

Practical Manual
B. Sc. (Hons.) Agriculture
Fundamentals of Genetics
Course No.: ASPG1101
Credit: 3(2+1)
Semester: 1



Centurion
UNIVERSITY
*Shaping Lives...
Empowering Communities...*

**Department of Genetics and Plant Breeding
& Seed Science and Technology**
M. S. Swaminathan School of Agriculture
Centurion University of Technology and Management, Paralakhemundi
www.cutm.ac.in

September, 2021

B. Sc. (Hons.) Agriculture
Fundamentals of Genetics
Course No.: ASPG1101
Credit: 3(2+1)
Semester: 1



Centurion
UNIVERSITY

*Shaping Lives...
Empowering Communities...*

**Department of Genetics and Plant Breeding
& Seed Science and Technology**
M. S. Swaminathan School of Agriculture
Centurion University of Technology and Management, Paralakhemundi
www.cutm.ac.in

September, 2021

**Department of Genetics and Plant Breeding
& Seed Science and Technology
M. S. Swaminathan School of Agriculture
Centurion University of Technology and Management
Paralakhemundi**

Certificate

Certified that this is a bonafide record of practical work done by Mr. / Ms.

_____ **Regd. No.** _____

**in B. Sc. (Hons.) Agriculture degree programme, Course No. ASPG1101 entitled
“Fundamentals of Genetics” during 1st Semester of the academic year 2021-2022.**

Date:

Faculty In charge

Evaluation System
Fundamentals of Genetics
Course No.: ASPG1101

Sl. No.	Marks distribution	Total marks	Marks obtained
1	Record		
2	Attendance		
3	Assignment		
4	Presentation		

Signature of Faculty In-charge

List of Experiments

Exp. No.	Date of Exp.	Date of Submission	Title	Page No.	Signature	Remark
1			Study of microscope and its parts			
2			Study of cell ultrastructure and its organelles			
3			Experiments on monohybrid, dihybrid, trihybrid, test cross and back cross			
4			Experiments on epistatic interactions including test cross and backcross and Solving problems on co-dominance and multiple allele			
5			Practice on mitotic cell division			
6			Practice on meiotic cell division			
7			Experiments on probability and Chi-square test			
8			Determination of Linkage and crossing over estimation (two point cross and three point cross data)			
9			Study on sex linked Inheritance in drosophila			
10			Study of models on DNA and RNA structure			

Experiment no. 1: Study of microscope and its parts

Aim: To study the parts of microscope

Objective:

- ❖ To study the principles and operation of a compound microscope
- ❖ To study the different types of microscopes

COPMPOUND MICROSCOPE

The word microscope has been derived from two Greek words: Mikros meaning small and Skopein means to see. A microscope is an optical instrument used for magnifying and resolving the image of any object, which cannot be seen by a naked eye. The first operational microscope was developed by Z. Janseen and H. Janseen of Holland in 1590. It is designed to obtain both magnification and resolving power; it utilizes visible light of wave length ranging from 380 nm to 740 nm as the source of illumination.

PARTS OF MICROSCOPE

1. **Eyepiece Lens:** the lens at the top that you look through. They are usually 10X or 15X power.
2. **Body Tube:** A hollow cylindrical tube that connects the eyepiece to the objective lenses and through which light passes from objective lens to the eye piece at the top.
3. **Arm:** it is a curved back that supports the upper parts of the microscope. It is useful to carry the microscope.
4. **Base or foot:** The bottom of the microscope that is U shaped and gives stability to the instrument.
5. **Illuminator:** A steady light source (110 volts) used in place of a mirror.
6. **Stage:** The flat platform where you place your specimen to be observed. Stage clips hold the slides in place.
7. **Revolving Nose Piece or Turret:** This is the saucer shaped plate that holds two or more objective lenses and this can be rotated easily to change power.
8. **Objective Lens:** Usually you will find 3 or 4 objective lenses on a microscope. They consist of 4X, 10X, 40X and 100X powers. When coupled with a 10X (most common) eyepiece lens, we get total magnifications of 40X (4X times 10X), 100X, 400X and 1000X respectively. The shortest lens is the one having lowest power; the longest one is the lens with the greatest power. The high-power objective lenses are retractable (i.e. 40 XR). This means that if they hit a slide, the end of the lens will push in (spring loaded) thereby protecting the lens and the slide.
9. **Iris Diaphragm:** Many microscopes have a rotating disk above the condenser and below the stage. Iris regulates the amount of light reaching the specimen.
10. **Condenser Lens:** The purpose of the condenser lens is to collect the light reflected by the mirror and focus the light onto the specimen. Microscopes with a condenser lens render sharper image than those with no lens.
11. **OBJECTIVE LENS SYSTEM:** The lens system near the object is called objective lens system. The objective is mounted on revolving nose piece and objective is the primary magnifying mechanism.
 - There are 3 types of objective lens are generally used
Low Power- 10X
Higher power-40X
Oil immersions Objective-100X

12. **Eye piece / ocular lens:** Placed on the top of body tube and magnifies the image formed by objective lens.
13. **FOCUSING KNOB:** Two types of adjustable knobs are useful to move the body tube upward and downwards in order to focus
 - A. **Coarse adjustment knob:** It is always used first for rough focusing. Coarse adjustment is done only with the low power objective.
 - B. **Fine adjustment knob:** Used after coarse adjustment for fine focusing. It is used with the high- power objective to bring the specimen into better focus.

For higher magnification (400X) an oil emission objective lens (100X) can be used. The light gathering properties of lens are greatly increased by placing a fluid between the objective lens and coverslip. The fluid must have some refractive index as the lens itself so the fluid generally used is wood Oil.

MAGNIFICATION: Magnification is an apparent increase in the size of an object achieved with the help of a lens.

Total magnification = magnification of eye piece \times magnification of objective.

- (a) **Effective Magnification (E M):** It is the minimum magnification to view a particular object.
Effective Magnification=Resolving power of a healthy human eye/resolving power of microscope
- (b) **Useful Magnification:** It is 2-4 times of the effective magnification
- (c) **Empty/ Useless Magnification:** It is over & above the useful magnification. It is than four times of effective magnification.

RESOLVING POWER: It is defined as the power of an instrument to distinguish two points or two lines that are close together. Greater the resolving power greater is the efficiency of microscope.

$$\text{RESOLVING POWER} = 0.61 \times \lambda / \text{Numerical Aperture}$$

Where, NA is the numerical aperture of objective. Smaller the numerical aperture, smaller will be the resolving power.

0.61 is the constant i.e., minimum detectable reference in contrast.

λ is wavelength of illumination. Shorter the wavelength of light, higher will be the resolving power.

Numerical Aperture: It is the light gathering/collecting capacity of objective lens. It indicates resolving power of lens. **NA=n \times sin θ**

Here 'n' is the index of refraction of the medium in which the lens is working (1.00 for air, 1.33 for pure water, and typically 1.6 for immersion oil); and

θ is the angle of the marginal rays formed with the axis..

TYPES OF MICROSCOPES:

Depending up on the source of illumination & principle of magnification, microscopes are of following types.

1. **Compound Microscope:** A compound microscope works by illuminating the slide from underneath with a light bulb. The specimen is then magnified by a series of lenses, one near the slide (called the objective lens) and one near the top (called the eyepiece). This results in a two-dimensional image. Other advantages of compound microscopes are that they can go up to a high magnification and are affordable for amateurs, students, and scientists. A disadvantage is that they have a lower resolution, so your image will never be as crisp and sharp as some more advanced types of microscopes (as described below).
2. **Dark-field microscope:** This microscope contains a special condenser that scatters light and causes it to reflect off the specimen at an angle. A light object is seen on a dark background. Live organisms such as spirochetes can be seen with this microscope.
3. **Phase-contrast microscope:** This microscope also contains special condensers that throw light “out of phase” and cause it to pass through the object at different speeds. It is commonly used to observe living cells without staining them. Internal cell parts such as mitochondria, lysosomes, and the Golgi body can be seen with this instrument.
4. **The fluorescent microscope** uses ultraviolet light as its light source. When ultraviolet light hits an object, it excites the electrons of the object, and they give off light in various shades of color. Since ultraviolet light is used, the resolution of the object increases. Fluorescent dyes are used to help identify unknown bacteria.
5. **Electron microscopy.** The energy source used in the **electron microscope** is a beam of electrons. Since the beam has an exceptionally short wavelength, it strikes most objects in its path and increases the resolution of the microscope significantly. Viruses and some large molecules can be seen with this instrument. The electrons travel in a vacuum to avoid contact with deflecting air molecules, and magnets focus the beam on the object to be viewed. An image is created on a monitor and viewed by the technologist. TEM and SEM are types of electron microscope.
6. **Scanning Electron Microscope (SEM):** The scanning electron microscope, or SEM, uses electrons rather than light for image formation. Samples are scanned in vacuum, so they must be specially prepared by first undergoing dehydration and then being coated with a thin layer of a conducive material, such as gold or palladium. After the item is prepared and placed in the chamber, the densely coated parts of the specimen deflect the electron beam, and both dark and light areas of image are seen on a computer screen. Although this microscope gives lower magnifications than the TEM, the SEM permits three-dimensional views of microorganisms and other objects.
7. **Transmission Electron Microscope (TEM):** Like the scanning electron microscope, the transmission electron microscope (TEM) uses electrons in creating a magnified image. To use this instrument, one place ultrathin slices of microorganisms or viruses on a wire grid and then stains them with gold or palladium to obtain a 2-D view of specimens. A TEM offers a high degree of both magnification and resolution than SEM.

Problems:

1. Draw a neatly labelled diagram of a compound microscope and it's working mechanism.
2. Calculate the resolving power of microscopes with the following data:
 - a. $\lambda = 1500$ angstroms and numerical aperture = 1.6
 - b. $\lambda = 2000$ angstroms and numerical aperture = 1.6
 - c. $\lambda = 4200$ angstroms and numerical aperture = 1.0

Fig.: Schematic diagram of compound microscope

Experiment No: 2: Study of cell Ultra-structure

Aim: To study about different types of cells and their structure..

PROCEDURE: The cell is the basic unit of organization or structure of all living matter. Loewy and Sickevitz (1963) defined the cell as a unit of biological activity delimited by a semi-permeable membrane and capable of self-reproduction in a medium free of other living systems. Cells will reproduce, assimilate, respire and respond to changes in environment and absorb water from internal and external sources. Cell consists of cell wall, cytoplasm, nucleus and cell organelles.

A. CELL WALL:

It is the membrane which converts the cell. It gives shape and structure to the cell. Protect the cell. It also provides mechanical support to the cell.

Structure:

The cell wall is complex in nature and is differentiated into middle lamella, primary cell wall and secondary cell wall. Middle lamella is the outer most layer of the plant cell wall and it connects the two adjacent cells. It is composed of calcium and magnesium pectate and does not contain any cellulose. Primary cell wall is thin and elastic and lies between middle lamella and secondary cell wall. It is mainly composed of cellulose. Secondary wall is the inner most layer of the cell wall and lies between primary cell wall and plasma membrane. Secondary cell wall is relatively thick and is primarily composed of microfibrils of cellulose. In some tissues, besides cellulose, lignin and suberin are also found in secondary cell wall.

B. PLASMA MEMBRANE:

The cytoplasm is surrounded on the outer side by a thin and flexible membrane known as plasma lemma, which is composed of lipids and proteins. It acts as a selectively permeable membrane such that it allows molecules like minerals into the cell and restricts their outward movement. Plasma membrane checks the entry of toxic substances from outside into the cytoplasm.

C. CYTOPLASM:

Cytoplasm is the jelly-like substance surrounding the nucleus within the cell membrane. It contains the cell organelles like mitochondria, ribosome etc. All the substance inside the cell except the nucleus is called cytoplasm. Cytoplasm is the main area of cellular activities in both plant and animal cells. Various reactions take place in it to keep the cell in a living stage.

PROTOPLASM = Cell membrane + Cytoplasm + Nucleus

HYALOPLASM = cytoplasm – Cell organelles – Nucleus

D. CELL ULTRA STRUCTURE (CELL ORGANELLES):

1. ENDOPLASMIC RETICULUM

Endoplasmic reticulum refers to a vast network of membrane enclosed tubules, vesicles and sacs in the cytoplasm. It is found in differentiated cells only.

Structure:

Endoplasmic reticulum appears as a double membrane with variable spaces. It forms a continuous system and is attached to the nuclear envelope on one side and cell membrane on the other. It is of two types viz. rough and smooth. The endoplasmic reticulum carrying ribosomes has granular surface and is therefore called rough endoplasmic reticulum, while the ribosome free region has smooth surface and is hence called smooth endoplasmic reticulum.

Functions:

1. Rough endoplasmic reticulum is associated with the synthesis of proteins.
2. Smooth endoplasmic reticulum is associated with synthesis of lipids and glycogen.
3. It acts as an inter-cellular transport system for various substances.
4. It contains many enzymes which perform various synthetic and metabolic activities..
5. It provides a passage for mRNA from nucleus to the cytoplasm.
6. It is involved in the formation of the new nuclear membrane after each nuclear division.

2. GOLGI COMPLEX

They are cellular organelles which consist of a series of concentric double membranes. They are located either near nuclear membrane or cell periphery. They arise from rough endoplasmic reticulum.

Sub-microscopic spaces or sacs are enclosed by the double membranes and are known as cisternae. Each golgi complex has 3 to 12 inter-connected cisternae which are composed of lipo-proteins.

Functions:

1. Packaging of food materials such as proteins, lipids and phospholipids for transport to other cells.
2. It plays a role in the formation of the plasma membrane.
3. It is involved in the formation of the cell wall of plant cells.

Question:

1. Draw a neatly labeled diagram of the Golgi complex.

3. LYSOSOMES:

- It originates from Golgibody.
- It is rich in hydrolyticenzymes.
- Its main function is digestion.

4. VACUOLE:

- Membrane bound space found in thecytoplasm.
- It contains water, sap, excretory product and some othermaterials.
- It is maintaining the internal pressure and avoids cells fromcollapsing.

5. MITOCHONDRIA

Mitochondria are rod shaped cytoplasmic organelles, which are the main sites of cellular respiration. Hence, they are often referred to as “**Power house**” of the cell.

Structure:

Each mitochondrion is enclosed by two concentric unit membranes comprising of an outer membrane and an inner membrane. The space between the two membranes is known as perimitochondrial space. The membranes are lipo-proteinaceous in nature. The inner membrane has a series of infoldings known as cristae. The inner space enclosed by cristae is filled by a relatively dense material known as matrix. The matrix contains several copies of round or circular DNA molecules.

Functions:

1. They are the sites of cell respiration. Oxidation of carbohydrates, lipids and proteins occurs in the mitochondria.
2. They contain some amount of DNA and are thus associated with cytoplasmic inheritance.

Problem:

1. Draw a neatly labelled diagram of a mitochondrion.

6. CHLOROPLAST

Chloroplasts contain green pigment chlorophyll and are sites of photosynthesis in plants. They are usually spherical, oval or disc shaped.

Structure:

Two concentric lipo-proteinaceous membranes enclose each chloroplast. The outer membrane is permeable, while the inner one is impermeable. The space between the two membranes is known as periplastidial space. The space inside the inner membrane is known as stroma. It contains enzymes related to dark reaction of photosynthesis. Chloroplasts contain some amount of DNA which is present in the stroma. In higher plants, the stroma contains small cylindrical structures called grana. Each granum contains 5 to 25 thylakoids placed one above the other. The grana are

inter-connected by fine tubules known as stroma lamella. Light reaction of photosynthesis takes place in the grana.

Functions:

1. To carry out the process of photosynthesis.
2. They contain some amount of DNA and are thus associated with cytoplasmic inheritance.

Question:

1. Draw a neatly labeled diagram of a chloroplast.

.

7. PEROXISOME:

- It is a lipid bilayer membrane and helps in glyoxylate metabolism.

8. SPHAEROSOME:

- A small organelle plant cell bounded by a single membrane.
- It helps in synthesis and storage of food.

9. NUCLEUS

Nucleus is called the “**controlling centre**” of a cell. It is usually of spherical or oval shape and is generally larger in active cells than in resting cells.

Structure:

A nucleus consists of three main parts viz. nuclear envelope, nucleolus and chromatin.

Nuclear envelope constitutes outer boundary of the nucleus and appears as a double layered membrane composed of lipo-proteins. The space between the outer and inner membrane is known as perinuclear space and is about 200 angstroms wide. The envelope has many small apertures known as nuclear pores which provide direct connection between nucleus and cytoplasm.

Nucleolus is a spherical, colloidal body found in the nucleus. It is associated with a specific region of a particular nucleolar organizing chromosome. It disappears during prophase of mitosis and meiosis and reappears during telophase.

Chromatin refers to DNA + Proteins such as histones. It is a basic unit of chromosomes and contains genes which play an important role in the inheritance of characters from the parents to offspring.

Functions:

1. To regulate growth and reproduction of cells.

Question:

1. Draw a neatly labeled diagram of a plant cell nucleus.

10. RIBOSOME

Ribosome's are spheroid structures occurring either freely in the cytoplasm or remain attached to the membranes of the endoplasmic reticulum. They are known as universal components of all biological organisms.

Each ribosome is composed of two subunits. One subunit is large in size and is dome shaped, while the other subunit is small in size and is placed under the larger sub unit, thus forming a cap like structure. In higher organisms, the large subunit is of 60s type and the smaller sub unit is of 40s type. The major components of ribosomes are RNA and proteins. There is no lipid content in ribosome.

Functions:

1. To carry out synthesis of proteins with the help of mRNA.

Question:

1. Draw a neatly labeled diagram of a plant ribosome.

Experiment no. 3: Experiments on monohybrid, dihybrid, trihybrid cross, test cross and back cross

Aim: To know about different types of crosses and calculation of genotypic and phenotypic ratio.

The term genetics was first given by Bateson in 1906. It is often described as a biological science which deals with heredity and variation. Gregor Johann Mendel is known as the father of genetics with the help of experiment on garden pea. He is able to formulate laws which explained the manner of inheritance of characters. He presented his findings before the Natural History Society of Brunn in 1865.

Mendes described his result in 1866, his work was recognized in 1900 when his laws were discovered by Hugo devries, E. V. Tschermak and Correns, Mendel's original paper was republished in 1901 in the journal Flora.

LAWS OF MENDEL:

1. Law of Segregation
2. Law of Independent assortment


1. The laws of segregation:

It states that when a pair of alleles or allelomorphs is brought together in a hybrid (F1) they remain together without contaminating each other and they separate or segregate from each other in to a complete and pure form during the formation of gamete.

Explanation of laws of Mendel:

Monohybrid ratio: Mendel's law of segregation explains the monohybrid ratio. "The two alleles present in (F1) don't contaminate each other; they separate and pass into different gametes in their original form producing two different types of gametes in equal frequencies". It can be explained by an example

In a pea plant, Round seed shape is dominant over wrinkled seed shape.

Parental generation	Female	×	Male
Phenotype	Round	×	wrinkled
Genotype	RR	×	rr
Gametes	R		r
			
F1 Generation	Rr (Heterozygous) All round		

On selfing:

Parents	Female	×	Male
Phenotype	Round	×	Round
Genotype	Rr	×	Rr
Gametes	Rr		Rr



Generation **F2**

	R	r
R	RR (Round)	Rr (Round)
r	Rr (Round)	rr (Wrinkled)

Genotypic ratio- 1RR :2Rr: 1rr

Phenotypic ratio - 3round :1wrinkled

Test cross: Crossing of F1 hybrid with recessive parent

Rr X rr

	R	r
r	Rr (Round)	rr (wrinkled)

Genotypic ratio-1:1 (RR : rr)

Phenotypic ratio- 1:1 (Round : Wrinkled)

Dihybrid Ratio

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

Parents	Female	X	Male
Phenotype	Yellow Round	X	Green Wrinkled
Genotype	YYRR	X	yyrr
Gametes	YR	X	yr



F1generation **YyRr (Yellow Round) (Heterozygous)**

On selfing / Inter-mating (F2)

Parent Female × Male

♂ ♀	YR	Yr	yR	yr
YR	YYRR Yellow Round	YYRr Yellow Round	YyRR Yellow Round	YyRr Yellow Round
Yr	YYRr Yellow Round	YYrr Yellow Wrinkled	YyRr Yellow Round	Yyrr Yellow, Wrinkled
yR	YyRR Yellow Round	YyRr Yellow Round	yyRR Green Round	yyRr Green Round
yr	YyRr Yellow Round	Yyrr Yellow wrinkled	yyRr Green Round	yyrr Green Wrinkled

Phenotypic ratio- 9 (Yellow round):3(yellow wrinkled):3(Green round):1(Green Wrinkled)

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

CLASS WORK:

1. Work out of given problem based on monohybrid, dihybrid, trihybrid cross, test cross and back cross

Experiment No: 4: Experiment in multiple alleles, co dominance and epistatic interaction

Aim: To know about ratio of multiple alleles, co dominance and epistatic interaction

MULTIPLE ALLELES:

Allele is the alternate form of gene. Alleles of a gene situated at the same locus of homologous chromosomes. According to Mendel, each gene had two alternate forms or allele morphs, one being dominant and the other being recessive. Dominant being the wild type from which recessive mutant evolved through mutation.

Alleles are alternative forms of a gene, and they are responsible for differences in phenotypic expression of a given trait (e.g., brown eyes versus green eyes). Instances in which a particular gene may exist in three or more allelic forms are known as multiple allele conditions.

EXAMPLES OF MULTIPLE ALLELES:

1. ABO blood group

Two human examples of multiple-allele genes are the gene of the ABO blood group system, and the human-leukocyte-associated antigen (HLA) genes.

The ABO system in humans is controlled by three alleles, usually referred to as I^A , I^B , and I^O (the "I" stands for iso-haemagglutinin). I^A and I^B are codominance and produce type A and type B antigens, respectively, which migrate to the surface of red blood cells, while I^O is the recessive allele and produces no antigen.

Genotype of Blood Group		
Blood Group	Antigen	Antibody
A	A	B
B	B	A
O	Nil	A, B
AB	A,B	Nil

2. Multiple alleles for Rabbit fur/coat colour:

CO DOMINANCE (co- (together) + dominance):

Codominance is a form of dominance where both the alleles of a gene pair in a F1 heterozygote are fully expressed. This results in offspring with a phenotype that is neither dominant nor recessive. A typical example showing codominance is the ABO blood group system. For instance, a person having A allele and B allele will have a blood type AB because both the A and B alleles are codominant with each other.

Codominance is different from incomplete dominance in a way that the former has both alleles expressing the phenotypes completely whereas the latter produces an intermediate phenotype because the dominant allele is incompletely dominant. In incomplete dominance, when the dominance is not complete, it is referred to as incomplete dominance. In this form of dominance, the dominant allele is only partially dominant giving rise to an intermediate phenotype in F1 heterozygote (Aa). In another form of dominance, i.e. codominance, the alleles of a gene pair in a heterozygote are fully expressed. This results in offspring that has phenotypes of both the alleles.

INCOMPLETE DOMINANCE:

Mendel's results were groundbreaking partly because they contradicted the (then-popular) idea that parents' traits were permanently blended in their offspring. In some cases, however, the phenotype of a heterozygous organism *can* actually be a blend between the phenotypes of its homozygous parents.

For example, in the snapdragon, *Antirrhinum majus*, a cross between a homozygous white-flowered plant (C^WC^WCWCW) and a homozygous red-flowered plant (C^RC^RCRCR) will produce offspring with pink flowers (C^RC^WCRCW). This type of relationship between alleles, with a heterozygote phenotype intermediate between the two homozygote

phenotypes, is called incomplete dominance.

Ex. Flower color RED \times WHITE, that gives pink flower on crossing that shows incomplete dominance.

COMPLETE DOMINANCE:

Complete dominance is a form of dominance in heterozygous condition wherein the allele that is regarded as dominant completely masks the effect of the allele that is recessive. For instance, an individual carrying two alleles that are both dominant (e.g. AA), the trait that they represent will be expressed. But if the individual carries two alleles in a manner that one is dominant and the other one is recessive, (e.g. Aa), the dominant allele will be expressed while the recessive allele will be suppressed. Hence, the heterozygote (Aa) will have the same phenotype as that of the dominant homozygote (AA). This condition is called complete dominance.

EPISTASIS- GENE INTERACTION RATIO:

1. Mendelian dihybrid ratio-	9:3:3:1
2. Supplementary gene interaction-	9:3:4
3. Complementary gene interaction-	9:7
4. Duplicate gene interaction-	15:1
5. Inhibitory gene interaction-	13:3
6. Masking gene interaction-	12:3:1
7. Polymeric gene interaction-	9:6:1
8. Additive gene action-	1:4:6:4:1

CLASS WORK

1. Work out of given problem on multiple alleles, co dominance and epistatic interaction

Experiment no. 5: Practice on mitotic cell division

Aim: To understand the process and different stages of mitosis and to visualize different phases of mitosis.

PRINCIPLE:

Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs. An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, like Feulgen stain and Acetocarmine stain.

MATERIAL REQUIRED:

- | | | |
|----------------------|--------------------------|--|
| 1. Onion root tip | 2. Acetoorcein (2%) | 3. 1 N HCl |
| 4. Scissors | 5. Forceps | 6. Razor blade |
| 7. Dropper | 8. Vial | 9. Dissection probe with wooden back |
| 10. Light Microscope | 11. 45% acetic acid sol. | 12. Microscopic slides and cover slips |

PROCEDURE:

- ❖ Present using a sharp blade.
- ❖ Grow root tips by placing the bulbs in a beaker filled with water.
- ❖ New roots may take 3–6 days to grow.
- ❖ Cut off 2–3 cm of freshly grown roots and let them drop into a watch glass.
- ❖ Using forceps transfer them to the vial containing freshly prepared fixative of aceto-alcohol (1:3: glacial acetic acid: ethanol).
- ❖ Keep the root tips in the fixative for 24 hours.
- ❖ Using forceps take one root and place it on a clean glass slide.
- ❖ Using a dropper, place one drop of 1N HCl on the root tip followed by 2–3 drops of Acetoorcein stain.
- ❖ Warm it slightly on burner. Care should be taken that the stain is not dried up.
- ❖ Carefully blot the excess stain using filter paper.
- ❖ Using a blade, cut the comparatively more stained tip portion of the root, retains it on the slide and discard the remaining portion.
- ❖ After that, put one drop of 45% acetic acid sol on the root tip.
- ❖ Mount a cover slip on it using a needle. Care should be taken to avoid bubble formation.
- ❖ Now, slowly tap the cover slip using the blunt end of a needle so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.
- ❖ This preparation of onion root tip cells is now ready for the study of mitosis.
- ❖ Place the slide under the compound microscope and observe the different stages of mitosis.

- ❖ Various stages of mitosis are prophase, metaphase, anaphase and telophase can be observed.

PROCEDURE:

Growing of root tips

Select a few medium-sized onion bulbs. Carefully remove the dry roots present. Grow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water. Care should be taken so that the stem portion of the bulb (basal part) just touches the water. A few drops of water may be added periodically to compensate evaporation losses. New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared fixative, i.e., aceto-alcohol (1: 3: glacial acetic acid: ethanol). Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future). Onion root-tip cells have a cell cycle of approximately 24-hour duration, i.e., they divide once in 24 hours, and this division usually takes place about two hours after sunrise. Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

STUDY OF CELL:

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells. Examine the dividing cells under higher magnification of the microscope to observe the detailed features of mitosis.

OBSERVATION:

The stages of mitosis can be broadly categorized into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell). Those cells, which are not in the phases of cell division, are considered to be in interphase. You may observe that most of the cells in a microscope field are in interphase

INTERPHASE

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (colored) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus

DISCUSSION

Mitotic index (MI) is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular focus chosen randomly under the microscope and is calculated as $MI = n/N \times 100$ By randomly selecting 5 to 10 such foci, one can estimate the mitotic index for a given type.

The effect of different samples of water (polluted or contaminated) can be assayed on the mitotic-index (an indicative feature of somatic growth rate in them). Further, the impacts of different types of pollutants on different phases of mitosis can also be assayed.

Fig.: Different stages of mitosis

Experiment no. 6: Practice on meiotic cell division

Aim: Study of different stages of meiosis

PRINCIPLE:

Meiosis cell division studies mainly in reproductive cell that can be found rapidly in mature onion buds. The anthers can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of meiosis present in the anther cells can be visualized by treating with DNA specific stains, like Acetocarmine.

MATERIAL REQUIRED:

- | | | |
|----------------------|-------------------|--|
| 1. Onion bud | 2. Acetocarmine | 3. Dropper |
| 4. Needle | 5. Forceps | 6. Razor blade |
| 7. Watch glass | 8. Brush | 9. Dissection probe with wooden back |
| 10. Light Microscope | 11. Canoy's fluid | 12. Microscopic slides and cover slips |

PROCEDURE:

Fixation

Flowers buds are fixed in canyons fluid (1 part acetic acid: 6 parts ethyl alcohol). The amount of fixatives must always be at least 10 times more than the volume of the plant tissue. After 48 hours, the material is transferred and preserved in 70% ethyl alcohol.

Staining

Wash the material with double distilled water twice. Remove the anthers from flowers buds. Keep an anther on a clean glass slide in a drop of acetocarmine. Squash it with the help of flattened needle and remove the empty anther sac. Place a glass cover slip over the smear.

Smearing

Keep the glass slide in between the folds of blotting paper and apply uniform pressure over it with thumb. Add a few drops of acetocarmine stain from the slides of coverslip carefully, without disturbing the coverslip and allow it to dry.

Observation

Place the glass slide under a microscope and observe the various stages of the meiotic cell cycle. If the cells are of desired stage, the cover glass is sealed with paraffin wax temporarily. This can then be made permanent as per method given below.

Making of permanent slide

1. Wax is removed carefully from the slide and cover glass with the help of a blade without disturbing the slide.
2. The slide is kept in a Petridis containing 45% acetic acid for separation of the cover glass and slide from each other.
3. They are then transfer to a mixture of acetic acid and Butanol (1:1) for 1 minute. Then they are transfer to an absolute Butanol for 30 seconds and then the slide and cover glass is mounted in eupatrid separately

Meiosis is the process of nuclear division which occurs during the final stage of gamete formation. Meiosis is consist of two cell divisions

- A. Meiosis I (Reduction phase)
- B. Meiosis II (Equational Phase)

STAGES OF MEIOSIS:

Meiosis I

- This is sometimes referred to as the reduction division because it is during the first meiotic division that the chromosome number is reduced from 46 to 23.
- Meiosis I consist of four stages: prophase I, metaphase I, anaphase I, and telophase

Meiosis I: Prophase I

Prophase stage of meiosis I is relatively long and can be subdivided into five stages.

- Leptotene
- Zygotene
- Pachytene
- Diplotene
- Diakinesis

Fig.: Different stages of meiosis cell division

Experiment no: 7: Experiment on chi -square test and probability

Aim: Solving problems on probability and chi square test

Probability:

Probability is the likelihood of occurrence of an event. In daily life, each one of us is often faced with decision about the likelihood that an event will or will not occur

E.g.: Before commencement of examinations .is student anxious about the likely hood of his doing well in the examinations. The likelihood of obtaining good marks in an examination may be influence by a number of factors

Events: An event is the occurrence of something. The occurrence of head or tail in a toss is an event. Similarly, new born baby being boy or girl is also an event

Formula: $p=1/n$

Q1. A pea plant is heterozygous for 2 genes (TtRRYy) where, T=Tall, t=dwarf, R=round seed r=wrinkled seed, Y=yellow seed, y= green seed, If this plant is self-fertilized what are the predicated phenotypes offspring what fractions of the offspring will occur in each category What fraction of progeny will be tall round and green?

Solution:

TtRRYy XTtRRYy

	TRY	TRy	tRY	tRy
TRY	TTRRYY (tall, round, yellow)	TTRRYy (tall, round, yellow)	TtRRYY (tall, round, yellow)	TtRRYy (tall, round, yellow)
TRy	TTRRYy (tall, round, yellow)	TTRRyy(tall, round, green)	TtRRYy (tall, round, yellow)	TtRRyy (tall, round, green)
tRY	TtRRYY (tall, round, yellow)	TtRRYy (tall, round, yellow)	ttRRYY (dwarf, round, yellow)	ttRRYy (dwarf, round, yellow)
tRy	TtRRYy (tall, round, yellow)	TtRRyy (tall, round, green)	ttRRYy (dwarf, round, yellow)	ttRRyy (dwarf, round, green)

tall, round, yellow-9/16

dwarf, round, yellow-3/16

tall, round, green-3/16

dwarf, round, green-1/16

Here, tall, round, green, are 3/16

Fraction of progeny tall, round, green will be 3/16

2. A pea plant is heterozygous for 3 genes (TtRrYy) where, T=Tall, t=dwarf, R=round seed r=wrinkled seed, Y=yellow seed, y= green seed. If this plant is self-fertilized what

are the predicated phenotypes offspring? What fractions of the offspring will occur in each category?

Solution: By applying alternative forked line method here, predicted phenotypes is (2^n) i.e. 8 and the fraction of the offspring is as follows

27/64 (tall, round, yellow) 9/64 (tall, round, green) 9/64 (tall, wrinkled, yellow) 3/64 (tall, green, wrinkled) 9/64 (dwarf, round, yellow) 3/64 (dwarf, round, green) 3/64 (dwarf, wrinkled, yellow) 1/64 (dwarf, wrinkled, green)

Chi-square:

Q 2. F1 generation, all offspring have straight wings and gray body of drosophila. In F₂ generation out of 352 total flies, 193 are straight wing and gray bodies, 69 straight wings, along body, 64 curved wings, gray body 26 curved wings boney body apply chi square test for the given data

Solution:

Given data,

Straight wing gray bodies =193

Straight wing ebony bodies = 59

Curved wing gray bodies = 64

Curved wing ebony bodies= 26

Total no of files =352

Expected values based on the Mendelian law of independent assortment (9:3:3:1) are as follows

Here straight wing gray bodies are dominant over curved wing on ebony bodies respectively

Straight wing gray bodies= $9/16 \times 352 = 198$

Straight wing ebony bodies

= $3/16 \times 352 = 66$ Curved wing gray

bodies = $3/16 \times 352 = 66$ Curved wing

ebony bodies= $4/16 \times 352 = 22$

Phenotype	Expected data (E)	Observed data (O)	O-E	(O-E) ²	χ^2
Straight, gray	198	193	-5	25	0.126
Straight, ebony	66	69	3	9	0.136
Curved, gray	66	64	-2	4	0.060
Curved, ebony	22	26	4	16	0.727

TOTAL	352	352			1.049
--------------	------------	------------	--	--	--------------

Hence, the value of χ^2 is 1.049

CHI-SQUARE TABLE WITH RESPECTIVE DEGREE OF FREEDOM

Degree of freedom	P=0.099	0.95	0.80	0.50	0.20	0.05	0.01
1	0.000157	0.00393	0.0642	0.455	1.642	3.841	6.635
2	0.020	0.103	0.446	1.386	3.219	5.991	9.210
3	0.115	0.352	1.005	2.366	4.642	7.815	11.354
4	0.297	0.711	1.649	3.357	5.989	9.488	13.277
5	0.554	1.145	2.343	4.351	7.289	11.070	15.086
6	0.872	1.635	3.070	5.348	8.558	12.592	16.812
7	1.239	2.167	3.822	6.346	9.803	14.067	18.475
8	1.646	2.733	4.595	7.344	11.030	15.067	20.090
9	2.088	3.325	5.380	8.343	12.242	16.919	21.666
10	2.588	3.940	6.179	9.342	13.442	18.919	23.209
15	5.229	7.261	10.307	14.339	19.311	18.307	30.578
20	8.260	10.851	14.578	19.337	25.038	24.996	37.566
25	1.524	14.611	18.940	24.337	30.675	31.410	44.314
30	14.953	18.493	23.304	29.336	36.750	43.773	50.892

Here the value of chi -square i.e. 1.049 is less than the table of 7.815 at 0.05 p and 3 degrees of freedom hence, the null hypothesis is accepted.

CLASS WORK

1. Work out of given problem on probability and chi square test.

Experiment no. 8: Determination of linkage and crossing over Estimation (two point and three point test cross data)

Aim: To study on linkage, crossing over and gene mapping

According to chromosome theory, many genes are located linearly in each chromosome. All the genes located in a chromosome tend to go together from generation to generation. The tendency of genes to be inherited together from generation to generation is termed as linkage. The first concept of linkage was demonstrated by T. H. Morgan in 1910 while studying the inheritance pattern of white eye (w gene) of drosophila (queen of genetics).

CONSEQUENCE OF LINKAGE: -

- ❖ Genes present in the chromosome tends to stay together during linkage inheritance.
- ❖ The distances between two genes are inversely proportional to the effect of linkage.
- ❖ All the genes present in a chromosome are in a linear form.
- ❖ There are two phases of linkage.
 - a) **Coupling Phase:** the dominant alleles of the linked genes are present in the same chromosome, while the recessive alleles of the same genes are present in the other chromosome of a homologous pair. Example is AB / ab
 - b) **Repulsion Phase:** the dominant alleles of some genes are present with recessive alleles of other genes in the same chromosome of a homologous pair. Example is Ab/aB
- ❖ Linkage does not follow independent assortment, so the ratio obtained in F₂ generation and test cross progeny is deviated from 9:3:3:1 or 1:1:1:1 respectively.

Map distance / Map unit: the distance between two given genes is calculated based on the percentage of crossing over, because the frequency of crossing over is directly proportional to the distance between two genes. A map unit is the distance between two linked genes and permits one percent recombination/crossing over between the two linked genes.

Formula for calculating Crossing over Frequency (%):

$$\text{Crossing Over Frequency (\%)} = \frac{\text{number of recombinants}}{\text{Total offspring}} \times 100$$

Two point test cross

What are two point tests cross?

Two point cross is used to determine the distance between the two linked genes. Single chiasma is observed in two point test cross.

Working example: AB/ab x ab/ab

Progeny:

AB/ab = 37 } Parental Type

ab / ab = 37 } Parental Type

Ab/ab= 13 } Recombinant types

aB/ab= 13 } Recombinant types

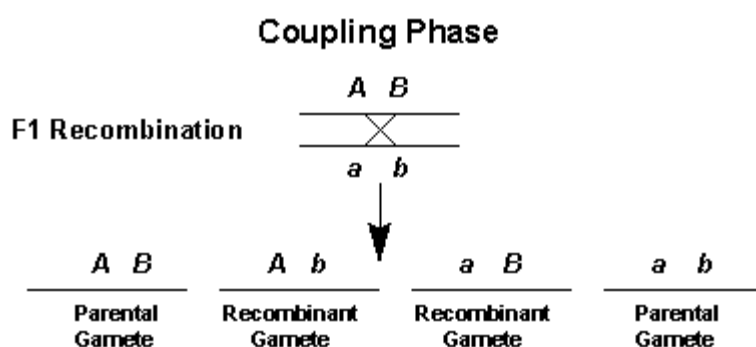
Total = 100

$$\text{Crossing Over Frequency (\%)} = \frac{\text{number of recombinants}}{\text{Total offspring}} \times 100$$

$$= 26 \times 100/100 = 26$$

Therefore the distance between the genes A & B = 26 map units.

The genes A and B are said to be linked in the coupling phase as seen below.



What is a three point cross?

Three point cross is used to determine the order and distance between the three linked genes. Two chiasma and double crossovers are observed in three point test cross.

Why are three point crosses important?

By solving a three point cross you can determine two important things:

- ❖ Order of the three genes on a chromosome.
- ❖ Determine the distance (in map units) between each pair of genes to construct linkage map.

PROCEDURE: Construction of linkage map and calculation of coefficient of coincidence and

interference can be illustrated by the following example.

Q. Draw a genetic map of these 3 genes (A, B and C) showing the distances between all pairs of genes and then calculate the value of interference

In a three point test cross, eight different phenotypic classes are obtained.

Parental types have the maximum phenotypic frequencies, double crossovers have the lowest phenotypic frequencies, and the single crossovers have phenotypic frequencies between these two classes. Suppose, ABC/abc are three linked genes located on two different chromosomes in F₁ of a cross between AABBCC and aabbcc parents.

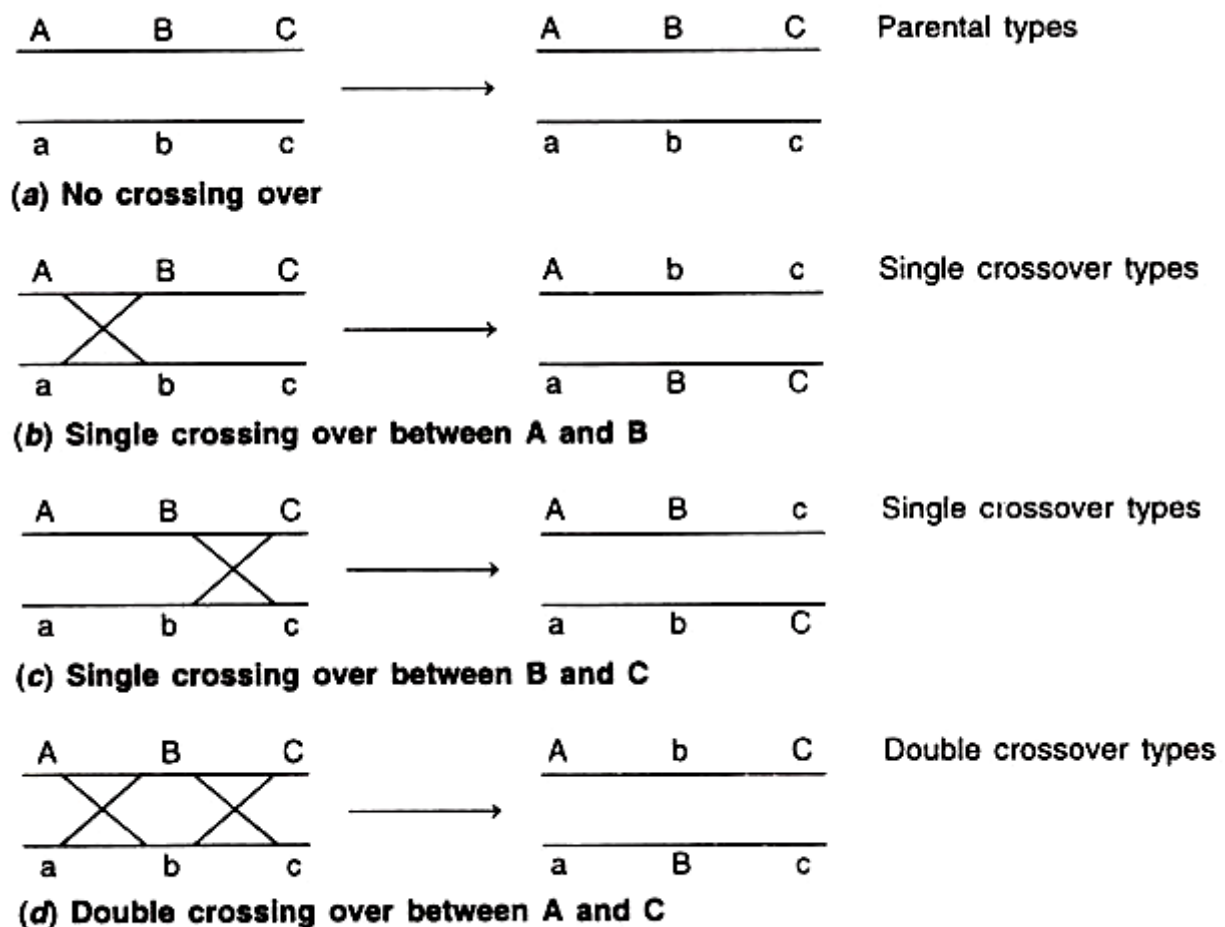


Fig. 9.3. Single and double crossing over between three linked genes.

1. Single crossover between A and B will alter the position of two genes, viz., B and C (Fig. 9.3).
2. Single crossover between B and C will alter the position of only one gene, i.e., C (Fig. 9.3).
3. Double crossover between A and C will alter the position of only middle gene, i.e., B (Fig. 9.3).

Thus eight types of gametes are produced by F₁ and only one type of gamete is produced by homozygous recessive parent. Union of male and female gametes will produce eight different phenotypic classes (Table 9.3).

TABLE 9.3. Summary of the results obtained from a three point test cross between $ABC/abc \times abc/abc$

<i>Genotypic classes</i>	<i>Phenotypic classes</i>	<i>Assumed frequencies</i>	<i>Remarks</i>
ABC/abc	ABC	349	} Parental types.
abc/abc	abc	360	
Abc/abc	Abc	114	} Single crossover between A and B.
aBC/abc	aBC	116	
ABc/abc	ABc	128	} Single crossover between B and C.
abC/abc	abC	124	
AbC/abc	AbC	5	} Double crossover between A and C.
aBc/abc	aBc	4	
Total		1200	

2. Recombination (%) of Three point Test Cross:

Crossing over percentage between genes A and B = $(114+116)+(5+4)/1200 \times 100 = 230 + 9/1200 \times 100 = 19.92\%$

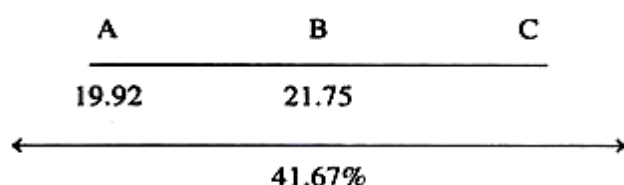
Crossing over percentage between genes B and C = $(128 + 124) + (5 + 4) / 1200 \times 100 = 252 + 9 / 1200 \times 100 = 21.75\%$

3. Gene Sequence of Three Point Test Cross:

Hence, distance between genes A & B = 19.92 map units

Distance between genes B& C= 21.75 map units

Distance between genes A & C = $21.75 + 19.92 = 41.67$ map units



4. Coefficient of Coincidence in Three point Test Cross:

Coefficient of coincidence =

Observed no. of double crossovers/Expected no. of double crossovers

1. Observed double crossovers = $9/1200 \times 100 = 0.75$

2. Expected double crossovers = Product of two single crossovers between AB & BC values/100

$$= 19.92 \times 21.75/100 = 4.33$$

Coefficient of coincidence = $0.75/4.33 = 0.17$

Coefficient of interference = $1 - \text{Coefficient of coincidence} = 1 - 0.17 = 0.83$

Experiment no 09: Study on sex linked inheritance in drosophila

Aim: To study on sex linked inheritance

Sex linkage:

This is an association during inheritance of a character with sex of the progeny. Sex linked is the consequence of a gene being located in the X or sex chromosome. In the early 1910, T. H. Morgan studied the inheritance pattern of White eye (w) gene in *Drosophila melanogaster* to try to disprove Mendel's theory of the 3:1 ratio

Inheritance of white eye in drosophila:

Case I

- ❖ While white eyed male was mated with red eyed female. All F₁ flies had red eyes and in F₂, 3 flies had red eyes and one had white eyes (3:1). This reveals that white eye is due to recessive gene.
- ❖ But when F₂ individuals were classified on the basis of their sex as well as eye color, all the F₂ female has red eyes, but half of the male had red and the other half had white eyes. It appears that the eye color of a fly depended on its sex.

Fig.: Sex linked inheritance of White eye (w) gene in *Drosophila melanogaster*

Case II

- ❖ But when white eyed female flies were mated with red eyed males, half the flies in F_1 were red eyed and the remaining half had white eyes. In F_2 from this reciprocal cross, red eyed and white eyed flies were obtained in a 1:1 ratio; which is same as in F_1 generation.
- ❖ The classification of F_1 and F_2 flies on the basis of their sex as well revealed that all the F_1 males were white-eye, while the females were red eyed and in F_2 both the male and female flies showed the ratio 1:1 (red: white)

Conclusion:

- ❖ Thus the hypothesis shows that the W gene is located in the X-chromosome and that it has no allele in the Y-chromosome is adequate to explain the peculiar inheritance pattern of the white eye trait in *Drosophila*.
- ❖ Female flies will have two copies of w gene (WW , Ww or ww), while male will have only one copy (W or w) i.e. hemizygous for w
- ❖ This was the first conclusive demonstration that a specific gene was located in a specific chromosome of an organism.

Experiment no. 10: Study of models on DNA and RNA structure

Aim: To study the different types of genetic material and their models

Nucleic Acid

Friedrich Miescher discovered nucleic acid (DNA) in 1869, and he first isolated it from collection of dead white blood cell, and called as nuclein. The term "nucleic acid" was given by Richard Altman. Nucleic acid was found to be associated with various proteins called nucleoprotein. There are 2 types of nucleic acid.

1. Deoxyribose Nucleic Acid(DNA)
2. Ribose Nucleic Acid(RNA)

Structure of nucleic acid:

- ❖ Nucleotides are the building block of DNA and RNA
- ❖ Polymers of nucleotides
- ❖ Each nucleic acid contains 4 different nucleotides
- ❖ A nucleotides consists of Sugar, Nitrogenous base and Phosphate group

Pentose sugar:

- ❖ Pentose sugar is the basis of nomenclature of nucleic acid (RNA or DNA)
- ❖ Each carbon atom of ring designated by a number with prime sign
- ❖ Deoxyribose sugar differ from ribose sugar in lacking an oxygen atom at 2' Carbon.

Nitrogenous bases:

- ❖ Mainly total five different types of nitrogenous bases found in nucleic acid viz. Adenine (A), guanine (G), cytosine (C), thymine (T) and Uracil (U).
- ❖ DNA and RNA can be differed in respect of nitrogenous bases as RNA contains Uracil in place of Thymine found in DNA.

Nucleotide (sugar + phosphate + nitrogen containing base):

Nucleoside (sugar + nitrogen containing base):

Structure of DNA:

In 1953 James Watson and Francis crick, based on the X-ray diffraction data produced by Maurice Wilkins and Rosalind Franklin, proposed a very simple but famous and universally accepted double helix model for the structure of DNA. The silent features of double helix structure of DNA are listed below.

Fig.: Double helix model of DNA molecule

Structure of RNA:

RNA is a polynucleotide and RNA nucleotides have ribose sugar, which participate in the formation of sugar phosphate backbone of RNA. Thymine is absent and is replaced by uracil. Usually RNA is a single stranded structure. Single stranded RNA is the genetic material in most viruses E.g. - TMV. Double stranded RNA is also found to be the genetic material in some organisms. E.g. - Plant wound and tumor viruses.

Types of RNA:

There are three main types or forms of RNA.

1. Messenger RNA(m-RNA)
2. Ribosomal RNA(r-RNA)
3. Transfer RNA(t-RNA)

1. Messenger RNA (m-RNA):

2. Ribosomal RNA(r-RNA):

3. Transfer RNA (t-RNA):

