genetics has never stopped expanding. Its growth, however, has been very rapid and phenomenal in the past four decades ever since the discovery of the structure of the genetic material by Watson and Crick. One has to be really alert and hardworking to keep pace with advances made in this branch of science. It is generally said that scientific knowledge doubles every ten years, but as far as genetics is concerned the doubling time is just about two years.

Modern genetics is divided into three main branches: i) **transmission genetics**, ii) **molecular genetics**, and iii) **population genetics**.

- i) **Transmission genetics** deals with the study of transmission of genes from one generation to the next. This first branch relies mainly on the same kind of experimental approach used by Gregor Mendel in the middle of the nineteenth century. Organisms with different traits (different phenotypes) are mated and transmission of these traits to the next generation is observed. Similarly, these progeny organisms can be mated with others of the same or different phenotypes, and again the transmission of traits can be observed. Since Mendel pioneered this approach of genetics, it is also frequently called **Mendelian** or **Classical genetics**. And as it deals with the transmission of genes to successive generations we also call it **Transmission genetics**.

- ii) **Molecular genetics** is the study of the molecular structure of genes as well as the nature, expression and regulation of these molecules. Whereas transmission genetic studies are mainly carried out with eukaryotic organism, viruses and bacteria are the source of study in molecular genetics. This branch of genetics paved the way for the development of genetic engineering and recombinant DNA studies which have **far-reaching** implications in the field of agriculture and medicine.
- iii) **Population genetics** deals with the study of behaviour of genes in populations. More importantly population genetics is concerned with changes in the gene frequencies of populations over a period of time. Processes such as mutation, recombination, selection etc. tend to affect gene frequencies of populations. In fact population geneticists define evolution as changes in gene frequencies.

These branches are not exclusive of one another, rather, they reinforce each other. In this course you would find an intergration on these three branches.

This course aims at presenting the subject matter of genetics in the broader context of study of heredity. It examines not only the inheritance of genes which affect the characters of organisms but also the developmental processes whereby the characters are produced. The major questions that have concerned geneticists, in the past and the present, are highlighted. Some of the important questions that this course would address are: What is the genetic material? How it is packaged, where is it located in a cell? How does it express itself in a coordinated and organised manner? What are the sources of similarity and variability among individuals of a species? What is the behaviour of genes in populations? And in the present context, what is the relevance of genetic studies to humans and so on and so forth.

Evolutionary Biology is the discipline that describes the history of life and investigates the processes that account for this history. Evolutionary Biology has two goals.

- (i) To discover the history of life on earth i.e., to determine the (a) phylogeny of species (b) of their origin and extinction (c) rate and course of change in their characteristics.
- (ii) To understand the causal processes of evolution i.e., to understand (a) origin of hereditary variations (b) how mutation natural selection and genetic drift have given rise to other characteristics (c) how populations become different species.

Second Volume entitled “Evolutionary Biology” consists of seven units in two blocks.

Evolution as defined by Darwin is descent with modification. But the definition of evolution has undergone changes after the principles and mechanisms of inheritance became better understood. Today, the Darwinian concept of natural selection is fully explained in terms of gene frequencies and the changes they undergo from one generation to the next. Although Darwin had no knowledge of genetics, he could predict that variability in populations is responsible for the origin of adaptations. Today we know that variability is generated continuously in populations by mutations and further enhanced by genetic recombinations. These processes, together with the action of natural selection, bring about changes in gene frequencies in populations.

Objectives

After studying this course on Genetics and Evolutionary Biology, you should be able to:

- interpret and explain the results of mono-, di- and tri-hybrid crosses based on Mendel's laws of inheritance,

- recognise and describe giving examples the extensions and modifications of Mendelian genetic analysis,
- illustrate and explain the concepts of linkage, crossing-over and genetic mapping,
- discuss extra-nuclear genetic systems present in chloroplast and mitochondria of eukaryotic cell and their cooperation with nuclear genetic system,
- describe the techniques employed to study the various aspects of human chromosomes,
- describe the normal human chromosome at morphological as well as molecular level,
- recognise and explain the various kinds of structural chromosomal anomalies in humans and their resultant effects,
- recognise and explain the different forms of numerical chromosomal anomalies in humans and their resultant effects,
- discuss the nature and structure of genetic material,
- describe the mechanisms of recombination and complementation in a bacteriophage,
- describe the different types of mutations that arise in the genetic material and the rates at which they occur,
- distinguish between the spontaneous and induced mutations and list the various mutagenic agents that would induce mutations,
- present a brief history of the origin and development of evolutionary thought from the time of early Greek philosophers to the present,
- interpret the evolution of adaptations through natural selection based on the Darwinian tenets,
- describe the sources of heritable variations and the manifestation of variability, and
- discuss the characteristics of different kinds of selective forces.

Block

1

HEREDITY AND PHENOTYPE

UNIT 1

Introduction to Genetics	11
---------------------------------	-----------

UNIT 2

Extension and Modifications of Mendelian Genetic Analysis-I	46
--	-----------

UNIT 3

Linkage, Crossing-Over and Chromosome Mapping	71
--	-----------

UNIT 4

Extra-Nuclear Inheritance	106
----------------------------------	------------

Course Design Committee

Prof. M.S. Nathawat
Former Director, School of Sciences
IGNOU, Maidan Garhi, New Delhi-110068

Prof. S. S. Hasan (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Prof. Jaswant Sokhi (Retd.)
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. A.K. Bali
Associate Professor in Zoology
Bhaskaracharya College of Applied Sciences
Sector 2A, Dwarka, New Delhi-110075

Dr. S.K. Sagar
Associate Professor in Zoology
Swami Shraddhanand College, Alipur Village
University of Delhi, Delhi- 110036

Dr. H.S. Pawar
Scientist D, NIMR
Sector 8, Dwarka, New Delhi-110077

Dr. Ranjana Saxena
Associate Professor in Zoology
Dyal Singh College, Lodhi Road, New Delhi-110003

Prof. Sarita Sachdeva
Head, Department of Biotechnology
Manav Rachna International Institute
of Research and Studies, Faridabad, Haryana-121004

Prof. Neera Kapoor
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Prof. Amrita Nigam
School of Sciences, IGNOU
Maidan Garhi, New Delhi-110068

Dr. Nisha
Consultant, School of Sciences
IGNOU, New Delhi-110068

Block Preparation Team

Dr. A.K. Bali
Associate Professor in Zoology
Bhaskaracharya College of Applied Sciences
Sector 2A, Dwarka, New Delhi-110075 (Unit 1)

Prof. Abhilasha Shourie
Department of Biotechnology
Manav Rachna International Institute of Research and
Studies, Faridabad, Haryana-121004 (Unit 2)

Dr. Nidhi Didwania
Associate Professor, Department of Biotechnology
Manav Rachna International Institute of Research and
Studies, Faridabad, Haryana-121004 (Unit 3)

Dr. S.K. Sagar
Associate Professor in Zoology
Swami Shraddhanand College, Alipur Village,
University of Delhi, Delhi- 110036 (Unit 4)

School of Sciences

Prof. Neera Kapoor (Units 1 to 4)

Prof. S.S. Hasan (Units 1 and 4)

Dr. Nisha (Units 2 and 3)

Course Coordinator	:	Prof. Neera Kapoor
---------------------------	---	---------------------------

Course Editor	:	Prof. P.C. Joshi Professor & Head, Department of Zoology Gurukul Kangri University, Haridwar Uttarakhand-249404
----------------------	---	--

Production

Mr. Hemant Kumar
SO (P), MPDD, IGNOU

Acknowledgement:

- Prof. Neera Kapoor and Mr. Ajit Kumar, Suggestions for figures and Cover Design.
- Mr. Vikas Kumar, JAT for word processing and CRC preparation.

February, 2021

©Indira Gandhi National Open University, 2021

ISBN : 978-93-89969-15-3

All rights reserved. No part of this work may be reproduced in any form, by mimeograph or any other means, without permission in writing from Indira Gandhi National Open University.

Further information on Indira Gandhi National Open University courses may be obtained from the University's office at Maidan Garhi, New Delhi-110 068 or IGNOU website www.ignou.ac.in.

Printed and published on behalf of Indira Gandhi National Open University, New Delhi by the Registrar, MPDD, IGNOU.

Printed at :

BLOCK 1: HEREDITY AND PHENOTYPE

This is the first block of the course that contains four units dealing with the basic concepts of transmission of genetic material. Unit 1 describes the classic work of Gregor Mendel and the basic principles of Mendelian genetics that resulted from his work. A thorough understanding of these principles is necessary for dealing with more complex phenomenon presented later. Also introduced in this unit are problem solving and data analysis as standard methods in genetic study.

Mendelian analysis cannot be applied to all situations. The exceptions to, and the extensions of Mendelian analysis, such as the existence of multiple alleles, lethal genes, modification of dominance relationships, epistasis, sex linkage and many others are discussed in Unit 2.

Unit 3 is about the classical genetic topic of linkage, crossing-over and genetic mapping. We deal with questions such as what is linkage and recombination? What are the principles of gene mapping? And how the order and the distance between genes in eukaryotic chromosomes is determined.

In Unit 4 of this block, we explain the non-mendelian mode of inheritance or more precisely the extranuclear inheritance. We begin with the early experiments that indicated the presence of genetic determinants outside the nucleus, chloroplast and mitochondria. Thereafter, the characteristics of chloroplast and mitochondrial genome and associated protein synthesising machinery are discussed. These two extranuclear genomes have been mapped in a few species. In the last section of the unit, we have discussed the interaction between genomes-nuclear and chloroplast and nuclear and mitochondria, suggesting the possible origin of these organelles.

Objectives

After studying this unit, you should be able to:

- interpret and explain the results of mono-, di- and tri-hybrid crosses based on Mendel's laws of inheritance,
- recognise and describe giving examples the extensions and modifications of Mendelian genetic analysis,
- illustrate and explain the concepts of linkage, crossing-over and genetic mapping, and
- discuss extra-nuclear genetic systems present in chloroplast and mitochondria of eukaryotic cell and their cooperation with nuclear genetic system.

UNIT 1

INTRODUCTION TO GENETICS

Structure

- | | |
|---|---|
| 1.1 Introduction | 1.4 Mendel's Classical Experiments with Pea |
| Objectives | Choice of material |
| 1.2 Development of Genetics – A Historical Perspective | Monohybrid Crosses and Mendel's Law of Segregation |
| Birth of Genetics | Dihybrid Crosses and Mendel's Law of Independent Assortment |
| Growth of Genetics : From Mendel to Genetic Engineering | Trihybrid Crosses |
| 1.3 Some Basic Genetic Terminology | 1.5 Summary |
| Gene | 1.6 Terminal Questions |
| Genes and Alleles | 1.7 Answers |
| Homozygous and Heterozygous | |
| Phenotype and Genotype | |

1.1 INTRODUCTION

Genetics is the study of heredity. It is an ancient discipline. At least 4000 years ago, in Sumeria, Egypt and other parts of the world, farmers recognised that they could improve their crops and their animals by selective breeding. Their knowledge was based on the experience and they recognised that many features of plants and animals were passed on from generation to generation. Furthermore, they were aware that desirable traits – such as size, speed and weight of animals could sometimes be combined by controlling mating, and that in plants, crop yield and resistance to arid conditions could be combined by cross pollination. The ancient breeding programmes were not based on experimental studies because nothing was known of genes or any of the principles of heredity.

In this first unit of the course, we begin with a brief outline of the history of development of modern genetics and then we would discuss Mendel's laws: law of segregation and independent assortment and the molecular basis of inheritance.

Objectives

After studying this unit you would be able to:

- ❖ describe the historical developments and the landmarks in genetics,
- ❖ outline the contributions of Mendel to the science of genetics,
- ❖ define and use correctly the terms gene, allele, locus, genotype, phenotype, dominance, recessiveness, homozygous, heterozygous and testcross,
- ❖ describe Mendel's breeding experiments,
- ❖ state and explain Mendel's laws of inheritance, and
- ❖ discuss the molecular basis of inheritance.

1.2 DEVELOPMENT OF GENETICS – A HISTORICAL PERSPECTIVE

As systematic study of genetics began, answers were sought to questions such as i) why are we like our parents? ii) why do organisms belonging to a group have an astonishing similarity to each other? The first geneticists were the persons who observed that "like begets like" and then used this basic observation to improve their strains. Domestication of animals like dogs, cats and cattle, was started when humans learnt how to control the matings of these species. Once animals were tamed, people could breed more of those which they found most useful and the control of lineage remained with humans. Cultivation of crops required that seeds of one generation were saved to produce the next generation. Therefore, the seeds that were saved were from the largest and the healthiest plants.

Next, scientists started seeking answers to the question as to why we are like our parents but are also different from them in many respects. It was only after the work of Mendel was published in 1860s that answers to these questions started to emerge.

1.2.1 Birth of Genetics

Modern genetics originated with Gregor Mendel's (Fig. 1.1) work. It is based on this paper entitled "Experiments in Plant Hybridisation" published in 1866 in the Proceedings of the Society of Natural History in Brno. Mendel carried out detailed investigations of inheritance in garden pea. He performed elaborate plant hybridisation experiments and kept accurate pedigree records of his results (Fig. 1.2). With the data obtained, he was able to formulate the basic principles of inheritance.

Mendel proposed the concept of hereditary units. According to him equal number of these units (factors) inherited from each parent determined the observable characters of the offspring. This was the first conceptualisation of what is now referred to as **particulate inheritance**. Characteristics themselves are not inherited but the particles, units or factors that determine or control the observable traits are transmitted from parents to offspring. The appearance of the character in the offspring is determined by the particular combination of factors inherited from the two parents. This was the beginning of the concept of a **gene**, which is the **modern term for the hereditary units or particles** originally described by Mendel.

Mendel's work was not appreciated by the rest of the scientific community until 1900, when three botanists **Carl Correns** in Germany, **Hugo de Varies** in the Netherlands and **Erich von Tschermak** in Austria, rediscovered his work after each had independently reached similar conclusions. They all found Mendel's report while scanning the literature for related work and cited it in their own publications. William Bateson (Fig. 1.3), an English scientist, coined the term "genetics" in 1905 for this developing science. The term was derived from Greek word which means "to, generate". Many consider Bateson as the real founder of genetics as he was the first to have Mendel's papers translated into English and the first one to show that Mendel's theory was also applicable to animals.



Fig. 1.1: Gregor Johaan Mendel – the father of the Science of Modern Genetics. Born – 22 July, 1822 in Czechoslovakia. Died – 6 January, 1884 Brno Czechoslovakia. He was born in a small village and was the only son of a peasant family. Mendel had an insatiable curiosity about the natural and the physical world and was keenly interested in the diversity of living beings. His parents could not afford his higher education. He joined a monastery of St. Thomas, which was at that time in Austria and now in Czechoslovakia. His interest in Botany began early in life, as farming and the development of new varieties of apples were his family's chief occupation. His early interest was further stimulated by his formal education which centred round mathematics, physics, botany and zoology. The monastery provided him a stimulating environment, as it was a centre of cultural, intellectual and religious life, and its members and visitors included many notable scholars and scientists of that period. In 1851, he joined the University of Vienna and upon completing his course he returned to his teaching responsibilities at Brno. His experiments in plant hybridization were carried out in the monastery garden for several years beginning from 1826. Mendel combined his talents, background and interests in a series of experiments with garden peas. His experiments are now recognized as classic example of carefully planned and executed scientific research.

1.2.2 Growth of Genetics: From Mendel to Genetic Engineering

Genetics has come a long way and at present is a mature and dynamic science. The science of genetics was built on the foundation laid by Mendel but it owes its present stature to the contributions of a large number of scientists. In fact, the history and development of genetics is a subject worthy of study. You will be studying about the important contributions of various researchers in different units of this course. Nevertheless, a list of salient developments/contributions in the field has been compiled in Table 1.1 to give you some idea of their chronology.



Fig. 1.2: Monastery garden where Mendel's experiments on peas were carried out.

Table 1.1: The Genetics time Line (Modified from Gardner et al., 1991).

1865	Mendel	read his paper to the Brunn Society for Natural History
1866	Mendel	his paper published in the Proceedings of the Brunn Society for Natural History
1868	Miescher	first study of DNA
1900	De Vries, Correns, and Tschermak	Mendel's work discovered
1902	Boveri & Sutton	demonstrated the presence of paired chromosomes (homologs) in diploid species
1905	Bateson	named the science genetics
1908	Hardy & Weinberg	formulated the "Hardy Weinberg Law" relating genotypic frequencies to gene frequencies in randomly mating populations
1909	Johannsen	introduced the term gene
1909	Garrod	book on Inborn Errors of Metabolism

1910	Morgan	established the sex-linked inheritance of white eyes in <i>Drosophila melanogaster</i> (Nobel Prize 1933)
1911	Morgan	postulated the chromosomal basis of linkage
1913	Sturtevant	construction of a genetic map
1927	Muller	reported the use of the <i>CIB</i> technique to demonstrate that X-rays are mutagenic (Nobel Prize, 1946)
1928	Griffith	discovery of transformation in <i>Diplococcus pneumonia</i>
1931	Creighton & McClintock	demonstrated that genetic recombination is correlated with the exchange of morphological markers on chromosomes
1940	Oliver	demonstration of recombination within the lozenge functional unit in <i>Drosophila</i>
1941	Beadle and Tatum	one gene one enzyme concept (Noble Prize 1958)
1944	Avery, MacLeod & McCarty	demonstrated that the pneumococcal “transforming principle” is DNA
1946	Lederberg & Tatum	discovered conjugation in bacteria (Nobel Prize 1958)
1950	McClintock	first to present a paper on “Transposable elements” in Maize (Nobel Prize 1983)
1952	Hershey & Chase	demonstrated that the genetic material of bacteriophage T2 is DNA (Nobel Prize 1969)
1952	Zinder & Lederberg	discovered the phage-mediated transduction in bacteria
1953	Watson & Crick	worked out the double-helix structure of DNA using the X-ray diffraction data of Wilkins and the base composition data of Chargaff (Nobel Prize 1962)
1955	Benzer	first to present a paper on the fine structure of the Phage T4 rII locus
1956	Tjio & Levan	established that the normal diploid chromosome number in human is 46
1957	Fraenkel-Conrat & Singer	demonstrated that the genetic information of tobacco mosaic virus is stored in RNA

1958	Meselson & Stahl	demonstrated that DNA replication is semi-conservative
1958	Kornberg	isolated DNA polymerase I from <i>E. Coli</i> (<i>Nobel Prize 1959</i>)
1959	Ochoa	discovered RNA polymerase (<i>Nobel Prize 1959</i>)
1961	Jacob & Monod	proposed the “Operon Model” for regulating gene expression (<i>Nobel Prize 1965</i>)
1964	Yanofsky & colleagues; Brenner & colleagues	established colinearity between polypeptide products
1964	Temin	proposed the DNA provirus from RNA tumour viruses (<i>Nobel Prize 1975</i>)
1965	Holley	worked out the first complete nucleotide sequence of a tRNA (<i>Nobel Prize 1968</i>)
1966	Nirenberg, Khorana & coworkers	established the complete genetic code (<i>Nobel Prize 1968</i>)
1970	Nathans & Smith	isolated the first restriction endonuclease (<i>Nobel Prize 1978</i>)
1970	Baltimore	identified reverse transcriptase of RNA tumour viruses (<i>Nobel Prize 1975</i>)
1972	Berg	produced first recombinant DNA (<i>Nobel Prize 1980</i>)
1976	Bishop & Varmus	demonstrated the protooncogene to oncogene relationship (<i>Nobel Prize 1989</i>)
1976	Hozumi & Tonigawa	demonstrated somatic rearrangements of gene encoding antibodies
1977	Breathnach, Mandel & Chambon; Jeffreys & Flavell	demonstrated the presence of introns in eukaryotic genes
1977	Maxam & Gilbert; Sanger, Nicklen & Coulson	description of the DNA sequencing techniques
1977	Sanger & colleagues	worked out the complete 5387 nucleotide sequence of phage ϕ X174
1978	Three different laboratories	discovered “splicing” of adenovirus RNAs
1982	Sanger & colleagues	worked out the complete 48,502 nucleotide-pair sequence of phage lambda

1983	Cech & Altman	established the existence of catalytic RNAs (<i>Nobel Prize 1989</i>)
1985	Jeffery	DNA finger-printing
1988	Watson	established the existence of catalytic RNAs (<i>Nobel Prize 1989</i>)
1989	NIH Recombinant DNA Advisory Committee	recommended approval of first-human “gene transplant” experiment
1989	Tsui, Collins & colleagues	coloned the “cystic fibrosis gene”
1990	Saiki	Polymerase chain reaction
1993	R. Roberts, P. Sharp, K.	RNA processing of split genes (Nobel Prize 1993)
1993	Mullis and M. Smith	Development of polymerase chain reaction (PCR) and site-directed mutagenesis (SDM)
1995	E. B. Lewis, C. Nusslein-Volhard and E. Wieschaus	Genetic control of early development of <i>Drosophila</i> (Nobel Prize 1995)
1997	S. Prusiner	Prions-a new biological principle of infection (Nobel Prize 1997)
1999	G. Blobel	Genetically encoded amino acid sequences in proteins that guide their cellular transport (Nobel Prize 1999)
2001	L. Hartwell, T. Hunt and P. Nurse	Genes and regulatory molecules controlling the cell cycle (Nobel Prize 2001)
2002	S. Brenner, H.R. Horvitz and J.E. Sulston	Genetic regulation of organ development and programmed cell death (Nobel Prize 2002)
2006	A.Z. Fire and C.C. Mello	RNA interference (RNAi) (Nobel Prize 2006)
2006	R. Kornberg	Molecular basis of eukaryotic transcription (Nobel Prize 2006)
2007	M.R. Capecchi, M.J. Evans and O. Smithies	Development of gene-targeting technology essential to the creation of knockout mice serving as animal models of human disease (Nobel Prize 2007)
2009	E.H. Blackburn, C.W. Greider and J.W. Szostak	The nature and replication of the DNA of telomeres, and the discovery of the telomere-replenishing ribonucleoprotein enzyme telomerase (Nobel Prize 2009)

The above table, however, is not complete as we intended to make it concise.

Presently, new information is accumulating at an unprecedented rate. Most of the important contributions were made between 1970s and 1990s. The next few years are going to be exciting as many scientists are working out details of the molecular mechanisms responsible for differentiation in eukaryotes that include humans as well. Several challenging areas in the field are open for research, and perhaps one day we would be able to understand the molecular basis of complex phenomena like learning, memory, cancer and aging.

1.3 SOME BASIC GENETIC TERMINOLOGY

Before we move on to discuss Mendel's experiments with pea in detail and the laws of inheritance formulated by him, let us first understand some basic genetic terminology.

1.3.1 Gene

You have studied that Mendel proposed the concept of hereditary units, which were later on identified as genes. We shall elaborate on it further. Let us consider different traits such as flower colour, seed shape or height in plants, each of these characters is controlled by a different gene.

1.3.2 Genes and Alleles

The inheritance of any character can be studied only when there are two contrasting conditions, such as yellow versus green seed colour (as observed by Mendel in peas), normal pigmentation versus absence of pigmentation (albinism) in humans and other animals, and brown versus black coat colour in guinea pigs. An individual expresses one or the other, but not both contrasting conditions at the same time. **Genes that govern variations of the same characteristic and that occupy corresponding loci on homologous chromosomes are termed alleles** (Fig. 1.3).

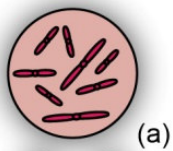
Geneticists use the term allele to emphasise that there are two or more alternative forms of a gene that can occur at corresponding specific loci.

The possible variants of a gene at any given locus are known as alleles, each of which is assigned a single letter (or a group of letters) as its symbol. In the example below, we shall consider the trait – height of pea plant. Tall forms are denoted by T and the short ones are denoted by t. T and t are alleles of the same gene. Since they are present in pairs they are represented as TT, tt or Tt.

Mendel's particulate hereditary elements or factors are called genes.

It is customary to indicate a dominant allele with a capital letter and a recessive allele with a lower case letter. The choice of the letter or letters themselves is generally determined by the first allelic variant in a particular locus. For example, Mendel studied the alleles that govern seed colour – yellow versus green. The allele that is responsible for the yellow colour of the seed is designated as Y, and the allele for the green colour y. Because discovery of the yellow allele made identification of this locus possible, we commonly refer to the locus as the yellow locus, although pea seeds are most commonly green. The term locus is used to designate not only a location on a chromosome but also a kind of “generic” gene controlling a particular kind of

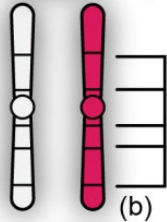
characteristic; thus Y (yellow) and y (green) represent a specific pair of alleles of a locus involved in determining seed colour in peas. To prevent confusion, the dominant allele is always designated first and the recessive allele second (Yy, never yY). Geneticists use the term gene sometimes to specify a locus and sometimes to specify one of the allelic variants at that locus. Usually the meaning is clear from the context.



(a)

Each diploid cell has two sets of chromosomes ($2n$)

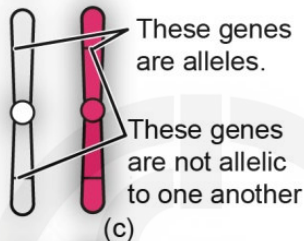
Members of one set can be paired with members of the other set. The members of a given pair correspond in shape, size, and type of genetic information, and are referred to as homologous chromosomes. Can you identify the homologous pair?



(b)

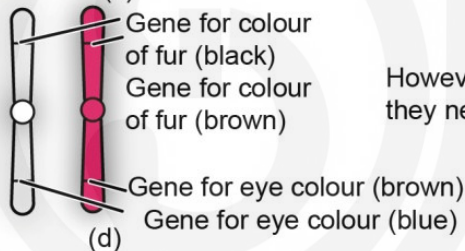
Gene loci

Each chromosome is made up of thousands of genes. The genes occupy definite physical locations on the chromosomes known as gene loci.



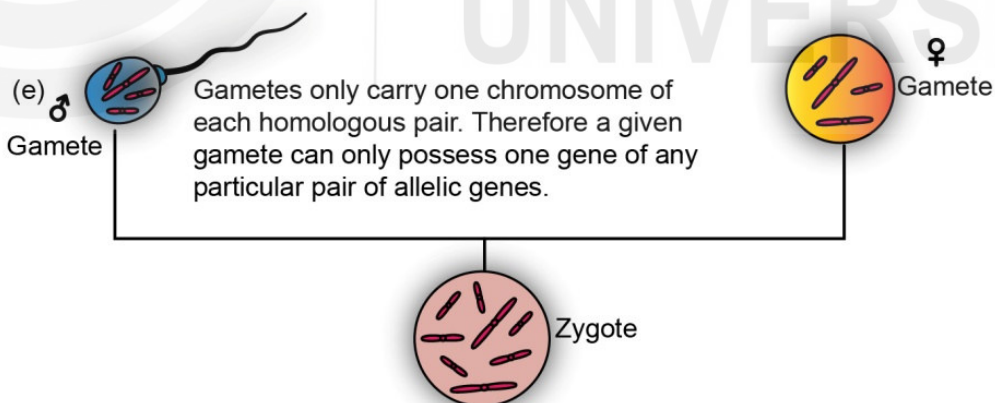
(c)

Since diploid organisms possess pairs of homologous chromosomes, the genes borne in corresponding loci of the pair also occur in pairs. If genes occupy the same locus on each of a pair of chromosomes, they are said to be alleles. Allelic genes code for variants of the same polypeptide and so govern the same characteristic of the organism.



(d)

However, even though allelic genes govern the same characteristics they need not necessarily contain the same information.



(e)

Gametes only carry one chromosome of each homologous pair. Therefore a given gamete can only possess one gene of any particular pair of allelic genes.

Zygote and the resultant embryo will have homologous pairs of chromosomes. In each homologous pair of chromosomes one member will be maternal in origin, and one paternal in origin. Each pair will bear allelic genes.

Fig. 1.3: Homologous chromosomes and alleles.

There are many genes within each chromosome, each generally different from the other and each controlling the inheritance of one or more characteristic(s). The members of a homologous pair of chromosome have similar set of alleles arranged in the same order. The regularity of the mitotic process ensures that

each diploid daughter cell receives a pair of each chromosome and therefore a pair of each gene. As the chromosomes separate during meiosis, and become associated with new partners at the time of fertilisation, the alleles also separate and associate with new partners. The entire concept of Mendelian Genetics depends on these simple facts.

1.3.3 Homozygous and Heterozygous

Homozygous: *Same factor.*

Heterozygous: *Different factors.*

In an individual two identical alleles may exist for a given character and, hence, the individual is referred to as Homozygous (e.g. AA and aa). If there are two non-identical or different alleles for a given character, the individual is referred to as heterozygous (e.g. Aa).

Let us examine a situation where both the parents are homozygous. The male is homozygous recessive aa, and female is homozygous dominant AA. During meiosis in the male the two 'a' alleles separate from each other so that each sperm has only a single 'a' allele. Similarly, in the female parent each egg has one 'A' allele. The fertilisation of the 'A' egg by 'a' sperm results in a heterozygous animal with 'Aa'.

Physical appearance: Phenotype.

Genetic constitution: Genotype.

The form of gene which occurs in an individual in nature is called the 'wild type' while a 'mutant type' is the one in which the genetic material is somewhat altered. Mutants arise due to various reasons.

The alleles which express themselves in both homozygous and heterozygous conditions are known as the dominant alleles. For example, TT represents tallness (homozygous). The individuals having the alleles TT, and Tt would be tall as T is a dominant allele and it can express itself in both homozygous and heterozygous conditions.

Some alleles express themselves only in homozygous condition and are referred to as recessive alleles. In the height characteristic, dwarfness can be seen in individuals that have the alleles 'tt', i.e., recessive alleles are expressed in homozygous condition only.

1.3.4 Phenotype and Genotype

From the above subsection it is clear that some alleles are dominant while others are recessive. This indicates that we cannot always deduce the alleles present in an organism by just looking at it. The **phenotype** is the term used to specify the appearance of an individual in a given environment with respect to a certain inherited trait. The genetic constitution of that organism, most often expressed in symbols, is its **genotype**.

Using the same example as above let us understand the concepts of phenotype and genotype. If the female parent has the genotype TT (homozygous, dominant) can you guess the phenotype? Yes! It is Tall. If the male parent is homozygous recessive 'tt' can you tell what is its phenotype? The answer is dwarf. If these two parents are crossed then what would be the genotype and phenotype of the offspring? The genotype would be 'Tt' and phenotype would be tall.

Similarly, an individual has alleles that govern hair colour. These specific alleles constitute the individual's genotype for hair colour. The actual hair colour that the individual exhibits is its phenotype (black, brown, red, blonde etc.). The crux of the above discussion is that the **genetic constitution of an individual is referred to as its genotype and the expression of these genes as its phenotype.**

Phenotype should be used in the broadest possible sense. Phenotype commonly refers to visible characteristics such as size, shape, colour of the parts of an organism, height, weight, length of limbs and so on. But other less obvious characteristics also comprise the phenotype: behavioural traits (twitches, yawns, posture, smiles per hour), physiology (heart rate, blood pressure, level of basal metabolism) and biochemical characteristics (cholesterol level, presence or absence of particular enzymes, blood type). In fact, any trait of an organism which can be described and/or measured is the phenotype of that character.

We hope that you have clearly understood the above basic terms. You would learn about many more terms at relevant places in the following units.

SAQ 1

From the list of the items given below, select all those which are: i) phenotype, ii) genotype, iii) homozygous, dominant, iv) homozygous, recessive, and v) heterozygous. Write your answers in the space provided.

- a) Rr
- b) TT
- c) Tt
- d) tall
- e) RR
- f) Yy
- g) rr
- h) terminal flower
- i) tt

SAQ 2

Make appropriate matches of the mentioned in **column A** with the explanation in **column B**. Write your answers in the boxes.

A		B	
a) Genes	() i)	any character of an organism that can be measured or described	
b) Phenotype	() ii)	different alleles for specific traits on homologous chromosomes	
c) Chromosomes	() iii)	contain hereditary units	
d) Heterozygote	() iv)	once called the inheritance "factors" by Mendel	

1.4 MENDEL'S CLASSICAL EXPERIMENTS WITH PEA

Mendel's studies provide an outstanding example of good scientific technique. He chose research material well suited for the study of the problem at hand, designed his experiments carefully, collected large amount of data and used mathematical analysis to show that the results were consistent with his explanatory hypothesis.

1.4.1 Choice of Material

Before Mendel, several investigators carried out research work to understand the principles of inheritance, but they failed to reach meaningful conclusions because of the unsuitability of the system they were studying. The garden pea with which Mendel worked has several suitable features. The foremost were that these plants were cheap, easy to obtain, required little space, had shorter generation time, produced many offspring and could be crossed easily. The pea flowers are bisexual and are usually self-fertilised, that is, the ovule (female gamete) is fertilised by pollen (male gamete) from the same flower as both the male and female parts of the flower are closed in a petal box or keel (Fig. 1.4). Pollen from another plant can be experimentally introduced to the stigma of a flower to bring about cross-pollination.

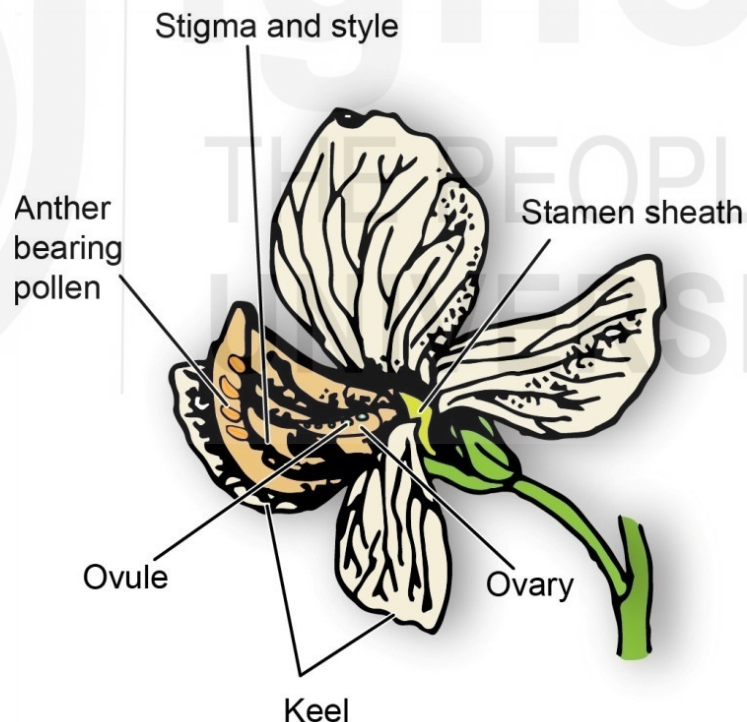


Fig. 1.4: A pea flower that is partially cut to show the reproductive parts.

Cross-pollination can be encouraged experimentally either by removing stamens from female parent and placing pollen from another variety on the stigma of its flowers (Fig. 1.5a), or by placing the pollen from the male parent on the stigma of a different plant, whose stamens have been removed (Fig. 1.5b). Therefore, with the common pea plant, the geneticists can perform crosses in the way they choose and can easily establish lineage or pedigree of each plant in a particular cross.

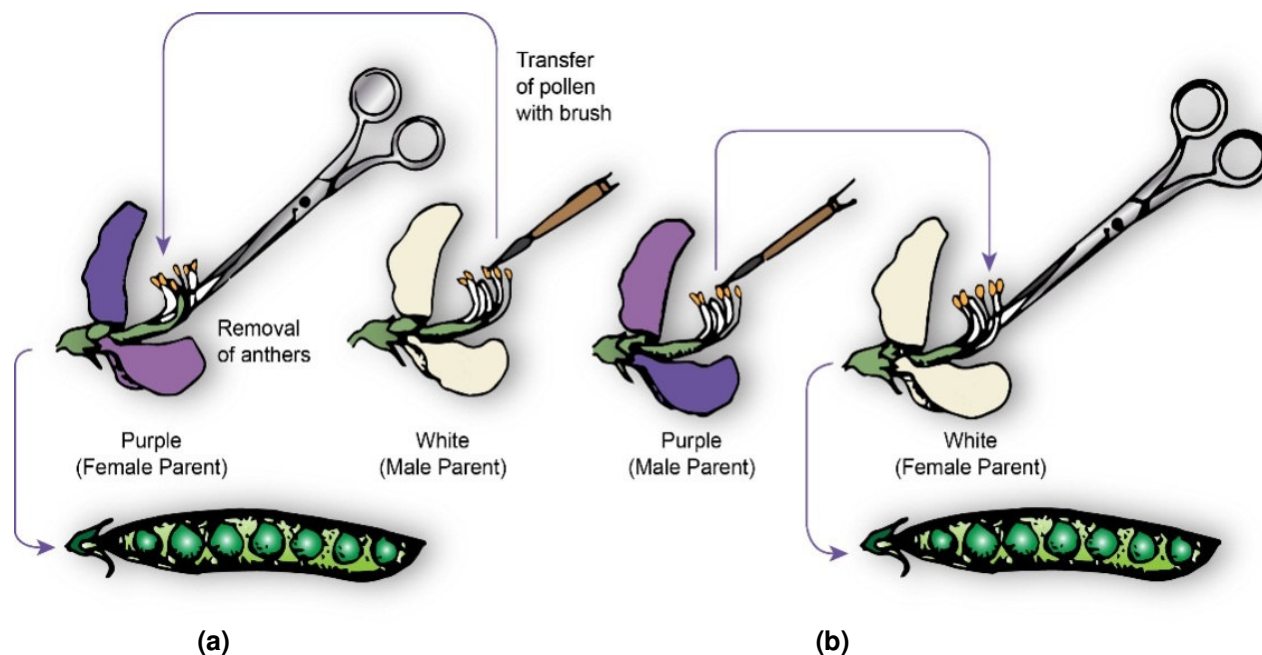


Fig. 1.5: Cross pollination in pea. In a) the anthers are removed in the purple flowers to prevent self-pollination. Similarly, b) anthers are removed in the white flowers and it is used for cross-pollination.

The pea plants varied with respect to a number of characteristics such as plant height, seed texture, seed colour and flowers colour. Such a variation is essential if anything at all is to be learnt about the inheritance of any character. If, for example, all pea plants were of the same height and had the same flowers colour generation after generation, information would be gained from following plant height and flower colour in genetic studies. A characteristic must have alternative traits or variant forms that can be followed if insight is to be gained regarding the inheritance pattern.

We have just discussed Mendel's choice of material for experimentation. You will find an explanation of Mendel's experiments and how he deduced the laws of inheritance from them in the following sections.

1.4.2 Monohybrid Crosses and Mendel's Law of Segregation

Before we discuss Mendel's experiment let us clarify the terminology encountered in breeding experiments. The Parental generation is called the **P** or **P₁ generation**. The progeny produced from the mating of individuals in the parental generation is called the **first filial generation** or **F₁**. The subsequent generation produced by breeding of the **F₁** offspring is termed the **F₂** generation. Selfing the offspring of each generation results in **F₃, F₄, F₅ generation** and so on.

Mendel's Experiments and Results

Mendel undertook the study of seven characters of pea plant and their variant forms as shown in Figure 1.6. Each trait was studied individually such as tall versus dwarf, red flower colour versus white, yellow seeds versus green and so on. He performed monohybrid crosses – crosses in which only one pair of contrasting or alternative trait is involved. A cross is a mating between two individuals leading to the fusion of gametes. For example, to study the inheritance pattern of height, he crossed true breeding tall plants with dwarf plants. True breeding lines are those plants which produce progeny exactly

like themselves when allowed to self pollinate normally. Thus, he obtained true breeding tall (TT) and dwarf (tt) plants. Using these true monohybrid crosses, homozygous tall and dwarf plants (P1 generation) were crossed to give offspring (F1) which were all tall (Fig. 1.7). The same results were obtained whether he used the tall plant as male or female parent, i.e.,

Tall female (♀) × male (♂) ----- All offspring tall

Dwarf female (♀) × tall male (♂) ----- All offspring tall

Such a cross is also called a reciprocal cross.

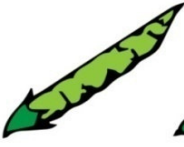
Characteristics		Traits	
a)	Height		
		Tall	Dwarf
b)	Seed shape		
		Round	Wrinkled
c)	Seed colour		
		Yellow	Green
d)	Flower colour		
		Red	White
e)	Flower position		
		Axial	Terminal
f)	Pod colour		
		Green	Yellow
g)	Pod shape		
		Inflated	Constricted

Fig. 1.6: The seven characteristics (a-g) in pea plant studied by Mendel. Each character has two well defined phenotypes that are easily recognized.

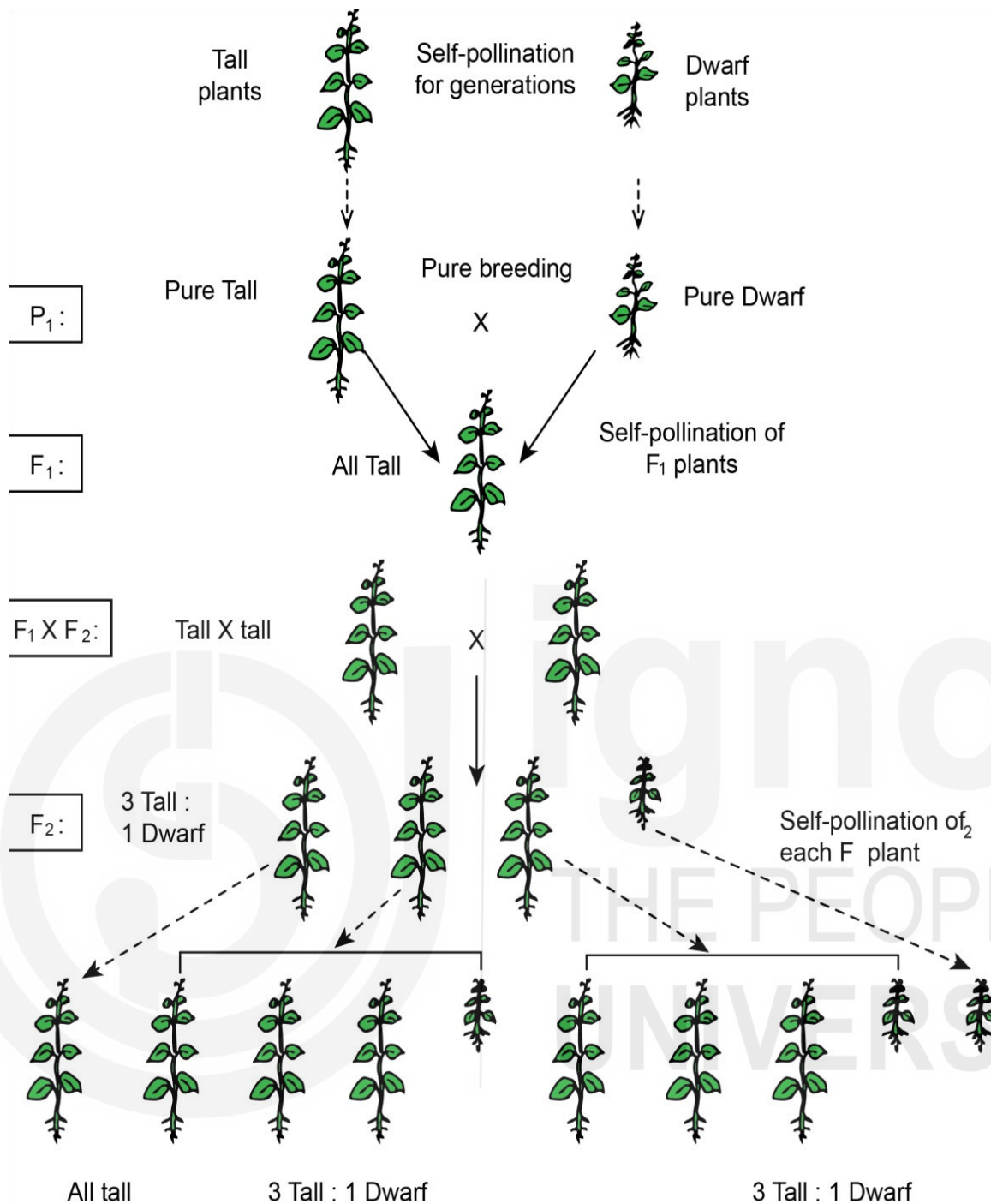


Fig. 1.7: Monohybrid cross in the pea plant followed through three generation.
The results of the reciprocal cross i.e., dwarf × tall are identical.

Since a cross was made between a pure breeding tall and pure breeding dwarf parent, Mendel surmised that these F₁ tall individuals were not the same as the P₁ tall parent because they had alleles from the dwarf parent as well. He allowed these F₁ individuals to self pollinate and studied the F₂ generation (Fig. 1.7 again). In one cross he actually obtained 787 tall and 277 short offspring. The ratio comes out to be 3:1. He further self pollinated (selfed) the offspring and found that the short plants continued to produce only short one (Fig. 1.7). On the other hand the tall plants were of two types: approximately one third of them produced only tall offspring upon self fertilisation; the remaining two thirds gave rise to both tall and short, again a ratio of approximately 3:1.

Using the same quantitative approach Mendel analysed the behavior of the six other pairs of traits. The results of such experiments were very similar to the ones obtained with reference to the height of the plant (Table 1.2).

Table 1.2: A Summary of Mendel's Results from the Seven Different Monohybrid crosses (modified from Ayala, F.J. and Kiger, J.A., 1984).

Character	The Phenotype of F ₁ Individuals	Number of F ₂ Individuals		
		Dominant	Recessive	Total
Seeds: smooth versus wrinkled	All smooth	5474	1850	7324
Seeds: yellow versus green	All yellow	6022	2001	8023
Flowers: purple versus white	All purple	705	224	929
Flowers: axial versus terminal	All axial	651	207	858
Pods: inflated versus pinched	All inflated	882	299	1181
Pods: green versus yellow	All green	428	152	580
Stem: tall versus short	All tall	787	277	1064
Total or average		14,949	5,010	19,959

Mendel's Explanation and Derivation of law of Segregation

Mendel questioned as to how a trait present in the P₁ generation could disappear in the F₁ and then reappear with full expression in the F₂ generation. Further, the F₁ progeny resembled only one of the parents in their phenotype and did not breed true. The F₁ progeny possessed the potential to produce F₂ progeny that resembled the parents of P₁ generation. Mendel concluded that each one of the alternate traits, the tallness and the dwarfness, was determined by a particulate factor. He reasoned that these factors which were transmitted from parents to progeny through the gametes, carried hereditary information. At present these factors are known as Genes. Since Mendel was examining pairs of contrasting traits each factor was considered to exist in alternate forms (which are known as alleles). Each of these alternate factors or alleles specifies a trait. For example, for the gene that controls the height of pea plant, there is one form or allele that results in the production of a tall plant and another allele that results in dwarf plant.

Mendel reasoned further that for pure breeding strains of the peas both egg and pollen must have carried or transmitted forms of the factor. Since both traits were seen in the F₂ whereas only one appeared in the F₁, each individual must have contained both factors, one for each of the alternate traits. Furthermore, since only one of the characters was seen in the F₁'s, the expression of the other trait must somehow have been masked by the other factor, a feature called dominance. For the tall × dwarf cross the F₁ plants were all tall. Thus, the allele for tallness is **dominant** to the allele for dwarfness. Conversely dwarfness is said to be **recessive** to tallness. Similar conclusions can be made for the other six pairs of characters. The dominant and recessive forms for each pair of traits are already indicated in Table 1.2.

We can understand the crosses more easily if we use symbols instead of pictures or Figures (Fig.1.7) for the alleles. In a cross between tall and dwarf plants, symbol T denotes the factor for tallness and the symbol t for dwarfness. You already know the convention that the dominant allele is denoted by the capital letters and the recessive allele is denoted by lowercase or small letters. Using these symbols we can depict the cross as shown in Figure. 1.8.

Since each parent is true breeding and each contains two copies of the same allele, thus, the genotype of the parental plant grown from the tall seeds is 'TT' and that of the dwarf plant is 'tt'. You may recall that the true-breeding individuals always carry a pair of same alleles, that is, they are homozygous genotypes.

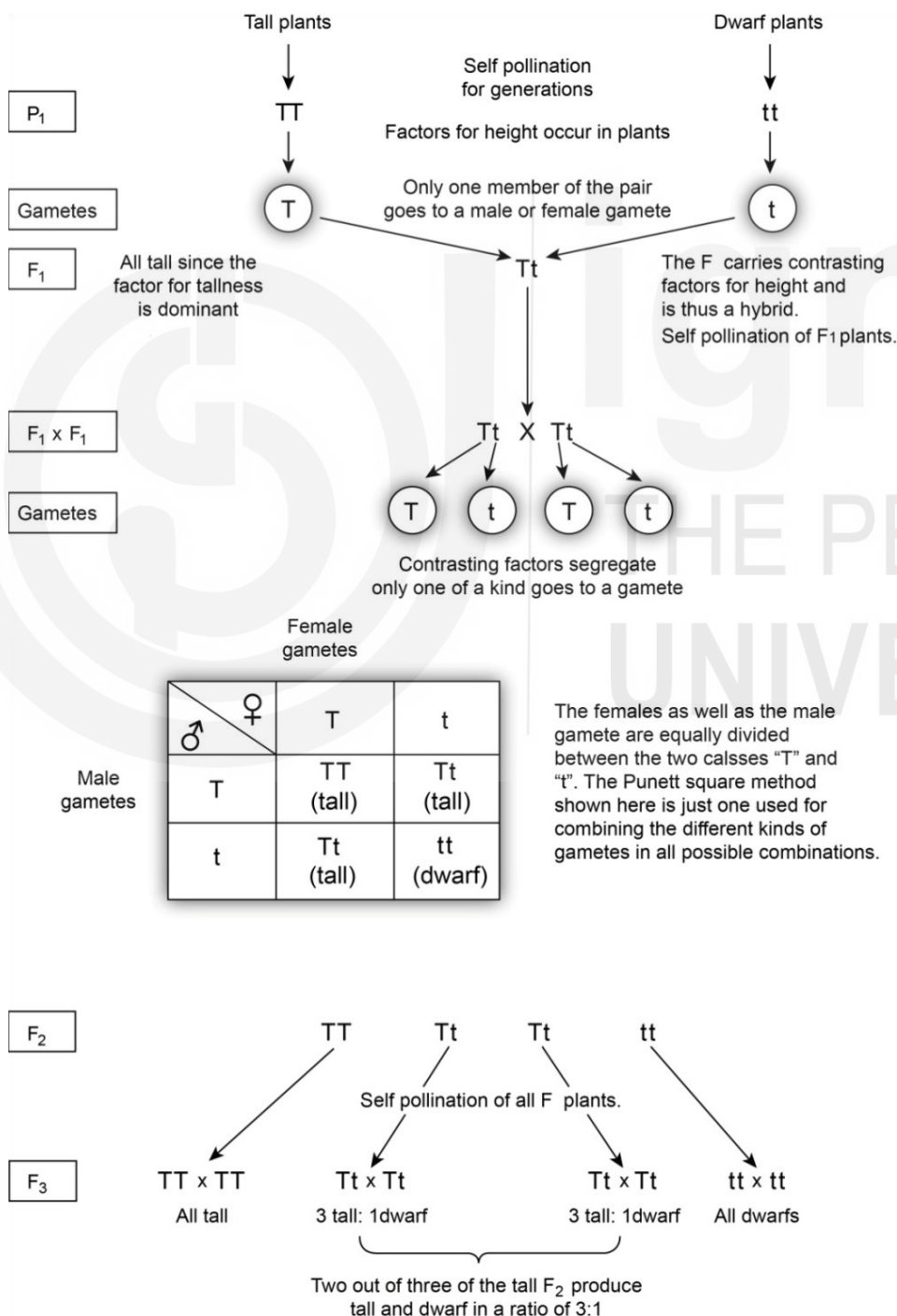


Fig. 1.8: Monohybrid cross of tall and dwarf pea plants. The results of the reciprocal cross are the same. Compare with Figure 1.8.

When these plants produce gametes by meiosis, each gamete contains only one copy of the gene (i.e., one allele), the plants from tall parents produce T-bearing gametes, and the dwarf plants produce t-bearing gametes. When these gametes fuse during the process of fertilization, the resulting zygote has both T and t factors, i.e., a genotype 'Tt' (Fig. 1.8). What are such plants that have two different alleles for a specific trait known as? Yes, it is heterozygous. Because of the dominance of the 'T' allele all the plants developed from the F₁ zygotes are phenotypically tall.

The plants derived from the F₁ generation differ from the tall parent in that they produce two types of gametes in equal numbers: T – and t – bearing. All the possible F₁ gamete combinations are represented in the matrix in Figure 1.8 called as a **Punnett square** after its originator R. Punnett. These gametic fusions/combinations give rise to the zygotes that produce the F₂ generation.

Three types of zygotes are produced : TT, Tt and tt. The relative proportion of these zygotes is 1:2:1 respectively, and the F₂ generation exhibits a phenotypic ratio of 3:1 (3 tall, 1 dwarf).

The results are the same for crosses involving the other six character pairs.

The Law of Segregation

Based on the results of the monohybrid cross, Mendel proposed his *first law*, known as the *law of segregation*. It states that the members of a gene pair (alleles) segregate (separate) from each other during the formation of gametes. As a result, half the gametes carry one allele and the other half carry the alternate allele. The progeny are produced by the random combination of gametes from the two parents. In proposing the law of segregation, Mendel clearly differentiated between the factors (genes) that determined the traits (the genotype) and the traits themselves (the phenotype). From modern perspective, this means that at the gene level the members of a pair of alleles of a gene on a chromosome, segregate during meiosis, so that any offspring receives only one member of the pair from each parent. This gene segregation parallels the separation of members of pairs of homologous chromosomes at anaphase I in meiosis.

To sum up, Mendel's first law states that the members of a gene pair (allele) segregate (separate) from each other while forming gametes; half the gametes carry one allele, and the other half carry the other allele.

Representing Crosses Using a Branch Diagram or Forked-line Method

The use of Punnett square to consider the pairing of all possible gametic types from the two parents (Fig. 1.9) is an acceptable way of predicting the ratios of genotypes in the next generation. There is, however, another method – known as the branch diagram or the forked-line method for representing the crosses. We require the application of our mathematical knowledge here.

Do you remember the basic principles of probability? If not, for your ready reference some basics of probability are given in Box 1.1.

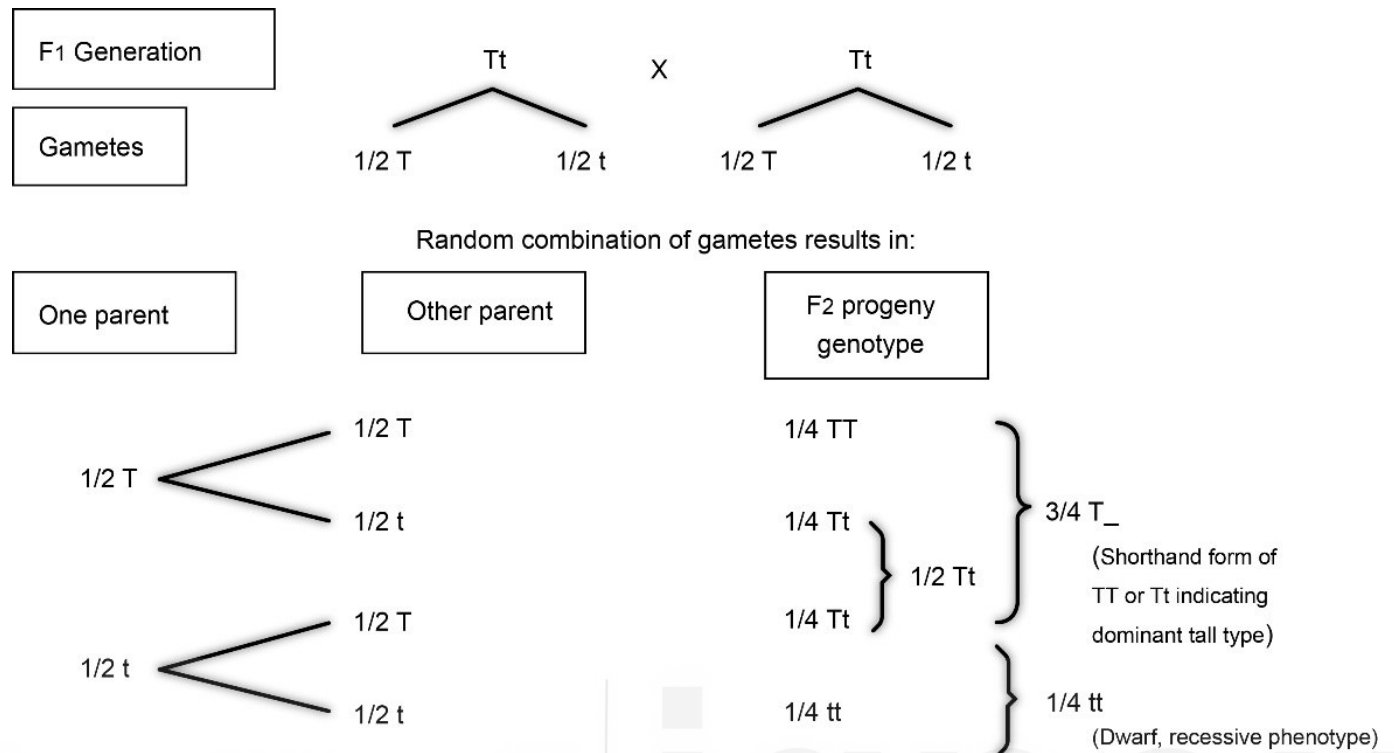


Fig. 1.9: Representing the cross as shown in Fig. 1.8 and 1.9 by a branch diagram method. This method helps in calculating the ratios of phenotypes of the F₂ generation in an easy way.

The F₁ plants have the genotype 'Tt'. Both eggs and pollen are produced in the flowers of the plants grown from these seeds. In the meiotic process an equal number of T and t gametes are expected to be produced so we can say that half the gametes are 'T' and the other half are 't'. Thus, 1/2 is the predicted frequency of these two types but just as tossing a coin many times does not always give exactly half heads and half tails, the gametic frequency may not be exactly realised. However, more the number of chances (e.g., tosses) the more likely you will obtain the true frequency.

From the rules of probability the relative expected frequencies of the three possible genotypes in the F₂ generation can be predicted. To produce a TT plant, an egg with T must fuse with a pollen grain carrying T. The frequency of T carrying eggs is 1/2 and the same holds good for the pollen grains also.

Therefore, the relative expected proportion of 'TT' plants in the F₂ is $1/2 \times 1/2 = 1/4$. A similar proportion is expected for dwarf or 'tt' progeny.

What about the Tt progeny? Again the frequency of 'T' in one gametic type is 1/2 and the frequency of t in the other gametic type is also 1/2. The first involves the fusion of an egg with T and a pollen with t, and the second involves the fusion of an egg with t and a pollen with T. Applying the product rule (Box 1.1) the probability of one or the other occurring is the sum of the individual probabilities, or $1/4 + 1/4 = 1/2$

From the above discussion, prediction is made that 1/4 of the F₂ progeny would be TT 1/2 would be Tt and 1/4 will be tt. These figures are exactly similar to those found with the method shown in Figure 1.9. Therefore, you can apply either of these methods for any monohybrid cross.

Box 1.1: Some Basics of Probability

A probability is the ratio of the number of times a particular event occurs to the number of trials during which the event could have happened. For example, the probability of picking a heart (from 13 hearts) from a deck of cards (52 cards) is $p(\text{heart}) = 13/52 = 1/4$. That is, we would expect, on the average to pick a heart from a deck of cards once in every four trials.

Probability and the *laws of chance* are involved in the transmission of genes. As a simple example, let's consider a couple and the chance that their child will be a boy or girl. Let us assume that an equal number of boys and girls are born. The probability that the child be a boy is $1/2$ or 0.5. Similarly, the probability that the child be a girl is $1/2$.

Let us see some rules of probability. Firstly, we take up the **product rule**. This rule states that the probability of two independent events occurring simultaneously is the product of each of their probabilities. Thus, the probability that a family will have two girls in a row is $1/4$. That is, the probability of the first child being a girl is $1/2$, the probability of the second being a girl is also $1/2$, and the product rule of the probability of the first and the second being girl is $1/2 \times 1/2$ or $1/4$. Similarly, the probability of having three boys in a row is $1/2 \times 1/2 \times 1/2 = 1/8$.

The **sum rule** of probability states that the probability, that one of two mutually exclusive events will occur is the sum of their individual probabilities. For example, if two dice are thrown, what is the probability of getting two sixes or two ones? The probability of getting two sixes is found by using the product rule. The probability of getting one six, $p(\text{one six})$ is $1/6$, since there are six faces to a dice. Therefore, the probability of getting two sixes, $p(\text{two sixes})$, when two dice are thrown is $1/6 \times 1/6 = 1/36$. Similarly, $p(\text{two ones}) = 1/36$. To role two sixes or two ones involves mutually exclusive events, so the sum rule is used to find the probability. The answer is $1/36 + 1/36 = 1/18$.

When a plant is crossed with any of its parents, it is called a **backcross**. And when a plant is specifically crossed **with** its homozygous, recessive parent, then it would be called a **testcross** or **test backcross**.

Confirmation of the Law of Segregation: The use of Test Crosses

Mendel did a number of tests to ensure the validity of his results while formulating his law of segregation. He continued the self-fertilisations to the F_6 generation and found that in every generation both the dominant and recessive characters appeared. He concluded that the law of segregation was valid no matter for how many generations it was carried out.

In another experiment, instead of allowing the F_1 heterozygous individuals to self-fertilise, he crossed them back to the recessive parent. Such a cross to a recessive is called a **test cross** or some times a **back cross** (see the adjacent Margin Remark) because it is used to test whether a dominant individual is heterozygous or homozygous. For example, let us consider the case of smooth and wrinkled seeds to understand it more clearly. The smooth (S) character is dominant over the wrinkled (s). In the Figure 1.11 see the two crosses (a & b).

In the Figure 1.10 you can see cross a. Here the gametes produced by parent 1 are of two types S and s, whereas the second parent produces only one kind of gametes, i.e., s. As a result of random fusion of gametes, half the progeny of the test cross are Ss heterozygotes having a smooth phenotype. Owing to the dominance of the S allele, and the other half are ss homozygotes and have a wrinkled phenotype. Test crosses are useful in determining the genotype of F₁ individuals as well as their parents.

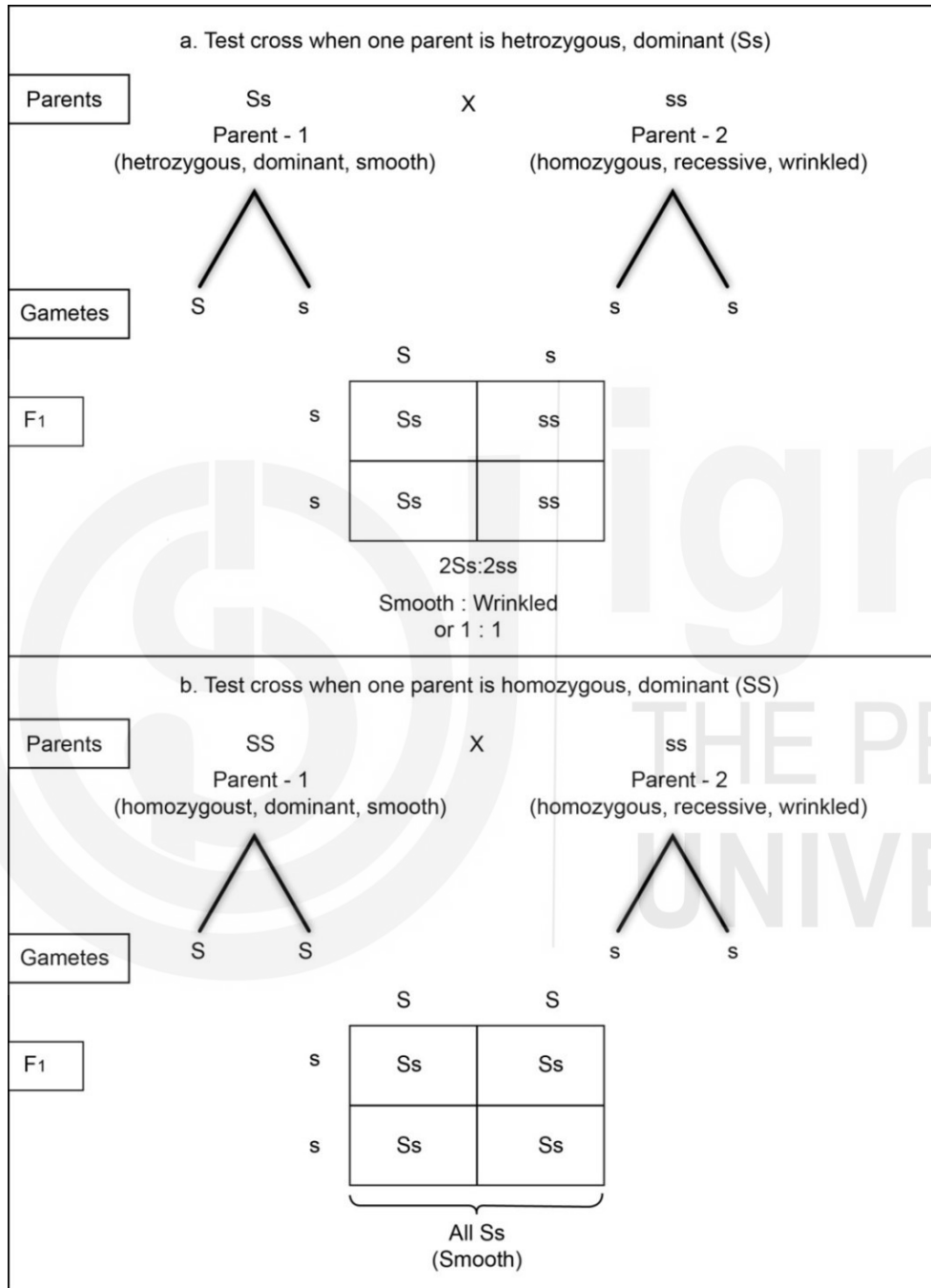


Fig. 1.10: (a & b) Cross with a recessive parent and to confirm the law of segregation.

The ratio 1:1 of dominant to recessive phenotypes would indicate that the F₁ smooth seeds used in the test cross are heterozygous. We can similarly predict the outcome of a test cross of a homozygous dominant (Fig 1.11, cross b). The first parent produces only one kind of gametes, that is, S, and so does the other, producing only s gametes. Thus, all the progeny are Ss and have smooth seed phenotype.

Therefore, we can conclude that if an organism in a test cross gives rise to progeny with the dominant phenotype, then the test-crossed plant must have been homozygous dominant (as shown in cross b above). For the Ss plant a 1:1 ratio of dominant to recessive phenotypes is expected as is seen in test cross 'a'.

The above discussion highlights the fact that the phenotypes of the progeny of the test cross indicates the genotype of the individual tested. If all the progeny show dominant phenotype, the individual tested is homozygous dominant. If there is an approximately 1:1 ratio of progeny with dominant and recessive phenotypes, then the individual is heterozygous.

Reasons for Mendel's Success

Mendel's success with the hybridisation experiments was mainly due to the following reasons:

First, careful choice and selection of experimental material, that is, garden pea. Besides several advantages it offers as an experimental material as described in Subsection 1.4.1, it must be mentioned that it is a diploid organism having two sets of chromosomes. Through many generations of natural self fertilization, garden peas had developed into pure lines, each of which could be differentiated by its own characteristic features. Furthermore, in the seven pairs of contrasting traits, Mendel chose to study the two parental plants exhibiting well-defined, visible, contrasting morphological traits. The factors responsible for the expression of the seven pairs chosen for study are located on seven different chromosomes which was a fortunate coincidence and this avoided the phenomenon of linkage.

Second, much of Mendel's success in his experiments may be attributed to his good judgement in making crosses between the parents that differed in only one trait. In other words, he considered only one trait at a time when analysing the progeny of a cross.

Thirdly, he methodically documented the results of his experiments.

Fourthly, by the application of mathematics he could analyse the segregating populations and make proper interpretations.

SAQ 3

Pollen from a tall pea plant was dusted on the stigma of a flower from a dwarf plant. What would be the reciprocal cross?

SAQ 4

Put a tick mark (✓) on the correct choice:

- (a) The offspring of mating between two pure strains are called:
- (i) hybrids
 - (ii) mutants

- (iii) the P_1 generation
- (iv) the F_2 generation
- (b) An organism with two copies of the same alleles is:
 - (i) homozygous for the trait
 - (ii) homozygous for the allele
 - (iii) heterozygous for the trait
 - (iv) heterologous for the allele
- (c) A woman without dimples marries a man who has dimples and who is known to be heterozygous for the trait. What is the chance their first child will have dimples?
 - (i) one in four
 - (ii) one in two
 - (iii) three out of four
 - (iv) it is certain
- (d) A test cross is done to find out:
 - (i) the genotype of an individual by examining the phenotypes of its offspring from a particular mating
 - (ii) the genotype of an individual by testing for its DNA content
 - (iii) whether a mating is fertile
 - (iv) whether two species can inter-breed
- (e) A test cross distinguishes between
 - (i) two homozygous forms
 - (ii) a homozygous dominant and the heterozygous form
 - (iii) two heterozygous forms
 - (iv) a homozygous recessive and a heterozygous form
- (f) The allele for black hair colour (B) is dominant over the allele for white hair colour (b) in guinea pigs. A test cross between a black male and a white female produced a litter of five black and one white guinea pigs. The genotype of the father is:
 - (i) unknown, due to the small sample size
 - (ii) BB
 - (iii) Bb
 - (iv) bb

1.4.3 Dihybrid Crosses and Mendel's Law of Independent Assortment

The experiments of Mendel that we have discussed so far have dealt with crosses between plants differing in only one character or alleles at one locus. The most logical extension of Mendel's experiments is to determine what happens when more than one pair of characters is simultaneously involved in the cross. He performed a number of dihybrid crosses in which the parents made different contributions for two traits. From these experiments he proposed his **second law, the law of independent assortment**. *This law states that the factors or genes for different traits assort independently of one, another.* In other words, the genes on different chromosomes behave independently for the production of gametes.

Let us examine the results of Mendel's experiments in which he crossed plants that differed in two characters. An example is mating of plants involving smooth or round (S)/wrinkled (s) and yellow (Y)/ green (y) seed traits. As you already know, yellow is dominant to green and smooth is dominant to wrinkled. Mendel made crosses between true breeding smooth, yellow plants (SSYY) and wrinkled, green plants (ssyy). The results are shown in the Figure 1.11.

All the F_1 seeds from this cross were smooth and yellow as predicted from the results of monohybrid crosses. The smooth yellow parents produce SY gametes which give rise to SsYy zygotes when fused with the sy gametes from other parent. Because of the dominance of the smooth and yellow traits, all F_2 seeds are smooth and yellow. As in the monohybrid crosses, the F_1 were selfed to give rise to the F_2 generation.

Mendel thought of two possible outcomes. One, that the genes for the traits from the original (P_1) parent would be transmitted to the progeny together. If this was the case then a phenotypic ratio of 3:1 smooth-yellow: wrinkled-green would be obtained. The second possibility was that each of the traits would be inherited independently of the other.

Let us examine the Figure 1.12 carefully. The dihybrid plants grown from the F_1 seeds produce four types of gametes: SY, Sy, sY and sy. Because of the independence of the two pairs of genes there is an equal frequency of each gametic type. When the F_1 plants are selfed, these four types of gametes fuse randomly in all possible combinations. All the possible 16 gametic fusions are represented on the Punnett square in the above figure. In the dihybrid cross, 9 different genotypes are obtained but because of dominance only 4 phenotypes are observed.

$$\left. \begin{array}{ll} 1 \text{ SSYY,} & 2 \text{ SsYY} \\ 2 \text{ SSYy,} & 4 \text{ SsYy} \end{array} \right\} = 9 \text{ smooth, yellow}$$

$$1 \text{ SSyy,} \quad 2 \text{ Ssyy} = 3 \text{ smooth, green}$$

$$1 \text{ ssYY,} \quad 2 \text{ ssYy} = 3 \text{ wrinkled, yellow}$$

$$1 \text{ ssyy,} \quad = 1 \text{ wrinkled, green}$$

Mendel's actual observations are shown in the Table 1.3.

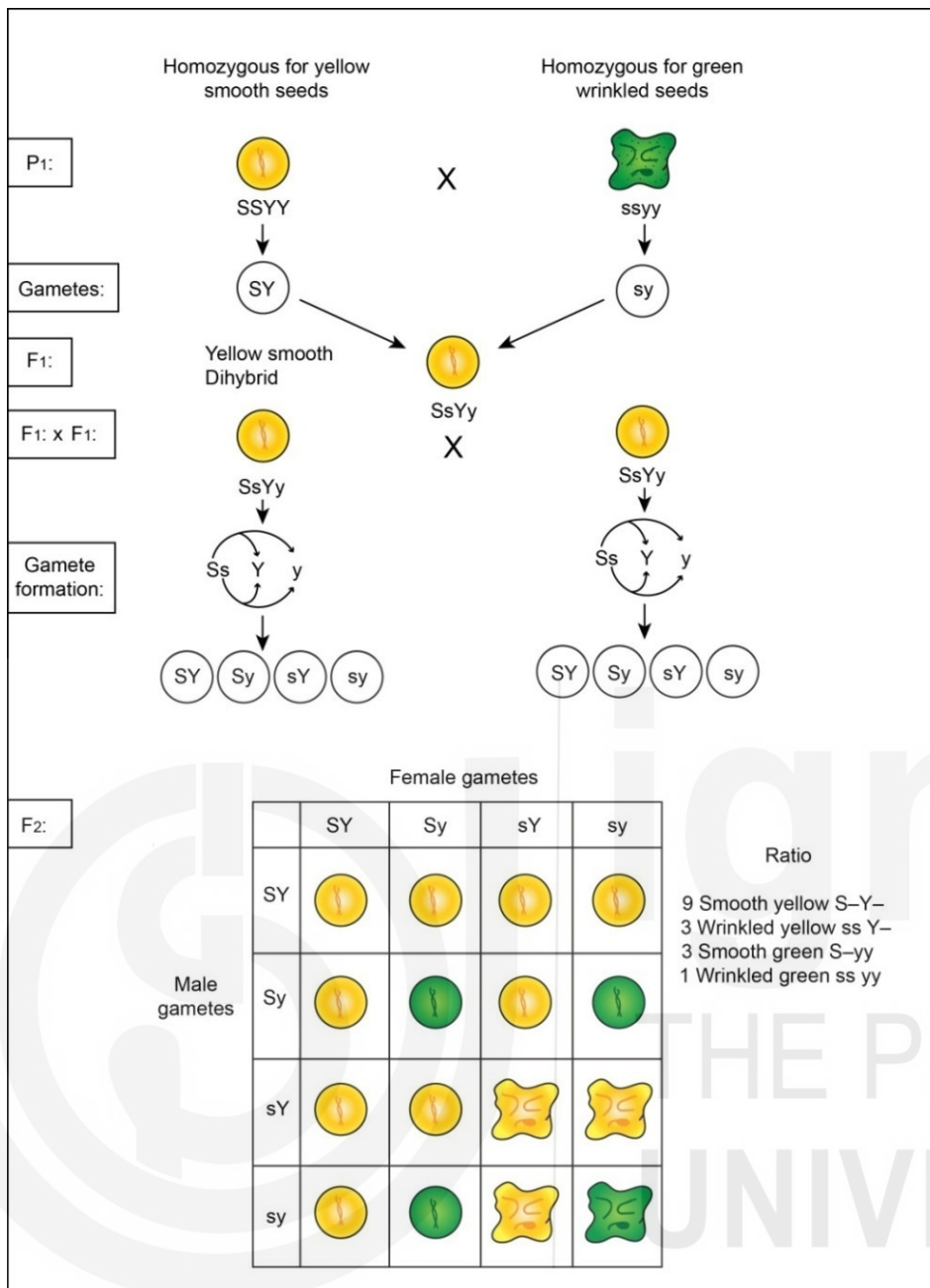


Fig. 1.11: A dihybrid cross followed through the second generation. On inspecting the Punnett square one can find nine different genotypes in the F₂ generation. Because of dominance in one member of each pair of alleles, only four phenotypes are expressed.

Table 1.3: Results of Mendel's Dihybrid Cross

Generation	Seed Type	Number	F ₂ Ratio
Parents	Round, green vs wrinkled, yellow		
F ₁	Round, yellow		
F ₂	Round, yellow	315	9.84
	Wrinkled, yellow	101	3.16
	Round, green	108	3.38
	Wrinkled, green	32	1.00
		556	

From this example, it is clear that if two pairs of characters are inherited independently then the selfing of F_1 individuals will yield a 9:3:3:1 ratio in the F_2 generation. This ratio arises as a result of independent assortment of two gene pairs into gametes and of the random fusion of these gametes. The 9:3:3:1 ratio is the product of two 3:1 ratios, that is, $(3:1)^2 = 9:3:3:1$. This prediction was met in all dihybrid crosses that Mendel performed. In every case the F_2 ratio was close to 9:3:3:1. Based on the results, Mendel concluded that the factors (genes) determining different traits were transmitted independently.

Let us now sum up as to what we have learnt in this section so far. *The principle of independent assortment states that genes on one chromosome segregate independently of those on another chromosome in the production of gametes.*

Branch Diagram of a Dihybrid Cross

As is already apparent, it is quite a tedious, time and space consuming job to construct a Punnett square of gametic combinations, then to count the numbers of each phenotypic class from all the genotypes produced. This exercise is not too difficult for dihybrid crosses, but for more than two gene pairs it becomes rather complex. So, it is better that we get into the habit of calculating the expected ratio of phenotypic classes by using a branch diagram.

We shall use the same example as the one used in Figure 1.12 where two gene pairs assort independently into gametes. As you have seen earlier that on selfing F_1 of an Ss heterozygote gave rise to progeny of which three fourths were smooth and one fourth were wrinkled. Genotypically, the former class had at least one dominant S allele, that is, its members were SS or Ss. A convenient way to signify this situation is to use a dash to indicate an allele that has no effect on the phenotype. *Thus, S-means that phenotypically the seeds are smooth and genotypically they are either SS or Ss.*

Now consider the F_2 produced from a selfing of Yy heterozygotes; again a 3:1 ratio is seen with three-fourth of the seeds being yellow and one-fourth of the seeds being green. The same holds good for the smooth vs wrinkled seeds in the F_2 ratio. Let us now find out the expected proportion of F_2 seeds that are smooth and yellow. This ratio can be obtained by the product of the probability that an F_2 seed will be smooth and the probability that it will be yellow, or $3/4 \times 3/4 = 9/16$ (Fig. 1.13, 1st ratio). Similarly, the expected proportion of F_2 individuals that are wrinkled and yellow is $3/4 \times 1/4 = 3/16$ (Fig. 1.13, 2nd ratio). Extending this calculation to all possible phenotypes, as shown in the Figure, we obtain the ratio 9:3:3:1.

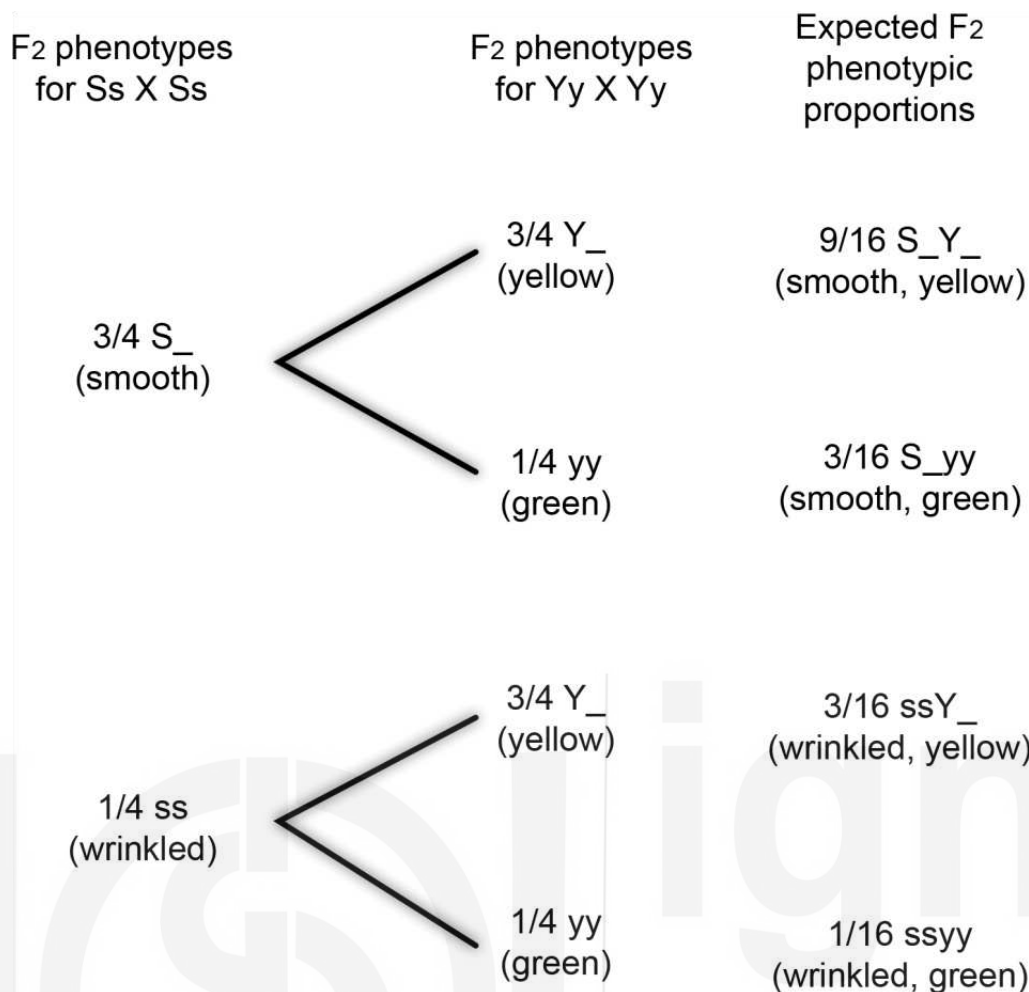


Fig. 1.12: The example used here is the same as that of Fig. 1.11. The above Figure shows the calculation of the F₂ phenotypic ratio by the branch diagram method.

If one wishes to know the genotype of the F₁'s and the F₂'s from a dihybrid cross, a test cross may be used. The example that we shall use to explain this point is already familiar to you that is of smooth/wrinkled, and yellow/green characters of pea seeds. The F₁ here is a double heterozygote (Fig. 1.13) SsYy. As you know this F₁ (Fig. 1.13). You may recall that in a test cross, we use a doubly homozygous recessive plant and in this case it is ssyy. A test cross like this one, shows a 1:1:1:1 ratio in the offspring of SsYy: ssYy:ssyy genotypes which means a 1:1:1:1 ratio of smooth-yellow:smooth-green:wrinkled-yellow:wrinkled-green phenotypes. This 1:1:1:1 phenotype ratio is diagnostic of test crosses in which the "unknown" parent is a double heterozygote.

In the F₂ of a dihybrid cross there are nine different genotypic classes but only four phenotypic classes. The genotypes can be ascertained by test crossing. Use Table 1.4 for obtaining the genotypes. Substitute A, a, B and b in the table with the respective character involved, i.e., with S, s Y or y. The test crosses in the table (from top to bottom) lists the 9 genotypic classes and the top column (from left to right) indicates the four phenotypic classes.

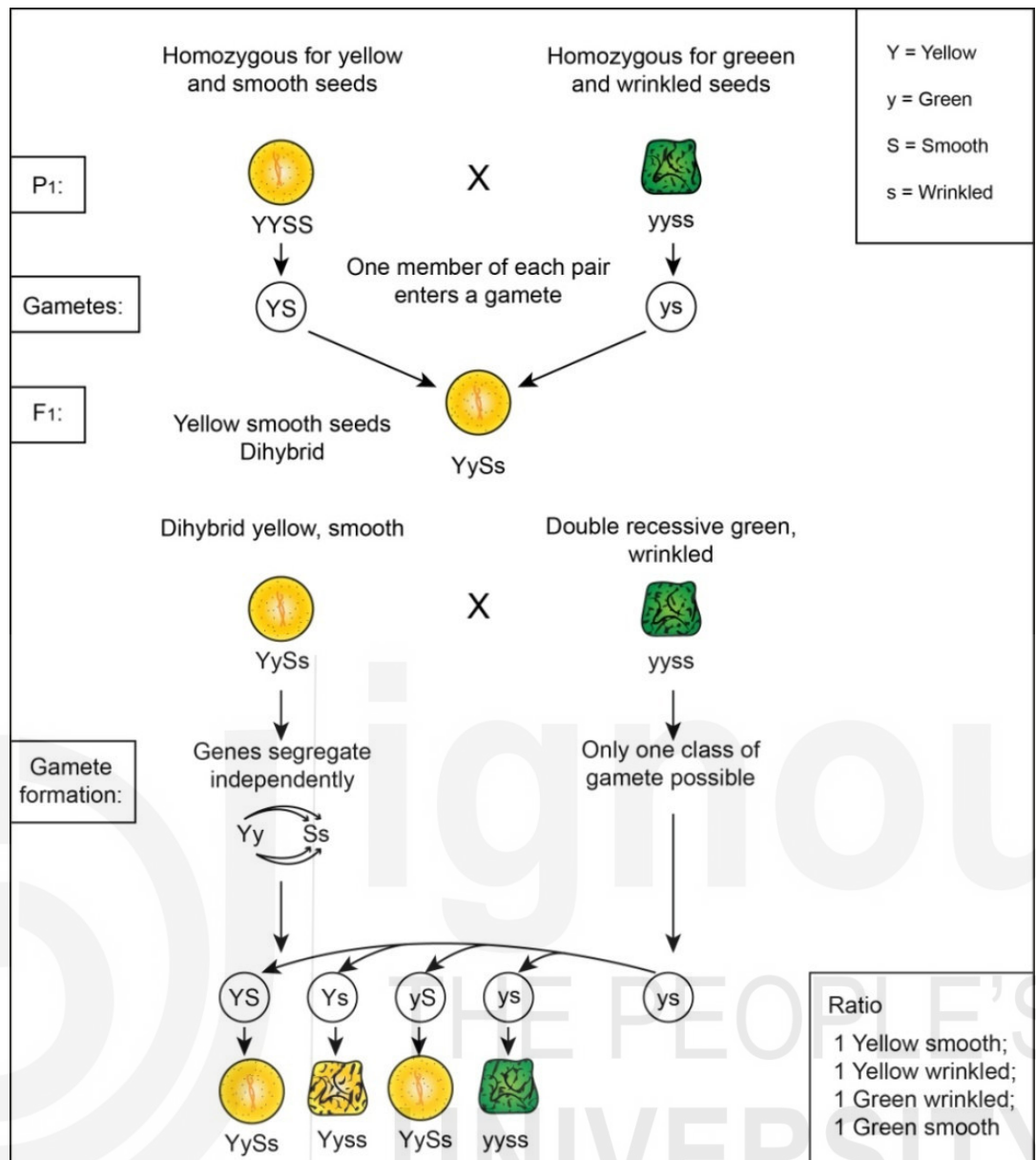


Fig. 1.13: A dihybrid (YySs) being test-crossed by double recessive, green wrinkled parent (yyss).

Table 1.4: Proportions of Phenotypic classes Expected from test crosses of strains with various genotypes for two gene pairs.

Test crosses	Proportion of Phenotypic Classes			
	A- B-	A- bb	aa B-	aa bb
AABB × aabb	1	0	0	0
AaBB × aabb	1/2	0	1/2	0
AABb × aabb	1/2	1/2	0	0
AaBb × aabb	1/4	1/4	1/4	1/4
AAbb × aabb	0	1	0	0
Aabb × aabb	0	1/2	0	1/2

aaBB × aabb	0	0	1	0
aaBb × aabb	0	0	1/2	1/2
aabb × aabb	0	0	0	1

From the above discussion it can be concluded that a test cross is a truly diagnostic approach to confirm the genotype.

SAQ 5

Given below is a Punnett square, go through it carefully and then answer the following question.

		Female			
		AB	Ab	AB	Ab
Male	AB	AABB	AABb	AABB	AABb
	Ab	AABb	1	AABb	AAbb
	aB	AaBB	AaBb	2	AaBb
	ab	AaBb	Aabb	AaBb	aabb

Key : A and B are dominant

a and b are recessive

- The genotype of the male parent is
- The genotype of the organism in box 1 is
- The organism in box 2 is for trait 'A'.
- The organism in box 2 exhibits dominant genetic traits.

1.4.4 Trihybrid Crosses

Mendel also applied his laws to the inheritance of three characters in other garden pea crosses. He found that both these laws hold good in this case also. Such crosses involving three characters are called trihybrid crosses. In such crosses too, the proportions of F₂ genotypes and phenotypes are predicted precisely with the same logic used before, with each character pair considered independently. You can try drawing a Punnett square to find out the genotypes as well as the phenotypic ratios, but here a branch diagram is given to show the derivation of the relative frequencies of the phenotypic classes. In the example illustrated in the Figure 1.14, the independently assorting character pairs in the cross are: smooth versus wrinkled seeds; yellow versus green seeds, and purple versus white flowers, there are 64 combinations of 8 maternal and 8 paternal gametes. Combination of these gametes give rise to 27 different genotypes and 8 different phenotypes.

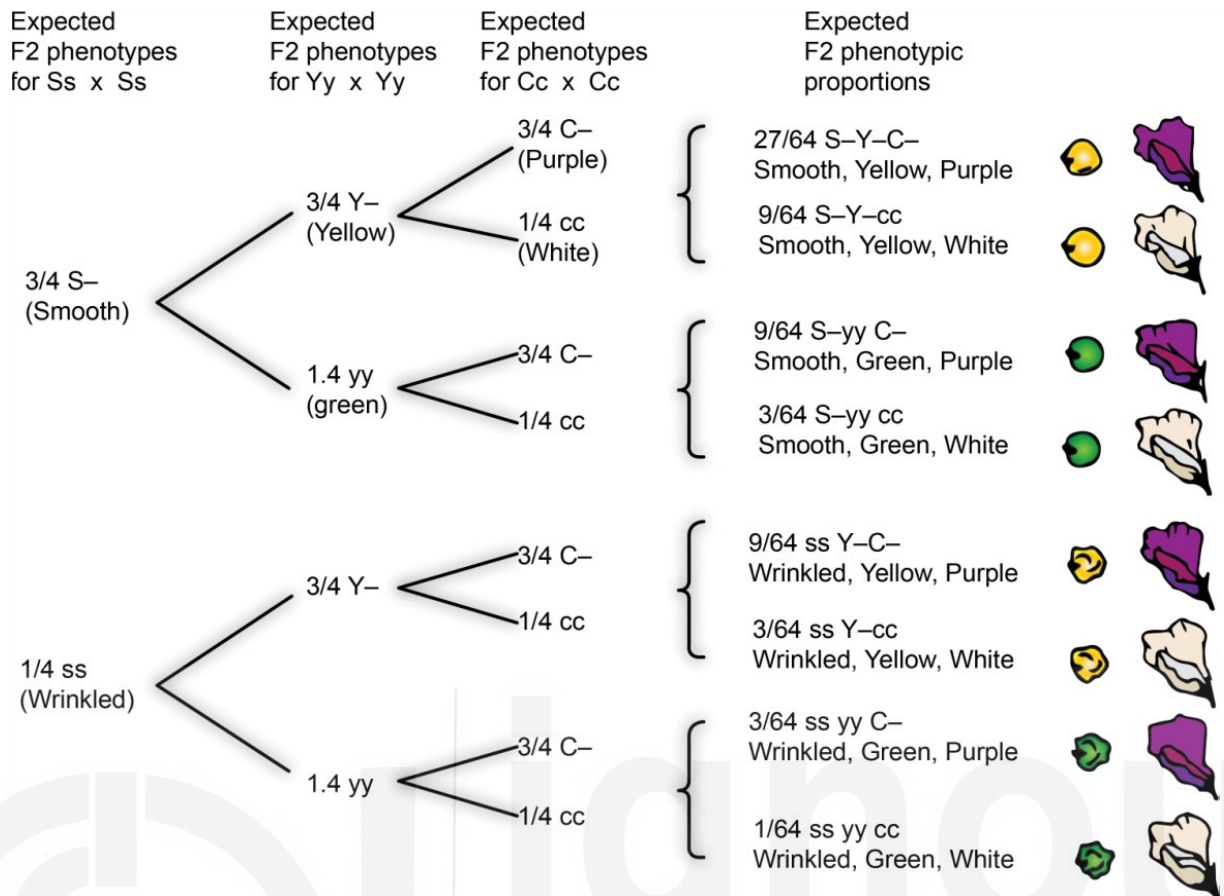


Fig. 1.14: Branch diagram of a trihybrid cross. The ratio of the eight phenotypic classes above are calculated in the same manner as you have learnt in Figs. 1.10 and 1.13.

1.5 SUMMARY

In this unit you have learnt:

- Genetics is a branch of biology concerned with the structure, transmission and expression of hereditary information. More than a century ago, an Austrian Monk, Gregor Mendel worked on garden peas. He analysed the data mathematically and laid foundation for the science of modern genetics.
- Genes are present on chromosomes. The term locus refers to the site a gene occupies in chromosome.
- Different alternate forms of particular gene are called alleles; they occupy corresponding loci on homologous chromosomes.
- An individual that carries two identical alleles for a given locus is said to be homozygous for that locus. If the two alleles are different then the individual is said to be heterozygous.
- According to Mendel's law of dominance, one allele (the dominant allele) masks the expression of the other allele (the recessive allele) in a heterozygous individual. For this reason an individual's appearance (phenotype) may be different from the genetic constitution (genotype).

- According to Mendel's law of segregation, during meiosis, the alleles for each locus separate or segregate, from each other as the homologous chromosomes separate. When haploid gametes are formed, each will contain one and only one allele for each locus.
- According to Mendel's law of independent assortment, during meiosis, each pair of alleles will segregate independently of the pairs. Alleles at different loci, therefore, assort randomly into the gametes.
- A cross between homozygous parents (P generation) that differ from each other with respect to their alleles at one locus is called a monohybrid cross; if they differ at two loci, it is a dihybrid cross. The first generation of offspring is heterozygous and is called the first filial or F_1 , generation; the generation produced by a cross of two F_1 individuals is the second filial or F_2 , generation. If a F_1 individual is crossed with a homozygous recessive individual the cross is known as a test cross.
- Mendel first demonstrated that inheritance was based on the segregation of alleles at a locus. Through self-fertilisation of F_1 garden pea plants, obtained from crosses between plants with different characteristics, he observed a 3:1 ratio of dominant to recessive phenotypes in the F_2 progeny. The law of segregation developed from these experiments states that heterozygous individuals produce equal proportions of gametes containing members of a pair of alleles. Mendel's second principle of independent assortment states that alleles at different loci assort independently of each other resulting in a 9:3:3:1 ratio in F_2 progeny from a dihybrid cross, and in a 27:9:9:9:3:3:3:1 ratio in a trihybrid cross.

1.6 TERMINAL QUESTIONS

1. Write whether the following statements are True (T) or False (F).
 - a) Mendel crossed different type of pea plants to blend their characteristics.
 - b) Homozygotes have identical alleles for a given trait on homologous chromosomes.
 - c) An organism having the genotype Tt makes only one type of gametes.
 - d) All organisms with identical phenotypes have identical genotypes.
 - e) The phenotype determines whether an organism is heterozygous for that trait.
 - f) Phenotype determines whether an organism is heterozygous or homozygous.
 - g) Any organism displaying a recessive trait must be homozygous for that trait.
 - h) A cross between a homozygous dominant tall pea plant and a heterozygous tall pea plant yields 100% tall plants.

- i) The inability for a person to roll his tongue represents a complete absence of the dominant gene (assuming tongue rolling is the dominant trait).
 - j) A cross between a homozygous dominant male and a heterozygous dominant female would not have any homozygous recessive.
2. Two long-winged flies were mated. The offspring consisted of 77 flies with long wings and 24 flies with short wings. Is the short-winged condition dominant or recessive? What are the genotypes of the parents?
 3. The ability to roll the tongue into almost circle is conferred by a dominant gene in humans, while its recessive allele fails to confer this ability. A man and a woman are heterozygous for tongue rolling, and have three sons. These three sons marry women who are not tongue rollers. Assuming that each of the three sons has a different genotype, what proportion of their children might have the ability to roll their tongues.
 4. Normal length of fur in rabbit is controlled by the dominant allele R, and a short type of fur called "rex" is determined by the recessive allele r. The dominant allele B is responsible for black fur colour; while the recessive allele b determine brown.
 - a) Diagram a dihybrid cross between a homozygous rabbit with normal-length black fur and rex rabbit with brown fur. What are the phenotypic ratio resulting from this cross?
 - b) What proportion of the normal, black rabbit in the F₂ generation of this cross can be expected to be homozygous for both pairs of genes?
 - c) What would be the expected phenotypic and genotypic results of a back cross between a member of the F₁ generation and a fully recessive rex, brown parent?

1.7 ANSWERS

Self Assessment Questions

1.
 - a) heterozygote, genotype
 - b) homozygous dominant, genotype
 - c) heterozygote, genotype
 - d) phenotype
 - e) homozygous dominant, genotype
 - f) heterozygote, genotype
 - g) homozygous recessive, genotype
 - h) phenotype
 - i) homozygous recessive, genotype

2. (a) iv, (b) i, (iii) iii, (iv) ii.
3. Pollen from dwarf plant dusted on the stigma of a tall plant.
4. a) i, (b) i, (c) ii, (d) i, (e) ii, (f) iii.
5. (a) AaBb, (b) AAbb, (c) heterozygous, (d) 2.

Terminal Questions

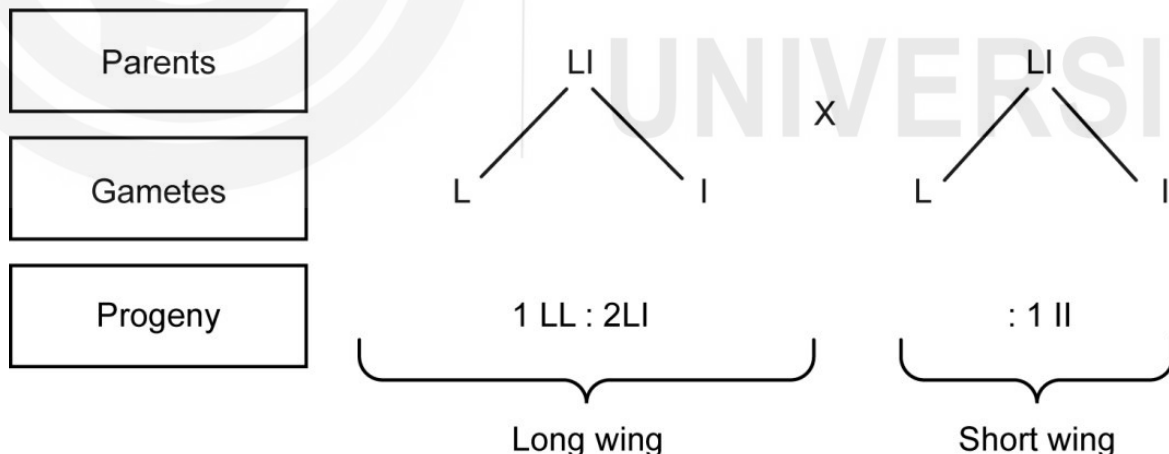
1. a) False, b) True, c) False, d) False, e) False,
f) False, g) True, h) True, i) True, j) True.
2. When we are not told which of the characteristics is dominant and which is recessive, we can deduce it from the ratio of phenotypes in the progeny. We know that 77 flies have long wings and 24 have short wings. This gives us an approximate ratio of 3 long-winged flies to every 1 short-winged fly.

$$77/24 = 3/1$$

As you know, the three-to-one ratio signifies that dominant and recessive characteristics are most likely involved. Since there are 3 long-winged ones, it suggests that short-wingedness is recessive.

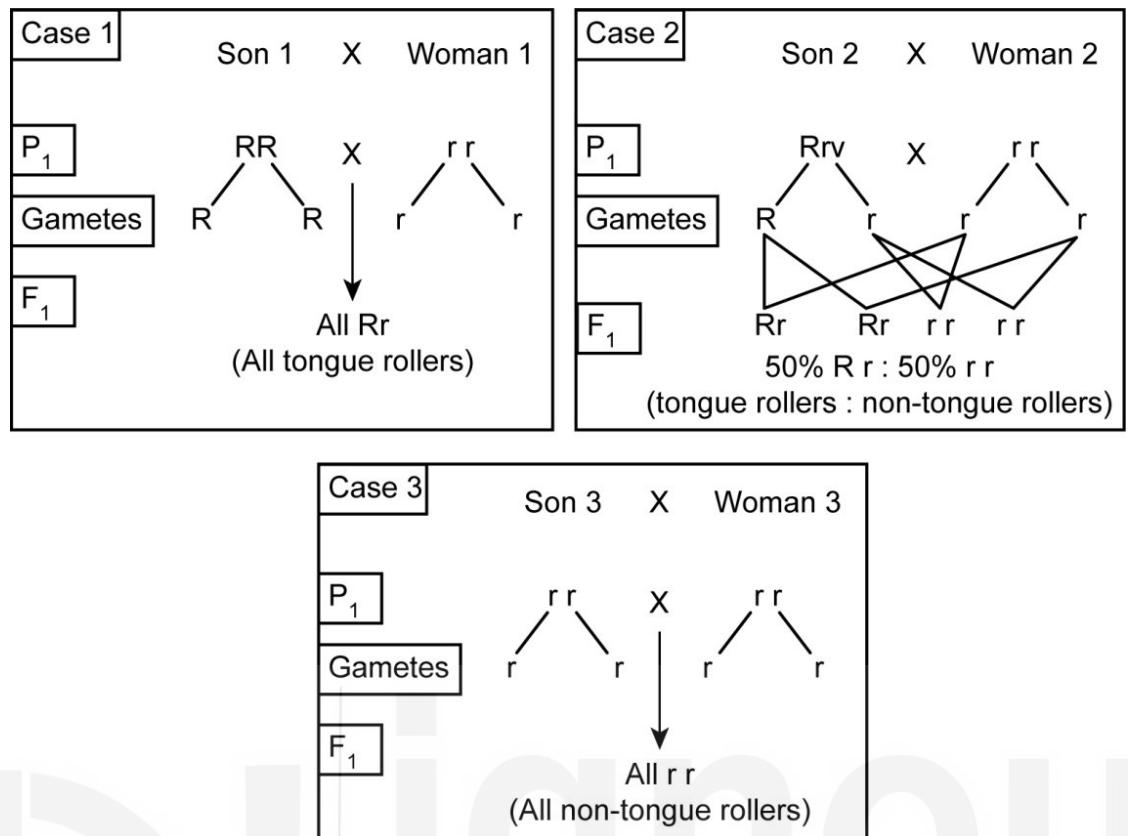
The presence of short-winged flies (homozygous recessive) in the progeny suggests that both parents carry the recessive gene and are thus heterozygotes.

Let L be the gene for long wings in flies and l be the gene for short wings. In the cross between two long-winged heterozygous parents:



3. Let us represent the dominant allele for the trait of tongue-rolling by R, and the recessive allele by r. Only three possible genotypes could result from the cross of the heterozygous parents. These alleles are RR, Rr and rr. Let one of the sons be RR, therefore, a tongue roller; the second son Rr and also a tongue roller; and the third son rr (homozygous recessive) and thus unable to roll his tongue. All three sons have married women who are not tongue rollers and who are, therefore, rr.

Let us now determine the proportion of the offspring who will be able to roll their tongues.



4. (a) First draw the Punnett square of this cross. The genotype of the homozygous black rabbit with normal fur is BBRR and the genotype of the homozygous brown rex rabbit is bbrr. The result of the cross is shown in the figure given below.

Gametes	BR	Br	bR	br
BR	BBRR	BBRr	BbRR	BbRr
Br	BBRr	BBrr	BbRr	Bbrr
bR	BbRR	BbRr	bbRR	bbRr
br	BbRr	Bbrr	bbRr	bbrr

Summary of F_2 phenotypes:

9 Black, long : 3 Black, rex : 3 Brown, long : 1 Brown, rex. This is an example of a typical dihybrid cross. The ratio 9:3:3:1 is expected from all dihybrid crosses involving dominance.

- (b) By scanning the above diagram, one can count 9 normal-furred black F_2 rabbits (either BB or Bb of fur colour and either RR or Rr for fur length, since normal length of fur black colour are dominant traits); of these only one is homozygous for both traits – BBRR

Total number of normal black F_2 rabbits expected = 9

Number of those expected to be homozygous for both traits = 1

Ratio = 1:9

- (c) Genotype of F_1 member – BbRr

Genotype of brown, rex parent – bbrr

The genotype and phenotypic results of this back cross are:

BbRr × bbrr



Gamete	BR	Br	bR	br
br	BbRr	Bbrr	bbRr	bbrr

Summary of back cross results : 1 Black, long : 1 black, rex : 1 black, long : 1 brown, rex



EXTENSION & MODIFICATIONS OF MENDELIAN GENETIC ANALYSIS-I

Structure

2.1	Introduction	Epistasis
	Objectives	Supplementary Gene Interaction
2.2	Dominance	Duplicate Genes (Pseudoalleles)
	Complete Dominance	2.5 Lethal Alleles
	Incomplete Dominance (Blending Inheritance or Semi/Intermediate Dominance)	2.6 Pleiotropy
	Codominance	2.7 Sex-Linked Genes
2.3	Multiple Alleles	2.8 Degrees of Gene Expression
	ABO Blood Type Alleles in Humans	Penetrance
	Rh Factor Alleles in Humans	Expressivity
	Incompatibility in Plants	2.9 Summary
2.4	Gene Interactions and Modified Mendelian Ratios	2.10 Terminal Question
	Complementary Gene Interaction (9:7 Phenotypic Ratio)	2.11 Answers

2.1 INTRODUCTION

In the previous unit you have been introduced to the concept of genetics and as to how it has developed unto full fledged discipline of genetics with the Mendel's work on garden pea. The secret of inheritance mechanism of all the

traits or characters in an organism, from parents to off-springs, lies in the genes. It is now an established fact that the segments of DNA express in a very well defined manner to produce a trait or character. Each gene has two alternative forms called alleles, each of which occurs at the same locus in each homologous chromosome. As you all know, the term 'allele' comes from 'allelomorph' and refers to the different forms of a gene which affect a phenotype in an organism. Thus number of allelic forms of a gene may be many in a population, but since each organism has only one pair of homologous chromosome of a kind, only two of its variants are present in an organism.

Various interactions may occur between alleles of same genes or alleles of different genes, which give rise to different phenotypes, making the inheritance patterns complex. The phenotypic expression of these characters could not be explained by Mendel's laws alone and thus this has opened a whole new myriad of allelic and non-allelic interactions. This unit explains the deviations from Mendelian principles, its extensions and modifications that have led to the occurrence of different phenotypes in a progeny as compared to their parents. Broadly categorizing, such interactions can be classified as-

- 1) **Intragenic interactions:** When the two alleles of same gene interact with each other and affect a phenotype, it is known as inter-allelic, allelic gene or intragenic interactions, for examples- incomplete dominance, co-dominance and multiple alleles.
- 2) **Intergenic interactions:** If the alleles of different genes, located on separate loci, interact with each other and influence a phenotype, it is known as non-allelic or intergenic interactions. For example- complementary gene interaction, epistasis, supplementary gene interaction and duplicate genes.

Objectives

After studying this unit, you should be able to:

- ❖ define various terms related to intergenic and intragenic interactions,
- ❖ understand the concepts underlying the modifications of Mendelian genetics,
- ❖ analyse the influence of various interactions among alleles and genes on a phenotype,
- ❖ distinguish among different allelic and non-allelic gene interactions, and
- ❖ explain the phenomena of production of new phenotypes.

2.2 DOMINANCE

We have studied in the previous unit that Gregor Mendel's experiments laid the very basis of the concepts of heredity, however, these studies were confined to the seven traits of pea plant, the conclusions were also restricted to the observations obtained thereof. The only relationship established by

Mendel between the factors or alleles of a trait was dominance and recessiveness. This simplicity of the Mendel's principles also come from the fact that the inheritance patterns of the seven traits selected by Mendel were fortunately all straight forward and showed no complexities. Later when the study was extended to other traits or organisms, it became evident that the inheritance patterns are far more complex than the simple genetic pattern described by Mendel. A variety of new traits and characters were investigated which were the result of some undefined genic/allelic interactions. Let us discuss the Mendelian concept of complete dominance and its modifications- **incomplete dominance** and **co-dominance**.

2.2.1 Complete Dominance

Complete dominance, as inferred by Mendel, refers to the type of dominance in which one allele completely masks the expression of other allele and is therefore said to be completely dominant. The allele which is masked or remains unexpressed is called recessive. The genotype of organism exhibiting complete dominance is heterozygous. A dominant feature of a trait is represented by allele 'A' and its recessive feature by allele 'a'. The homozygous condition 'AA' is responsible for its prominent phenotype while 'aa' gives its contrasting phenotype. When both the alleles appear together in heterozygous condition (Aa), the expression of 'a' is completely masked and only the phenotype of 'A' is visible. In such a case the allele 'A' is said to be completely dominant over allele 'a', and thus 'a' is said to be recessive. Phenotypically, dominant characters can be easily recognized because they are expressed even if one dominant allele is present. Recessive alleles need to come in pair for expression, therefore, they can be recognized only in homozygous individuals (aa). Thus the heterozygous individuals, who are the carriers of recessive allele, are not recognizable on the basis of phenotype, however, can be identified through test cross. In test cross potential carriers are crossed with homozygous recessive individuals to know their in genotype (Fig. 2.1).

The relationship of dominance and recessiveness, as deduced by Mendel, can be explained by taking example of seed shape in pea plants. The seeds in pea plant can be of any of the two shapes- round or wrinkled. The allele assigned to round seed shape is 'R' while allele for wrinkled seed shape is 'r'. If a monohybrid cross is performed between a plant homozygous for round seeds (RR) with another plant homozygous for wrinkled seeds (rr), the F₁ generation completely resembles the parents with dominant phenotype i.e. round seeds. In heterozygous plants 'Rr', the round shape of seeds completely masks the wrinkled phenotype. Thus allele 'R' for round shape was said to be dominant over wrinkled 'r'.

The dominant- recessive pattern as inferred by Mendel was too straightforward and does not always exhibit as such. The observable phenotype may occur due to the influence of different environmental factors or other genic or intergenic interactions. In case of inheritance of seed shape in peas, the round and wrinkled phenotypes are acquired due to biosynthesis of amylopectin, a branched chain component of starch. The biosynthesis of amylopectin occurs by the action of starch-branching enzyme I (SBE-I), and

the activity of this enzyme affects the amount of amylopectin deposition in seeds. The presence of amylopectin helps in adequate retention of water so that while maturation of seeds the shrinkage is uniform and they acquire a round shape. In homozygous RR seeds gene for SBE-I enzyme at 'r' locus was found to be fully functional, and in heterozygous Rr seeds it was partially functional. However, in homozygous rr seeds, the SBE-I gene function was found to be interrupted due to insertion of a small DNA sequence and these seeds completely lacked amylopectin. This led to an increase in amount of free carbohydrates and higher osmotic pressures. Therefore, when shrinkage started on maturation, abrupt loss of large amount of water led to acquisition of wrinkled phenotype in 'rr' seeds. Deep observation of all the phenotypes also revealed the amount of amylopectin and thereby the round shape of seeds in Rr was somewhat intermediate between RR and rr. The shape of starch grains was also quite distinct from each other in all the three genotypes.

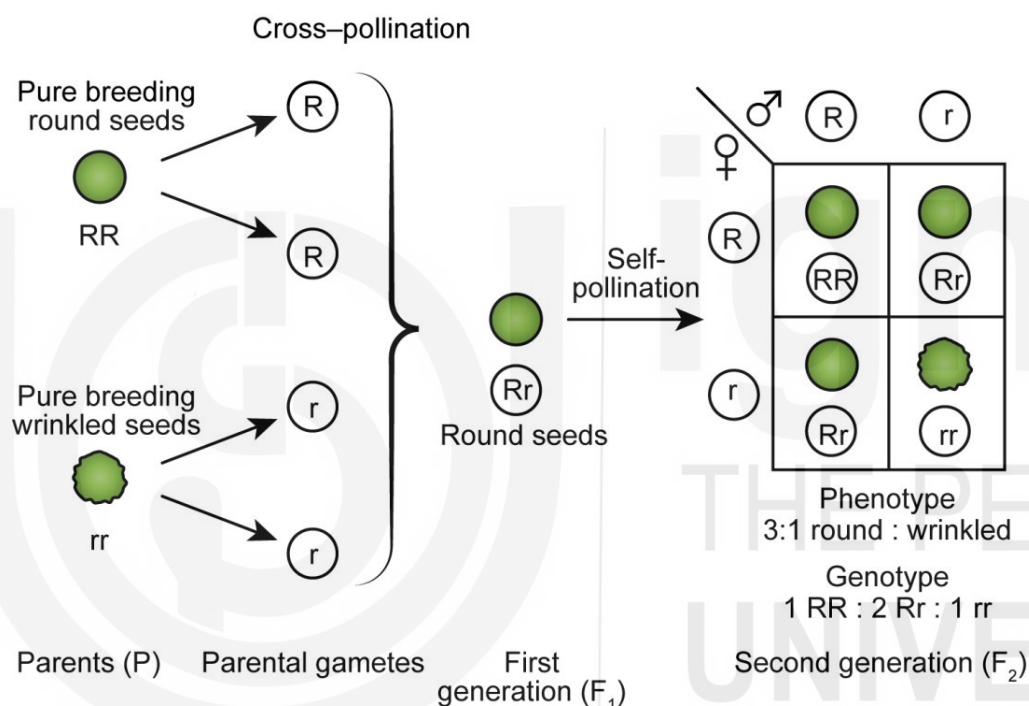


Fig. 2.1: Complete Dominance in inheritance of seed shape phenotype in Pea.

Thus simply rendering the round / wrinkled phenotypes in pea seeds to the phenomenon of dominance was not justifiable and suggested for more insights into the genetic mechanisms.

2.2.2 Incomplete Dominance (Blending Inheritance or Semi/Intermediate Dominance)

Another type of interaction between two alleles was discovered by Karl Correns in 1900, while experimenting with Four O' Clock plant (*Mirabilis jalapa* belonging to family Nyctaginaceae). When he crossed the homozygous plant with **red flowers** (RR) with homozygous recessive plant with **white flowers** (rr), he noticed a strange phenotype in F₁ hybrids. The flowers of heterozygous (Rr) plants were **pink**, instead of being red by virtue of dominance. He concluded that this was due to an intra-allelic interaction in which the dominant allele could express itself partially in heterozygous condition. Since the character appeared to be intermediate between the dominant and recessive phenotypes, the phenomenon was called '**incomplete dominance**'.

Further, if selfing is done among F_1 hybrids, the phenomenon of incomplete dominance persists and phenotypic and genotypic ratios obtained in F_2 generation are Red1: Pink2: White1 (Fig. 2.2).

2.2.3 Codominance

Codominance is a phenomenon in which both the alleles are completely expressed in the heterozygous condition, the phenotype of the heterozygous individual is a mixture of both. Since the characters expressed by both the alleles exist simultaneously in equal amount, there is no appearance of intermediate phenotype in heterozygotes as is seen in incomplete dominance. Codominance is observed in the MN blood groups of humans. This classification of human blood is based on the presence of M and N antigens on the surfaces of red blood cells. The M and N antigens are produced by a pair of codominant alleles designated as L^M & L^N . The homozygous condition of allele L^M produces marker antigen M, while an L^N produces marker antigen N, on the surface of red blood cells. Homozygotes $L^M L^M$ have only M while $L^N L^N$ have only N markers, however, heterozygotes $L^M L^N$ have both types of marker antigens in equal amounts on the cell surface. If a cross occurs between individuals with $L^M L^N$ genotypes, the probability of occurrence of M, MN, and N blood types would be as given below:

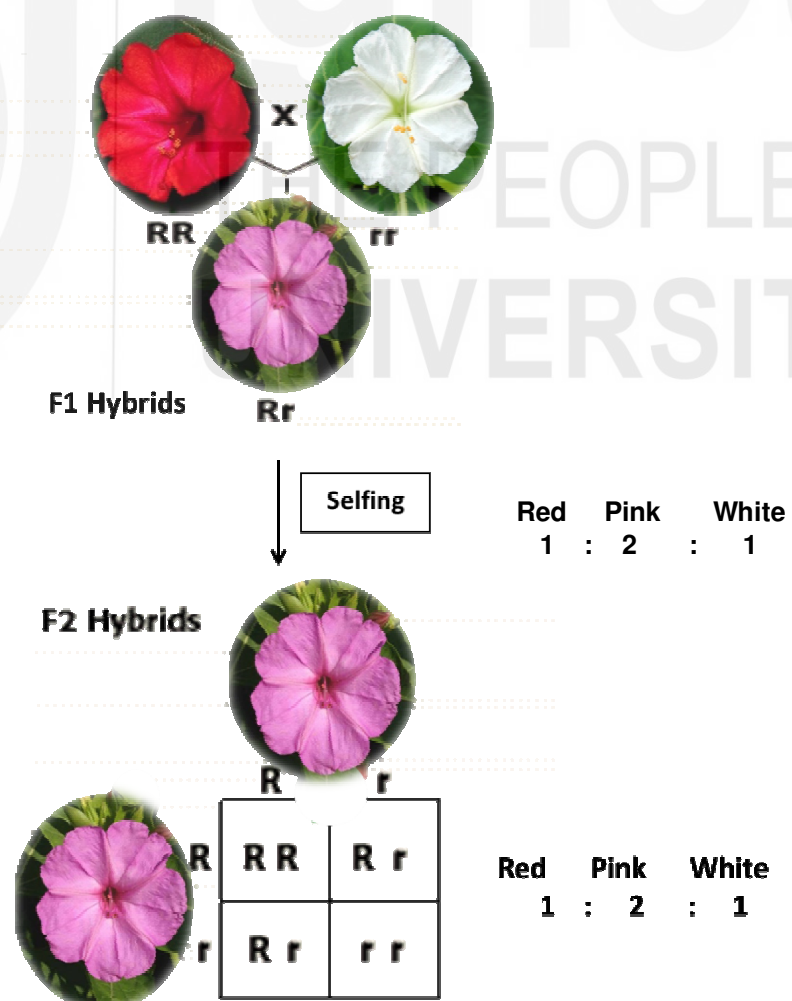


Fig. 2.2: Incomplete Dominance in flowers of *Mirabilis jalapa*.

Table2.1: Codominance in the MN blood groups in humans.

Genotype	Phenotype	Antigen present on RBCs
$L^M L^M$	M	M
$L^M L^N$	MN	M N
$L^N L^N$	N	N

Another classical example of codominance is inheritance of colour of flower in *Camellia* plants. If white *Camellia* are crossed with red *Camellia*, the flowers produced in F_1 generation produce flowers with mixed patches of red and white colour. The alleles for both red and white petal colour are codominant and both the colours are expressed simultaneously in equal amounts.

Fig. 2.3: Codominance in *Camellia* flowers.

SAQ 1

- The allele which is masked or remains unexpressed is called
- The flowers of heterozygous (Rr) plants were pink instead of being red by virtue of
- is a phenomenon in which both the alleles are completely expressed in heterozygous condition, the phenotype of heterozygous individual is a mixture of both.

2.3 MULTIPLE ALLELES

Multiple alleles refer to the existence of more than two forms of an allele in a species, which can give rise to a number of variations for a particular phenotype. Multiple alleles exist within a population and an individual possesses only two of its forms. Multiple alleles have been found in most populations including humans and have significant role in genetic stability of the population. Different allelic forms of a gene differ very minutely in their nucleotide sequence and thereby exhibit different levels of activity of their respective gene products. The phenotypes produced by multiple alleles may thus range from wild type (normal) to mutant. Most of the genes in populations of most organisms have multiple alleles. The 'normal' or 'wild-type' allele is thus not characterized by a single nucleotide sequence, rather it is a set of different nucleotide sequences, each of which is capable of carrying out the normal function of the gene.

A classic example of multiple alleles is coat colours in rabbits (Fig. 2.4). This phenotype is determined by a gene that has four alleles denoted differently i.e. allele C is for wild-type i.e. full colour, c^h is for Himalayan characterized by white coat with black tips, c^{ch} is for chinchilla having mixed coat colour and white hair, and c stands for albino. The range of coat colours also exhibits a kind of gradation in the order of dominance of the concerned genes, as shown below-

$$C > c^{ch} > c^h > c$$

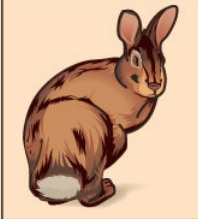
Allele			
C	c^{ch}	c^h	c
Genotype			
CC	$c^{ch}c^{ch}$	c^hc^h	cc
Phenotype			
WILD TYPE: Brown fur	CHINCHILLA: Black-tipped white fur	HIMALAYAN: White fur with black paws, nose, ears, tail	ALBINO: White fur
			

Fig. 2.4: Expression of multiple alleles in coat colour of rabbits.

According to this phenomenon, full colour C is dominant to all other coat colour alleles, chinchilla is dominant to Himalayan and albino, and Himalayan is dominant only to albino. This gradation has profound effect on the phenotypic and genotypic ratios of successive generations. The occurrence of homozygous dominant individuals (CC) is highest and this allele is called 'wild type'. Rest other alleles (c^{ch} , c^h and c) are called mutants (Table 2.2).

Table 2.2: Different Genotypes and their respective Phenotypes for coat colour in rabbit.

Genotypes	Phenotypes
CC, Cc^{ch}, Cc^h, Cc	Full Colour (Wild type)
$c^{ch}c^{ch}, c^{ch}c^h, c^{ch}c$	Chinchilla
c^hc^h, c^hc	Himalayan
cc	Albino

The inheritance pattern of multiple alleles of coat colour in rabbit is explained on the basis of two crosses between individuals of different phenotypes. When wild type homozygous full colour (CC) rabbit is crossed with homozygous recessive albino (cc), the phenotype of F_1 heterozygous offspring (Cc) resembles dominant parent because allele C is dominant over c . The cross between two heterozygous (Cc) individuals yields coloured phenotype in 3:1 ratio in F_2 generation. In another cross, when Himalayan (c^hc^h) individual is crossed with albino type (cc), the F_1 heterozygous offspring (c^hc) show Himalayan phenotype. The cross between individuals of F_1 generation again yields 3:1 phenotypic ratio in F_2 generation (Fig. 2.5).

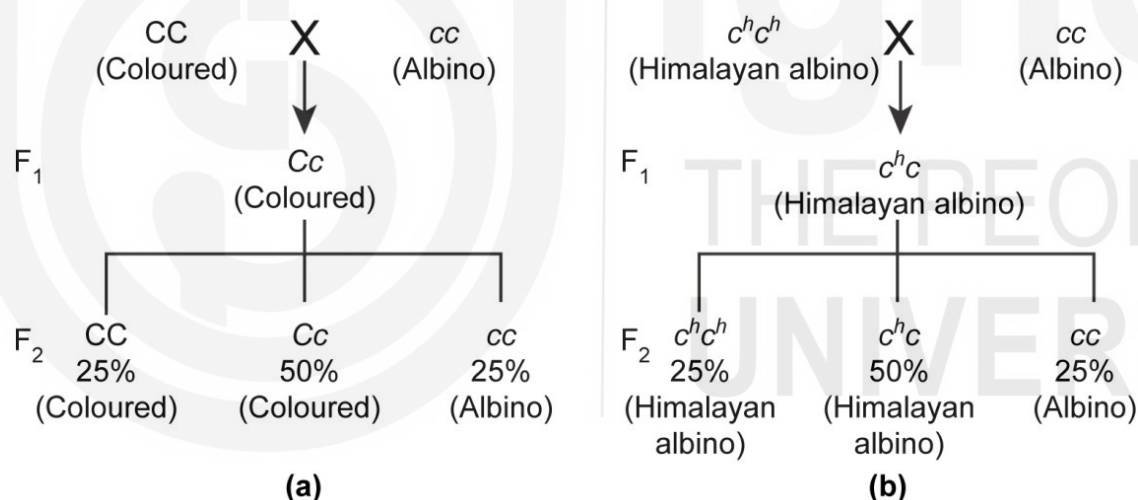


Fig. 2.5: Cross showing inheritance of coat colour phenotype in rabbits. (a) Cross between coloured and albino, (b) Cross between Himalayan and albino.

2.3.1 ABO Blood Type Alleles in Humans

Another common example of multiple alleles is ABO blood types in humans (Table 2.3). The blood groups in humans are classified as A, B, O, or AB, depending upon the three types of alleles I^A , I^B , and I^O . Here, 'I' denotes an antigen 'isoagglutinin' which is present on the surface of red blood cells. There are two types of oligosaccharides, either of which are attached to the common acceptor isoagglutinin by the action of enzyme glycosyltransferase. This enzyme is the product of ABO gene having three alleles. Allele ' I^A ' encodes for A- glycosyltransferase which converts isoagglutinin into A antigen, and allele ' I^B ' encodes for B- glycosyltransferase which converts isoagglutinin into B antigen. Allele ' I^O '

does not encode any of these enzyme, instead produces a different protein, therefore no oligosaccharide component is attached to its corresponding isoagglutinin and it is left unaltered.

The blood group of an individual is determined by the type of allelic pair present. Both the alleles of blood types i.e. A and B, are codominant and express completely. Individuals having the genotype $I^A I^A$ or $I^A I^O$ have type A blood group and the ones having the genotype $I^B I^B$ or $I^B I^O$ have type B blood group. Heterozygous individuals $I^A I^B$ have both A and B types of antigens therefore their blood group is designated as AB. People having homozygous allele combination $I^O I^O$ lack either of the two antigens and their blood group is designated as O.

Table 2.3: Genotypes and blood groups arising from different allelic combinations

Genotype		Blood Type	Antigen on red blood cells	Antibodies in plasma	Can receive blood from	Can donate blood to
AO, AA	$I^A I^O$, $I^A I^A$	A	A	anti-B	O and A	A and AB
BO, BB	$I^B I^O$, $I^B I^B$	B	B	anti-A	O and B	B and AB
AB	$I^A I^B$	AB	A and B	neither	O, A, B, and AB	AB only
OO	$I^O I^O$	O	neither	anti-A and anti-B	O only	O, A, B, and AB

The ABO antigens are the most immunogenic of all other blood group antigens, therefore, ABO system of classification of blood group has foremost clinical significance. The agglutination reactions arising due to incompatibility of A, B and O antigens are most common cause of death following error or mismatch in blood transfusions (Fig. 2.6).

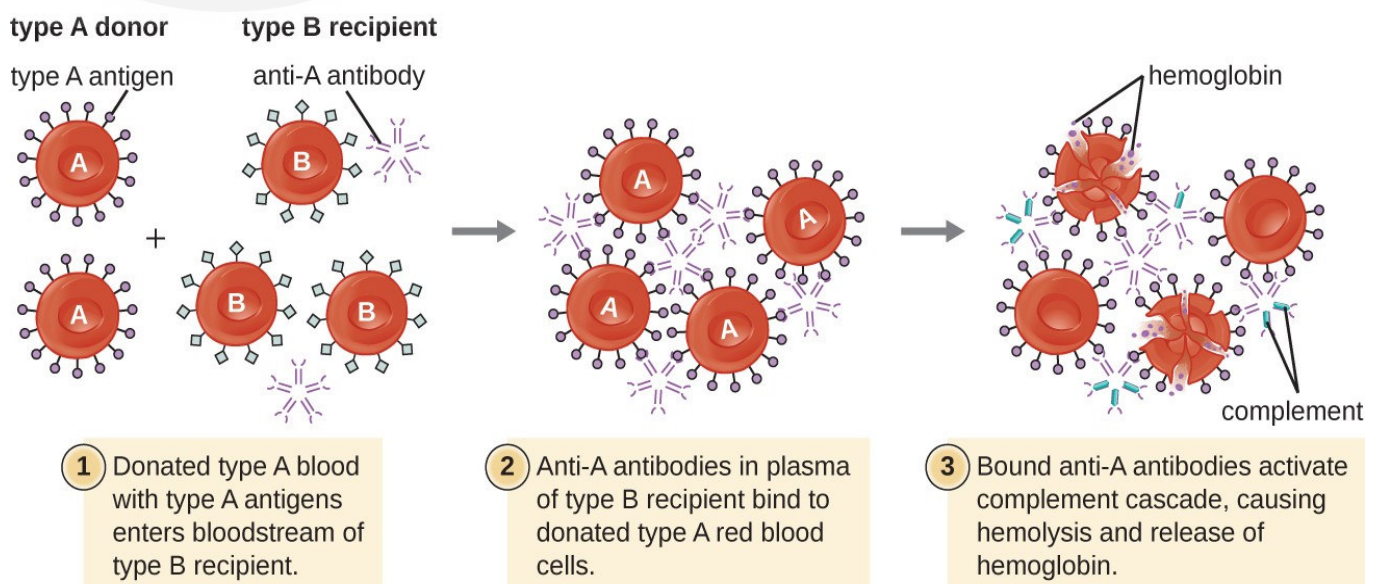


Fig. 2.6: Agglutination reaction and subsequent hemolysis due to incompatible blood transfusion.

The type of blood group is determined genetically and is, therefore, inherited by a child from his parents. Since the two alleles I^A and I^B are codominant, while i represents a recessive allele, there are number of possibilities arising for blood type of children. The inheritance patterns can be followed by making probable parental combinations of alleles (Table 2.4).

Table 2.4: Inheritance pattern of Blood Groups in Humans.

PARENT GENES	AA	BB	AB	OO	AO	BO
AA	AA	AA	AA, AB	AO	AA, AO	AA, AB
BB	AB	AB	AB, BB	BO	AB, BO	AB, BB
AB	AA, AB	AA, AB	AA, BB, AB	AO, BO	AA, AO, BO, AB	AA, BB, AB
OO	AO	AO	AO, BO	OO	AO, OO	AO, BO
AO	AA, AO	AA, AO	AA, AB, AO, BO	AO, OO	AA, AB, AO, BO	AA, AB, AO, BO
BO	AO, AB	AO, AB	AB, AO, BB, BO	BO, OO	AB, AO, BB, BO	AB, AO, BB, BO

2.3.2 Rh Factor Alleles in Humans

Rh Factor or Rhesus factor is second most clinically significant blood group system of ABO blood group system. It was discovered by Karl Landsteiner and Alexander Wiener in 1937 (published in 1940), long after the discovery of ABO system, when the two biologists carried out immunization experiments on rabbit using blood of rhesus monkey. They found that the antibodies produced after immunization, agglutinate the blood of monkey and also the blood of large number of human subjects. Rh incompatibility can be tested by reacting a blood sample with anti Rh serum and look for occurrence of agglutination as a positive response. An individual is either Rh + if his blood agglutinates, or is Rh- if there is no agglutination in such reaction.

Rh factor is based upon the presence of certain antigens on the surface of RBCs. Until now, about 49 antigens have been identified, encoded by RHD and RHCE genes, making it genetically very complex. The D antigen encoded by RHD is said to be most immunogenic, therefore Rh + phenotype simply refers to the presence of D antigen while Rh- indicates its absence in the blood, usually due to deletion of the concerned gene.

Alexander Wiener, discovered a number of Rh antigens and also proposed their nomenclature known as **Rh-hr nomenclature**. He postulated a theory for inheritance of Rh factor, later called Wiener's theory. The theory suggests the presence of an Rh gene at a particular locus which has eight multiple alleles which produce this large array of Rh antigens. The five major 'blood factors' or antigens identified by Wiener were Rh_0 , rh' , rh'' , hr' and hr'' , of which Rh_0 , rh' , rh'' are encoded by gene R1 and hr' and hr'' are encoded by gene r. This system of nomenclature is not followed now and is replaced by the one developed by Ronald Fisher and R.R. Race. It is known as the Fisher-Race system or **DCE nomenclature**, and it recognizes five most common antigens D, C, c, E and e. The antigens C, c, and E, e are antithetical; antigen

D lacks any antithetical antigen therefore 'd' is used to indicate absence of D antigen. In Weiner's nomenclature, the antigens D, C and E correspond to Rh₀, rh', rh'', while c and e antigens correspond to hr' and hr''. The antigen D is encoded by gene RHD and rest others are encoded by gene RHCE, located on a separate locus. The two genes constitute eight haplotypes -Dce, dce, DCe, dCe, DcE, dcE, DCE, and dCE, which are analogous to R₀, r, R₁, r', R₂, r'', R_z, and r_y respectively in Weiner's system of nomenclature.

The Rh factor is clinically the second most important blood group system after ABO blood group and is essentially checked for incompatibility to avoid agglutination reactions after blood transfusion. It is also responsible for haemolytic disease in fetus and new born, a condition arising due to incompatibility between the fetal blood and maternal blood. When Rh- mother carries Rh+ fetus (gene for Rh+ blood group inherited by fetus from Rh+ father), she is immunized to Rh antigens due to exposure to these antigens. The first child is not affected, but during subsequent pregnancy, the second fetus may be exposed to maternal anti-Rh antibodies. These are anti-D IgG, which can cross the placenta, destruct the antigens on fetal RBCs, and cause hemolysis. The disease is called hemolytic disease of the newborn (HDN) or **erythroblastosis fetalis**, may range from mild to severe, even causing the death of fetus.

The hemolytic disease of the newborn (Fig. 2.7), which is likely to occur due to RhD incompatibility, is prevented by injecting anti-Rh immunoglobulin Rho (D) in mother at certain stages of pregnancy. IgG Rho (D) binds to fetal Rh antigens that enter the blood stream of mother and prevent the elicitation of primary antibody response. This preventive treatment reduces the risk of HDN in subsequent pregnancy.

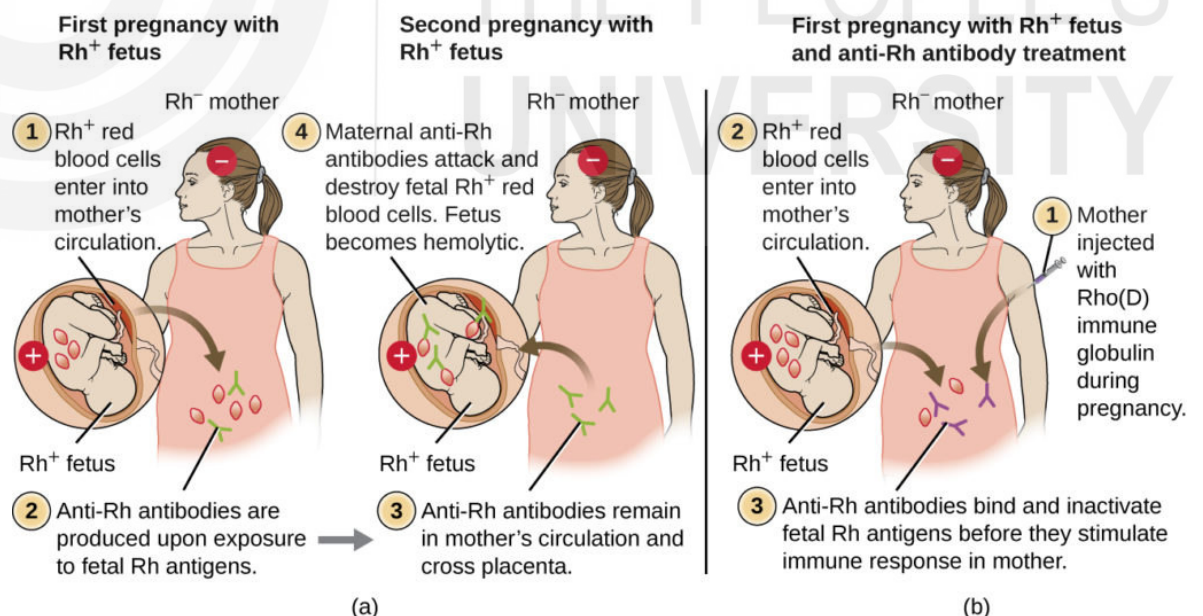


Fig. 2.7: Hemolytic disease (Erythroblastosis Fetalis) of the newborn and its prevention in Rh- mother

2.3.3 Incompatibility in Plants

Incompatibility refers to the prevention of fertilization of male and female gametes in bisexual plants, despite the gametes are functional. Incompatibility may be seen between the gametes of same species (intraspecific) or between

different species (interspecific). Intraspecific incompatibility, also known as self-incompatibility, may operate to prevent fertilization between the gametes of same plant or the gametes of different plants of same species. The mechanisms of self-incompatibility are different in case of pre-fertilization and post-fertilization barriers. The pre-fertilization involves pollen pistil interactions such as pollen-stigma interaction, pollen-style interaction and pollen-ovule interaction, in which the pollens may fail to germinate on stigma, if they germinate the growth of pollen tube may be inhibited or may fail to enter the ovule for gametic fusion.

Self-incompatibility is controlled by a single gene (designated as S) with multiple alleles (Fig. 2.8). It was first discovered in tobacco (*Nicotiana tabacum*). The multiple alleles of gene S are represented by S₁, S₂, S₃, S₄ S_n. All the alleles are codominant and either of the two alleles may be present in a plant. A plant with S₁S₂ genotype will produce pollens with either S₁ allele or S₂ allele, which will be able to pollinate the pistil of all other genotypes except S₁S₂. In other words, even if single allele is common in both male and female parent plants, fertilization will not take place (Table 2.5).

Table 2.5: Multiple alleles responsible for Self-incompatibility in plants.

Male Genotype	Female Genotype	Compatibility	Fertilization and Seed Production
S ₁ S ₂	S ₁ S ₂	Incompatible	No
S ₁ S ₃	S ₁ S ₂	Incompatible	No
S ₃ S ₄	S ₁ S ₂	Compatible	Yes
S ₃ S ₄	S ₂ S ₅	Compatible	Yes

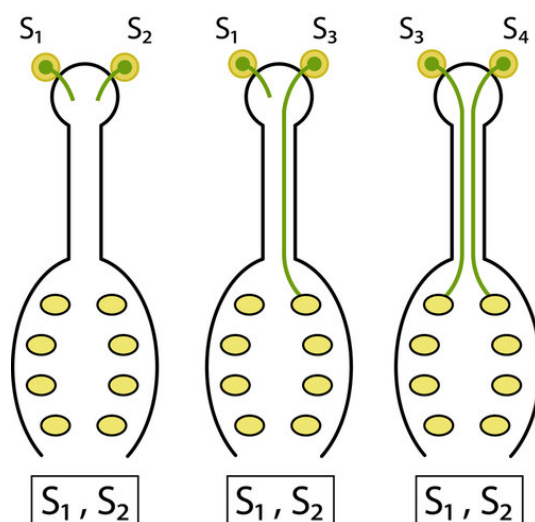


Fig. 2.8: Self-incompatibility in plants.

SAQ 2

Read the following statements and write True (T) or False (F) against each:

- i) Most of genes in populations of most organisms have multiple alleles.
 - ii) ABO antigens are the most immunogenic of all other blood group antigens.
 - iii) Rh factor is the clinically most insignificant blood group system of ABO blood group system.
 - iv) Self incompatibility is controlled by a double gene with multiple alleles.
-

2.4 GENE INTERACTIONS AND MODIFIED MENDELIAN RATIOS

The gene interactions studied above are allelic or intragenic, i.e. different alleles of single gene interact and affect the expression of each other, and may also lead to the development of a new phenotype. However, there are interactions in which two or more genes located at different loci are responsible to produce a phenotype. The expression of these genes is influenced by each other and interactions among these genes may create new phenotypic combinations exhibiting modified Mendelian ratios. Such interactions are called non-allelic or inter-genic interactions.

The different non-allelic interactions discussed here are complementary gene interaction, epistasis, supplementary gene interaction and duplicate genes.

2.4.1 Complementary Gene Interaction (9:7 Phenotypic Ratio)

Complimentary genes are a pair of non-allelic genes that are together responsible to produce one phenotype. Both are dominant but neither is able to produce the phenotype alone. At least one dominant allele from both the gene pairs is required to produce the concerned phenotype. In the absence of any of the dominant alleles, i.e. when any of the recessive pairs is present, mutant phenotype is expressed. If parents heterozygous for both the genes are crossed, a phenotypic ratio of 9:7 is obtained in F_2 generation.

This interaction is seen in sweet pea (*Lathyrus odoratus*) in which two genes designated as C and P contribute in synthesis of anthocyanin to give purple colour to flowers (Fig. 2.9). The mutant phenotype in this case is white colour of flowers which appears due to non functioning of enzymes involved in biosynthesis of anthocyanin.

If a homozygous pea plant with coloured flowers (CCPP) is crossed with homozygous recessive with white flowers (ccpp), the F_1 generation have coloured flowers (CcPp). However, on selfing the F_1 plants, the normal Mendelian dihybrid ratio (9:3:3:1) is not obtained, instead the interactions of complementary nature between the two genes C and P genes gives a

modified phenotypic ratio (9:7). A similar cross is described in Figure-2.9 in which a cross between two homozygous parents with white flowers CCpp and ccPP yields F₁ progeny with purple flowers (CcPp), which on selfing also exhibit complementary gene action. This cross infers that the anthocyanin pigment is biosynthesized only when a dominant allele from both the gene pairs is present, giving purple phenotype. Recessive pair of either of the two genes renders the enzymes of anthocyanin biosynthetic pathway non-functional and white flowers are produced.

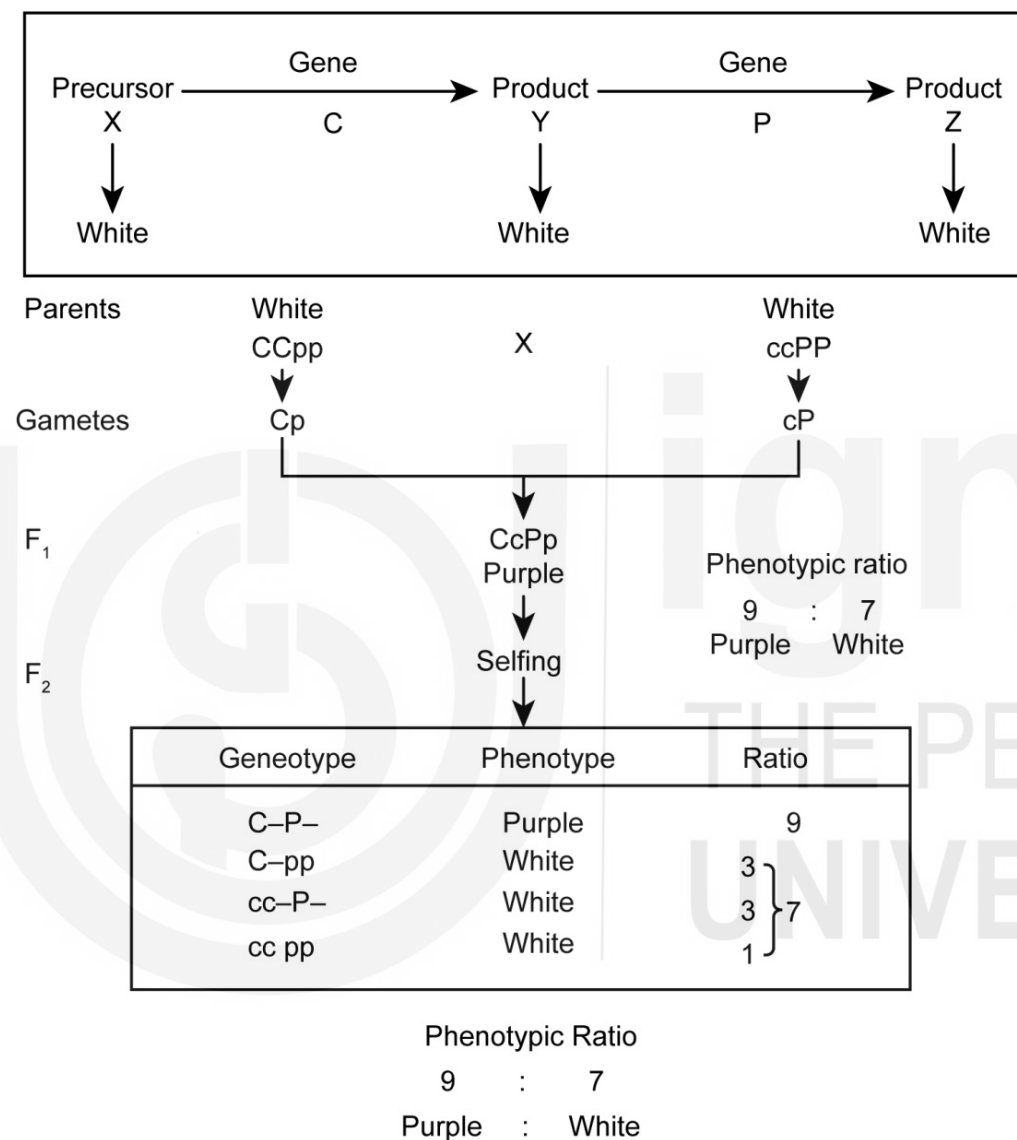


Fig. 2.9: Complementary Gene Interaction in *Lathyrus odoratus*.

2.4.2 Epistasis

Epistasis is another kind of gene interaction **in which one gene masks the expression of other non-allelic gene**. The gene that shows the masking action is called **epistatic gene** while the one whose expression is masked is called a **hypostatic gene**.

On the basis of the effect exerted on another gene, epistasis can be of two types- dominant and recessive epistasis.

- Dominant Epistasis:** This type of gene interaction occurs when a dominant gene suppresses the expression of a gene at some other

locus. For example, in *Cucurbita pepo* (summer squash), gene for white fruit colour is dominant and is designated as 'W' (Fig. 2.10). Another gene 'Y' controls the expression of yellow colour in fruit. The gene W exerts epistatic effect on the gene Y, therefore, the yellow colour is not expressed in fruits in presence of gene W. When both the genes (W and Y) are absent, green phenotype appears. The F₂ phenotypic ratio in dominant epistasis is **12:3:1**.

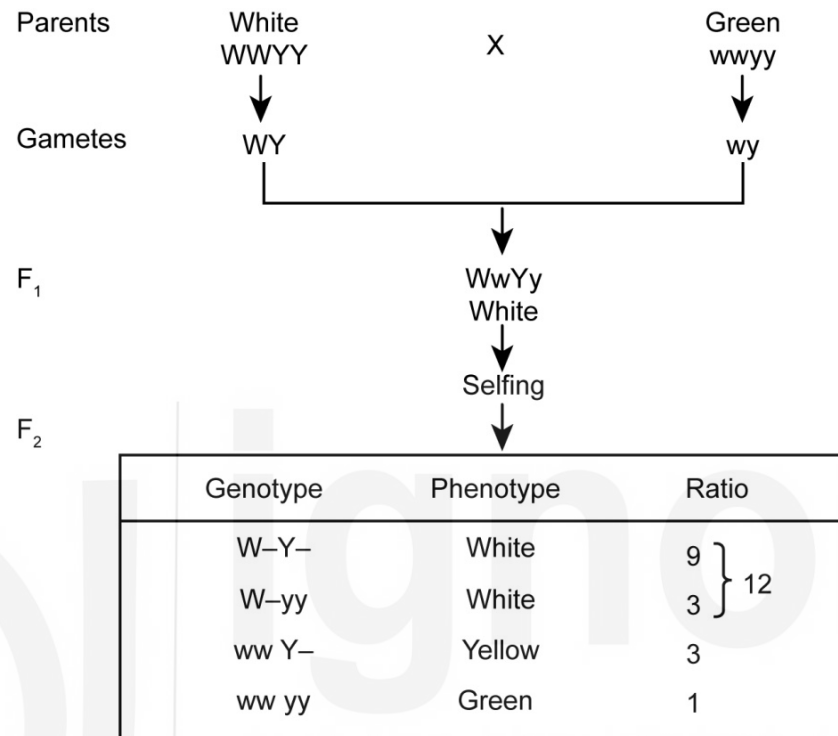


Fig. 2.10: Dominant Epistasis in *Cucurbita pepo*.

- b) **Recessive Epistasis**- In this type of gene action, a recessive pair of alleles inhibits or masks the expression of a gene at another locus. The epistatic action is exerted by the homozygous recessive gene pair and its own expression by its dominant form occurs only in the presence of other dominant gene.

Coat colour in mice is a common example of recessive epistasis. The coat colour in mice can be black, agouti or albino, and the phenotype is controlled by two pairs of genes- 'A' and 'C', both of which are non-allelic. The agouti coat colour in mice is expressed by gene 'A' only in presence of another dominant gene 'C'. However, the recessive pair of alleles 'cc' masks the expression of the gene A, for both the genotypes AA and Aa. The black coat colour in mice is expressed by gene 'C' only in absence of dominant gene A. Recessive homozygous forms of both the gene pairs produce albino phenotype. The F₂ phenotypic ratio in such a case is **9:3:4** (Fig. 2.11).

2.4.3 Supplementary Gene Interaction

Supplementary gene interaction also occurs between two non-allelic genes, each of which is responsible for same trait. This type of gene interaction is very similar to recessive epistasis. In supplementary gene action, a dominant gene is able to express its character, while another dominant gene, at different

locus, is able to express itself only in presence of the first one. For example, coat colour in mice is controlled by two pairs of genes- A and C. Gene C expresses black coat colour in mice. All the black mice consist of at least one dominant allele 'C' but no 'A'. Whenever dominant gene 'A' is present along with 'C', agouti phenotype appears, i.e. A is expressed only in presence of C. Presence of either only dominant gene A or recessive forms of both (aa and cc) produce no colour and give albino phenotype. The F₂ phenotypic ratio is same as in recessive epistasis i.e. 9:3:4 (Fig. 2.11).

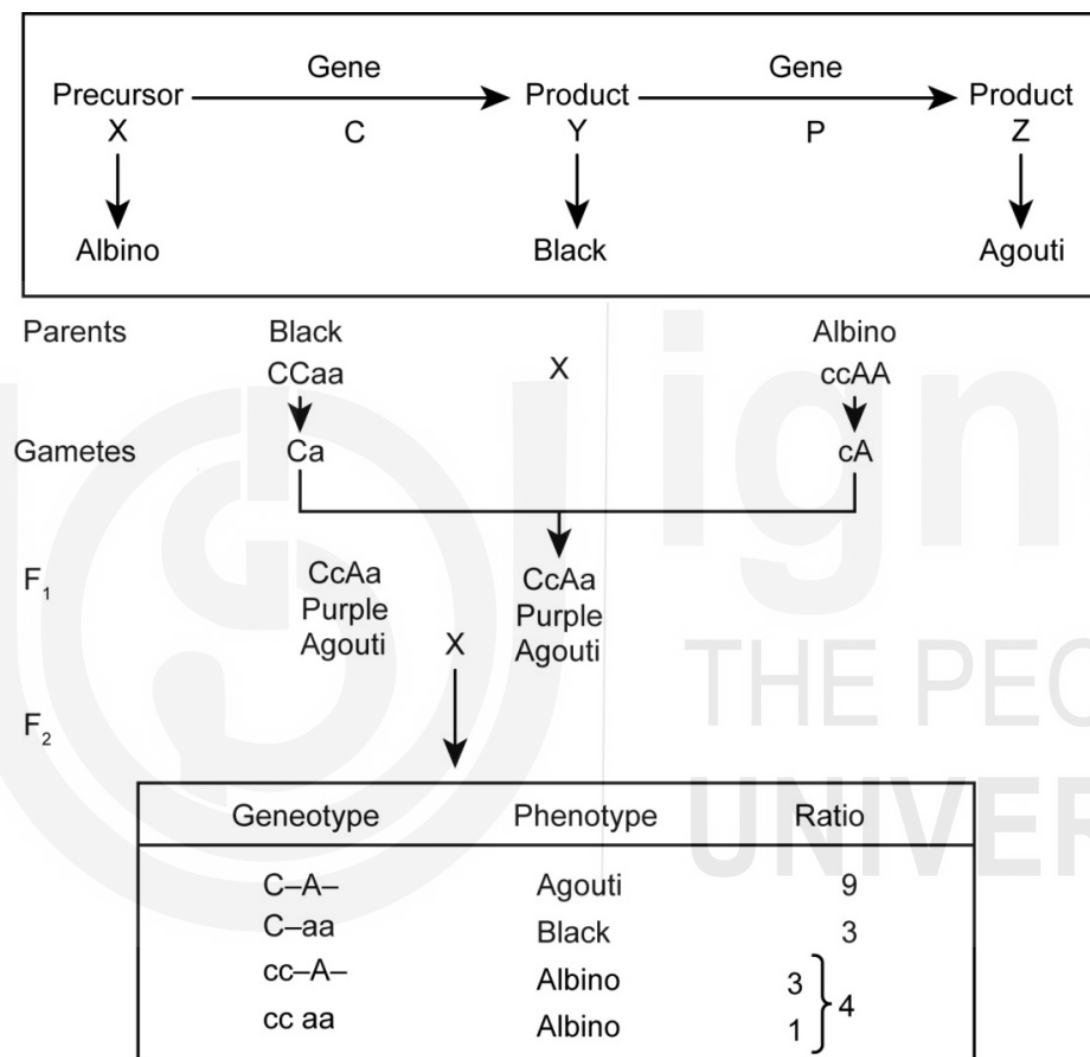


Fig. 2.11: Inheritance of coat colour in mice.

2.4.4 Duplicate Genes (Pseudoalleles)

When two non-allelic pairs of genes are able to produce a phenotype, whether they are alone or together, are called duplicate genes. In such case, the mutant phenotype is produced by double homozygous recessive condition only.

Example of duplicate genes is the presence of awn in the spikelet of rice, which is due to two dominant duplicate genes (A and B). The presence of awn is a dominant character and can be produced by any of the two genes.

Therefore, the awn is absent in only those plants which are homozygous recessive (aabb). The F₂ phenotypic ratio in such a cross is 15:1 (Fig. 2.12).

Parents		Awned Rice AABB		X	Awned Rice aabb	
F ₁		AaBb		↓	Awned Rice	
		AB	Ab	aB	ab	
F ₁	AB	AABB [A]	AABb [A]	AaBB [A]	AaBb [A]	
	Ab	AABb [A]	AAbb [A]	AaBb [A]	Aabb [A]	
	aB	AaBB [A]	AaBb [A]	aaBB [A]	aaBb [A]	
	ab	AaBb [A]	Aabb [A]	aaBb [A]	aabb [a]	

A = Awned Rice, a = Awnless Rice

Fig. 2.12: Duplicate Genes in rice.

SAQ 3

Match **Column A** with **Column B**.

Column A	Column B
i) Complementary Gene Interaction	a) A recessive pair of alleles inhibit or makes the expression of a gene at another locus.
ii) Dominant Epistasis	b) Occurs between two non-allelic genes for same trait.
iii) Recessive Epistasis	c) Two non-allelic pairs of genes are able to produce a phenotype.
iv) Supplementary Gene Interaction	d) Dominant gene suppresses the expression of a gene.
v) Duplicate genets	e) Genes are a pair of non allelic genes.

2.5 LETHAL ALLELES

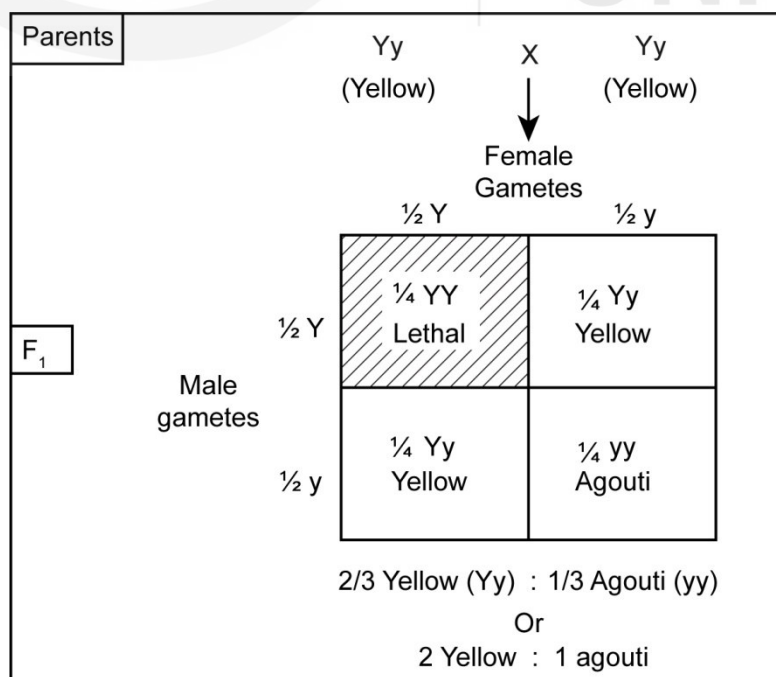
In the previous section, you studied the various gene interactions resulting in the modification of the ratio of F₂ individuals. The genetic ratios are also affected by several other factors. One of them is a class of genes - lethal genes, which you will study in this Section.

Genes may affect viability as well as visible traits of an organism. The living beings carrying certain genes are disadvantaged as they have impaired structural as well as, biochemical functioning. For example, *Drosophila* flies having white eyes and vestigial wings have lower viability than their wild types. The detrimental physiological effects are apparently associated with the genes involved, that is, **w** and **vg** respectively. Some other genes have no effect on the appearance of a fly but do influence viability in some ways. Other genes have such serious effects that the organisms is unable to live. These are called **lethal genes** and the alleles involved in the situation are termed as **lethal alleles**.

If the lethal effect is dominant and immediate in expression, all individuals carrying the gene will die and the gene will be lost. Some **dominant lethals**, however, have a delayed effect so that the organism lives for some time.

Recessive lethals present in the heterozygous condition have no effect but may come to expression when matings between carriers occurs.

We shall now take up an example for discussion, that clearly illustrates the functioning of these genes. In 1904, shortly after the rediscovery of Mendel's principles, a French geneticist, Lucien Cuénot, while carrying out experimental crosses on coat colour in mice, found that a gene was not consistent with the mendelian predictions. He observed from his experiments that the yellow body colour allele (Y) was dominant and agouti allele (y) was recessive. The crosses between two yellow mice (see Fig. 2.13) yielded approximately a 2:1 ratio of yellow to agouti mice rather than the expected ratio of 3: 1. Further, when the yellow individuals (Yy) are crossed to the agouti (yy) Cuénot found that some agouti progeny are produced. He, therefore, concluded that yellow mice were heterozygous (Yy) and there were no yellow homozygotes (YY) in the progeny. Later, it was suggested that the yellow homozygotes were actually lethal, and they died while still in the uterus. It was found that approximately $\frac{1}{4}$ of the embryos from yellow by yellow crosses failed to develop. Therefore, the observed ratio of phenotypes differs from the expected ratio, as they die very young - much before reaching the reproductive age.



Tay-Sachs disease (amaurotic idiocy):

A genetic recessive disorder that affects the central nervous system. Its clinical symptoms are: cherry-red spot in eye macula (the visible white portion of the eye); after 6-9 months rapid degeneration of vision and motor skills; death at about 2-4 years of age.

Huntington's

disease (HD): A fatal neurological disorder which is dominant. It is normally manifested after the age of thirty, but has been reported to occur at all ages, HD is characterised by mental and physical deterioration. There is progressive change in personality. As the disease progresses, the HD patients demonstrate twitching and uncontrollable muscle spasms.

There is degeneration of Central Nervous System. (CNS) and loss of brain cells. This leads to fits of depression, insanity and suicide. At the time of death, the patients have lost about 25 per cent of their brain weight.

Fig. 2.13: A cross between two yellow mice, yielding a 2:1 ratio in the offspring.

Such lethals are by no means exceptional and must always be considered in populations of plants and animals. Many lethals produce no pronounced effect at all on the phenotype, but they may make their presence known by a decrease in the life span or the very elimination of the carrier. It has been estimated that each human carries, on the average, about six lethal alleles.

How can an allele have a killing action? This perhaps may be the question arising in your mind. You may remember that metabolism is the result of many interlocked biochemical pathways. A defect in just one step can upset several others. Just one defective step can alter the entire chemistry of the body. A lethal, by blocking a critical reaction, can interfere with normal embryological development of any organ, SAY heart. The death of embryo may then follow. Lethals can thus decrease the chances of survival by causing various kinds of abnormalities in development and physiology.

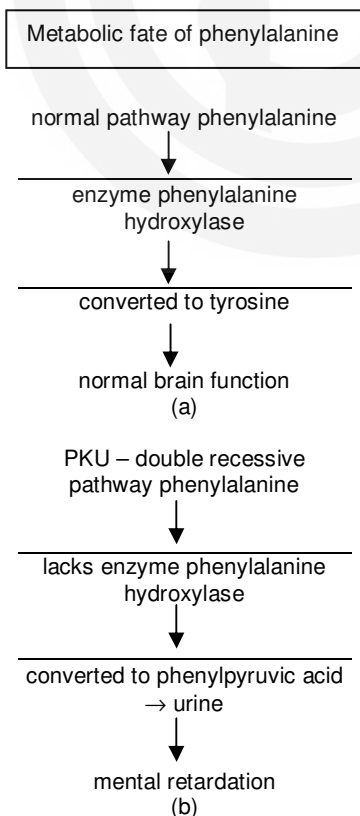
Different lethals eliminate individuals at different stages of the life cycle. The **complete lethal** removes the carrier before the reproductive age so that those affected have no offspring, e.g., the allele for yellow coat colour in mice; in humans the recessive factor for **Tay-Sachs disease** which kills in infancy, In humans, the dominant factor for **Huntington's disease**, a fatal, deterioration of the nervous system, does not usually express itself before the age of **30**. Such genetic determinants which can result in death but permit the carrier to live to reproductive age, are often grouped as **sublethals**. There is actually no sharp boundary during the life cycle at which lethals act.

2.6 PLEIOTROPY

The action of a gene at the cellular level is unitary, that is, one gene one action. Sometimes the presence of a gene results in a broad spectrum of phenotypic changes, so that it appears that the **gene has multiple action**. This phenomenon is called **pleiotropy**, and is found primarily in higher organisms where complex and interrelated developmental events occur.

Many lethal alleles are pleiotropic. For example, the **yellow coat colour in mice**, just discussed, is an allele that affects more than one character, that is, it produces yellow colour of the coat in heterozygotes, and it also affects survival, causing lethality in homozygotes. Another example of multiple effects is the gene affecting **seed shape in garden peas**; this gene also affects starch grain morphology. In fact, many genes affect more than one trait. Mendel also noticed that genes causing the flower colours, like violet and white, also influenced seed colour and caused the presence or absence of coloured areas on the leaves. This is due to pleiotropy as a single gene affects more than one character.

Pleiotropic traits also occur in humans. One such disease is **phenylketonuria**, abbreviated as **PKU**. This occurs in individuals that are homozygous for a defective, recessive allele. The diseased people lack the enzyme necessary for the metabolism of the amino acid phenylalanine. When normal and PKU individuals are compared, the level of phenylalanine is much higher in diseased group. In addition to this basic biochemical difference, a number of other features are seen in the untreated PKU patients, such as lower IQ, similar head size and lighter hair.



2.7 SEX-LIKED GENES

In the crosses we have discussed so far, it has not mattered which parent carried a particular allele in question. Reciprocal crosses gave identical results. For example, in all the Mendel's monohybrid crosses, F_1 and F_2 were the same regardless of which P_1 parent exhibited the recessive trait.

This, however, is not the case in crosses involving genes located on chromosomes involved in the determination of sex. Such genes are said to be **sex-linked**. The first thorough study of the sex-linked gene was conducted by T.H. Morgan in 1910, when he was studying the inheritance of eye colour in *Drosophila*. The wild-type flies have brick red eye colour. Morgan crossed mutant white-eyed flies with red-eyed flies. He found that the result of a cross between a white eyed male (rl) and red-eyed female ($RIIR$) were different from those of a cross between white-eyed male. Both of these reciprocal crosses are illustrated in Fig. 2.14. (crosses a and b).

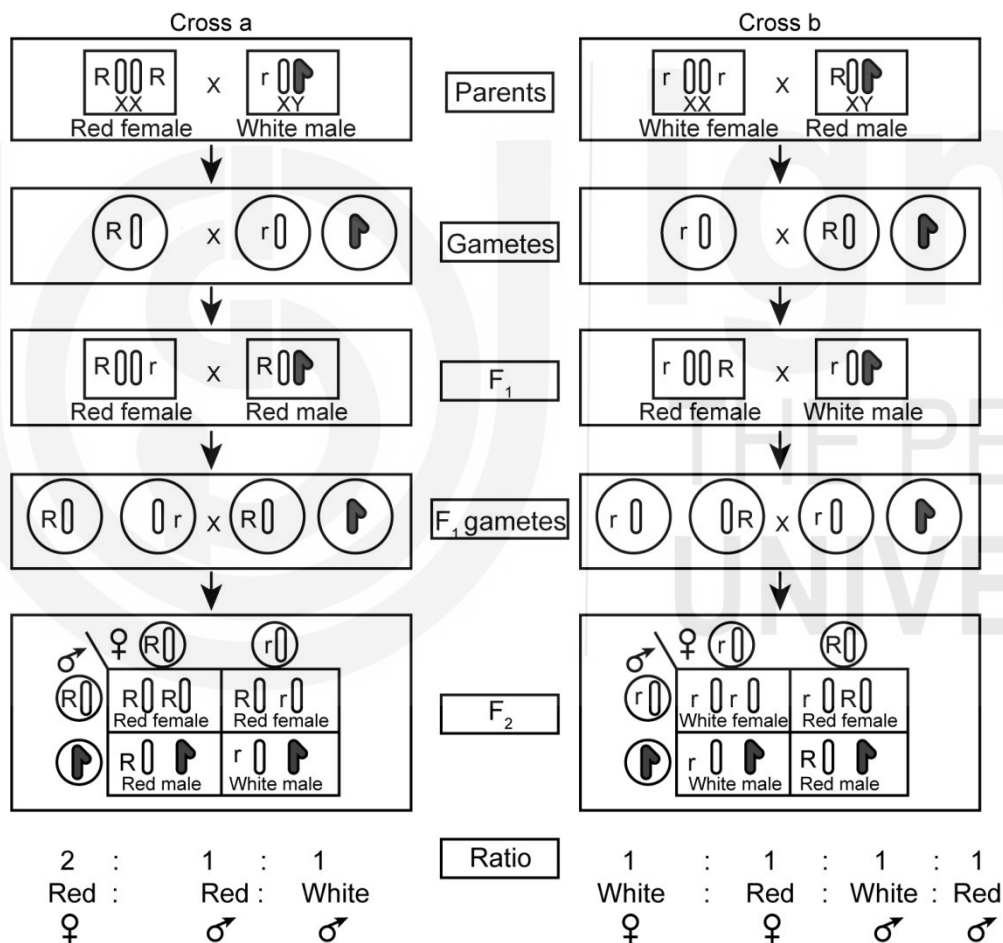


Fig. 2.14: Crosses a and b illustrate inheritance of sex-linked genes in *Drosophila*. a) is a cross between red-eyed female and white-eyed male. And b) is the reciprocal cross of a). In the figure the long, rod-shaped structure (I) represents the X-chromosome and the inverted J-shaped structure (l) stands for the Y chromosome.

Compare the phenotypic ratios of F_2 generations of cross a with b. Is there any difference? Yes, there is a clear difference between the F_2 , individuals of the two crosses. The phenotypic ratios thus depend on whether the P , white-eyed parent was male and female. Morgan was able to correlate these observations

with a difference found in chromosome composition between male and female *Drosophila*. It has *four* chromosomes and one of the chromosome varies between sexes (see Fig. 2.15). This chromosome pair (see XX or XY in the figure) is involved in sex determination mechanism and constitutes the **sex chromosomes**. The remaining chromosomes are called **autosomes**. The females possess two, rod-shaped homologs called the X chromosomes which are designated as XX. Males possess a single X chromosome and J-shaped Y chromosome, which are designated as XY.

On the basis of this correlation, Morgan postulated that the gene for white eye is present on the X chromosome but is absent from the Y chromosome. Females thus have two available genes, one on each X chromosome while males have only one available gene on their single X-chromosome. This explanation supposes that the Y chromosome lacks homologous loci to these on the X chromosome. However, the X and Y chromosomes still behave as homologues in that they do partially synapse with each other and segregate into gametes during meiosis.

Many species, including humans, possess an arrangement of sex chromosome as in *Drosophila*. Many sex-linked genes in humans have now been identified, e.g., the gene controlling one form of haemophilia and muscular dystrophy. We stop further discussion on this topic for the time being and shall resume it in an elaborated manner in Unit 4, that is, Sex Linkage and Dosage Compensation.

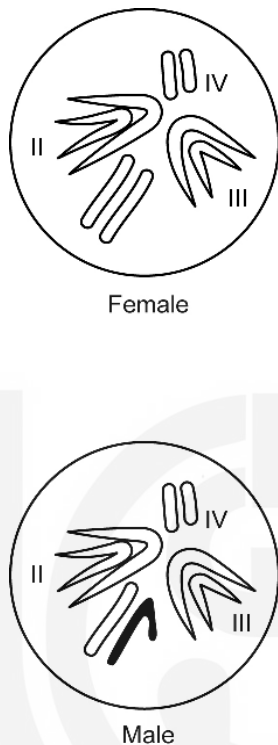


Fig. 2.15: Chromosomes composition in *Drosophila melanogaster*.

2.8 DEGREES OF GENE EXPRESSION

In observing offsprings from a cross, we tend to think of a phenotype in terms of all or none of the phenomena. A trait is expressed or it is not - and we draw conclusions as to what are the types of genotypes based on that form of expression. While it is true that some phenotypes are always certain, such as, in pea plants the genotype *ss* will produce always the wrinkled seeds. Similarly, all of the Mendel's genes and all blood group types show an absolute and clear cut pattern. However, for some genes, the expression is variable.

2.8.1 Penetrance

Some individuals fail to show particular trait, even though genetic analysis indicates that the controlling gene for that trait must be present in them. This aspect of gene action, that is *the frequency with which a genotype is expressed in the phenotype is called Penetrance*. For example, out of the eight individuals of a particular genotype, five express the diseased phenotype. The penetrance is $5/8 = 0.625$ or 62.5%.

A completely penetrant gene is always expressed; an incompletely penetrant gene may be expressed in some individuals, but not in others.

Incomplete penetrance of a gene may cause a trait to skip a generation; that is, a dominant trait present in a given generation may skip the first generation but appear again in the second generation. Such a case is illustrated in Figure 2.16. It shows a human pedigree where unaffected individual II-4 is the daughter as well as the mother of an affected individual each. This indicates she has the genotype *Aa*, but because of incomplete penetration, does not show the dominant phenotype.

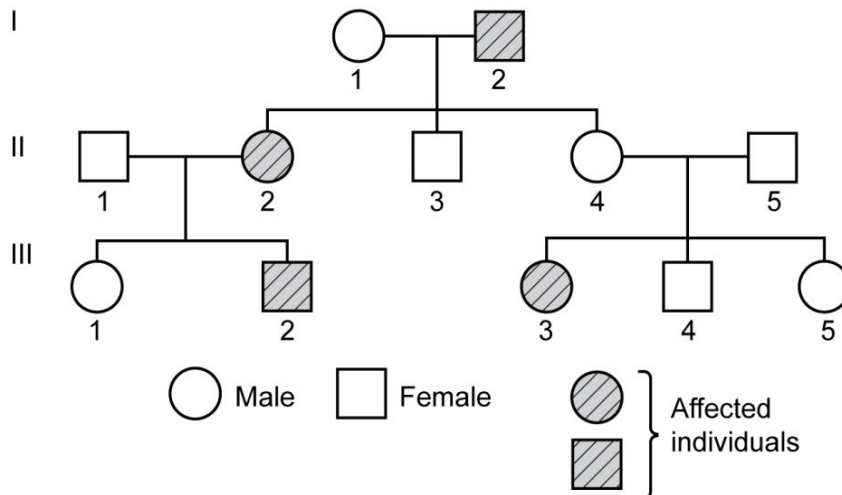


Fig. 2.16: A pedigree illustrating the pattern of phenotypes with an incompletely penetrant dominant allele.

2.8.2 Expressivity

Expressivity refers to the *intensity or range of expression of a trait in different parts of the same individual or different individuals*. This aspect of gene action differs from penetrance in that it describes the level of phenotypic expression, whereas penetrance refers to whether the phenotype is affected or not. Both penetrance and expressivity originate from variations in the degree to which a gene is manifested in the phenotype. These manifestations can be absent or be so slight as to pass unnoticed; in such individuals, a trait would be considered **non-penetrant**. However, when apparent in the phenotype, the same trait may vary in its effects from mild to severe, in which case the trait would be described as exhibiting not only variable penetrance, but **variable expressivity** as well.

Let us consider the example of *Drosophila*. In this the recessive allele *eyeless* (*e*) when present in homozygous condition causes a reduction in the size of the compound eye. However, the extent to which the eye is reduced varies considerably from individual to individual. In some, only slight decrease in eye size occurs, while in others the eye may be completely absent. There are also cases in which the fly has one normal eye and the other eye drastically reduced in size. Phenotypic variation can also be observed in humans. Polydactyly exhibits variable penetrance, it is also characterised by differences in expressivity. In different individuals showing this trait, extra digits may be present on the hands or on the feet or both hands and feet.

The source of this variation is partly genotypic and partly environmental. The genotype contains thousands of genes, and the actions of many of them are interrelated so as to modify one another's effects. For example, the level of expression of a trait is generally more similar among relatives than among unrelated individuals, provided that the relatives and unrelated individuals are raised in fairly similar environments. Such *genes that have secondary effect on a trait are called modifier genes*, and can sequentially influence the phenotype. The modifier genes can be seen in animals like house cat, where a 'dilute allele' reduces the intensity of pigmentation from black to grey. Another example of modifier genes is seen in *D. melanogaster* mutants when these are kept in laboratory culture for many years, sometimes they do not have as extreme a phenotype as was first observed.

2.9 SUMMARY

Let us sum up what we have learnt in this unit:

- The dominance is a phenomenon of suppression of expression of one allele by another. The allele that is able to express its phenotype is called dominant, while the one whose expression is suppressed is called recessive.
- In heterozygous condition, i.e. when the dominant and recessive alleles occur together, if an intermediate phenotype appears due to blending of traits of both alleles, it is called incomplete dominance.
- Further in heterozygous condition if the phenotype of both dominant and recessive alleles express equally, the phenomenon is called co-dominance.
- Certain phenotypes are controlled by more than two alleles, known as multiple alleles, for example the range of coat colour phenotype in rabbits, ABO blood groups in humans and self- incompatibility in plants.
- There are some non-allelic gene interactions which may give rise to new phenotypes and are exemplified by modified mendelian ratios. These are complementary gene interaction, epistasis, supplementary gene interaction and duplicate genes.
- In complementary gene interaction, neither of the two dominant alleles of complimentary gene pair is able to produce the phenotype alone. At least one dominant allele from both the gene pairs is required to produce the concerned phenotype. The F_2 phenotypic ratio in this case is 9:7.
- In another kind of gene interaction, one gene masks the expression of other non-allelic gene, known as epistasis. If the epistatic gene is dominant, it is called dominant epistasis, while if the masking action is due to a recessive gene, it is called recessive epistasis. The F_2 phenotypic ratio in both the cases is 12:3:1 and 9:3:4 respectively.
- The gene interaction similar to recessive epistasis is supplementary gene action in which a dominant gene is able to express its character, while another non allelic dominant gene is able to express itself only in presence of the first one. The F_2 phenotypic ratio in this case also is 12:3:1.
- A non-allelic gene interaction is observed in which the presence of any one dominant allele of the two gene pairs also produces a phenotype and the mutant phenotype appears only in double homozygous recessive condition.

2.10 TERMINAL QUESTIONS

1. Discuss the genetic basis of seed shape in peas.
2. State reason for appearance of pink flowers as an intermediate phenotype in *Mirabilis Jalapa*.

3. How is co-dominance different from incomplete dominance?
4. Explain the occurrence of a range of coat colour phenotypes in rabbits.
5. Predict the blood group of a child if blood group of mother is 'AB' and that of father is 'O'.
6. Explain the clinical significance of Rh antigens in human blood.
7. Describe the role of multiple alleles in self-incompatibility in plants.
8. Explain the complimentary gene action with example.
9. Explain the phenomenon of masking the expression of gene by another in epistasis.
10. Do you opine that the phenomena of recessive epistasis and supplementary gene interaction are same or different, explain.
11. Attribute the reason to obtain F_2 phenotypic ratio 15:1 in appearance of awn character in rice.

2.11 ANSWERS

Self Assessment Questions

1. i) recessive, ii) dominance, iii) co-dominance.
2. i) T, ii) T, iii) F, iv) F.
3. a) v, b) iv, c) i, d) ii, e) iii.

Terminal Questions

1. Refer to Sub-Section 2.2.1.
2. Refer to Sub-Section 2.2.2.
3. Refer to Sub-Section 2.2.3.
4. Refer to Section 2.3.
5. Refer to Section 2.3.1
6. Refer to Sub-Section 2.3.2.
7. Refer to Sub-Section 2.3.3.
8. Refer to Sub-Section 2.4.1.
9. Refer to Sub-Section 2.4.2.
10. Refer to Sub-Section 2.4.3.
11. Refer to Sub-Section 2.4.4.

FURTHER READINGS

1. Principles of Genetics by Gardner, Simmons, Snustad, 8th edition – John Wiley and Sons, Inc., 2003.
2. Genetics: a Conceptual Approach, 3rd edition, Peter J. Russell. Pub: WH Freeman & Co.
3. Genetics: Principles and Analysis, 4th edition. D.L. Hartl, D.W. Jones. Pub: Jones and Barlett Publishers
4. Genetics, 9th revised multicolor edition. P.S. Verma & V.K. Agarwal. Pub: S. Chand & Co.

Acknowledgement of Figures

Fig. 2.1: http://legacy.biotechlearn.org.nz/layout/set/lightbox/themes/mendel_and_inheritance/mendel_s_principles_of_inheritance

Fig. 2.3: <https://biologywise.com/codominance-explained-with-examples>

Fig. 2.4: <https://courses.lumenlearning.com/bio1/chapter/reading-multiple-alleles/>

Fig. 2.5: http://www.eplantscience.com/index/genetics/multiple_alleles_based_on_classical_concept_of_allelomorphism/skin_colour_in_rodents.php

Fig. 2.6: <https://courses.lumenlearning.com/microbiology/chapter/hypersensitivities/>

Fig. 2.7: <https://courses.lumenlearning.com/microbiology/chapter/hypersensitivities/>

Fig. 2.9: <http://www.biologydiscussion.com/genetics/gene-interactions/gene-interactions- allelic-and-non-allelic-cell-biology/38795>

Fig. 2.10: <http://www.biologydiscussion.com/genetics/gene-interactions/ gene-interactions -allelic-and-non-allelic-cell-biology/38795>

Fig. 2.12: <http://www.biologydiscussion.com/genetics/gene-interactions/top-6-types-of-epistasis -gene-interaction/37818>

UNIT 3

LINKAGE, CROSSING-OVER AND CHROMOSOME MAPPING

Structure

- | | |
|--------------------------------------|-------------------------------------|
| 3.1 Introduction | 3.4 Chromosome Mapping |
| Expected Learning Outcomes | Three-Point Crosses |
| 3.2 Linkage | Interference and Coincidence |
| Discovery of Linkage | 3.5 Why Didn't Mendel Find Linkage? |
| 3.3 Crossing-Over | 3.6 Summary |
| The Concept of Crossing-Over | 3.7 Terminal Questions |
| When does Crossing-Over Occur? | 3.8 Answers |
| Cytological Basis of Crossing-Over | |
| Molecular Mechanism of Crossing-Over | |

3.1 INTRODUCTION

The independent assortment of genes during meiosis at the time of gamete formation, is one of the fundamental processes of genetics. There are, however, situations where independent assortment of certain genes does not occur. These genes are transmitted together enblock because they are located on the same chromosome. Genes which are located on the same chromosome and transmitted together are called *linked genes* and are said to belong to a *linkage group*. Since it is the chromosome and not the gene which is the unit of transmission at meiosis, linked genes are not free to undergo independent assortment. How does one determine as to which genes on a chromosome are linked together? *Test crosses are useful for identifying linked gene*. This knowledge of linked genes and linkage group are used to construct a *linkage map* or *genetic map* of each chromosome.

The information obtained from genetic mapping is very useful in many aspects of genetic analysis. For example, chromosome maps can tell us whether certain genes that produce a particular phenotype are located together on the same chromosome. The knowledge thus obtained can be used in understanding; the regulation of gene expression in an organism; the DNA sequences in and around a particular set of genes, and it can also be applied to DNA recombinant research/technology.

In many instances, the linked genes, contrary to the expectations, may not be transmitted together. This is because, during the first meiotic prophase when homologous chromosomes undergo pairing, there is a reciprocal exchange of chromosome segments. This process is known as crossing-over. Crossing-over is responsible for the non-transmission of linked genes together and generation of recombinants.

In this unit you would learn about the concept of linkage, the evidence for the physical exchange of chromosome segments (crossing-over) resulting in recombination, and the construction of genetic map of a chromosome.

Objectives

After studying this unit you should be able to:

- ❖ explain the concept of genetic linkage and the basic terminology involved,
- ❖ contrast between linked and unlinked genes giving examples,
- ❖ state the importance of testcross in understanding/detecting linkage,
- ❖ describe the experiments of Bateson et al. and of Morgan, and relate how their analyses contributed to the development of the concept of linkage,
- ❖ describe the cis-, and trans-configuration of linked genes, and show how these affect the phenotypic ratios,
- ❖ identify from a given data, whether it is a case of linkage,
- ❖ describe the concept of crossing-over highlighting its main features;
- ❖ describe the cytological basis of crossing-over,
- ❖ illustrate the molecular mechanisms of crossing-over,
- ❖ explain how linkage maps are constructed, and their utility in genetic analysis,
- ❖ comment on why Mendel did not find linkage in his experiments with pea, and
- ❖ solve genetic problems involving linkage, crossing-over and gene mapping.

3.2 LINKAGE

All organisms have a large number of genes, their number being much more than the number of chromosomes. All genes on the same chromosome do not assort independently and therefore, provide another exception to Mendel's laws of inheritance. Genes whose patterns of inheritance deviate from that of independent assortment are often **linked**.

You have already studied about a dihybrid cross between a true-breeding strain of peas with round, yellow seeds (SSYY) and one with wrinkled, green seeds (ssyy). Independent assortment during gamete formation in the F_1 hybrid (SsYy) is expected to yield four types of gametes in equal proportions (SY, Sy, sY and sy), resulting in four phenotypes in the F_1 progeny in ratios of 9:3:3:1. But if parental association of alleles (i.e., S with Y, and s with y) are maintained during gamete formation in F_1 hybrid, only two types of gametes would be produced ; SY and sy. In other words, the genes for seed shape and seed colour would be **completely linked** in the F_2 progeny and would then be round, yellow seeds (SSYY and SsYy) and wrinkled, green seeds (ssyy) in a phenotypic ratio 3:1. You should note a point here that the individuals of F_2 progeny are classified as round, yellow seeds or wrinkled, green seeds based on their phenotypes. **Complete linkage** of these genes makes it appear that round-yellow and wrinkled-green are inherited as **single trait**.

One of the most preferred methods to study linkage is by making test crosses in which one parent is homozygous for the recessive genes under study. The phenotypes of progeny in a test cross directly reflect the gametic types produced by the heterozygous parent. Figure 3.1 shows the use of test cross for studying independent assortment and complete linkage.

In the first case, where the independent assortment of alleles of two genes occurs, the four phenotypes are produced in equal numbers. Two classes of progeny exhibit the same association of alleles as seen in the parents (a^+b^+ and ab), and the other two classes of progeny exhibit new (recombinant) association of alleles (a^+b and ab^+). If parental-type and recombinant-type progeny occur in equal numbers, the genes a and b assort independently during meiosis in the heterozygous parent, and are said to be not **linked**. The **recombination frequency** between the two genes is defined as the summed frequency of recombinant types among the total progeny (In the example shown in Fig. 3.1 it is $50/100 = 0.50$ or 50%). *Independent assortment is characterised by a recombination frequency of 50% and may indicate that the two genes possibly reside on different chromosomes.*

In the second case shown in Fig. 3.1, the complete association of alleles of two genes results in two genotypes among the progeny of a test cross. These progeny exhibit genotypes as those of the parents. Such complete linkage also indicates that the two genes may be located on the same chromosome.

Parent cross		$\frac{a^+ b^+}{a^+ b^+}$	X	$\frac{ab}{ab}$				
Test cross		$\frac{a^+ b^+}{ab}$	X	$\frac{ab}{ab}$				
(i) If independent assortment occurs					the progeny types are:			
					$\frac{a^+ b^+}{ab}$	Parental Types	Expected Number	Total Number
					$\frac{ab}{ab}$		25	100
					$\frac{ab}{ab}$		25	
					$\frac{a^+ b}{ab}$	25		
					$\frac{ab^+}{ab}$	25		
					$\frac{ab}{ab}$	Recombinant Types		
					$\frac{ab}{ab}$			
(ii) In case of complete linkage					the progeny types are:			
					$\frac{a^+ b^+}{ab}$	Parental Types	50	100
					$\frac{ab}{ab}$		50	
					$\frac{ab}{ab}$			
					$\frac{a^+ b}{ab}$	Recombinant Types	0	
					$\frac{ab^+}{ab}$		0	
					$\frac{ab}{ab}$			

Fig. 3.1: Progeny genotypes from a test cross involving alleles of two genes a and b. The observed number of each progeny genotype are those expected among 100 progeny if (i) genes a and b assort independently, or (ii) they are completely linked. The bar ($\frac{ab}{a^+ b^+}$) separates alleles of one homolog from those of the other.

In the above test cross (Fig. 3.1), we have not considered in a test cross whether the tester (homozygous, recessive) is the ♀ parent or ♂ parent. We shall now examine two situations: one in which the ♀ parent is the tester and the ♂ parent is doubly heterozygous (Fig. 3.2); and the second in which ♂ parent is the tester and the ♀ parent is doubly heterozygous (Fig. 3.3). In these two crosses we shall see if there are any differences in the ratios of progenies of these two test crosses.

The first cross in *Drosophila* (Fig. 3.2) shows *complete linkage* of the recessive autosomal traits for black body (*b*) and purple eyes (*pr*). When the male is the double heterozygous parent, and there is complete linkage, then only sperms with the parental combinations of alleles are produced and transmitted to the progeny resulting in half black-body, purple-eyed flies, and half wild-type flies. Recombinations of parental alleles are not observed, so no black-body normal eye flies, or normal-body purple eye flies are produced. The data shown in the Fig. 3.2 indicates that in a cross where is ♀ the tester and is doubly heterozygous, the progeny exhibits parental phenotypes which indicates the occurrence of complete linkage in this case.

Parental cross	$\frac{b\ pr}{b\ pr} \text{♀} \times \frac{b^+\ pr^+}{b^+\ pr^+} \text{♂}$	Allele	Phenotype
Test Cross	$\frac{b\ pr}{b\ pr} \text{♀} \times \frac{b^+\ pr^+}{b\ pr} \text{♂}$	b	black body (recessive)
		b ⁺	wild type (dominant)
		pr	purple eye (dominant)
		pr ⁺	wild type
Progeny Type		Observe Number	Number expected from independent assortment
Parentals	$\frac{b\ pr}{b\ pr}$ Black body Purple eye	374	193
	$\frac{b^+\ pr^+}{b\ pr}$ wild type	398	193
Recombinants	$\frac{b\ pr^+}{b\ pr}$ Black body Normal eye	0	193
	$\frac{b\ pr}{b\ pr}$ Normal body Purple eye	0	193
	Total	772	772

Fig. 3.2: A test cross with F₁ progeny of *Drosophila* in which ♀ is the homozygous recessive (tester) and ♂ is the doubly heterozygous parent. Complete linkage is observed.

In the second cross in *Drosophila* (see Fig. 3.3) four types of progeny are observed; two parental types (black body, purple eyes; and wild type) and two recombinant types (black body, normal eyes; and normal body, purple eyes). There are the four types of progeny one would expect, if the genes for body colour eye colour segregate independently. However, the observed ratio of recombinant to parental types $\frac{14 + 16}{210 + 240} = \frac{30}{450}$ is very different from that expected for independent ($\frac{120 + 120}{120 + 120} = \frac{240}{240}$).

Such a deviation from the ratio expected for independent assortment indicates there is linkage but is partial and not complete. The degree of linkage exhibited in such a cross (showing *partial or incomplete linkage*) is measured by the frequency of recombination. The recombination frequency in this case is $30/480 = 0.0625$ or 6.25%. The unlinked genes, or the ones assorting independently exhibit a recombination frequencies of 50% ($240/480 = 0.50 = 50\%$).

Parental cross	$\frac{b}{b} \frac{pr}{pr} \text{♀} \times \frac{b^+}{b^+} \frac{pr^+}{pr^+} \text{♂}$	Allele	Phenotype
Test Cross	$\frac{b}{b} \frac{pr}{pr} \text{♀} \times \frac{b^+}{b^+} \frac{pr^+}{pr^+} \text{♂}$	b	black body (recessive)
		b ⁺	wild type (dominant)
		pr	purple eye (dominant)
		pr ⁺	wild type
Progeny Type	Phenotype	Observe Number	Number expected from independent assortment
Parentals	$\frac{b}{b} \frac{pr}{pr}$ Black body Purple eye	210	120
	$\frac{b^+}{b^+} \frac{pr^+}{pr^+}$ Wild type	240	120
Recombinants	$\frac{b}{b} \frac{pr^+}{pr^+}$ Black body Normal eye	14	120
	$\frac{b^+}{b^+} \frac{pr}{pr}$ Normal body Purple eye	16	120
Total		480	480

Fig. 3.3: A test cross with the F₁ progeny of *Drosophila* in which the ♂ parent is homozygous recessive and the ♀ parent is doubly heterozygous. Partial linkage is observed.

So far we have discussed the concept of linkage explaining the basic terminology involved. We would now review two pioneering works: one by Bateson, Saunders and Punnett and the other by Morgan, which demonstrated linkage experimentally.

3.2.1 Discovery of Linkage

Partial Linkage in Sweet Pea: The effects of linkage were first evident in the results of dihybrid cross in sweet peas (*Lathyrus odoratus*) that were reported by W Bateson, E. Saunders and R.C. Punnett in 1905. This cross is represented diagrammatically in Fig. 3.4.

Parents	PPLL (Purple, long) × ppll (Red, round)	Gene for:	Alleles
		Flower colour	P (purple), p (red)
		Pollen grain shape	L (long), l (round)
F ₁	PpLl All purple, round		
Cross F ₁ × F ₁ :			
F ₂		Numbers obtained	Expected Numbers
	Purple, long (P-L-)	4831	3915
	Purple, round (P-l)	390	1305
	Red, long (pL-)	393	1305
	Red, round (ppl)	1338	435

Fig. 3.4: The Bateson, Saunders and Punnett cross. The expected numbers of the F₂ plants are calculated on the basis of the same total number of progeny (6952) and the 9:3:3:1 ratio obtained in a dihybrid cross by Mendel.

The sweet peas with purple flowers and long pollen grain (*PPLL*) were crossed with red-flowered plants with round pollen grains (*ppll*). Nothing was unusual about the F_1 progeny as all were purple and long (*PpLl*), showing these to be dominant traits. When the F_1 was inbred and each pair of alleles was examined separately, each one showed segregation like the Mendelian genes, that is, purple and red flowers were present in a 3:1 ratio, so were the long and round pollen traits.

But when the traits were considered together, then partial linkage was seen. Among the 6952 F_2 plants, 4831 were purple long (*P-L-*); 390 were purple, round (*P-l*) 393 were red, long (*ppL-*); and 1338 were red, round (*ppll*). Compare in the Fig. 3.4, numbers of plants obtained with the expected numbers. You can see that this was predicted about independently assorting genes. If these two traits had been located on different chromosomes, perhaps we would have got a 9:3:3:1 ratio of these F_2 progeny. In other words, 3915 purple, long; 1305 purple, round; 1305, red, long; and 435 red, round.

The data in Fig. 3.4 clearly shows that the two traits did not show complete linkage, had they done so a 3:1 ratio or 5214 purple, long and 1738, red, round progeny would have been expected. This clearly was a case of partial linkage. However, Bateson, Saunders and Punnett did not interpret their results in terms of behaviour of genes located on the same chromosome of linkage. Thomas Hunt Morgan was the first to relate linkage to the segregation of homologous chromosomes, and the occurrence of crossing-over between homologous chromosomes during meiosis. Morgan's interpretation of linkage was published in 1911 in a paper, where he reported the results of crosses involving linked genes in the fruit fly, *Drosophila melanogaster*. Many of our current concepts of linkage, crossing-over, and chromosome mapping have evolved from the work of Morgan and his students; C.B. Bridges; H.J. Muller and A.H. Sturtevant.

Morgan's Linkage Experiments with *Drosophila*: Morgan demonstrated the effects of linkage by two genes located on the second chromosome of *D. melanogaster* (see Fig. 3.5 and 3.6). In both these crosses, the recessive gene *b* in homozygous condition results in black colour of the body. The presence of its dominant allele *b*⁺ results in grey bodies. The second gene affects the phenotypes of the wings. The recessive allele *vg* in homozygous condition results in vestigial or underdeveloped wings (Fig. 3.7b); and its dominant allele *vg*⁺ leads to the development of long normal wings (Fig. 3.7a).

First let US consider a cross (Fig. 3.5) between homozygous flies with long wings and grey bodies and homozygous flies with vestigial wings and black bodies. This cross produces F_1 , flies with long wings and grey bodies. In the progeny of this test cross, 82 per cent exhibited one or the other (41 per cent each) of the parental combinations of traits. The other 18 per cent of the progeny have new or recombinant combinations.

Next study, the second cross as shown in Fig.3.6. This cross is between homozygous flies with long wings and black bodies and homozygous flies with vestigial wings and grey bodies. In this cross, 82 per cent of the test cross progeny have parental phenotypes, i.e., phenotypes identical to one or the other original parent and 18 per cent had new or recombinant phenotypes.

From the results of these experiments three points emerge: **First**, we find that in the progeny of the F_1 test cross in both the cases, the original parental combinations (long wings, grey body and vestigial wings, black body in the first cross – Fig. 3.5; long wings, black body and vestigial wings, grey body in the second cross – Fig. 3.6) are the most prominent phenotypes in the F_2 . As we have seen in the sweet pea experiments, an excess of parental phenotypes in the F_2 generation, strongly suggests the presence of linked genes.

Second, in both the crosses, the F_1 flies are phenotypically same, that is, they all have long wings, grey bodies (see Figs. 3.5 and 3.6). But on comparing the test cross progeny of the F_1 female flies in the two crosses, we can see that they contain very different frequencies of the four phenotypic classes. For example, in the 1st cross (Fig. 3.5), 41% of the testcross progeny show long wings, grey body phenotype and in the 2nd cross (Fig. 3.6) only 9% progeny show this phenotype.

You should note that, so far we are only looking at this point from the phenotypic angle. Let us now examine it from the viewpoint of arrangement of genes on the chromosomes. We break this discussion here, to quickly look into the basic terminology regarding gene arrangement. We shall resume our discussion after that.

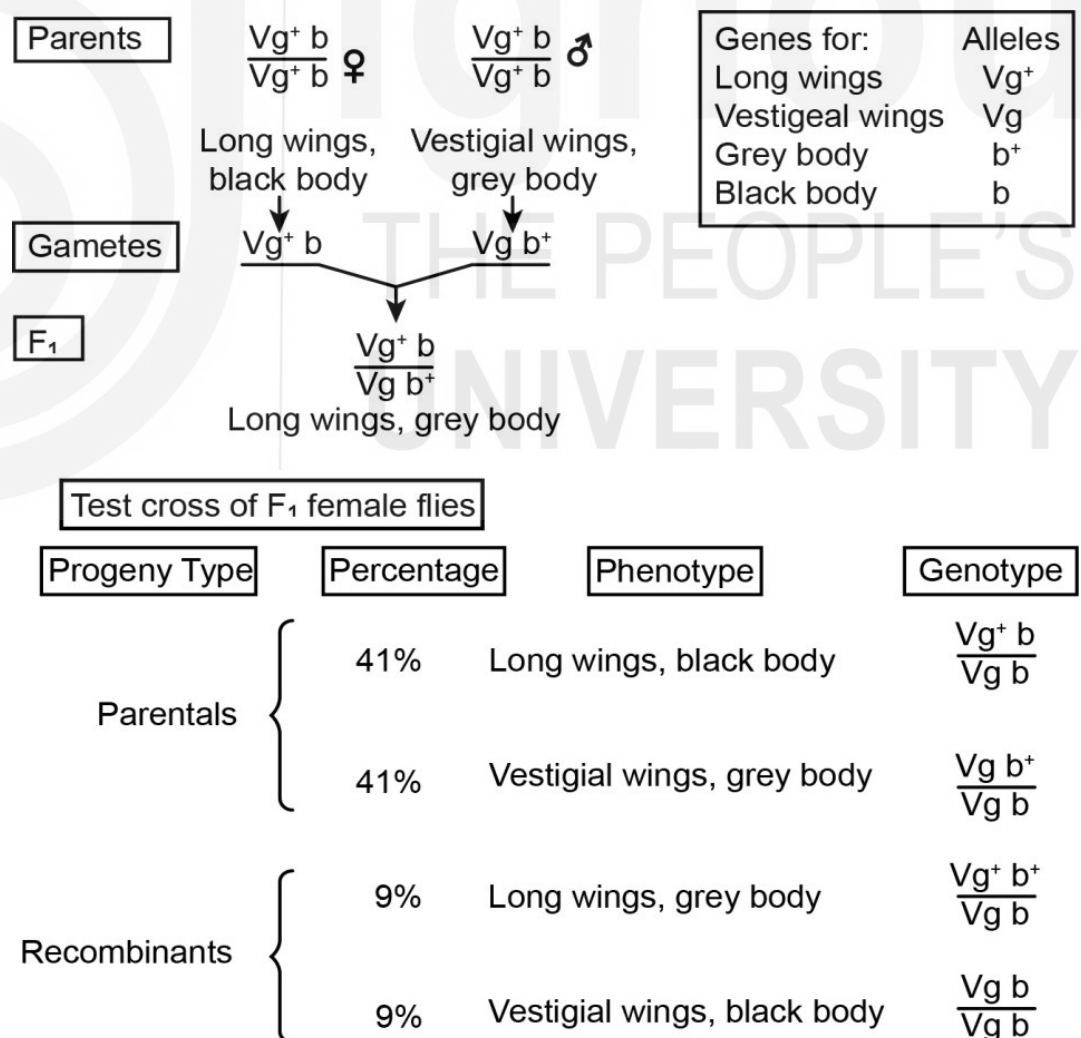
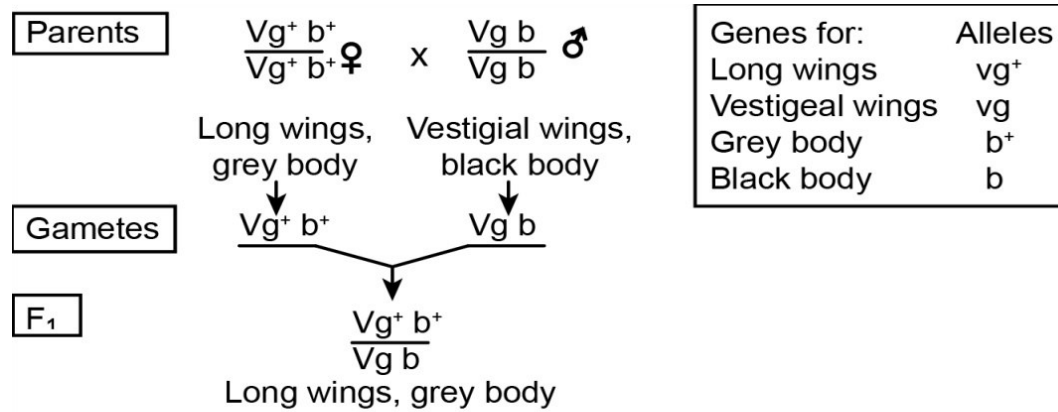


Fig. 3.5: A cross between parents with long wings grey bodies and vestigial wings, black bodies. A test cross with the F_1 individual shows 82% parental and 18 recombinant phenotypes.



Test cross of F ₁ female flies			
Progeny Type	Percentage	Phenotype	Genotype
Parentals	41%	Long wings, grey body	$\frac{Vg^+ b}{Vg b}$
	41%	Vestigial wings, black body	$\frac{Vg b}{Vg b}$
Recombinants	9%	Long wings, black body	$\frac{Vg^+ b}{Vg b}$
	9%	Vestigial wings, grey body	$\frac{Vg b^+}{Vg b}$

Fig. 3.6: A cross between parents with long wings black bodies and vestigial wings grey bodies. Just like the previous cross, the F₁ individuals show 82% parental and 18% recombinant phenotypes.

Arrangement of Genes: In the cross shown in Fig. 3.5, the F₁ flies carry the alleles ($vg^+ b^+$) on one homolog, and the recessive alleles ($vg b$) on the other homolog. The genotype of the F₁ heterozygote of this type is usually written as:

$vg^+ / vg b$ or simply $vg^+ / vg b$.

This arrangement of genes, i.e., dominants on one homolog and the recessives on the other, is called **coupling state** or **cis-configuration**. The alternate arrangement as shown in Fig. 3.6, where in the F₁ heterozygote, each homolog contains one dominant and one recessive gene such as $vg^+ / vg b^+$. This is called the **repulsion state** or **trans-configuration**.

We now resume our discussion. We were discussing the reason why we get different percentages of a particular phenotype, in the two crosses. You have seen above, though phenotypically the F₁ heterozygotes are same, but they show different arrangement of genes in the two cases. In the cross in Fig. 3.5, it is cis arrangement- $vg^+ b^+ / vg b$, whereas in the other case (Fig. 3.6) it is trans arrangement – $vg^+ b / vg b^+$. The arrangement of the genes on the homologs, too is an important factor. This is precisely the reason for occurrence of different ratios of a particular phenotype (long wings, grey body example discussed here).

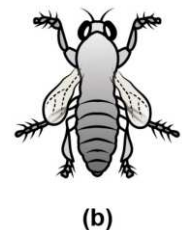
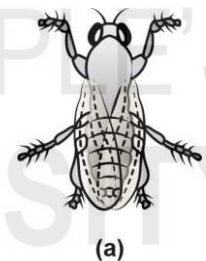


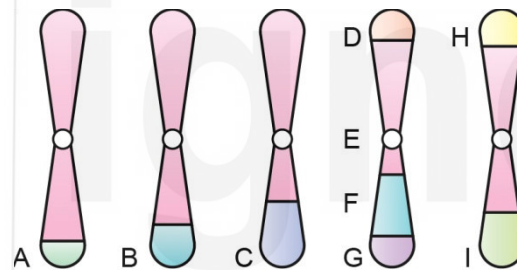
Fig. 3.7: Drosophila phenotypes a) Normal wings, b) Vestigial wings.

Another important feature of the two crosses just described is that regardless of whether the cross was done in *cis* (coupling, Fig. 3.5) or in *trans* (repulsion, Fig. 3.6) – the F_1 flies produced gametes 185 of which carried recombinant combinations and 82% were parental.

Third, Morgan also interpreted the results of these two crosses on the basis of physical phenomenon that were actually taking place. He interpreted these experiments in context of a study of meiosis in Salamander made by F. Janssens in 1909. Janssens had described chiasmata (Fig. 3.7) and suggested that these might represent sites of physical exchange between maternal and paternal homologues. Morgan hence proposed that partial linkage was observed whenever two markers in the same chromosome were separated from one another by a chiasma. He coined the term crossing-over for this process.

SAQ 1

Given below are five chromosomes (1-5) each having one or more genes. Observe them carefully and answer the following questions:



- Fill in the blanks:
 - The genes A, B, and C are genes.
 - The genes D, E, F and G are genes.
 - The genes H and I too are genes.
- How many linkage groups are seen in the above figure?
- Write the linkage groups that are shown in the figure.
- Write a statement that explains the situation in 1 to 5.

SAQ 2

Given below are three crosses – a, b and c. Study them carefully and answer the following questions.

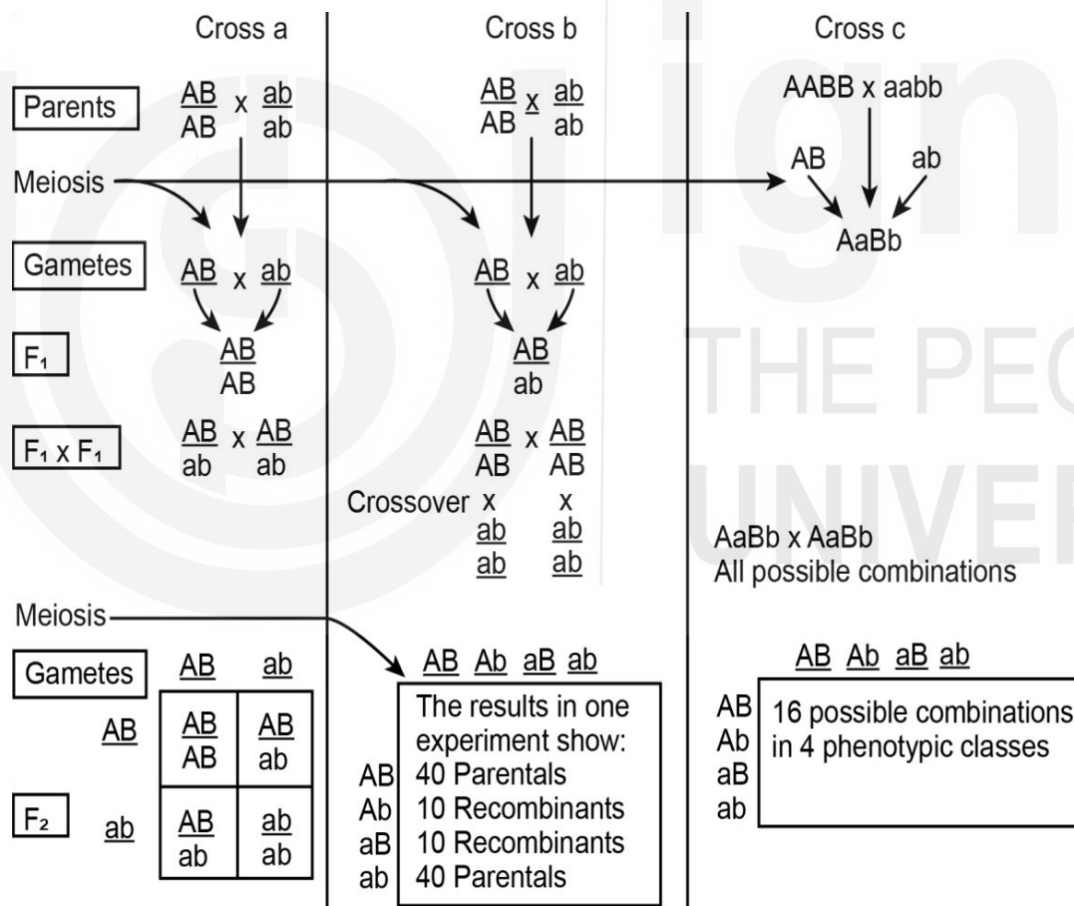
- Cross a**
 - The genes in meiotic gametes are
 - The phenotypic ratio of F_2 is
 - The genotypic ratio of F_2 is
 - This cross shows

b) **Cross b**

- The genes in meiotic gametes are
- F_1 inbreeding shows a special feature that is
- The F_2 genotypes constitute basically Classes of individuals that are and
- This cross shows

c) **Cross c**

- The genes in meiotic gametes are
- The F_1 progeny is
- The phenotypic ratio of F_2 progeny is
- This cross shows

**SAQ 3**

Which one of the following arrays represents the cis-configuration of the alleles A and B?

$\frac{ab}{AB}$ or $\frac{aB}{Ab}$

3.3 CROSSING-OVER

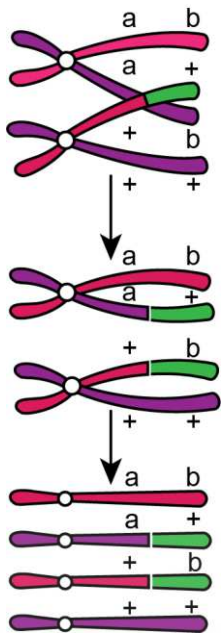


Fig. 3.8: Diagram illustrating the occurrence of crossing-over between two chromatids of homologous chromosomes. The exchange between two of the four chromatids results in two recombinants and two parental combinations at meiosis.

Crossing-over is a physical exchange between chromatids in a pair of homologous chromosomes. It results in a new association of genes in the same chromosome. The role of crossing-over is important for evolution to take place. In fact, **crossing-over and independent assortment are mechanisms that produce new combinations of genes.** Natural selection can then act to preserve those combinations that produce organisms with maximum fitness, that is, maximum probability of perpetuation of the genotype.

3.3.1 The Concept of Crossing-Over

Following are the important features of crossing-over:

- A gene is located on chromosomes at a particular site called a locus (plural-loci). The loci of the genes on a chromosomes are arranged in a linear sequence.
- In a heterozygote, the two alleles of a gene occupy corresponding positions in the homologous chromosomes, that is, allele *A* occupies the same position in homolog 1 that allele *a* occupies of a species is fixed or constant.
- Crossing-over involves the breakage and rejoining of two chromatids (of homologous chromosomes), resulting in reciprocal exchange of equal and corresponding segment between them (Fig. 3.8).
- Chromosomes with recombined or new combinations of genes are formed by the occurrence of crossing-over.
- Crossing-over occurs more or less at random along the length of a chromosome pair. Thus, the probability of its occurrence between two genes increases with increasing physical separation of the genes along the chromosome.

3.3.2 When does Crossing-Over Occur?

Crossing-over begins at pachytene stage, after the synapsis of the homologous chromosomes has occurred in zygotene stage of prophase-I of meiosis. Since chromosome replication occurs during interphase, meiotic crossing-over occurs in the post-replication four strand or tetrad stage. That is, after each chromosome has doubled such that four chromatids are present for each pair of homologous chromosomes.

3.3.3 Cytological Basis of Crossing-Over

Based on his results (Fig. 3.5 and 3.6), Morgan suggested that recombinations are formed as a result of pairing of homologous chromosomes during meiosis. A physical exchange of chromosome part takes place by a process called crossing-over. In the germs cells of many organisms at the time of meiosis, one can actually see certain cross-shaped structures in which two of the four chromatids of homologous chromosome pairs appear to exchange parts (see Fig. 3.8). These cross-shaped structures are called *Chiasmata* (singular *Chiasma*).

The first direct evidence that relates the occurrence of physical exchange between chromosomes to the genetic recombination was provided by C. Stern (1931) in *Drosophila*, and by H.B. Creighton and B. McClintock in maize. Normally, the two chromosomes constituting a homologous pair are morphologically, alike and are therefore, indistinguishable. But Stern, Creighton and McClintock, however, found 'homologs' that were not alike, and could be easily distinguished. Because at their ends they carried distinct morphological features that made them easily observable (see Figs. 3.9 and 3.12). These chromosomes are homologous along most of their length but differed in a small portion only. They, however, paired and segregated normally during meiosis. These structural alterations of the chromosomes permitted the microscopic recognition of parental and recombinant chromosomes. We shall now briefly discuss the experimental work carried out by Stern (in *Drosophila*) and McClintock (in maize using these genetic markers).

Stern discovered two X chromosomes in a *Drosophila* female strain that had undergone structural changes, that made them distinguishable from each other and from a normal X chromosome. One X chromosome had part of a Y chromosome attached to one end. The second X chromosome was shorter than normal, as its piece had broken off, and was translocated to one of the small, fourth chromosome (Fig. 3.9). These female flies were heterozygous for alleles of two genes located on the X chromosome. One gene affects eye shape, and other eye colour. The recessive allele *car* of the first gene results in carnation coloured eyes; its dominant allele *car*⁺ yield red eyes. The dominant mutant allele *B* of the second gene results in bar-shaped eyes (Fig. 3.10) and the recessive allele *B*⁺ produces round eyes (Fig. 3.10b) when homozygous. The females used in Stern's study carried the allelic pairs in **Cis-configuration** (see Fig. 3.9 and 3.11). Stern crossed such heterozygous females with males having carnation-coloured, round eyes, i.e., *car B*⁺ males. The cross and results Stern obtained are shown in Fig. 3.11. Cross a shows a situation where meiosis without crossing-over occurs during the gamete formation. And in cross b, there is meiosis with crossing-over between the *car* and *B* loci, during gamete formation in the female parent. The male *Drosophila* is unique as no crossing-over takes place. Now, look at the figure carefully.

Stern analysed the genotypes of the progeny to see whether there were only recombinations (see Cross b). Not only did he find the recombinant gene types but also found that the alleles on the X chromosomes of each recombinant progeny were precisely the same as predicted if crossing-over had occurred. For example, the recombinant male progeny with the bar-shaped red eyes (*car*⁺ *B*/Y) were found to carry the short X chromosomes, but now with the translocated piece of the Y chromosome at one end (see Cross b, 4). The male flies with normal-shaped, carnation eyes (*car B*⁺/Y) contained long X chromosomes, but without the attached piece of the Y chromosome (see Cross b, 2).

You should note that the male flies were analysed and classified as a particular type on the basis of their phenotypes - the new combinations of the two phenotypic characteristics. The progeny had parental combinations of these traits. And also the progeny flies had X chromosomes that were produced by a cross-over event between the *car* and *B* loci.

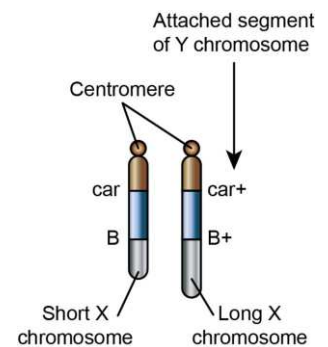


Fig. 3.9: Outline diagram showing the special features of the X chromosome, that were used in Stern's experiments.

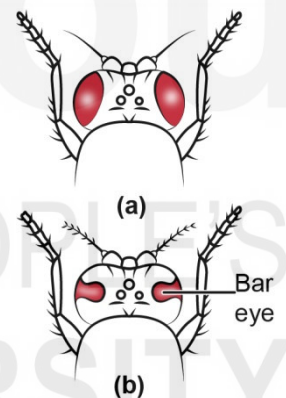


Fig. 3.10: Two phenotypes of eye shape in *Drosophila*. a) normal, round yes. b) bar-shaped eyes.

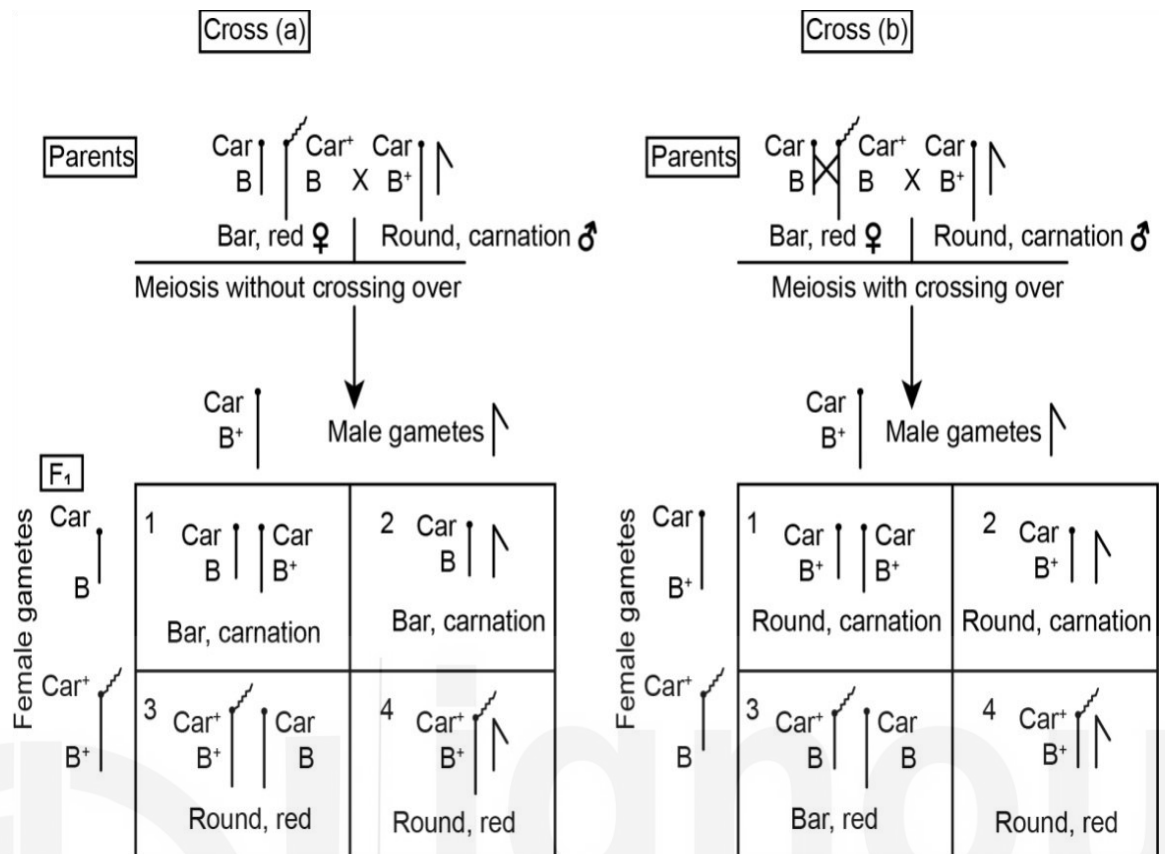


Fig. 3.11: The classic experiment in linkage, by Stern demonstrating that cross-over involves interchange of parts of homologous chromosomes. The cross b indicates the occurrence of crossing-over between the *car* and *B* loci. The knob-like structures on the chromosomes represent the centromere. You can see in the figure that the X chromosomes in the female parent are morphologically distinguishable. The short one on the left is missing the distal end, its homolog (one the right) has an extra piece of Y chromosome attached to the centromere end, which is shown here as a horizontal zig-zagged line.

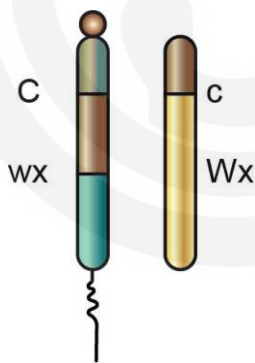


Fig. 3.12: Chromosomes 9 of maize, showing a knob at one end and a translocated piece at the distal end. Note, the genes are arranged in trans-configuration.

Creighton and McClintock also experimentally demonstrated that there was a correlation between crossing-over and exchange of homologous chromosomes. They made crosses involving two loci on chromosome 9 and analysed their results. On this chromosome one gene controls aleurone colour (*C* - coloured; *c* - colourless) and the other controlled the type of carbohydrate in the endosperm (*Wx*-starchy; *wx*-waxy). They made use of a chromosome with densely staining knob at one end and a translocated piece from chromosome 8, at the other end (see Fig. 3.12). These two features made the chromosome visually identifiable.

They made a cross between heterozygous female – coloured, starchy and a males – colourless, starchy, and examined their progeny. The entire cross is illustrated in Fig. 3.13. Note the arrangement of alleles and cytological markers in the unique parent (Fig. 3.12). If cross-over occurs in this parent then the arrangement of alleles changes (see Fig. 3.14). Now examine the phenotypes of the recombinant (cross-over) offspring (cross b). This shows a new phenotype - colourless, waxy. Creighton and McClintock on observing this new phenotype found a rearrangement of the cytological markers.

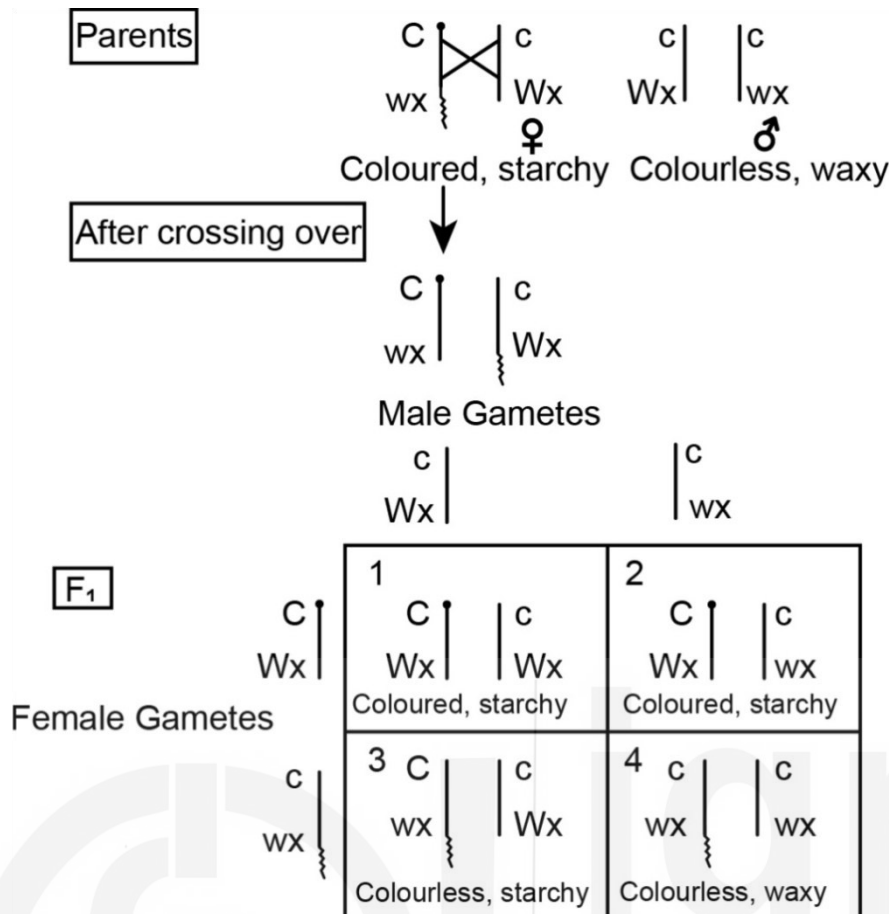


Fig. 3.13: Cross between heterozygote female and male with colourless aleurone, starchy endosperm in maize.

Both the above experiments provide cytological proof of crossing-over and leave no doubt that an actual physical exchange between homologs occurs during crossing-over.

These two experiments by Stern and by Creighton and McClintock are classics in genetic studies. They confirmed Morgan's hypothesis that **crossing-over involves the interchange of parts of homologous chromosomes**. They also provided strong evidence that **genes are located on chromosomes**.

3.3.4 Molecular Mechanism of Crossing-Over

The classic experiments of Stern, and Creighton and McClintock confirmed the correlation between the formation of recombinants and crossing-over, but still several questions remained to be answered, one of them was the nature of crossing-over.

A cross-over as you have seen above (Fig. 3.8) involves a reciprocal, physical exchange between homologous chromosomes. This suggests that a reciprocal exchange essentially occurs between the double helices of the DNA molecules found in each non-sister chromatids. For this process to take place, two homologous chromosomes come close to one another. In eukaryotes, crossing-over has been associated with the formation of a structure (or set of structures) called the synaptonemal complex (Fig. 3.15) which forms during prophase of the first meiotic division. This structure is composed primarily of

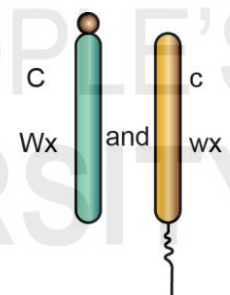


Fig. 3.14: Chromosome of maize, after crossing over between the C and Wx loci. Now the genes are arranged in cis-configuration from the earlier trans-configuration.

proteins and RNA, and has been identified in a large number of eukaryotic species. Very little information is available about the functions of the various components of the synaptonemal complex. It is known that small amount of DNA synthesis occurs at the time when the synaptonemal complex forms. This amount, however, is very small and is equivalent or even less than 1 per cent of the total DNA in the genome. It is believed that this DNA synthesis is involved in synapsis and/or crossing-over.

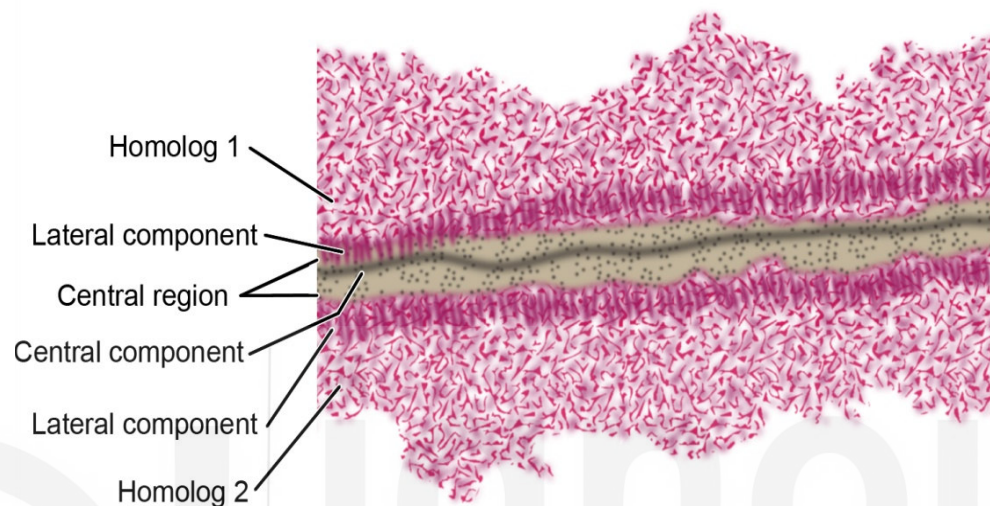


Fig. 3.15: Diagrammatic representation of the synaptonemal complex in *Neottiella rutilans* (Ascomycetes). A dense, central component is surrounded by a less dense space; together these make up the central region which is about 1000Å in width. The lateral components each about 500 Å in width are present on each side of the central region. The two homologous chromosomes are juxtaposed to the two lateral elements. (Redrawn from: M. Westergaard and D. Von Wett Stein, 1972 *Ann. Rev. Genet.* 6 : 71-110.

To explain the mechanism of crossing-over at molecular level, a number of models based on breakage and reunion of a DNA molecule have been proposed. The breakage of two homologous chromatids and the reunion of the parts in new arrangements (recombination) is the main feature of these models. One model for eukaryotic DNA recombination that survives was proposed by Robin Holliday in 1964. The important features of this model are illustrated in Fig. 3.16. This pathway begins when an *endonuclease* cleaves single strands of each of the two parental DNA molecules (*breakage*). Segments of the single strands on one side of each cut are displaced from their complementary strands. In this process *DNA-binding proteins*, *helix destabilisation proteins*, and *DNA unwinding proteins* or *DNA helicases* are also said to be involved.

The displaced strands then exchange pairing partners; base-pairing with the intact complementary strands of the homologous chromosomes. This process is also aided by certain proteins such as **rec A protein**. The **rec A** protein mediates such a reaction by binding to the unpaired strand of DNA, searching for a homologous DNA sequence. A homologous double helix is found, promoting the displacement of a segment of one strand of the double helix by the unpaired strand.

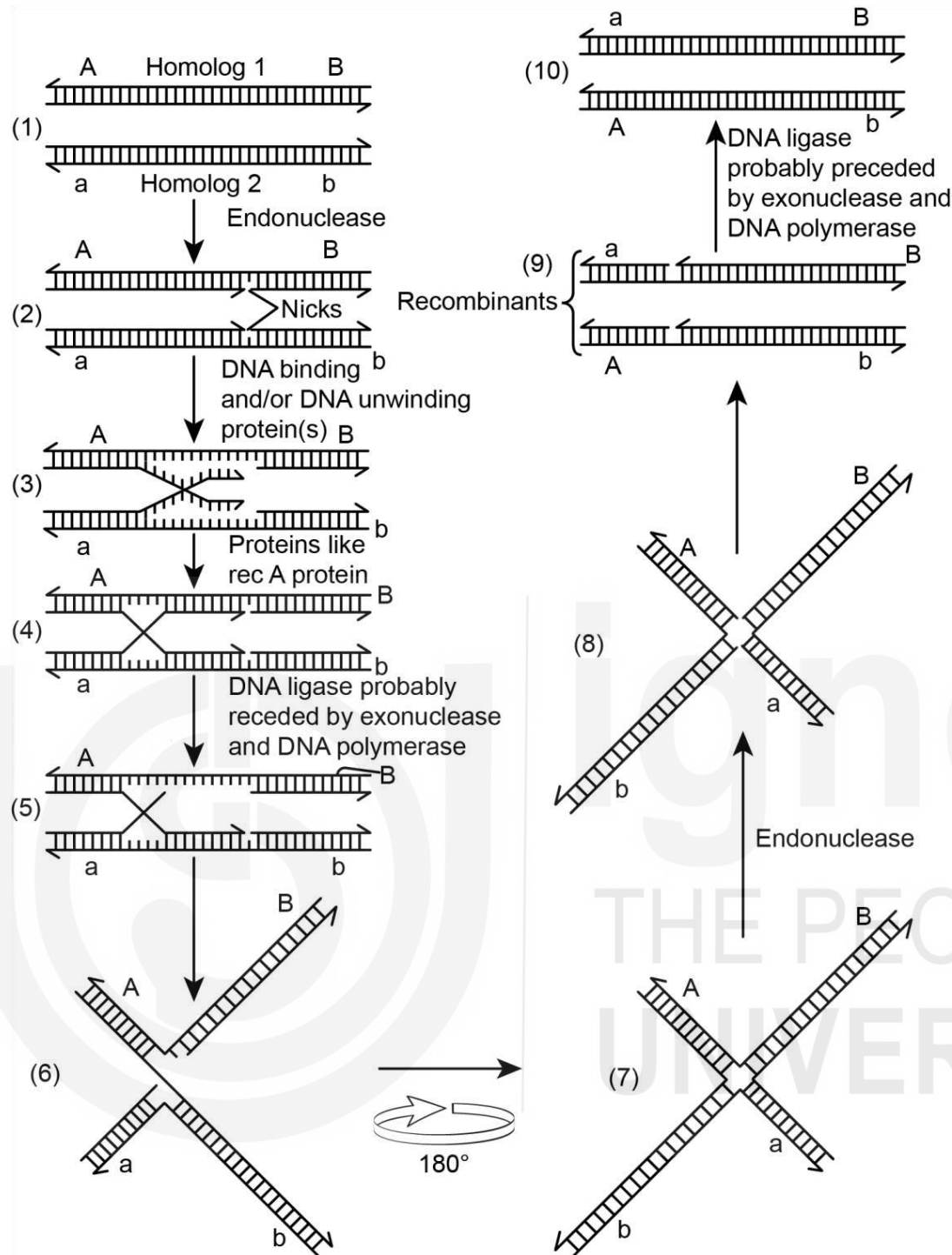


Fig. 3.16: Pathway for the occurrence of crossing-over by breakage and reunion based on the model of Robin Holliday. 1) shows two homologs 1 and 2. Homolog 1 carrying *A* and *B* genes and homolog 2 carrying *a* and *b*, genes. 2) Initiation of cuts on single strands due to endonuclease 3) displacement of one side of the free ends of the cuts, with the help of DNA binding, helix-destabilisation, and/or DNA-unwinding proteins, 4) The cleaved single strand of homolog 1 base-pairs with the complementary intact strand of homolog 2 and vice versa. 5) The cleaved strands are then rejoined in recombinant combinations by single stranded bridge, by the enzyme DNA ligase. 6) and 7) X-shaped intermediates also known as Holliday intermediate or chi forms [after the Greek letter chi (χ)] in different planar views. 8) The intact strands are cleaved at the intersection by endonuclease, and stage 9) is formed. Covalent closure of single stranded interruptions yields the intact recombinant chromosomes as shown in (10).

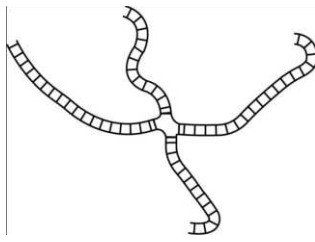


Fig. 3.17: Outline diagram of x-shaped recombinant intermediate as seen under the electron microscope.

The cleaved strands are then covalently joined in recombinant arrangements (*reunion*) by the enzyme DNA ligase. If the original breaks in the two strands do not occur at exactly the same site in the two homologs some “tailoring” will be required before DNA ligase catalyses the reunion process. This tailoring involves excision of a limited number of bases by an *exonuclease* and repair synthesis by a DNA *polymerase*. By this time an X-shaped recombination intermediate called a “chi” form or *Holliday intermediate* is formed. Such intermediates have been observed by electron microscopy (see Fig. 3.17) in several prokaryotic systems. A similar sequence of enzyme catalysed breakage and reunion events, involving the other two single strands, occurs to complete the process of crossing-over.

A current model that accounts for the formation of parentals as well as recombinants is shown in Fig. 3.18. It is based on the works in several laboratories including those of Meselson and Radding (1975); Potter and Dressier (1976); and Das Gupta et al. (1981). Up to stage 7, that is till the formation of “chi form” or Holliday intermediate, it is the same as the Holliday model described above. The difference is ‘from stage 8 onwards.

The joined homologs are separated from each other by endonuclease attack on either pair of opposite strands (see 8a, b). Following the endonuclease cleavages, the strands separate. Each strand contains a small gap in one strand of DNA, that is, closed by the action of the ligase. Which pair of strands is cleaved determines whether the molecules are recombinants or not. If the two strands are cleaved on the sides then the parentals — *AB* and *ab* are formed (9a, 10a). And if the cleavage occurs on top and bottom, then recombinants — *Ab* and *aB* (9b, 10b) are formed.

Along with the modifications of the above model, evidences have accumulated that indicate homologous recombination occurs by more than one mechanism — very possibly by several different mechanisms. A double-strand break model was proposed by Szostak et al. in (1983). The major difference between this model and the Holliday model is that, recombination is mediated by a double-strand break in one of the parental double helices, not by just single-strand breaks (see Fig. 3.19). These initial breaks in the two strands are enlarged further and gaps are formed in both the strands. The two single-stranded termini produced at the double stranded gap of the broken double helix invade the intact double helix, displacing the homologous strand in this region. Repair DNA synthesis then takes place filling the gaps using complementary strands of the other chromosome as templates. This process yields a recombination intermediate with the two double helices joined by two single-stranded bridges (chi structures). The bridges are resolved by endonuclease cleavages by the same process as for chi structures formed by the Holliday mechanism. This model, like the Holliday model, explains the production of chromosomes that are recombinant for genetic markers flanking the region in which cross-over occurs.

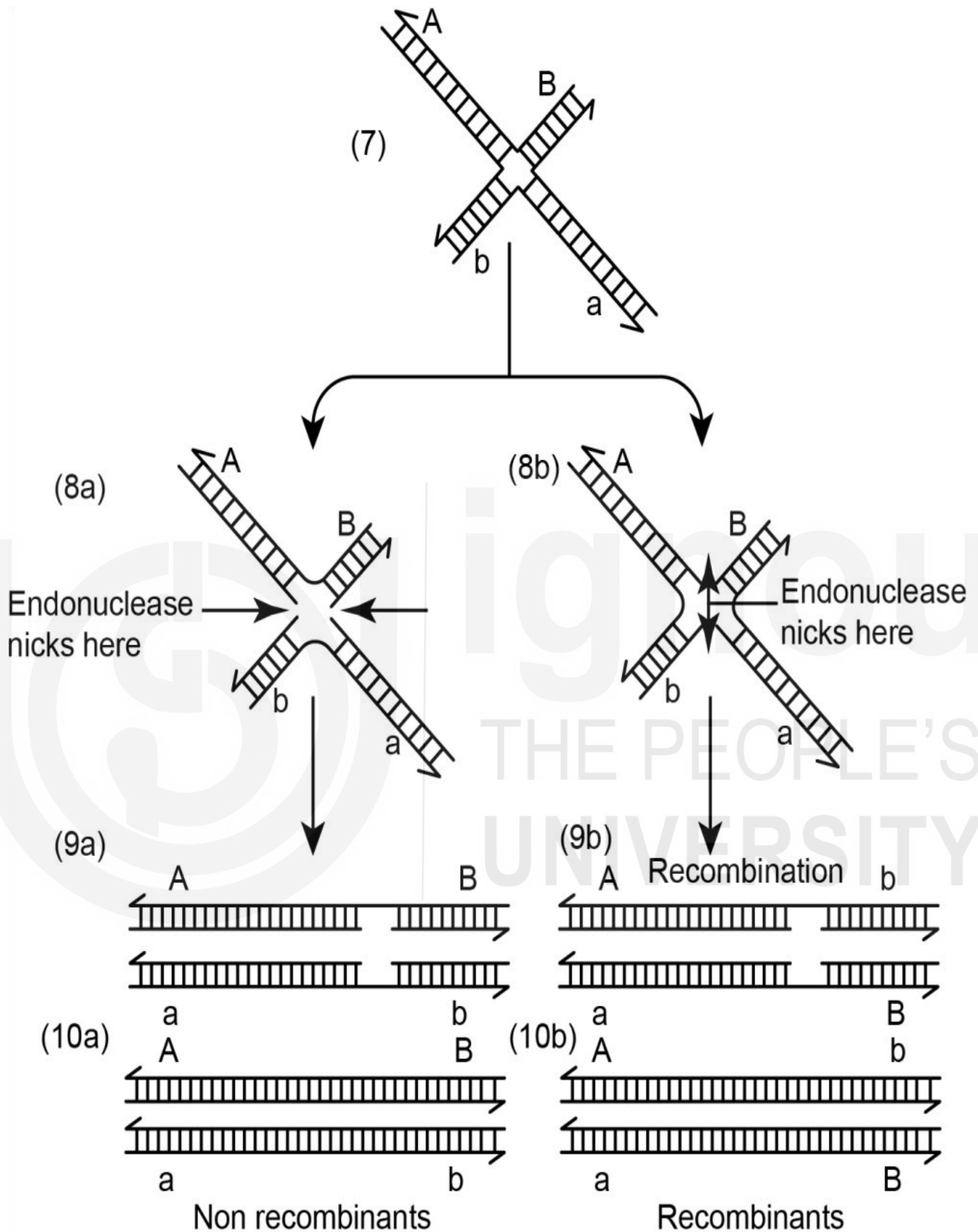


Fig. 3.18: Uptil stage (7) it is the same as Fig. 3.12 (Robin Holliday's model). In (8) there are two possibilities, first, endonuclease acts on sides (see 8a) resulting in nonrecombinants or parentals (9a and 10s). Second, if endonuclease nicks the opposite end then recombinant; (9b and 10b) are formed.

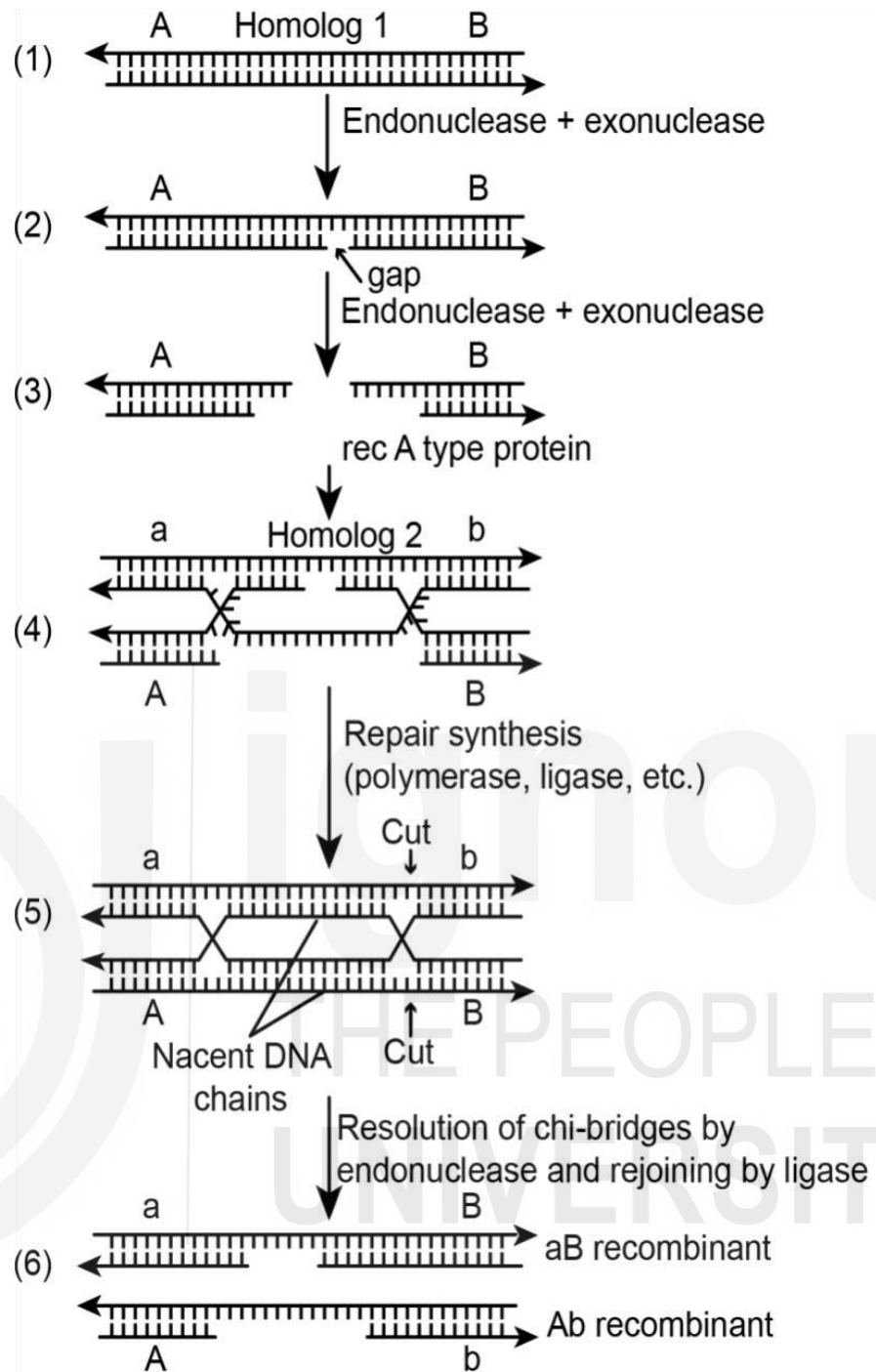


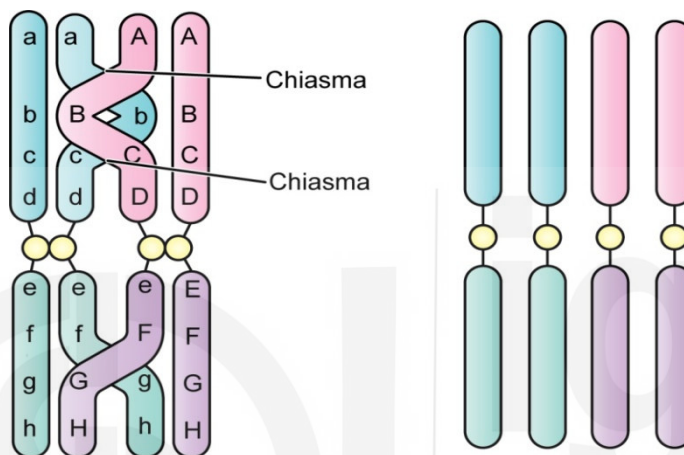
Fig. 3.19: A double-strand-break model of recombination (1-3) Gaps are produced in two strands of DNA of one chromosome (homolog 1) by sequential endonuclease and exonuclease activities. (4) the free single-stranded ends formed at the gaps invade the second DNA molecule (homolog 2) and with the help of rec A type proteins, and helicase displace the homologous strand of the intact double helix. (5) the gaps are filled by repair synthesis of DNA using the complementary strands of the intact, but locally unwound, double helix as templates. Ligation produces two double helices joined at two sites by single-stranded bridges (chi structures). (6) the bridges are resolved by the action of endonucleases, with subsequent rejoining catalysed by DNA ligase, and the recombinant chromosomes are produced (From Gardner et al. 1991 John Wiley and Sons Inc, New York.

SAQ 4

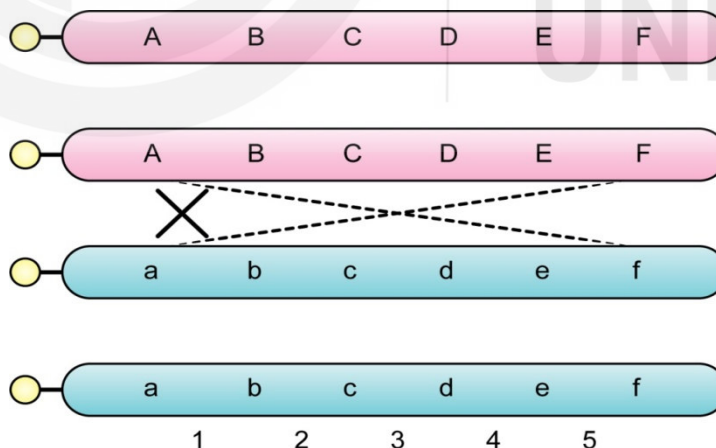
- a) When does chiasmata formation and crossing-over occur?
- b) Between which structure does exchange of genes occur?

SAQ 5

The figure given below shows chiasma formation. The two chromosomes involved have the genes *a, b,.....h* and *A,B,.....H*. Fill in the blank spaces as to what genes the resultant separated chromosomes have, and shade the areas where genetic exchange has taken place.

**SAQ 6**

In the cross-over given below, is there a greater chance of genes *A* and *B* being separated or genes *A* and *F*? Explain your answer.

**3.4 CHROMOSOME MAPPING**

The linkage of the genes in a chromosome can be represented in the form of a **genetic map**. It shows the linear order of the genes along the chromosome with the distances between adjacent genes proportional to the frequency of recombination between them. A genetic map is also called a **linkage map** or a **chromosome map**.

The unit of distance in a genetic map is called a **map unit (mu)**. One **map unit** is equal to 1 per cent recombination or one centimorgan (cM), named in honour of T.H. Morgan. For example, two genes that recombine with a frequency of 4.5 per cent are said to be located 4.5 map units apart.

Alfred H. Sturtevant, a student of T.H. Morgan, was the first one to construct a genetic map based on his data on recombination frequencies that shows the location of various genes on a chromosome. Sturtevant also pointed out that the map is linear or one dimensional. The first genetic map of the X chromosome of *Drosophila* constructed from his data in Table 3.1 is shown in Fig. 3.20.

Table 3.1: Recombination Frequencies for Some Sex-Linked Mutations in *Drosophila melanogaster*.

Genes	Recombination Frequency
yellow (y) and white (w)	0.010
yellow (y) and vermilion (v)	0.322
yellow (y) and miniature (m)	0.355
vermilion (v) and miniature (m)	0.030
white (w) and vermilion (v)	0.300
white (w) and miniature (m)	0.327
white (w) and rudimentary (r)	0.450
vermilion (v) and rudimentary (r)	0.269

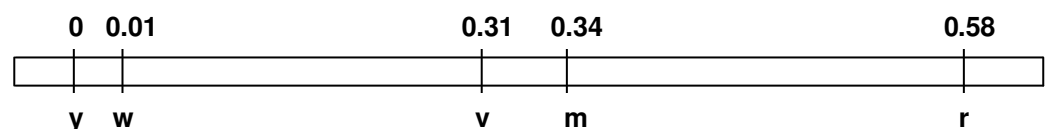


Fig. 3.20: The first genetic map of the X-chromosome of *Drosophila melanogaster*, showing the relative map positions of the genes *yellow*, *white*, *vermilion*, *miniature*, and *rudimentary*. The *yellow* gene was arbitrarily chosen as zero on the genetic map.

Sturtevant reasoned that if genes were distributed along a chromosome, then the farther apart two genes lie the greater the likelihood that a cross-over would occur between them. Thus, the frequency of recombination between widely separated genes should be greater than that between closely located genes.

Let's have a look at the data in Table 3.1 once again. The *yellow* and *white* mutations exhibit a recombination frequency of 0.010 and therefore, must be fairly close to each other compared to *yellow* and *vermillion*, which exhibit a recombination frequency of 0.322. Moreover, *white* and *vermillion* show a recombination frequency of 0.300, indicating that *white* must be closer to *vermillion* than is *yellow*. Therefore, the data indicates that *white* must lie between *yellow* and *vermillion*. Similarly, it has been found out that the *vermillion* is located quite close to *miniature* (recombination frequency 0.030) and *vermillion* is more closely linked to *white* than is *miniature* (compare 0.300 to 0.327); therefore, *rudimentary* must be between *white* and *miniature*. The *rudimentary* appears to be almost unlike to *white*, as shown by its recombination frequency 0.450. This approaches the value of 0.50 expected for independent assortment. The location of *rudimentary* which might be on the left or the right of *white* on the map, was based on the observation that *vermillion* is closely linked to *rudimentary* than is *white* and therefore, must lie between the two.

The case of *white* and *rudimentary* (recombination frequency of 0.450) illustrates the point that two genes may reside on the same chromosome and yet assort independently, if they are very distantly located. For example, if a gene is located some distance right of *rudimentary* it would then assort independently of *white*. Such a gene would have a recombination frequency about 0.50 and yet could be shown to be on the same chromosome as *white* through common linkage to *rudimentary*.

In Fig. 3.20, the distance between adjacent genes is based on the observed recombination frequency. And you know that recombination frequency is defined as one map unit, or one centimorgan (cM). The location of each gene is designated by the map distance from one end of the set of linked genes. The *yellow* gene is arbitrarily chosen as the left end of the map with a position of 0.0, and the positions of the other genes are assigned by summing recombination frequencies between the nearest genes. Thus, *yellow* and *rudimentary* are separated by 58 cM on the map of the X chromosome.

3.4.1 Three- Point Crosses

Sturtevant presented his proof of the linearity of genetic map based on the analysis of data from crosses in which alleles of three different X chromosome genes were segregating – the three point crosses. We shall continue using the same example of the X-linked genes in *Drosophila* as above to describe these crosses.

You already know, that a test cross is very useful in studying linked genes, and it helped immensely in our understanding of linkage. Sturtevant test crossed a female heterozygous for the mutations *yellow* body, *white* eye, *miniature* wings with a *ywm* male (see Fig. 3.21).

Test cross:			
	$\frac{+++}{y\ w\ m}$ ♀	$\times$	$\frac{y\ w\ m}{Y}$ ♂
Progeny	Genotype of maternal X chromosome present in sons or daughters		Observed Numbers
<u>Class</u>			<u>Numbers</u>
Parental	{	$\frac{+++}{y\ w\ m}$	6972
m Recombined with y and w	{	$\frac{+++}{y\ w\ +}$	3454
y Recombined with w and m	{	$\frac{y\ ++}{+ w\ m}$	60
w Recombined with y and m	{	$\frac{y\ +\ m}{+ w\ +}$	9
Recombination frequency between:			10,495
w and m	$\frac{3454 + 9}{10,495} = 0.330$		
y and w	$\frac{60 + 9}{10,495} = 0.007$		
y and m	$\frac{3454 + 60}{10,495} = 0.335$		

Fig. 3.21: A test cross involving three loci on the X chromosome of *Drosophila melanogaster*.

Consider the types of eggs produced by such a female. In addition to the parental combination of alleles, six other combinations are expected to occur because for each gene either of the two alleles may be present on one X chromosome. The total number of possible combinations is therefore, $2 \times 2 \times 2 = 8$ (also Fig. 3.21). Since these genes are linked, the different combinations do not occur with equal frequencies but are dictated by the association of alleles and by the frequency with which crossing-over occurs between each pair of genes. These recombination frequencies are shown in Fig. 3.21. If these genes are arranged in a linear order on the chromosome, then three different arrangements are possible (see Fig. 3.21).

You should note that the reciprocal recombinant types cannot arise from the two cross-overs occurring during meiosis. In other words, if three genes are present linearly, the recombinant types cannot be produced independently of one another.

Only the other order I showing the *y-w-m* gene arrangement is consistent with the data in Fig. 3.21. The observed recombination frequency between *y* and *w* is 0.007 and that between *w* and *m* is 0.330. Therefore, the frequency of double cross-over recombinant class should be approximately $0.007 \times 0.330 = 0.00231$.

In Fig. 3.22, you can see that the most infrequent recombinant class is *w* recombined with *y* and *m*. Its recombination frequency being $9/10,495 = 0.00086$ and therefore, this is the class produced by double cross-over, Sturtevant showed that this type of relationship existed for alleles of any three genes on the X chromosome and only a linear map could account for the recombination data from many different crosses involving different sets of three genes.

Sturtevant's discovery of linear arrangement of genes on the chromosome is accorded an importance second only to Mendel's discovery of the gene. The linear nature of the chromosome provided a framework for all future work in genetics and presaged 'the discovery of the linear nature of the DNA molecule.

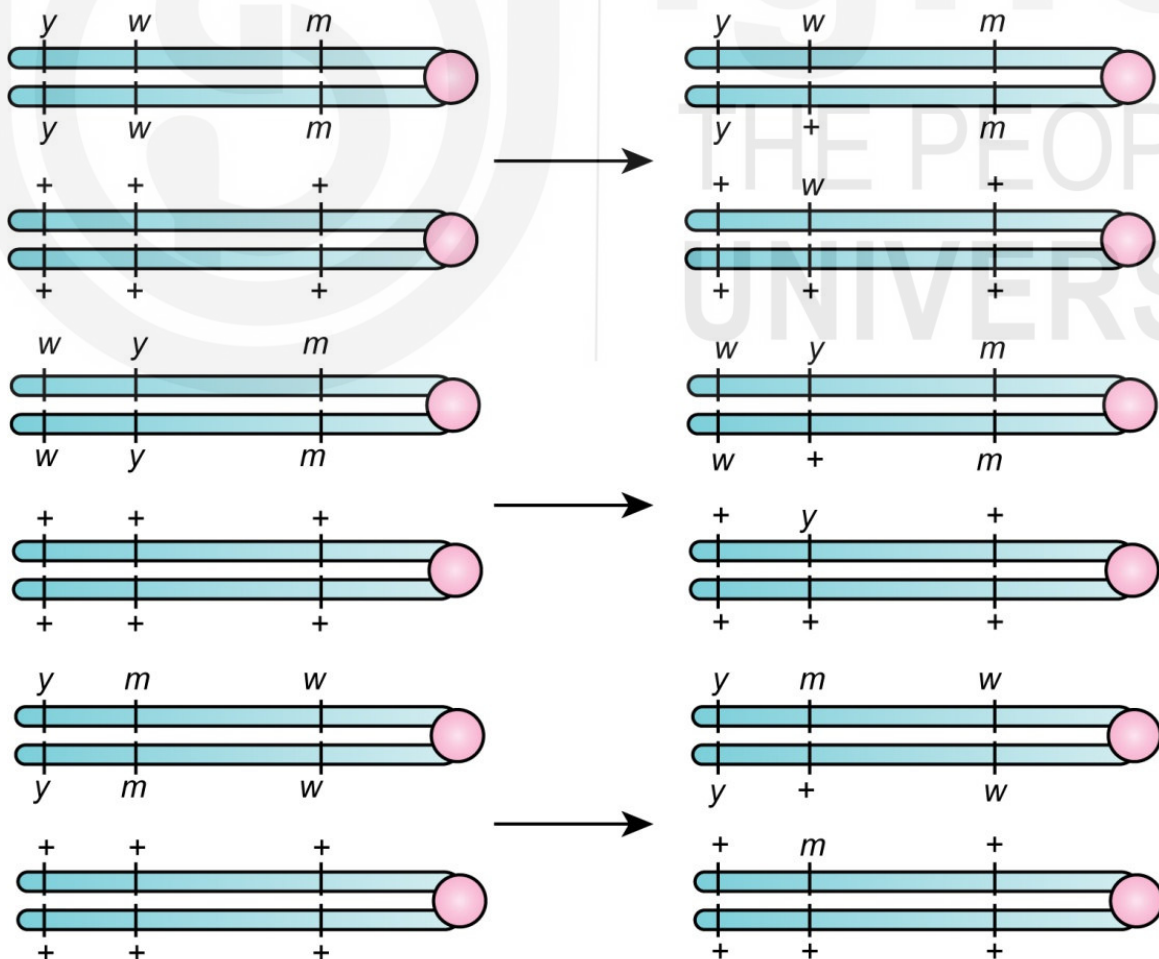


Fig. 3.22: The three possible orders of genes *y* and *m* with gametes produced from double recombination.

Thus, *the three-point crosses provide information regarding both the order of the genes involved, and the recombination frequencies among them.* This type of analysis, first done by Sturtevant, forms the basis of all genetic mapping. The genetic map of the *Drosophila melanogaster* genome established by Sturtevant and other students in Morgan's laboratory is shown in Fig. 3.22.

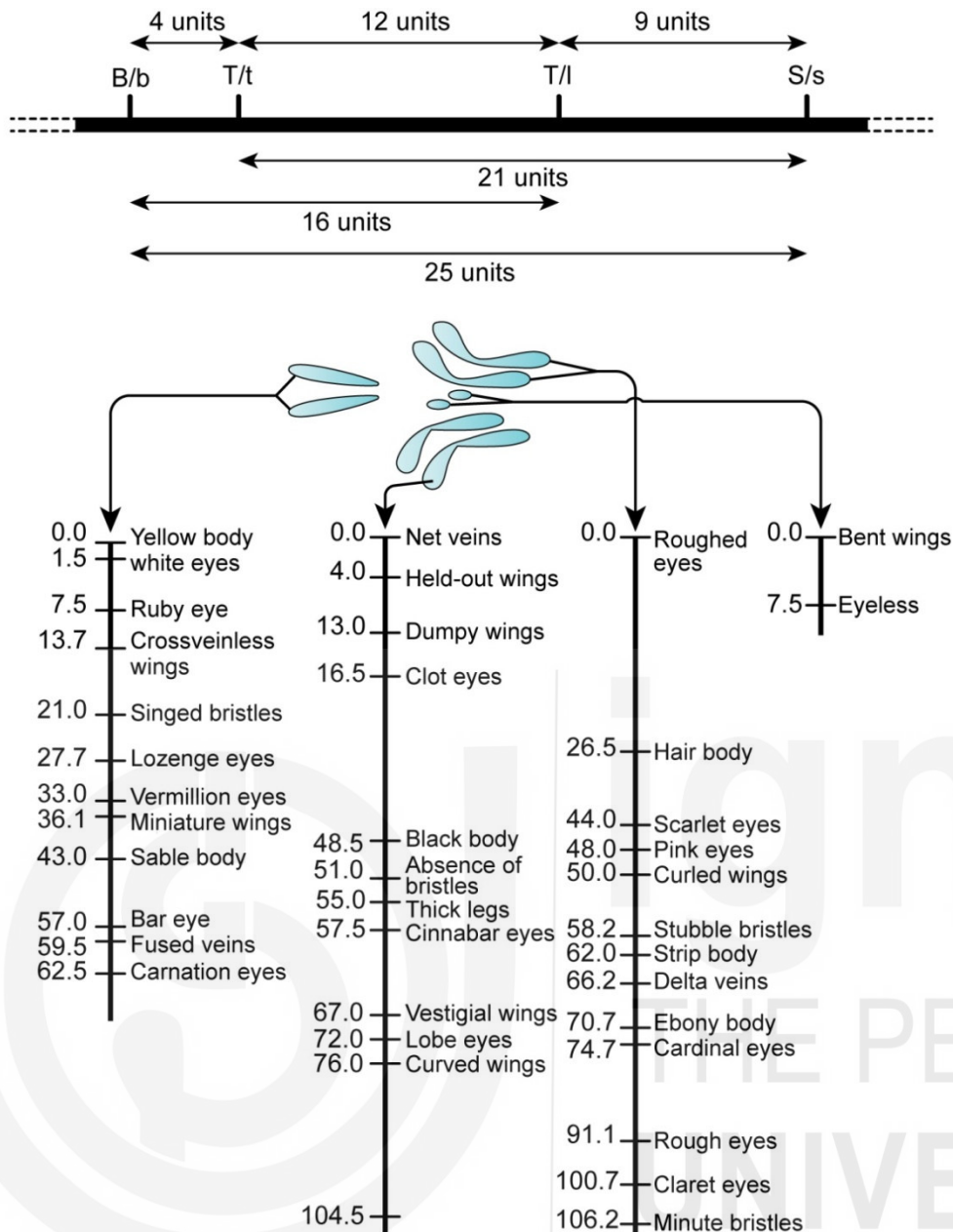
3.4.2 Interference and Coincidence

The detection of double crossing-over has made it possible to determine whether exchanges in two different regions of a pair of chromosomes occur independently of each other. Using the information from the above example of *Drosophila* (data given in Fig. 3.21), we know from the recombination frequencies that the probability of exchange is 0.330 between *w* and *m*, and 0.007 between *y* and *w*. If crossing-over occurs independently in the two regions (that is, if the occurrence of one exchange does not alter the probability of the second exchange), the probability of the occurrence of an exchange in both regions is the product of these individual probabilities, that is, $(0.330 \times 0.007 = 0.00231)$ or 0.231 per cent. This means that in a sample of 10,495 the **expected** number of double cross-overs would be $10,495 \times 0.00231 = 24.2$ or 24. Whereas the number observed was only 9. Such deficiencies in the observed number of double cross-overs are common and are said to be due to **Interference** (*I*). That is, the occurrence of crossing-over in one region of a chromosome reduces the probability of a second cross-over in a nearby region.

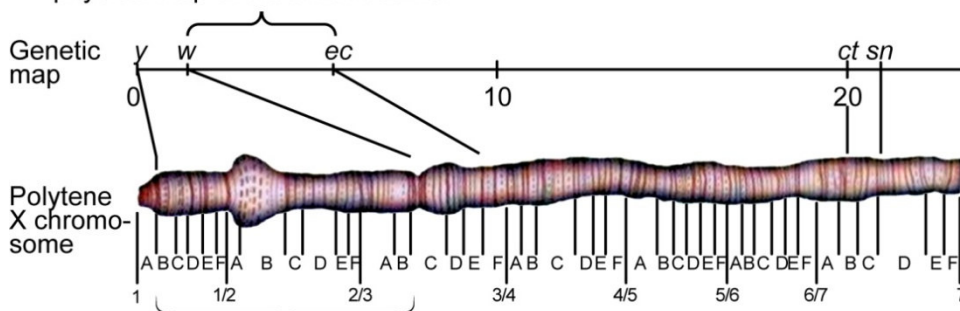
The **coefficient of coincidence** (*c*) is the observed number of double recombinants divided by the expected number; its value provides a simple measure of the degree of interference, which is measured as:

$$\text{Interference} = I = 1 - \text{coefficient of coincidence or } I = 1 - c$$

From the data in our example, the coefficient of coincidence is $9/24 = 0.375$, meaning that the number of double cross-overs that occurred was only 37 per cent of the number expected if crossing-over in the two regions were independent. It has been found experimentally that interference usually increases as the distance between the two markers becomes smaller, until a point is reached at which double crossing-over does not occur; that is, no double cross-overs are found and the coefficient of coincidence equals 0 (or the interference equals 1). The distance is about 10 map units in a variety of organisms. Conversely, when the total distance between the gene loci is greater than about 45 map units, interference disappears and the coefficient of coincidence becomes 1 (Fig. 3.23).



The genes *w* and *ec* are far apart on the genetic map, but close together on the physical map of the chromosome.



The genes *y* and *w* are far apart on the physical map of the chromosome, but close together on the genetic map.

Fig. 3.23: An abbreviated genetic map of *D. melanogaster* showing the correspondence between each of the four linkage groups and a pair of chromosome. The map position of the genes in a chromosomes are in map units from the gene closest to one end of the chromosome. Only a few of the many genes that have been snapped as shown.

3.5 WHY DIDN'T MENDEL FIND LINKAGE?

It is often said that Mendel had extremely good fortune in his experiments with garden pea as in none of his crosses did he come across any apparent linkage between any of the seven traits he studied. Had Mendel obtained highly variable data characteristic of linkage and crossing-over, these unorthodox ratios may have hindered his successful analysis and interpretation of data.

One of the common and simplest explanation for the absence of linkage is that each of the seven genes was located on different linkage group or chromosome. As *Pisum sativum* has a haploid number of 7, so this speculation was widely accepted.

Stig Blixt, however, demonstrated the inadequacy of this hypothesis and explained why Mendel did not encounter ratios, characteristic of linkage and crossing-over (see Box 3.1).

Box 3.1: Why Didn't Gregor Mendel Find Linkage?

It is quite often said that Mendel was very fortunate not to run into the complication of linkage during his experiments. He used seven genes, and the pea has only seven chromosomes. Some have said that had he taken just one more, he would have had problems. This, however, is a gross oversimplification. The actual situation, most probably, is that Mendel worked with three genes in chromosome 4, two genes in chromosome 1, and one gene in each of chromosomes 5 and 7. (See Table 3.1). It seems at first glance that, out of the 21 dihybrid combinations Mendel theoretically could have studied, no less than four (that is, *a-i*, *v-fa*, *v-le*, *fa-le*) ought to have resulted in linkages. As found, however, in hundreds of crosses and shown by the genetic map of the pea, *a* and *i* in chromosomes 1 are so distantly located on the chromosome that no linkage is normally detected. The same is true for *v* and *le* on the one hand, and *fa* on the other, in chromosome 4. This leaves *v-le*, which ought to have shown linkage.

Table 3.1: Relationship between Modern Genetic Terminology and Character Pairs Used by Mendel

Character Pair Used by Mendel	Alleles in Modern Terminology	Located in Chromosome
Seed colour, yellow-green	<i>I-i</i>	1
Seed coat and flowers, coloured-white	<i>A-a</i>	1
Mature pods, smooth expanded-wrinkled indented	<i>V-V</i>	4
Inflorescences, from leaf axis-umbellate in top of plant	<i>Fa-fa</i>	4
Plant height, 0.5-1 m	<i>Le-le</i>	4

Unripe pods, green yellow	<i>Gp-gp</i>	5
Mature seeds, smooth wrinkled	<i>R-r</i>	7

Mendel, however, does not seem to have published this particular combination and thus, presumable, never made the appropriate cross to obtain both genes segregating simultaneously. It is therefore not so astonishing that Mendel did not run into the complication of linkage, although he did not avoid it by choosing one gene from each chromosome.

Stig Blixt, 1975

From: Nature, Vol. 256, p.206.

3.6 SUMMARY

In this unit you have learnt that:

- Certain non-allelic genes located on the same chromosome tend to remain together during meiosis than undergoing independent assortment. This phenomenon is called linkage. The indication of linkage is deviation from the 1:1:1:1 ratio of phenotypes in the progeny of a cross of the form *AaBb* X *aabb* when genes in a cross with two unlinked genes segregate, more than 50 per cent of gametes produced have parental combinations of the segregating alleles and less than 50 per cent have nonparental (recombinant) combination of alleles.
- The recombination of linked genes occurs by crossing-over, a process by which non-sister chromatids of the homologous chromosomes exchange corresponding segments. Crossing-over involves the breakage of individual chromatids and exchange of parts. This process of breakage and reunion is usually associated with a small amount of DNA repair synthesis. Crossing-over occurs after chromosome duplication, in the four-chromatid stage of meiosis. A given cross-over involves any two of the four chromatids.
- The frequencies of crossing-over between different genes can be used to determine the relative order and locations of the genes in chromosomes. This is called genetic mapping. Distance between adjacent genes in such a map (a genetic or linkage map) is defined to be proportional to the frequency of recombination between them. The unit of map distance (one map unit - mu or cM) is defined as 1 per cent recombination. One map unit corresponds to a physical length of the chromosome in which a cross-over event will occur. For short distances map units are additive.
- The four haploid products of individual meiotic divisions can be used to analyse linkage and recombination in some species of fungi and unicellular algae. The method is called tetrad analysis.

3.7 TERMINAL QUESTIONS

1. From the data given below answer i) and ii)

Genotype	Number of Progeny
<i>OR</i>	327
<i>Qr</i>	61
<i>qR</i>	56
<i>qr</i>	342

- i) The above data indicates that genes:

- a) are completely linked
- b) are not linked
- c) are partially linked
- d) no conclusion can be made

- ii) The per cent recombination that has occurred is:

- a) 10%
- b) 15%
- c) 30%
- d) 50%

2. Fill in the blank spaces with appropriate words:

- i) Two events that take place during meiosis contribute to genetic variability: and The general term for production of new gene combination is genetic
- ii) The more chromosomes an organism has, the more genetic variability it gets from
- iii) The longer the chromosomes of an organism, the more genetic variability it gets from
- iv) Assume that the genes for hair colour and eye colour are closely linkage, w... the alleles for brown hair and brown eyes on one chromosomes of a homologous pair; and the alleles for blonde hair and blue eyes on the other chromosome of the pair. A cross-over between these two loci would produced a child with hair and eyes, or hair and eyes.
- v) Independent assortment recombines genes from chromosomes, and crossing-over recombines genes from chromosomes.

3. Tick mark the correct answer for the following:

- i) Independent assortment during meiosis produces gametes with all possible combinations of maternal and paternal chromosomes. How many different kinds of haploid gametes does a diploid cell with four chromosomes produce?
- a) Two
 - b) Four
 - c) Eight
 - d) Sixteen
- ii) Assuming there is an independent assortment of chromosomes and no crossing-over in a cell with n pairs of chromosomes. How many different kinds of gametes is the organism expected to produce?
- a) n
 - b) n^2
 - c) 2^n
 - d) $\frac{1}{2}n$
- iii) During which phase of meiosis crossing-over take place?
- a) Prophase of meiosis I
 - b) Prophase of meiosis II
 - c) Anaphase of meiosis I
 - d) Anaphae of meiosis II
- iv) During crossing-over, genetic information is exchanged between two:
- a) chromatids of a chromosome
 - b) long arm of a chromosome
 - c) chromatids of two homologous chromosomes
 - d) chromatids of two nonhomologous chromosomes
- v) The probability of cross-over occurring between two gene loci is proportional to:
- a) the activity of the two loci
 - b) the distance between the loci from the centromere
 - c) the distance between the two loci
 - d) the length of the chromosome

- vi) The maximum frequency of a recombination at two loci is:
- a) 25%
 - b) 50%
 - c) 75%
 - d) 100%
- vii) The recombination frequency between gene *A* and *C* is 17 per cent and between *B* and *C* is 26 per cent. What is the sequence of genes in the chromosome?
- a) *ABC*
 - b) *ACB*
 - c) *BAC*
 - d) cannot be determined from the information provided.
- viii) In a test cross between two linked genes having 50% crossing-over between them, what phenotypic ratio of the progeny should be expected?
- a) 9:3:3:1
 - b) 1:1:1:1
 - c) 1:2:1
 - d) 2:2
- ix) In order to calculate map distances of genes on a chromosome, one must know the:
- a) number of mutant genes
 - b) cross-over percentage
 - c) recombination frequency of each locus
 - d) a and c
- x) In order to determine the gene sequence in a three-point cross, you must know which of the progeny are:
- a) parental (non cross-over) types
 - b) single cross-over types
 - c) double cross-over types
 - d) a, b and c
4. In a plant, long leaves (*S*) and green veins (*Y*) are dominant over short leaves (*s*) and yellow veins (*y*). The cross *SSYY* × *ssyy* produced an *F*₁ *SsYy*. When *F*₁ plants were inbred, the *F*₂ consisted of 570 long, green individuals and 190 short, yellow. Are the genes *S* and *Y* linked?

5. In *Drosophila*, the recessive, sex-linked genes abnormal eyes facet (*fa*) and singed bristles (*sn*) show 18 percent recombination.
- If a singed male is crossed to a $\frac{fa^+}{fa^+}$ female, what phenotypes are expected in the F_1 ?
 - If the F_1 males and females are inbred what phenotypic proportions would be expected to occur in F_2 males and females?
6. Two recessive genes *ds* and *mp* are present in corn. These are linked and are 20 map units apart. From the cross:

$$\frac{ds\ mp}{++} \times \frac{ds\ +}{+mp}$$

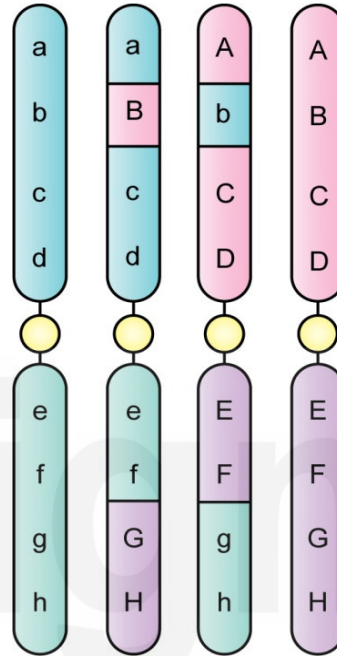
What percentage of the progeny would be expected to be both *ds* and *mp* in the phenotype?

3.8 ANSWERS

Self Assessment Questions

- unlinked
 - linked
 - linked
 - 2
 - [D-E-F-G], [H-I]
 - Genes located on the same chromosome are called linked genes and belong to a linkage group.
- linked
 - 3:1
 - 1:2:1
 - complete linkage
 - linked
 - exchange between chromatids
 - 2, parentals, recombinants
 - partial linkage
 - independent
 - heterozygous
 - 9:3:3:1
 - nonlinkage

- 3) $\frac{ab}{AB}$
- 4) a) During the first prophase of meiosis.
b) Exchange occurs between the chromatids of homologous chromosomes.
- 5)



- 6) There is a greater chance of genes *A* and *F* being separated by crossing-over. For *A* and *B* to be separated, chiasma formation must occur at position 1. For *A* and *F* to be separated, the chiasma can form at any one of the five position (1, 2, 3, 4 or 5).

Terminal Questions

- 1) i) c, ii) b.
- 2) i) independent assortment, crossing-over, recombination
ii) independent assortment
(iii) crossing-over
(iv) brown, blue, blonde, brown
(v) non-homologous, homologous
- 3) i) b, ii) c, iii) a, iv) c, v) c, vi) b, vii) c, viii) b,
ix) c, x) d.
- 4) The genes *S* and *Y* are linked; the ratio is 3:1 indicating complete linkage that no crossing-over. If not linked, a ratio of 9:3:3:1 would have been obtained
- 5) a) F_1 females: $fa^{+}/+Sn$ (wild type)
 F_1 males fa^{+}/Y (facet)

- b) From the data, the cross-over frequency between *fa* and *sn* is 18 per cent. Therefore, cross-over eggs will be *++* (9 per cent) and *fa sn* (9 per cent), and noncross-over eggs would be *fa +* (41 per cent) and *+ sn* (41 per cent). Union of eggs with *fa +*, X-bearing sperm will produce females in the phenotypic proportions of 50 per cent wild type and 50 per cent facet. Union of eggs with Y-bearing sperm will produce males in the phenotypic proportion of 41 per cent facet, 41 per cent singed, 9 per cent facet, singed and 9 per cent wild type.
- 6) Forty percent of the gametes of $\frac{ds\ mp}{++}$ parents will carry both *ds* and *mp*; 10 per cent of the gametes produced by the $\frac{ds\ +}{+ mp}$ parent will carry both recessive genes. Therefore, $0.4 \times 0.1 = 0.04$ or 4 per cent of the progeny would be homozygous for these genes.



ignou
THE PEOPLE'S
UNIVERSITY

EXTRA-NUCLEAR INHERITANCE

Structure

4.1	Introduction	4.5	Organelles Inheritance
	Objective		Dependent on Nuclear Genome
4.2	Extra-Nuclear (cytoplasmic) Inheritance		Chloroplast Proteins
	Early Experiments		Mitochondrial Proteins
4.3	Maternal VS. Extra-Nuclear Inheritance	4.6	Organelle-Associated Linear Plasmid DNA
4.4	Systems of Extra-Nuclear Inheritance	4.7	Possible Origin of Organelles
	Chloroplasts	4.8	Summary
	Mitochondria	4.9	Terminal Questions
		4.10	Answers

4.1 INTRODUCTION

In the earlier units on Mendel's laws of inheritance, you have learnt that genes follow the basic laws formulated by Mendel. Genes which follow such a pattern, are said to be governed by mendelian inheritance.

The notion that genes reside in the nucleus of eukaryotic organisms has become a firm part of general dogma of genetics. In fact, the mechanics of the nuclear processes of meiosis and mitosis, together with the understanding that genes are located on chromosomes, provide the basic set of operational rules for genetics. However, like most fundamental truths, this one does have exceptions and these exceptions form the basic frame-work of this unit. In eukaryotes, the special inheritance patterns of some genes reveal that these genes are located outside the nucleus. Such an inheritance called the **extra-nuclear, extrachromosomal, cytoplasmic, uniparental** or **maternal inheritance**, has been exemplified in a variety of eukaryotic systems. We would mostly refer to this kind of inheritance in this unit as extra-nuclear inheritance.

The phenomenon of extra-chromosomal inheritance operates in all the eukaryotic organisms. Since there is invariably a preference in favour of the organisms which are easily amenable to experiments, certain species, the green alga, *Chlamydomonas* and fungi, *Neurospora* and *Saccharomyces* (yeast) have served good experimental systems of this type of inheritance.

In the first section of this unit we will discuss the basic nature of non-mendelian inheritance and mechanism of its transmission to the next generation. We would also see that not all maternal effects are due to non-mendelian inheritance. The non-mendelian inheritance is due to the genes residing on cytoplasmic organelles, chloroplasts and mitochondria. You will be introduced to certain well-studied cases of such an inheritance controlled either by mitochondria or chloroplasts. You would see how inferences drawn from various experiments lead to certain concepts. Finally, we would also discuss the interdependence of nuclear and cytoplasmic genetic systems.

Objectives

After studying this unit you should be able to:

- ❖ discuss the involvement of chloroplast and mitochondria in extra-nuclear inheritance,
- ❖ explain the nature of extra-nuclear inheritance,
- ❖ give evidences that genes are present in mitochondria and chloroplasts,
- ❖ appreciate the complexity of genetic systems residing in various cellular compartments of a cell, and
- ❖ discuss the possible origin of organelles involved in extra-nuclear inheritance.

4.2 EXTRA-NUCLEAR (CYTOPLASMIC) INHERITANCE

Let's recall that genes in the case of diploid organisms exist in pairs and two members or alternative forms of a single gene are called alleles. The alleles are located at the same position (locus) on homologous chromosomes. Law of segregation, as you have studied earlier, deals with the separation of the alleles at the time of gamete formation. In terms of chromosomes, it implies the separation of two homologues from each other during the first meiotic division. During the cross and in the formation of F_1 heterozygote, one allele is contributed by the female parent and the other comes from the male parent. The outcome of the cross in terms of phenotype of the F_1 is identical irrespective of which of the alleles, recessive or dominant comes from the female parent. Therefore, we say that in a typical Mendelian inheritance, the phenotypic outcome of reciprocal crosses is identical as shown in Fig. 4.1.

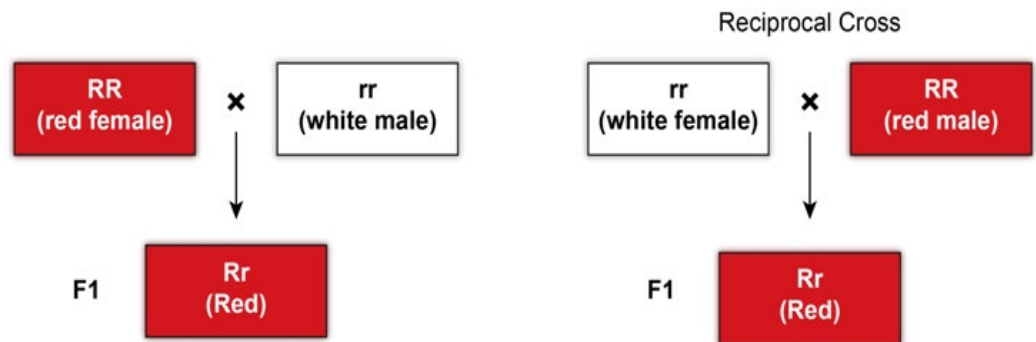


Fig. 4.1: Typical Mendelian pattern of inheritance in reciprocal crosses.

The implication of this observation is that there are no differences whether gamete (R) is contributed by the female parent or by the male parent. The genetic contribution of the two parents is identical and resulting is the same phenotype in reciprocal crosses. However, there are exceptions to this general rule.

It has been observed in certain cases that the phenotype of F_1 in the reciprocal crosses is different. When phenotypes of the reciprocal crosses are not identical, it is the first indication that the inheritance of the particular character may not follow Mendelian pattern. Such a phenotype is invariably influenced by maternal parent. Broadly, this pattern of inheritance is termed non-Mendelian inheritance.

4.2.1 Early Experiments

Soon after the rediscovery of Mendel's law of inheritance in the beginning of this century, the results of various experiments were not in conformity with what was to be expected on the basis of nuclear inheritance. We will discuss some experiments which provides proof for the existence of extra-nuclear mode of inheritance.

Variegated Plants

One of the first convincing examples of extra-nuclear inheritance was found in higher plants. In 1990, a scientist named Carl Correns observed some strange variegation in Four O'clock (*Mirabilis jalapa*) plant. It had branches containing patches of both green and white tissues but some branches carried green leaves and others only white leaves. Figure 4.2 shows such a variegated plant. In fact, you must have seen many such variegated plants in your surroundings.

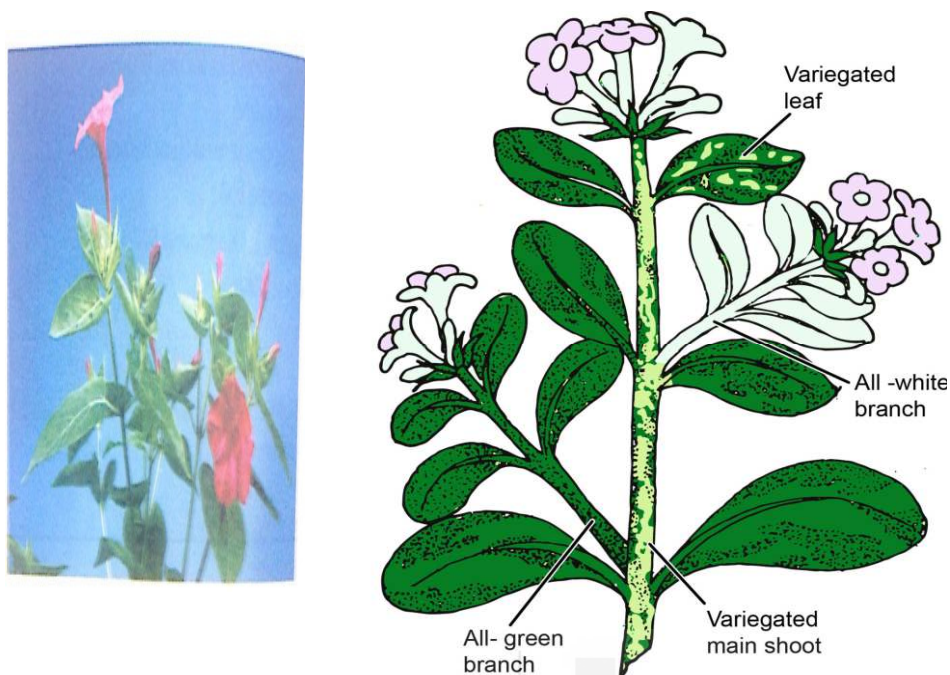


Fig. 4.2: Four O'clock (*Mirabilis jalapa*) plant.

Flowers appeared on all types of branches. He intercrossed different combinations by transferring pollen from one flower to another and obtained the following results (Table 4.1).

Table 4.1: Results of the crosses of different phenotypes in *Mirabilis jalapa*.

Phenotype of branch bearing egg parent (female)	Phenotype of branch bearing pollen parent (male)	Phenotype of progeny
White	White	White
White	Green	White
White	Variegated	White
Green	White	Green
Green	Variegated	Green
Green	Green	Green
Variegated	White	Variegated, green or white
Variegated	Green	Variegated, green or white
Variegated	Variegated	Variegated, green or white

Two basic features are obvious from these results.

- (i) There is a difference between these results.
- (ii) The phenotype of the maternal parent is solely responsible for determining the phenotype of all progeny. The contribution of the male parent appears to be nil.

The results are summarized in Fig. 4.3.

Since there is no contribution of the male parent, this phenomenon has also been termed maternal inheritance. White plants lacking chlorophyll do not survive but other types i.e. variegated and green survive and can further be tested. This type of pattern is not transitional but keeps on persisting in the subsequent generations. How can such results be explained? You know that the green leaf colour is due to the presence of chloroplasts. The colourless sectors or branches contain colourless plastids and variegated sectors will have green as well as colourless plastids. The inheritance patterns can easily be explained with the diagram shown in Figure 4.4 if chloroplasts contain genetic determinants and pollens do not contribute any chloroplast to the zygote. It is reasonable to assume that the pollen parent does not transmit any organelles since bulk of the cytoplasm of the zygote is known to come from the maternal parent via pollen parent cytoplasm of the egg. The green branches produce eggs with only green chloroplasts and white branches produce eggs with colourless chloroplasts. Variegated branches apparently produce three kinds of eggs, some contain only white chloroplasts, some contain only green chloroplasts and some contain both kinds of chloroplasts. The egg containing both types of plastids (chloroplasts) will produce a zygote containing both the types of chloroplasts. In subsequent mitotic divisions, chloroplast sorting occurs, whereby some cells contain only green chloroplasts and others colourless ones, resulting in variegated phenotype in the progeny individuals. Following these observations, various other cases of this type of inheritance have been observed in a variety of other plant species.

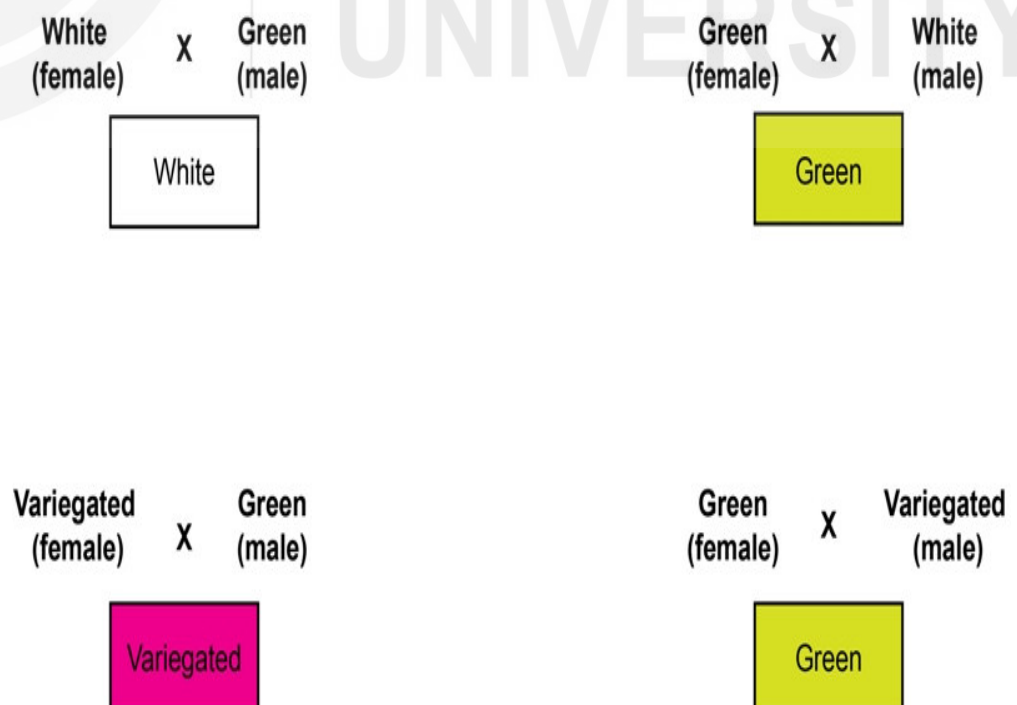


Fig. 4.3: Non-mendelian pattern of inheritance in the reciprocal crosses of *Mirabilis jalapa*.

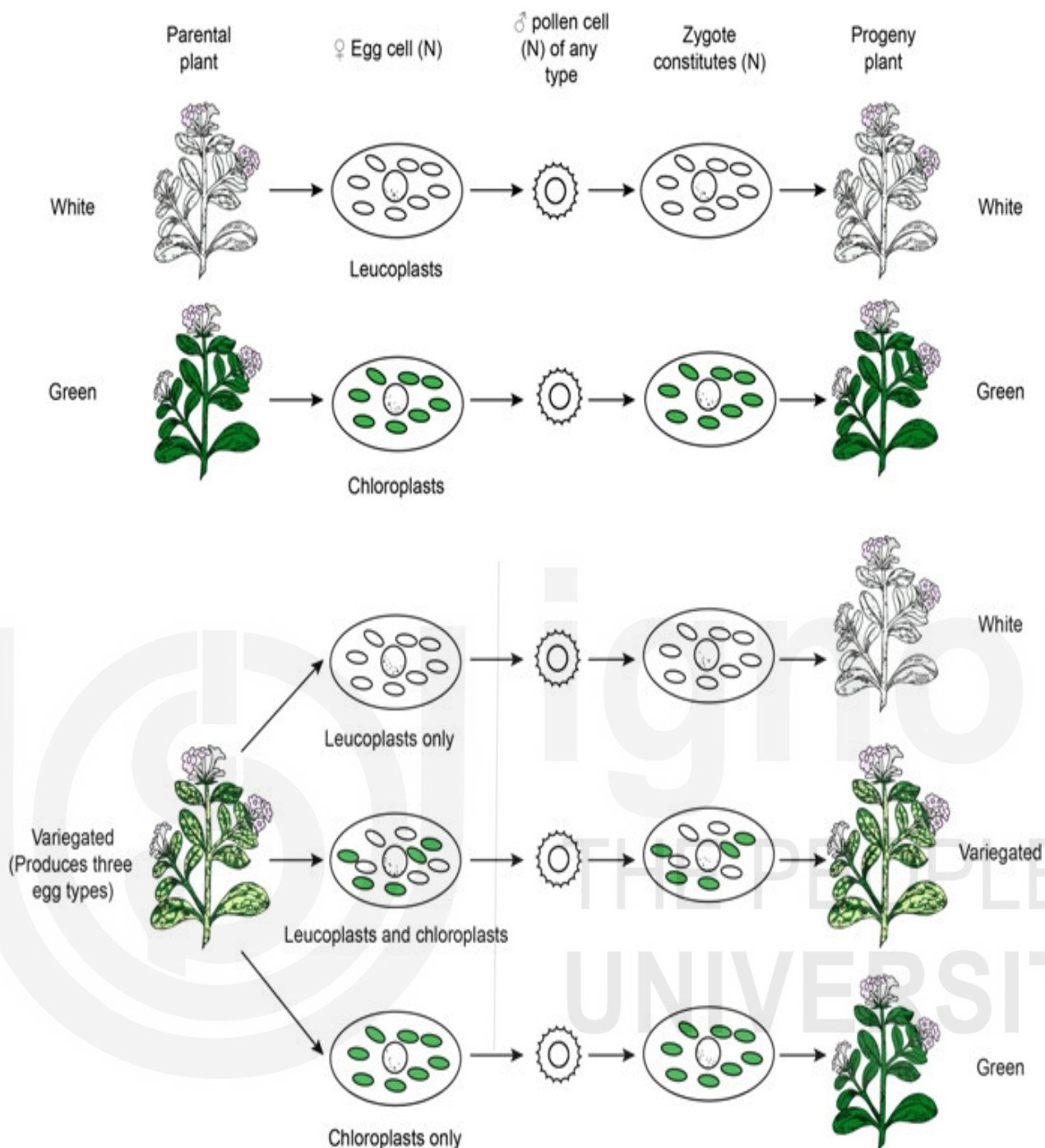


Fig. 4.4: A model explaining the results of *Mirabilis jalapa* crosses in terms of autonomous chloroplast inheritance. The first two crosses exhibit strict maternal inheritance. If the maternal branch is variegated three of zygotes can result, depending on whether the egg cell contains only white, only green or both green and white chloroplasts. In the last case, the resulting zygote can produce both green and white tissue, so a variegated plant results.

Poky Mutants

In 1952, a mutant strain of fungus, *Neurospora* called poky was identified. The poky mutant is slow growing and has abnormal amounts of cytochromes. You have already learnt that cytochromes are mitochondrial electron transport proteins necessary for oxidative phosphorylation. In the poky strains, of the three main types of cytochromes, cytochrome *a* or *b* are absent but an excess

of cytochrome *c* is present, *Neurospora* is a haploid fungus, but a eukaryote. It has two different mating types and the strains with differing mating types can be crossed. The crosses of poky to wild type produced the following results (Fig. 4.5).

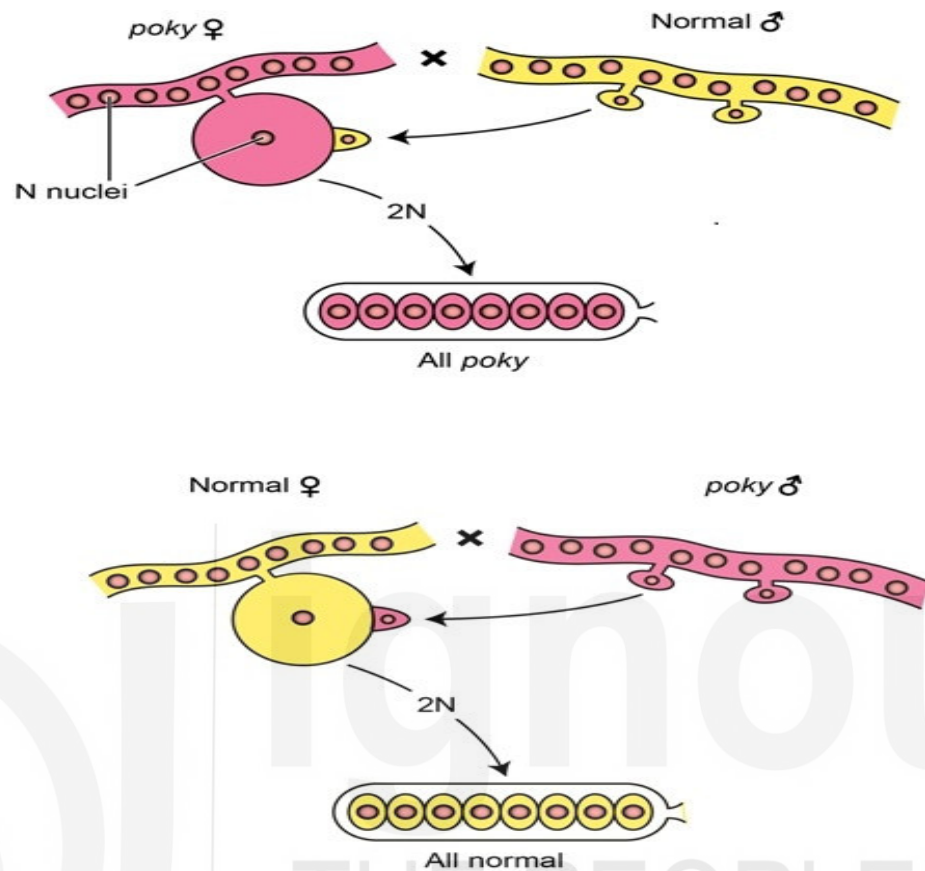


Fig. 4.5: Results of reciprocal crosses of poky and wild *Neurospora*, (a) poky ♀ x wild type ♂ (b) wild type ♀ x poky dl.

These results again show that there are differences in reciprocal crosses and the phenotype of the F_1 solely controlled by the maternal parent.

SAQ 1

Which of the following statements are correct:

- i) The mode of inheritance of extranuclear genetic system is distinct from mendelian inheritance. ☐
- ii) The phenotype of reciprocal crosses are identical in extra-nuclear inheritance. ☐
- iii) In cytoplasmic inheritance the phenotype of reciprocal crosses are invariably influenced by the male parent. ☐
- iv) Extra-chromosomal inheritance is recognized by uniparental (usually maternal) transmission through a cross. ☐
- v) In cytoplasmic inheritance the egg cell would be expected to influence non-mendelian traits. ☐

4.3 MATERNAL VS. EXTRA-NUCLEAR INHERITANCE

Usually but not always, it is possible for a maternal inheritance pattern of reciprocal crosses to be due to nuclear genes or due to mendelian inheritance. One of the prominent example in this category is the direction of shell coiling in the water snail *Limnaea*. The coiling of the shell in snails, is of two types. Some snails coil to the right (dextral) while others coils to the left (sinistral coiling). All the F_1 progeny of the dextral female and sinistral male are dextral but all the progeny of the cross between sinistral female and dextral male are sinistral (Fig. 4.6).

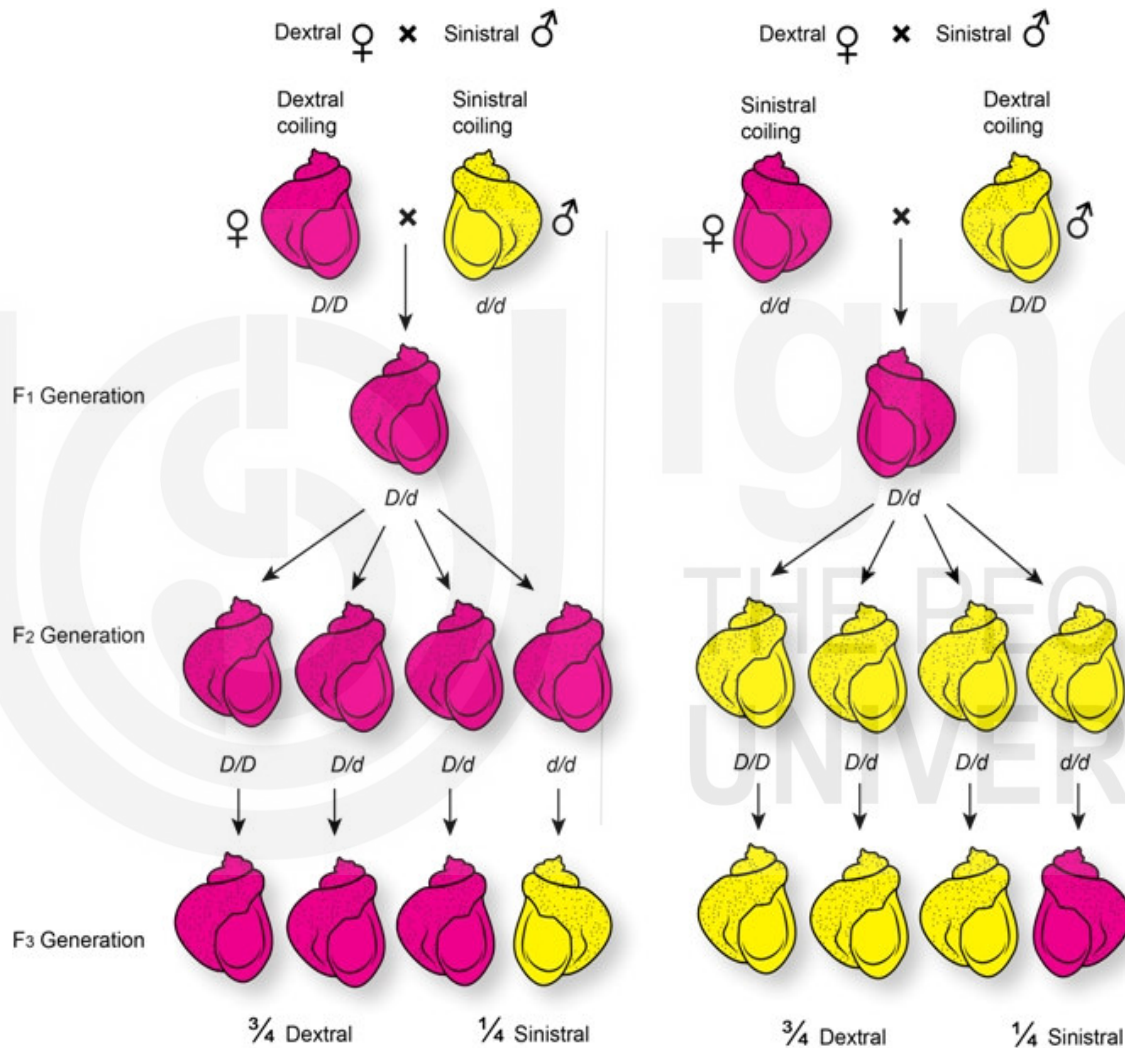


Fig. 4.6: The inheritance of dextral (D) and sinistral (d) nuclear genes for shell coiling in a species of water snail. The direction of coiling is determined by the nuclear genotype of the mother, not the genotype of the individual involved.

This appears similar to the observations made in the case of chloroplast inheritance or with the one observed with the poky mutant in *Neurospora*. However, in the F_2 generation the shell coiling is all dextral from both the pedigrees. The F_3 generation in this case reveals that inheritance of the direction of coiling is controlled by nuclear genes rather than extranuclear genes. It was found that $3/4$ th of F_3 are dextral and $1/4$ th are sinistral. The ratio reveals a mendelian segregation, 3:1 of the F_2 generation. Apparently dextral

is dominant over sinistral. It was found that the coiling pattern is conditioned by a biochemical compound present in the maternal cytoplasm. Subsequently, this biochemical compound gets diluted due to somatic divisions, and the new compound is synthesised depending on the genes present in the female parent. It is apparent from the foregoing example that whether an inheritance is controlled by extra-chromosomal genes, can only be confirmed if the inheritance pattern persists in subsequent generations.

4.4 SYSTEMS OF EXTRA-NUCLEAR INHERITANCE

We have seen earlier that genes residing in cytoplasm are responsible for extra-nuclear inheritance. The experiments with Four O' clock plants were conclusive enough to implicate chloroplasts in the inheritance of variegation. Similarly, the experiments with poky mutant in *Neurospora* show that the defect is in one of the components of mitochondria. When we talk about genes or genetic material we invariably imply DNA (deoxyribonucleic acid). You already know that DNA is the store-house of genetic information. Whatever information an organism requires to develop from a single-celled zygote to an adult is encoded in its DNA.

The experiments with plants and *Neurospora* and also with *Chlamydomonas* and yeast, clearly establish following:

- (i) Certain characters are not governed by the principle of Mendelian inheritance but follow extra-nuclear inheritance and
- (ii) In the case of *Mirabilis*, chloroplasts also determine the inheritance pattern, and in the case of *Neurospora*, the inheritance is also controlled by mitochondria. Thus, the extra-nuclear inheritance implies the presence of genetic determinants in chloroplasts, mitochondria or both. Since the genetic determinants or genes invariably mean DNA (in certain cases RNA) the search continued for showing the presence of DNA in these organelles. Using both electron microscopic and biochemical studies it was established that both of these organelles contain DNA and also possess the machinery necessary to decode the information contained in their DNA.

4.4.1 Chloroplasts

In 1950's Ruth Sager isolated a mutant of *Chlamydomonas reinhardtii* which cannot grow in the presence of an antibiotic, streptomycin. *Chlamydomonas* is a fresh water unicellular eucaryotic alga. It does not have different sexes but have mating types (mt^+ and mt^-). The haploid cells under conditions of nitrogen starvation behave like gametes and gametic fusion between dissimilar mating types occurs. The diploid cells after fusion, undergo meiosis and release four haploid cells (tetrads) as shown in Fig. 4.7.

When a strain, which can grow in the presence of streptomycin (sm^r) was crossed (Fig. 4.8) with a strain which cannot grow in the presence of streptomycin (sm^s), the following pattern of inheritance was observed:

$$sm^r mt^+ \times sm^s mt^- \rightarrow \text{all } sm^r$$

$$sm^s mt^+ \times sm^r mt^- \rightarrow \text{all } sm^s$$

we can draw two kinds of inferences from these crosses with respect to the inheritance of the *sm* gene:

- (i) There are differences in reciprocal crosses and
- (ii) The phenotype of F_1 is determined by mt^+ parent. It is similar to the pattern expected for a maternal inheritance and it is a case of uniparental inheritance. Using the analogy of higher system mt^+ is referred to as the female and mt^- as male. The mating type genes, mt^+ segregate in 1:1 ratio, as expected for true mendelian inheritance.

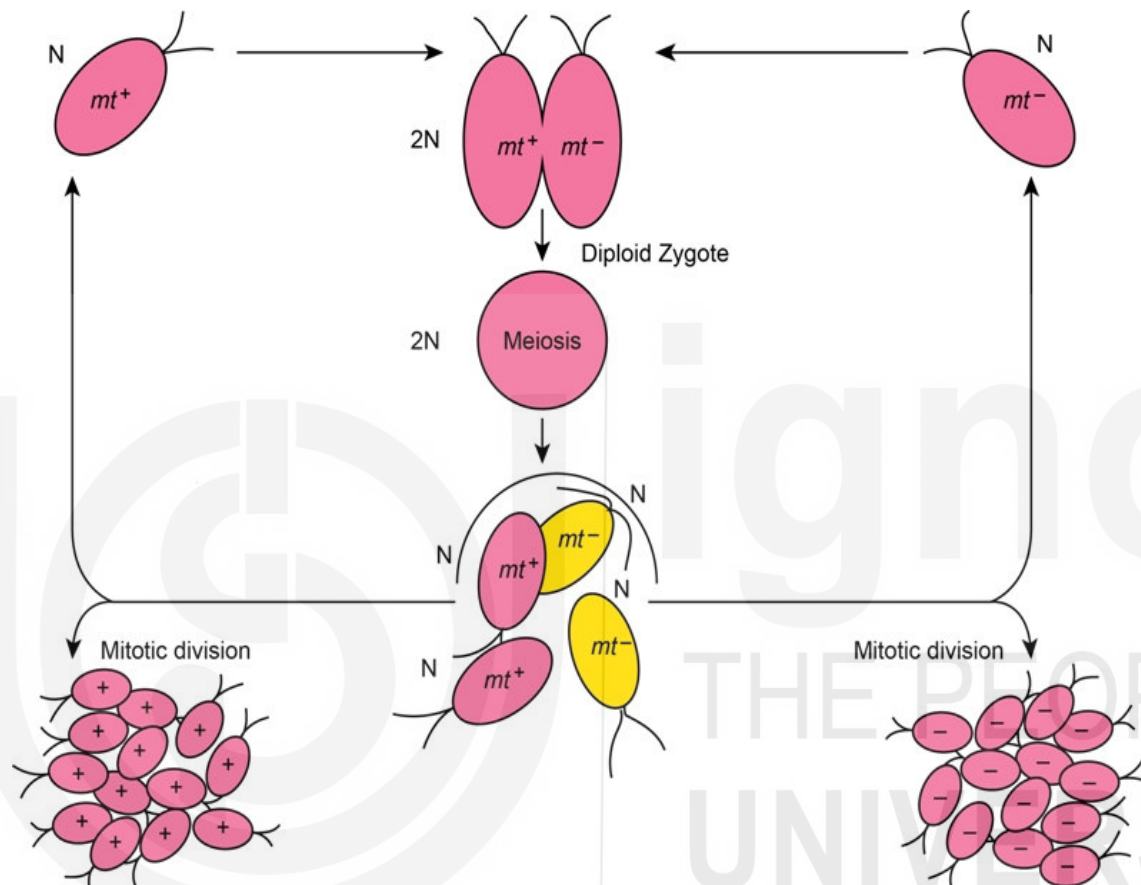


Fig. 4.7: Diagrammatic representation of the life cycle of *Chlamydomonas*. The nuclear mating-type alleles mt^+ and mt^- must be heterozygous in order for the sexual cycle to occur.

In the case of higher organisms, where a zygote is formed by fusion of egg with sperm, the cytoplasmic contribution of the male gamete is almost negligible. Under such conditions, it is easy to comprehend the mechanism of maternal inheritance. In case of *Chlamydomonas*, the cytoplasmic contribution of the two gametes, mt^+ and mt^- is almost identical. However, a uniparental inheritance is still observed and the cytoplasmic contribution of the mt^- parent, somehow has to be eliminated from the progeny. Therefore, a basic question arises, what happens to the cytoplasmic determinants of mt^- gametes? This puzzle was solved by Ruth Sagar, who discovered that on gametic fusion, mt^- chloroplast DNA gets degraded. The mt^+ gene or a gene very closely linked to it, specifies a restriction-modification system. Restriction, in simplistic terms, means degradation and modification that imply protection. The system, encoding the DNA modifying enzyme, modifies its own DNA which cannot be degraded by the restriction system. However, the mt^- chloroplast which is not modified and thus not protected, is degraded by the restriction system

specified by the mt^+ cells. It has been shown that an mt^+ linked gene encodes an endonuclease (enzyme which degrades DNA) which can differentiate DNA of its own cell from that of the mt^- cell due to modification. Since the mt^- cell with mt^+ cells, no expression of mt^- chloroplast DNA is observed, leading to the uniparental pattern of inheritance. Therefore, nature in this case has conspired to make mt^+ cells mimic the female gametic functions. However, in a fraction (1 in 100) of cases, mt^- chloroplast DNA does escape the degradation by the mt^+ restriction system. Such biparental zygotes containing the chloroplast DNAs from both cells are called **cytohets** (cytoplasmic heterozygotes). The cytohets, in fact, are very useful in studying the recombination of cytoplasmic genes. Ruth Sagar by using the analysis of cytohets constructed a genetic map of *Chlamydomonas* chloroplast DNA.

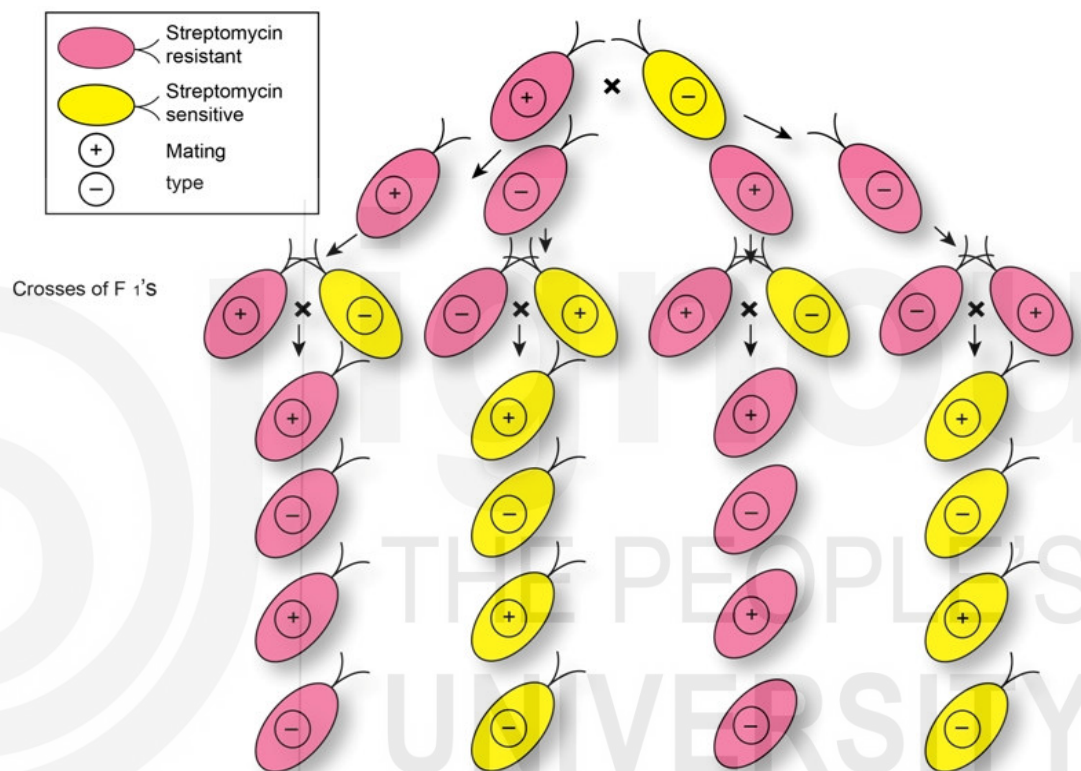


Fig. 4.8: Inheritance of streptomycin resistance in *Chlamydomonas*.

SAQ 2

Fill in the blank spaces in the following statements by appropriate words.

- The genetic determinants of maternal inheritance are present in and
- The biparental zygote containing chloroplast DNAs of both parents in *Chlamydomonas* is called
- In *Chlamydomonas* crosses, the chloroplast DNA the mt^+ strain survives because it has gene for restriction modification enzyme to its own *cpDNA* from
- The DNA fibrils in chloroplast have been observed by studies and have also been and characterised by biochemical studies.

4.4.2 Mitochondria

In support of extra-nuclear inheritance, we have already discussed the involvement of mitochondria in the poky mutants in *Neurospora*. However, the best studied examples of such a pattern of inheritance come from genetic as well as molecular studies of mitochondrial genetic system of baker's yeast, *Saccharomyces cerevisiae*. Yeast is a haploid organism and has two kinds of mating type alleles, α and a . The gametes carrying different mating type alleles, α and a , fuse leading to the formation of a zygote.

The diploid zygotes undergo meiosis and result in the formation of four ascospores. The ascospores germinate and form haploid yeast cells. The life cycle of yeast is given in Figure 4.9.

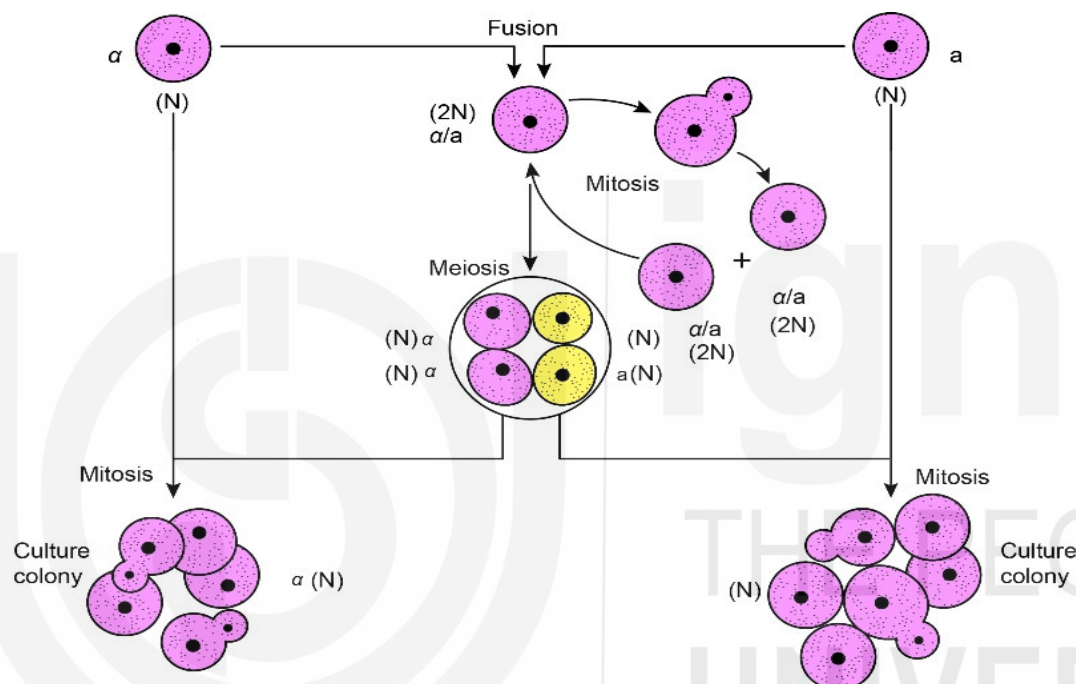


Fig. 4.9: The life cycle of baker's yeast (*Saccharomyces cerevisiae*).

Yeast cells normally form large colonies on agar plates which are called '**grande**'. Occasionally minute colonies, called '**petite**' (meaning small) also appear amongst them. When petites were crossed grande strains, following three different kinds of results, as outlined in Figure 4.10 were obtained:

If the inheritance of a nuclear gene, such as the mating type locus is followed, the progeny ascospores segregate in the expected 2:2 ratio. The similar results (2:2 ratio with respect to grande and petite phenotype) were obtained in the first category of crosses indicating that this petite phenotype is controlled by nuclear gene. Such petites governed by the mendelian inheritance are called **nuclear** or **segregational petites**. Therefore, we are not going to discuss them here.

The other two crosses produce 0:4 or 4:0 with respect to grande and petite phenotypes indicating the extra-nuclear pattern of inheritance. In one case, all the progeny is normal (grande) and in the other, all are petites. In the former case, it appears that the more functional mitochondria take over resulting in all the normal progeny. The petites falling into this category are called **neutral petites**. The last category of cross, petite x grande producing all petite

progeny, are called **suppressive petites**. Since the inheritance pattern of neutral and suppressive petites is extra-nuclear, they are also referred to as cytoplasmic petites. In a yeast cross, the two parental haploid cells fuse and apparently contribute equally to the cytoplasm of the resulting diploid cell. In extra-nuclear inheritance in yeast, the phenotype of the F_1 is not dependent on a particular mating type. In this respect yeast is clearly different from *Chlamydomonas*.

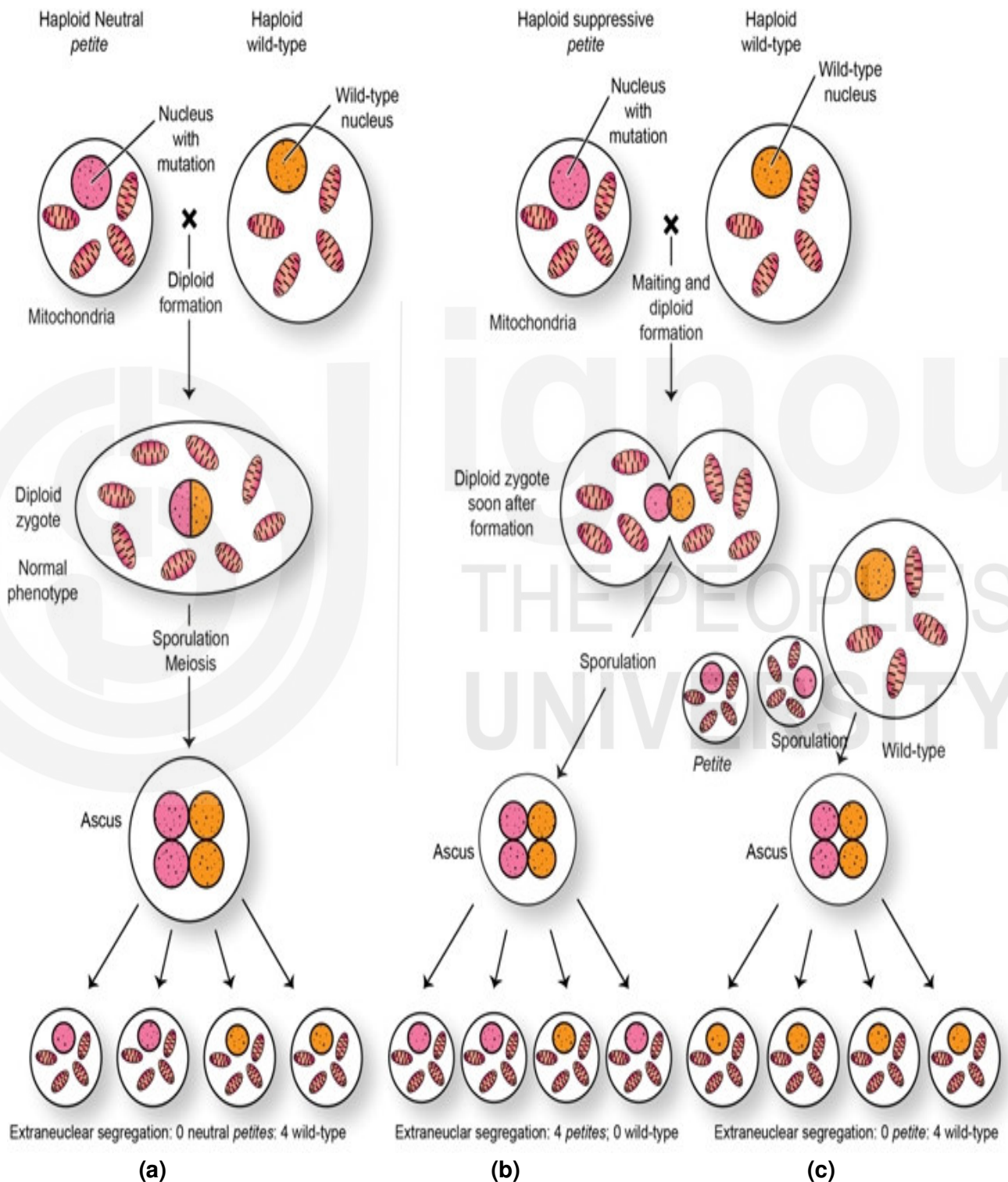


Fig. 4.10: *Petite* mutations in genetic crosses. The nuclear *petites* (a) show mendelian segregation; the neutral *petites* (b) show 4+ : 0 *petite* non-mendelian segregation; the highly suppressive *petites* (c) show 0+ : 4 *petite* non-mendelian segregation.

Several properties of the cytoplasmic petites which are listed below point to the involvement of mitochondria in their phenotype.

- (i) In cytoplasmic petites, the mitochondrial electron transport chain is defective. Therefore, the petites have to rely on the less efficient process of fermentation for providing ATP. If they are placed in a medium containing non-fermentable energy source such as glycerol, they do not grow.
- (ii) Mitochondria normally possess their unique protein-synthesizing apparatus, which is different from the non-organellar or cytosolic system. The cytoplasmic petites, are therefore, defective in protein synthesis.
- (iii) The suppressive petites have altered mitochondria DNA (*mtDNA*) and the neutral petites contain no *mtDNA* at all.

During the derivation of petite mutants from grande strains, certain other mitochondrial genes are also lost. The coincidental or simultaneous loss of several determinants is indicative of deletion. This led to a proposition that petite phenotype may possibly be a consequence of deletion in mitochondrial DNA (*mtDNA*). It is now established that the petite phenotype in fact results from deletion of *mtDNA*. However, the size of *mtDNA* isolated from petite mitochondria was found to be similar to that of the *mtDNA* isolated from normal mitochondria (~78kb). Moreover, different petites have been shown to contain different genetic determinants indicating the deletions of different regions in *mtDNA*. To compensate the deleted segments of *mtDNA*, the DNA retained by the petite is amplified through tandem duplication to provide a chromosome of approximately the normal length. This phenomenon of deletion-amplification can be explained diagrammatically (Fig. 4.11).

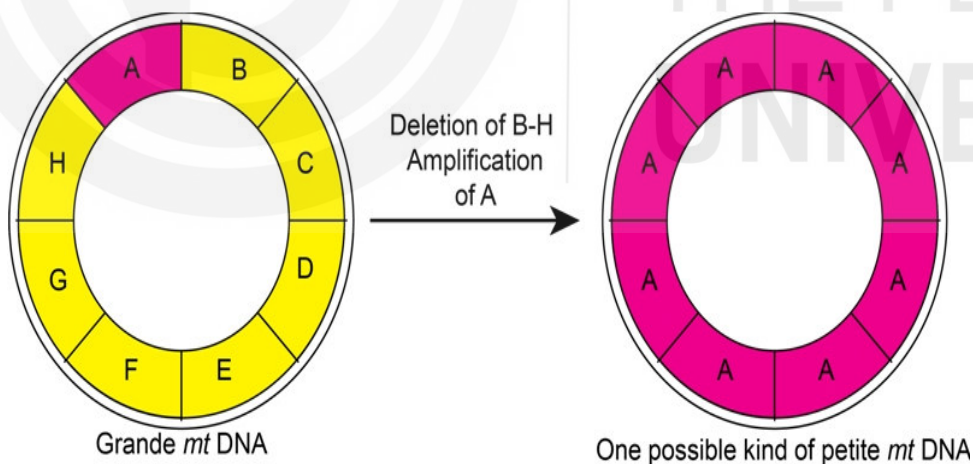


Fig. 4.11: When a petite is produced from a grande cell, a large region of *mtDNA* may be deleted. Apparently, the DNA region retained by the petite (in this example A) is amplified through tandem duplication to provide a chromosome of approximately the normal length.

Beside petites, several other mutations showing the patterns of extra-nuclear inheritance have been analysed in yeast. Prominent among them are

- (i) Mitochondrial mutations conferring resistance to various antibiotics such as chloramphenicol (*cap^R*), erythromycin (*ery^R*), spiramycin (*spi^R*), paramomycin (*par^R*), and oligomycin (*oli^R*). These mutants as a class are called antibiotic resistant (*ant^R*), mutants.

- (ii) The other category of mitochondrial mutants called *mit* are defective in mitochondrial electron transport chain and they also produce small colonies. However, unlike petites they have normal protein synthesis and are able to revert back to normal. The reversion indicates that they resemble point mutations. The inheritance pattern of the *mit* mutants is comparable to antibiotic mutants of *Chlamydomonas*, mentioned earlier, showing uniparental inheritance at meiosis.

SAQ 3

In the following statements choose the alternate correct word given in the parenthesis.

- (i) Resistance to antibiotic in some strains of yeast is due to (chloroplast / mitochondrial / nuclear) genes.
- (ii) The *mit* mutant strain of yeast shows (uniparental / biparental) inheritance.
-

4.5 ORGANELLES INHERITANCE DEPENDENT ON NUCLEAR GENOME

It is apparent from the foregoing discussion that animal and non-photosynthetic eukaryotic cells contain two genetic systems i.e. nuclear and mitochondrial; and plant and other eukaryotic photosynthetic cells contain three different genetic systems namely nuclear, chloroplast and mitochondrial. The presence of more than one genetic systems, each having its own distinct machinery for transcription and translation, requires an active cooperation and coordination between the genetic systems for various cellular functions. The systems of cooperation, as we will discuss in this section, is apparent in the synthesis and assembly of the chloroplast or mitochondrial proteins. We will describe the examples of organelle-proteins in which one or more subunits are encoded by nuclear genome and the others by the organelle genome.

4.5.1 Chloroplast Proteins

We have mentioned earlier that chloroplast DNA codes for several proteins which are involved in photosynthesis and also in translational machinery of chloroplasts. Some examples of the genes encoding the chloroplast proteins are large subunit of the enzyme, ribulose biphosphate carboxylase (*rbc*), photosystem 1 (*psa*), photosystem 2 (*psb*), ATP synthetase (*atp*), RNA polymerase (*rpo*), permease (*mbpx*), 30 and 50 S ribosomal subunit proteins (*rps* and *rpl*) and ribosomal and various transfer RNA genes. Nuclear genes also code for many of the chloroplast proteins or subunits of some proteins involved both in photosynthesis. The mRNAs of these proteins is translated on cytosolic ribosomes (80 S) and these proteins are then transported to chloroplasts. The cooperation of nuclear and chloroplast gene, each encoding a subunit of a single protein is well carboxylase-RuBP subunits: large and small. The large subunit is encoded by chloroplast DNA and synthesised on chloroplast ribosomes (70 S) while the small subunit encoded by nuclear genes is synthesised in cytosol and is then transported into chloroplasts, where it assembles with the large subunit to produce a functional RuBP carboxylase.

4.5.2 Mitochondrial Proteins

Overall function of the mitochondrial genome shows that it codes for some proteins that are actually involved or associated with the electron transfer chain, and all tRNAs and rRNA's necessary for mitochondrial protein synthesis. As you know that the three main components of the electron transfer chain of mitochondria—cytochrome *c* oxidase, cytochrome *b* and ATPase complex are located in its inner membrane. For these three functional units, *mtDNA* supplies six proteins, three subunits of cytochrome *c* oxidase, two subunits of ATPase, and cytochrome *b*. The remaining necessary components are encoded by nuclear genes and the products are transported to mitochondria where they assemble into functional complexes.

It is apparent that all the genes required to fulfil either chloroplast or mitochondrial functions are not present in these organelles. The cytosolically synthesised subunits contain specific signal sequences which direct their transport to either of the organelles, where the signal peptides are cleaved off and the subunits are assembled to form a functional protein. The photosynthetic eukaryotes have three genetic systems i.e. mitochondrial, chloroplasts and nuclear and other eukaryotes have two i.e. mitochondrial and nuclear. It is clear from the foregoing discussion that in spite of the existence of a compartmentalisation in a eukaryotic cell, a degree of interdependence of chloroplasts and mitochondrial systems cannot be considered autonomous. With the same logic and considering limited genetic potential of the organelles they cannot possibly survive outside the cellular system, even though they have their own distinct genetic system and protein synthesising machinery.

4.6 ORGANELLE-ASSOCIATED LINEAR PLASMID DNA

Plasmids are autonomously replicating extra-chromosomal DNA molecules commonly occurring in bacteria. Occasionally they have also been found in eukaryotes. For example certain strains of yeast, *Saccharomyces Cerevisiae* contain 2 micron (μ) plasmid circles which are present in nucleoplasm. It has been found that in certain cases, plasmid DNA is also associated with *mtDNA*. Two interesting cases of plasmid DNA associated with *mtDNA* are (i) Cytoplasmic male sterility in maize and (ii) Senescence (aging) in the fungus, *Neurospora*.

- (i) Male sterility in plants has a great economic importance in agriculture, since male sterile plants eliminate the necessity of emasculation (removal of anthers) for producing hybrid plants. A male sterile plant does not produce functional pollen. If a male sterile plant is used as a female parent in a cross with a normal fertile plant as a pollen parent, all the progeny is male sterile (Fig. 4.12). Thus, the inheritance pattern is exclusively maternal, indicating that the determinants of male sterility are located in organelles. It has been found that male sterile lines of maize have two linear plasmids, S1 and S2 within mitochondria. The presence of these plasmids makes the plants male sterile. They are also found to recombine with *mtDNA*. The exact role of S1 and S2 in male sterility is not yet fully understood.

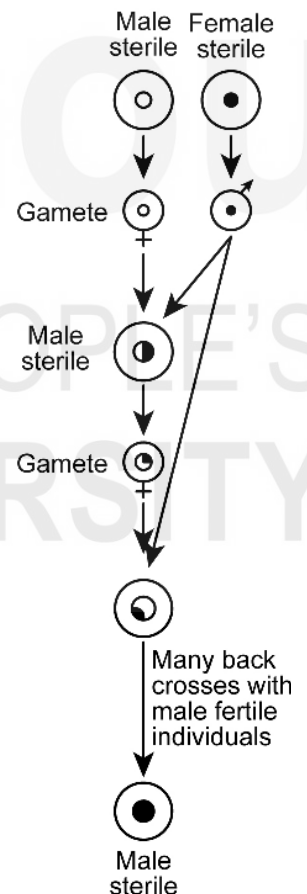


Fig. 4.12: Maternal inheritance of male sterility in maize.

- (ii) Most *Neurospora* strains do not senesce and if nutrients in the medium are not limiting they will keep on growing for ever. However, a Hawaiian strain called “Kalilo” does senesce. Kalilo strain will grow only certain distance through the culture medium before it dies. This senescent property is maternally inherited or shows extra-nuclear inheritance. It has been established now that senescence is determined by a linear plasmid called *Ka1* DNA. This plasmid exists autonomously and the onset of death is preceded by insertion of this plasmid DNA into *mtDNA*. At death, most *mtDNA* molecules contain full length plasmid DNA inserts. Presumably, the insertion of plasmid DNA into mitochondrial genome inactivates the genes affecting certain essential mitochondrial functions and thus results in cell death.

SAQ 4

- a) In the following statements choose the alternate correct word given in parenthesis.
- i) The DNA regions in chloroplast could be observed under (light microscope/electron microscope).
 - ii) The regions containing *cpDNA* are called (nucleoids/celluloids).
 - iii) Each nucleoid contains (a single/a few) copies of DNA.
 - iv) The *cpDNA* and *mtDNA* are usually (circular/linear) in nature.
 - v) Both chloroplast and mitochondria contain (a few/many) copies of DNA.
 - vi) The *mtDNA* of yeast is (bigger/smaller) than *mtDNA* of humans.
 - vii) The occurrence of introns is discovered in (yeast/human).
- b) Which among the following statements are correct.
- i) Mitochondria contain 80 S ribosomes.
 - ii) The mRNA encoded by nuclear genes for the smaller subunit of rubisco is translated in the chloroplast.
 - iii) The inheritance of chloroplast genome is independent of nuclear genome.
 - iv) The chloroplast proteins or their subunits synthesised in the cytoplasm are transported to chloroplast.

4.7 POSSIBLE ORIGIN OF ORGANELLES

We know that both chloroplasts and mitochondria perform certain essential functions for the cell. They contain own distinct genetic system and protein synthesising apparatus. However, the genetic system, and transcription and translational machinery is similar to prokaryotes. You may recall that the eukaryotic ribosomes are of 80S whereas the prokaryotic ribosomes are of

70S type. The protein synthesis in both the organelles is similar to the prokaryotic system and can be inhibited by **chloramphenicol** whereas the cytoplasmic protein synthesis is inhibited by **cycloheximide**. The inhibition of protein synthesis by different sets of antibiotics, in fact, reflects the differences in the nature of ribosomal subunits present. The existence of genetic and protein synthetic machinery similar to prokaryotes within chloroplasts and mitochondria led to the question of their possible origin from a prokaryotic source i.e. the theory of endosymbiosis.

It has been widely conjectured that during pre-evolutionary time certain bacteria-like organism (cyanobacteria-like organism in the case of chloroplasts) invaded eukaryotic cell and established a symbiotic (endosymbiosis) like relationship within the cell. The chloroplasts conferred on the cell the photosynthetic ability and mitochondria endowed the cell with energy-transducing properties. The relationship was reinforced when the system became permanently interdependent; i.e. the cell cannot survive without mitochondria and/or chloroplasts and these organelles cannot survive outside the cell as discussed earlier.

The above endosymbiotic theory has received a certain degree of experimental support from recent molecular studies. We have discussed in the earlier section that subunits of several enzymes of chloroplast and mitochondria are encoded by nuclear genes. These enzymes and other organelle proteins such as RNA polymerase and ribosomal proteins are very similar to bacteria or cyanobacteria. A certain degree of similarities in sequences, both at the protein and DNA level, between organelles and bacteria exists. If chloroplast and mitochondria were indeed prokaryotes during a pre-evolutionary period, then they certainly have lost a considerable amount of genetic information. It appears that a massive transfer of genetic information from organelles to nuclei might have occurred during their long co-existence. The transfer would have resulted in certain modifications or prokaryotic genes in order for them to function in a eukaryotic manner.

Recent studies have shown that certain common DNA sequences are present between chloroplasts and nuclei; between chloroplasts and mitochondria; and between mitochondria and nuclei in plants. The results of these studies provide evidence for the inter-organellar movements of DNA sequences. Many of the such DNA sequences are not functional now but they certainly appear to have left their evolutionary footprints.

Other indications in support of the distinct origin of mitochondria are their unique genetic code and phenomenon of RNA editing. The genetic code is considered universal, however, certain variations exist in mitochondria. In fact, it is one of the exclusive property of mitochondria and it is the only exception to the universality of genetic code. A very recent discovery is related to RNA editing in plant mitochondria which has also been observed in certain parasites. After the synthesis of mRNA, certain nucleotides are edited and replaced by others in mitochondria.

The evidences discussed above provide support to the endosymbiotic theory. Basically many processes which are characteristics of prokaryotic systems and unique to the organellar system and not present in the nuclei support the distinct origin of the organelles.

4.8 SUMMARY

Let us summarise what we have learnt so far:

- The study of inheritance pattern of certain traits in plants and animals show non-conformity with Mendel's laws of heredity.
- The phenotype of the progeny of reciprocal crosses of variegated plant *Mirabilis jalapa*, resembled the phenotype of maternal parent. The inheritance pattern of variegation in this case is not influenced by nuclear chromosomes but by the genetic contribution of female parent. Such type of inheritance called extranuclear inheritance.
- The resemblance of phenotype to the maternal parent in reciprocal crosses is not always due to extra-nuclear inheritance. A trait controlled by extrachromosomal inheritance can conclusively be established if the inheritance pattern persists in subsequent generations.
- The specific characters maternally inherited in plants, fungi and other organisms suggest that the genetic determinants are present in the cytoplasmic organelles—chloroplast or mitochondria. The DNA containing regions have been observed in both the organelles by electron microscopy. The DNA molecules have been isolated and characterised by biochemical studies.
- Chloroplast and mitochondria contain distinct machinery for the transcription and translation of information encoded in their DNA.
- In *Chlamydomonas* where fusion is between isogamous gametes the pattern of inheritance remains uniparental (maternal) because the female gamete has restriction modification system which protects its own chloroplast DNA but degrades that of the male gamete.
- The mitochondrial genetic system is best studied in yeast. The cross between grandis and petite strain show both types of inheritance—the ones governed by mendelian inheritance are called nuclear petites, the other two neutral and suppressive show cytoplasmic inheritance. The defective electron transport chain, protein synthesising machinery and altered DNA point to the involvement of mitochondria in neutral and suppressive petites.
- Both chloroplast and mitochondria contain several copies of DNA molecules which are distributed in certain regions called nucleoids. In most of the cases the DNA molecules are circular.
- EM studies show that chloroplast contain circular DNA which is located in several regions in chloroplasts called nucleoids. Each nucleoid has about 4 to 8 copies of DNA. Likewise, mitochondria also have nucleoids. In all, both the organelles have many copies of DNA molecules. Chloroplast DNA of majority of plants is made of 120-200 kb. The *cpDNA* of liverwort (*Marchantia polymorpha*) and rice have completely been sequenced and *mtDNA* of human and liverwort are also completely been sequenced. Based on the information derived from DNA sequences as well as from other studies, the genetic maps of these organelle DNAs have been constructed.

- Both organelles have genes to code for tRNA, rRNA and several proteins required for the organelles function and to carry out RNA and protein synthesis.
- The two different genomes—nuclear and chloroplast or nuclear and mitochondria cooperate in the synthesis of proteins, each coding for certain subunit(s) of it. The subunit coded by nuclear genome is synthesised on ribosomes of the cytoplasm. It is transported to the organelles and assembles with its complementary subunits synthesised on chloroplast ribosomes.
- In certain species, plasmids have also been found to be associated with mitochondria.
- Since the two organelles contain DNA, ribosomes and protein synthesising machinery similar to prokaryotes, a theory has been proposed that they were at one time prokaryotes, and during the course of evolution became endosymbiont by invading a cell, thus the present day eukaryotic cells were evolved.

4.9 TERMINAL QUESTIONS

1. A scientist carried out reciprocal crosses between two species A and B of the evening primrose known to have same chromosome constitution. When the seed parent was A, the plastids of the progeny were green and when the seed parent was B, the plastids of the progeny were yellow. How might this difference in the results of reciprocal crosses be explained?
2. Compare the features listed below among the eukaryotic, prokaryotic cells with chloroplast and mitochondria.

Eukaryotes	Prokaryotes	Chloroplast	Mitochondria
i) DNA			
ii) Ribosomes			
iii) Ribosomal subunits			
iv) Effect of antibiotics on protein synthesis			

4.10 ANSWERS

Self Assessment Questions

1. i) T, ii) F, iii) F, iv) T, v) T.
2. i) chloroplast, mitochondria

- ii) cytochets
- iii) protect, degradation
- iv) electron microscopic studies, isolated
- 3. i) mitochondrial
- ii) uniparental
- 4. a) i) electron microscope, ii) nucleoids, iii) a few, iv) circular,
v) many copies, vi) bigger, vii) yeast
- b) i) F, ii) F, iii) F, iv) T.

Terminal Questions

1. The result of the crosses indicate that the plastid of the offspring are the same as female parent suggesting that maternal inheritance is involved.
2. Eukaryotes:
 - i) linear
 - ii) 80 S
 - iii) 60 S and 40 S
 - iv) cycloheximideProkaryotes, Chloroplast and Mitochondria:
 - i) circular
 - ii) 70 S
 - iii) 40 S and 30 S
 - iv) Chloroamphenicol