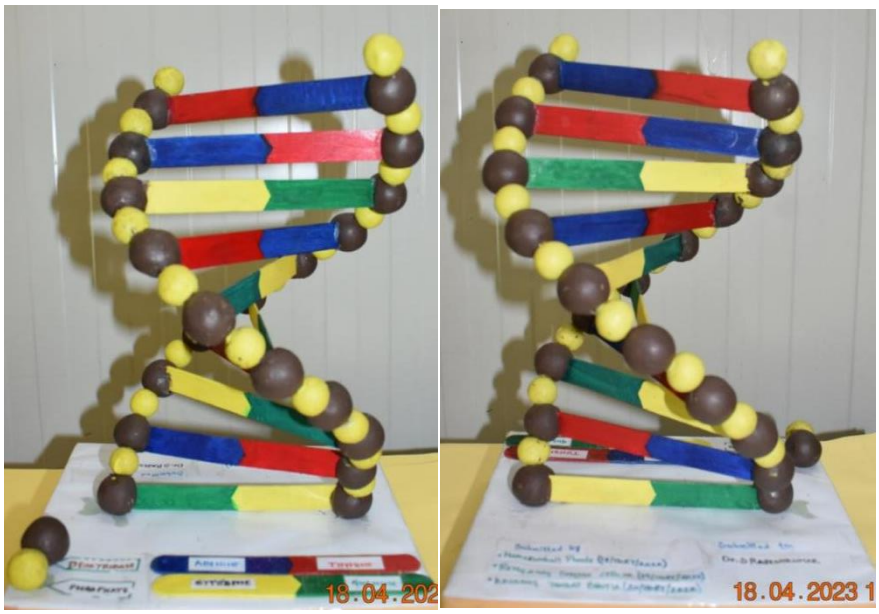
PRINCIPLES OF GENETICS AND CYTOGENETICS

BSH 115

PRACTICAL MANUAL



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FOREWORD

The principles of genetics describe how traits are passed from one generation to the next, and how genetic variation arises within populations. Some of the key principles of genetics include: Mendelian Inheritance, Dominance and Recessiveness, Segregation, Independent Assortmen Genetic Variation. Genetic variation provides the raw material for evolution to act upon, and is important for the adaptation of populations to changing environments. Overall, the principles of genetics help us to understand how traits are inherited and how genetic variation arises, which has important implications for fields such as medicine, agriculture, and evolutionary biology.

This manual provides information on how to study Medelian genetics, knowledge on fixatives and Stains and its use to observe mitotic and meiotic phases of cell cycle in meristematic cells and Pollen grains, construction of linkage map grom three point test cross and Chi square test for various observed genetic rations. There are 13 practicals in this book to impart practical knowledge to the students.

Anupam Mishra

PREFACE

Genetics is the study of genes, heredity, and genetic variation in living organisms. It involves understanding the mechanisms of inheritance, how traits are passed down from one generation to the next, and how genetic information is expressed and regulated within cells. Some of the key areas of study in genetics include: Molecular genetics: This involves the study of the structure and function of genes, as well as the regulation of gene expression. Population genetics: This involves the study of how genetic variation is distributed within and between populations, and how it changes over time. Evolutionary genetics: This involves the study of how genetic variation contributes to the evolution of species and the development of new traits over time. Medical genetics: This involves the study of genetic disorders and how they are inherited, diagnosed, and treated. Genomics: This involves the study of the complete set of genes in an organism's genome, and how they interact with each other and with the environment. Overall, genetics is a rapidly evolving field that has significant implications for many areas of science, including medicine, agriculture, and conservation biology.

Cytogenetics is the branch of genetics that deals with the study of chromosomes and their behavior during cell division and inheritance. It involves the analysis of the structure, function, and abnormalities of chromosomes, as well as their relation to genetic diseases and disorders.

Overall, the study of cytogenetics is essential in understanding the genetic basis of various diseases and disorders, and it continues to advance our understanding of genetics and genomics.

Practical applications of genetics are vast and varied, and they have significant implications in various fields, including medicine, agriculture, biotechnology, forensics, and ecology. In Agriculture, Genetics plays a crucial role in plant and animal breeding, which is vital for improving crop yields, disease resistance, and food production. The use of genetic engineering and biotechnology has also led to the development of genetically modified crops that are more resistant to pests and environmental stresses.

The authors

SYLLABUS

Study of fixatives and stains. Squash and smear techniques. Demonstrations of permanent slides and cell division, illustration in plant cells, pollen fertility and viability, determination of gametes, Solving problems of monohybrid, dihybrid, and test cross ratios using chi-square test, gene interactions, estimation of linkages using three point test cross from F2 data and construction of linkage maps. Genetics variation in pea.

CERTIFICATE

This is to certify that Mr./Ms …….…….....……. …..

Reg. No…….……has performed Practical for the semester ……

B. Sc. (Hons) Agriculture in the Course No…….…….

Title……..……..

During the academic year……

He/She has performed……. practical out of ……

US ID:……..

Course Teacher

CONTENT

Experiment No: 1 **Date:_____**

Aim: To study the compound microscope

Principle

A microscope is a laboratory instrument used to examine objects that are too small to be seen by the naked eye. Microscopy is the science of investigating small objects and structures using a microscope. Technically, microscope is a device used for magnification of the microscopic samples.

Different types of microscopes

a. Simple microscope

A simple microscope is defined as the type of microscope that uses a single lens for the magnification of the sample. It is a convex lens with a small focal length. It was invented by 17^{th} century by Antony van Leeuwenhoek. It is common among the watchmakers as they can view a magnified image of the smallest parts.

b. Compound microscope

A compound microscope is a high power (high magnification) microscope that uses a compound lens system. It has multiple lenses: the objective lens (typically 4x, 10x, 40x or 100x) is compounded (multiplied) by the eyepiece lens (typically 10x) to obtain a high magnification of 40x, 100x, 400x and 1000x. Higher magnification is achieved by using two lenses *viz*., the eyepieces and the objective lenses create high magnification, a condenser beneath the stage focuses the light directly into the sample. It is sometimes referred as biological microscope. Compound microscopes are used in laboratories, schools, wastewater treatment plants, veterinary offices, and for histology and pathology.

c. Electron microscope

The electron microscope (EM) is among the most powerful magnification tools. It is a scope that instead of light uses a flow of electron to produce an image. Moreover, this microscope enhances the images of viruses, protein, lipids, ribosomes, and even small molecules. The transmission electron microscope, or TEM, can display images of samples as small as 1 nanometer across. Scanning electron microscopes, or SEMs, are about 1/10 as powerful as a TEM. They can produce black and white and 3D images with sharp resolution. Both TEMs and SEMs are widely used in biology, chemistry, gemology and metallurgy, and in other industries requiring microscopic topology, morphology and similar observations.

d. Fluorescence microscope

This type of microscope uses ultraviolet light to illuminate specimens that fluoresce. Besides, mostly, a fluorescent antibody or dye is added on the viewed specimen.

e. Digital microscope

The digital microscope was invented in Japan in 1986. It uses the power of the computer to view objects not visible to the naked eye. The computer software allows the monitor to display the magnified specimen. Moving images can be recorded or single images captured in the computer's memory.

f. Stereomicroscope

The Stereo microscope, also called a dissecting microscope, has two optical paths at slightly different angles allowing the image to be viewed three-dimensionally under the lenses. It have lower magnification, typically between 10X and 200X, generally below 100x, but longer working distances.

g. Scanning probe microscope

A scanning probe microscope is a type of microscope in which the examination of specimens is done at the nanoscale level. This type of microscope helps to study the specimen's properties, reaction time, and also its behaviour when stimulated.

Parts of the compound microscope and their uses

The part of the microscope is grouped under two categories:

I. Mechanical parts
II. Optical parts

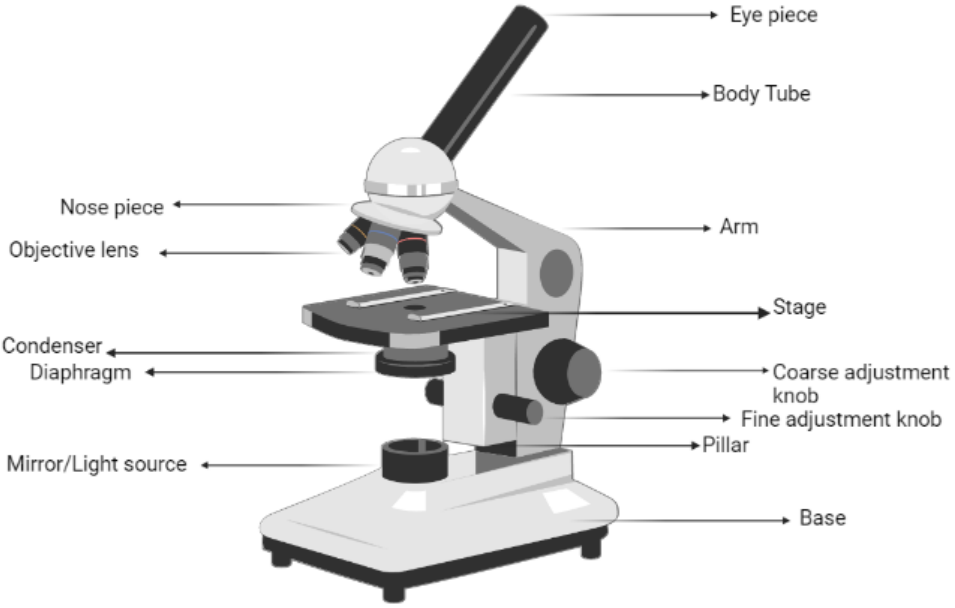


Fig 1.1. Parts of a compound microscope

I. Mechanical parts of a compound microscope

i. Base or Foot

It is a U-shaped structure and supports the entire weight of the microscope.

ii. Arm

This is the part connecting the base and to the head and the eyepiece tube to the base of the microscope. It gives support to the head of the microscope and it is also used when carrying the microscope.

iii. Pillar

It is a vertical projection. This stands by resting on the base and supports the stage.

iv. Inclination joint

A joint at which the arm is attached to the pillar of the microscope is called inclination joint. It is used for tilting the microscope.

v. Coarse adjustment knob

It is a large knob that is used for moving the body tube down and up for bringing the object to be examined under exact focus.

vi. Fine adjustment knob

It is the smaller knob, which is used for fine focusing of the object.

vii. Body tube

The upper part of the arm of the microscope comprises a hollow and tubular structure known as the body tube. The body tube can be shifted down and up using the adjustment knobs.

viii. Nose piece

The nose piece is circular and a rotating metal part that is connected to the body tube's lower end. The nose piece has three holes wherein the objective lenses are embedded.

ix. Stage

The flat and rectangular plate that is connected to the arm's lower end is called the stage. The specimen is placed on the stage for studying and examining the various features. The centre of the stage has a hole through which light can pass.

x. Mechanical stage

It allows the control of the slides by moving the slides using the mechanical knobs on the stage instead of moving them manually.

II. Optical parts of a compound microscope

i. Eyepiece or Ocular

This is the part used to look through the microscope. It is present at the top of the microscope. Its standard magnification is 10x with an optional eyepiece having magnifications from 5X to 30X.

ii. Objective lens

These are the major lenses used for specimen visualization. They have a magnification power of 40x-100X. There are about 1- 4 objective lenses placed on one microscope, in that some are rare facing and others face forward. Each lens has its own magnification power. Oil immersion objective lens magnifies 100X and is used for observing structures and organisms at the sub-cellular level. Oil is placed between the slide and the lens to prevent additional light refraction as it passes from one medium (glass) into another (oil). The objective produces an enlarged and inverted projection of the object on the other side of the lens. This first image (a real image) serves as the object for the ocular. The ocular produces a final image (a virtual image) that is greatly enlarged and still inverted.

iii. Condenser

These are lenses that are used to collect and focus light from the illuminator into the specimen. They are found under the stage next to the diaphragm of the microscope. They play a major role in ensuring clear sharp images are produced with a high magnification of 400X and above. The higher the magnification of the condenser, the more the image clarity. Abbe condenser is specially designed for high-quality microscopes, which makes the condenser to be movable and allows very high magnification of above 400X. Its numerical aperture is 1.25

iv. Reflecting mirror/ Light source

A mirror is found attached wither to the pillar or the lower end of the arm. It consists of a concave mirror on one side and a plain mirror on the other side. It can be used for reflection of light rays into the microscope.

v. Diaphragm

It's also known as the iris. It is found under the stage of the microscope and its primary role is to control the amount of light that reaches the specimen. It's an adjustable apparatus, hence controlling the light intensity and the size of the beam of light that gets to the specimen.

Working conditions of compound microscope

- Firstly, view into the eyepiece. The mirror is rearranged such that adequate light passes into the microscope.
- The mirror, lenses, stage, and slides should be cleared of dust and be clean.
- The slide is placed in the middle of the stage.
- The slide is firmly secured with clips at two edges of the slide to ensure that the slide cannot move.
- The nose piece is adjusted in such a way that the low power objective is aligned with the object of focus placed on the slide.
- The coarse adjustment knob can be shifted upwards or downwards such that the slide is well under focus.
- the fine adjustment knob is turned by moving upwards or downwards to get a clear and sharp image of the object under focus.
- All minute details of the object are observed under low power objective. Necessary diagrams are sketched.
- The nose piece is now turned to bring the high power objective aligning with the object. The fine adjustment knob is tuned as much as possible to get a bright and precise view of the object.
- In high power, the details of the object are observed. Draw the necessary diagrams. The coarse adjustment knob should not be used when the object is being examined in high power as it can crush the slide.

Precautions to be taken while working with microscope

- ✓ The objective lenses and eyepiece should be cleaned with the help of silk cloth and cleaning liquid before use.
- ✓ The microscope should not be tilted when working or using it.
- ✓ When an object needs to be studied, focus on the low power objective first and then move to high power.

- ✓ The lower power needs to be left in place after all observations are completed.
- ✓ When focusing, care needs to be taken to ensure that the objective lens never strikes the stage or the slide.
- ✓ Only the fine adjustment knob should be used when the high power objective is employed.
- ✓ Coverslip should always be used to cover well-mount preparations before observation under the microscope is made.
- ✓ Do not dismantle the microscope.
- ✓ When carrying the microscope, always use both hands and support the base of the microscope.
- ✓ Try to place it in a box after using the microscope.
- ✓ The concave part of the mirror should be used under dim light.
- ✓ An oil immersion lens should never be used without the use of oil.

Exercises

1. Draw and label a compound microscope.

Experiment No: 2 **Date:_____**

Objective: To study the cell structure and its organelles

Principle

Cell is the basic functional, structural and biological unit of living organism. The study of cells is called as cell biology. An organelle is a specialized subunit, usually within a cell, that has a specific function.

The cell consists of the following components; cell membrane, cytoplasm and the nucleus.

The structures present in the plant cell wall are: cell wall, plasmalemma, endoplasmic reticulum (ER), ribosomes, golgi bodies, lysosomes, spherosomes, chloroplast, mitochondria and nucleus whilst the animal cells lack cell wall and chloroplast having centrioles not found in the plant cells.

Plant cell is the structural and physiological unit of plant, which have protoplasm.

Cell organelles and its function

Cell wall:

It is the outermost part of the cell and always non living, tough produced and maintained by living protoplasm

Cell wall always found in plants cell and absent in animal cell

Functions

1. To protect inner parts of the cell
2. To give a definite shape to the cell
3. To provide mechanical support Cytoplasm

Variety of structure remain suspended such as living and non living

Non living: Non membrane bounded – lipid, starch granules

Living membrane bounded

Golgi complex

Golgi body first described by Camilo Golgi in 1822 in nerve cell of cat and owl

It is a structure like stalk of filaments arranged one above the other

Composed –Lamellae, tubules, vesicles and vacuoles

Functions :

1. Packaging food materials such as proteins, lipids and phospholipids for transport to other cells
2. It secrete many granules and lysosomes

Lysosomes :

- The term lysosome was first used by Dave in 1955
- In plant cell they are bounded storage granules and containing hydrolylic digestive enzymes

Functions :

1. It is responsible for digestion of intracellular substances and foreign particles.
2. When cell dies lysosomes releases its enzymes, which digest the dead cell resulting in cleaning of debris.

Ribosomes :

Small cellular particles composed of RNA + Protein

Site of protein synthesis

They contain nearly 40-60 per cent RNA and other several kinds of protein

In young actively dividing cell they are usually free in the cytoplasm but in the mature cells, they are attached with ER.

The size or weight of the ribosomes molecules is expressed in S units (sedimentation rate or coefficient)

Mainly three kinds –

(1) Mitochondrion – 70 S (2) Chloroplastic – 70 S (3) Cytoplasmic – 80 S

Functions :

1. To carry out protein synthesis with the help of m-RNA

Mitochondria :

Mitochondria are the rod like cytoplasmic organelle, which is the main site of cellular respiration

They are the source of energy and known as the power house of the cell

Their average number is vary from 200 to 800 per cell
Functions:
1. It involved in respiration, oxidation and metabolism of energy (Power house of the cell)
2. They contain circular DNA and ribosomes, so they are capable of synthesis of certain proteins
3. They contain DNA, so also contribute to heredity by the way of cytoplasmic inheritance
Nucleus and its structure:
First discovered by Robert Brown in 1833
Nucleus contains chromosomes and genes, so it known as controlling center of cell
Generally single nucleus per cell
Multi nucleus per cell – protozoa and some fungi (repeated division of nucleus without cytoplasmic division)
They are spherical or oval shaped
Large in size in active cell than in resting cells
Store house of all genetic information
It consist four parts
1. Nuclear membrane
2. Nucleoplasm
3. Nuclear reticulum
4. Nucleous

Nuclear membrane :
1. Nucleus is enclosed by two membranes of lipo proteins, which separate nucleus and cytoplasm
2. They are not continuous but having several nuclear pores in between
3. Having space between two membranes, which is known as peri nuclear space
4. Outer membrane is attached with ER on which ribosomes are attached
Functions:
It protects the chromosomes from cytoplasmic effects
It permits transport of electrons and exchange of materials between nucleus and cytoplasm
It gives rise to some cell organelles
Nucleolus
A spherical body found in the nucleus is called nucleolus
It is found in the higher organisms and is attached with specific region of a particular chromosome.
It disappears during prophase of mitosis and meiosis and reappears during telophase.
Chemically it is composed of ribosomal proteins and RNA
Functions:
1. Formation of ribosome and synthesis of proteins
2. It provide energy for all nuclear activities

Exercise:
Draw a neat diagram on Cell Structure and Label the different cell organelles and their functions:

Exercise 3 **Date: _____**

STUDY OF FIXATIVES AND STAINS

Fixatives:

A large number of chemicals such as ethyl alcohol, formalin, acetic acid, chloroform, mercuric chloride, chromic acid, picric acid, osmic acid, etc. are used singly or in combinations as fixatives for anatomical studies. Amongst these, formalin and formalin-aceto-alcohol (FAA) are most commonly used in Anatomy.

Formalin:

A 5% to 10% aqueous solution of commercial formalin (35% to 40% concentration) is used for fixation. For making a 5% solution, 5 ml of comm. formalin is taken and dist. water is added to make the volume 100 ml (Plant Micro-technique by Johansen).

Formalin, as a rule, penetrates slowly and is one of the best hardening agents. It is a powerful reducing agent. It does not precipitate proteins or render them insoluble in water. Formalin neither destroys not preserves fats but, more or less, preserves phospholipids.

Formalin-Aceto-Alcohol:

This fixative, commonly called FAA, is the standard fixative for plant tissues meant for anatomical studies.

A number of variations have been proposed, but the standard proportions are:

70% (or 50%) ethyl alcohol — 90 ml

Glacial acetic acid — 5 ml

Formalin (40% formaldehyde i.e. comm. formalin) — 5 ml

or,

70% ethyl alcohol — 85 ml

Glacial acetic acid — 5 ml

Formalin — 10ml

The lower percentage of alcohol is more suitable for delicate materials, such as bryophytes. For hard and woody materials, it is better to decrease the amount of acetic acid and to increase the amount of formalin, i.e. use the second mixture, since formalin penetrates more slowly. The material may be left in FAA almost indefinitely without any appreciable damage.

The minimum time of fixation is 18 hours. Materials to be sectioned are removed with forceps and washed in running water 1/2 to 1 hour and then handled freely. This is a corrosive liquid, and if it comes in contact with the skin it should be washed-off immediately.

Stains:

A large number of natural and synthetic dyes are used as anatomical stains, such as hematoxylin, brazilin, hematein, Bismarck brown, eosin, fast green, gentian violet, light green, safranin, Sudan IV, etc..

Hematoxylin:

Hematoxylin is a chromogen obtained from the heartwood of the plant Hematoxylin campechianum. The dye solution itself has no affinity for tissues, unless iron or aluminium is present as a mordant. The colour effect of hematoxylin depends upon the pH of the solvent and after-treatment of the tissue.

In acidic medium the colour is red and in alkaline medium it is blue. Hematoxylin stain can be prepared according to various schedules, such as Heidenhain's iron hematoxylin, Delafied's hematoxylin, Ehrlieh 's hematoxylin, etc.

Heidenhain's Iron Hematoxylin:

0.5% solution of hematoxylin in distilled water is used. Practically, a 10% solution in absolute ethyl alcohol is prepared and then diluted with distilled water to 0.5% when required. The solution is allowed to stand for a few days to ripen (into hematein). The solution may also be ripened more quickly following the method described under Ehrlieh's hematoxylin. On ripening, the solution attains a rich wine-red colour. A more stable solution can be made by adding 5 ml of 10% absolute ethyl alcohol solution to 100 ml methyl cello solve, 50 ml distilled water and 50 ml tap-water that contains calcium compounds in solution.

This is used in mixture with safranin for staining woody plant tissues. To 400 ml of a saturated aqueous solution of ammonium aluminium sulphate add drop by drop a solution of 4 gm. of hematoxylin crystals in 25 ml of 95% ethyl alcohol. Expose this to light and air for 4 days.

Then add 10 ml G.P. glycerin and 100 ml methyl alcohol. Allow this solution to stand for 2 months, exposed to air, till the colour turns sufficiently dark. Alternatively, expose the solution to a quartz mercury lamp for 2 hours as described above.

Bismarck Brown Y:

This is a synthetic dye. It is obtained from coal-tar and belongs to the azo group. Bismarck brown is a basic dye. Its solubility is 1.36% in water and 1.08% in alcohol. A 1% solution in 70% ethyl alcohol is used. Cellulose and mucin walls are stained bright brown. The stain is permanent and it rarely over-stains. It is generally used for relatively soft plant tissues without lignin, such as bryophytes.

Eosin:

Eosin is available both in bluish and yellowish form. Yellowish eosin is more frequently used. It is a coal-tar dye, acidic and is a fluorine derivative. Solubility in water is 44.2% and in alcohol 2.18%. It is a valuable cytoplasmic stain, although not much used by botanists. Usually a 1% aqueous solution is used.

Light Green:

It is a coal-tar dye, acidic and belongs to diamino-triphenyl methane group. Solubility is 20.35% in water and 0.82% in alcohol. It is a very good stain for cytoplasm and cellulose walls. Staining occurs very rapidly. Irrespective of the solvent, a 0.2% to 0.5% solution is used.

Usually the dye is dissolved in 90% or 95% alcohol. Sometimes it is dissolved in absolute alcohol and diluted with clove oil. By combining light green with alcoholic Sudan IV, cutinized and suberized tissues can be differentiated from lignified tissues.

Safranin O:

It is a coal-tar dye, basic in reaction and belongs to the azin group. Its solubility is 5.45% in water and 2.41% in alcohol. It is a very important stain for botanists. It stains lignified, suberized, cutinized and chitinized structures as also nucleoli, chromosomes and centrosomes.

Various formulae are available for making safranin solution; but generally a 1% solution in 50% alcohol is good enough for most plant materials. A 1% aqueous solution can be used for temporary staining and for this special water solution safranin is available; because although it is soluble in both water and alcohol, it dissolves better in alcohol than in water. Other stains will be mentioned as and when required.

Precautions for Staining:

1. While staining free-hand section, take a few drops of the required reagent in a watch glass staining cube) and, after transferring the sections, always keep it covered by another watch glass.

2. Transfer the section from one reagent to another with a section lifting spatula or a scalpel. Use of brush for transferring sections is not advisable because it causes transference of the reagent too. However, a fine brush may be used, blotting it each time after use. Needles should not be used as they may prick the sections and damage them.

3. Dehydration is influenced by atmospheric humidity. Hence it often becomes difficult to completely dehydrate sections during the rainy season. Fans should be switched-off during staining in order to reduce evaporation of alcohol during staining.

4. The watch glass should be placed on a piece of white paper while taking out sections from stain. They can be easily located against a white background.

5. If sections turn whitish on being transferred to xylol or balsam from absolute alcohol, it indicates incomplete dehydration. If staining is inadequate (either excess or less), it can be re-stained. In all such cases the sections should be passed down through the alcohol grade in the opposite order as it has come up.

6. While mounting the sections in Canada balsam or any other mounting medium, the cover glass should be dipped in xylol and then placed over the section gradually with the help of a needle.

If the balsam appears to be too thick, it should be diluted first with a little xylol. Ordinarily, balsam is neutral, and has a light straw colour. If the balsam is reddish, it indicates acidity. Sections should not be mounted in acidic balsam, because it fades the stain quickly.

7. After mounting, the sections should be kept on a hot plate (37° to 45°C) for at least 24 hours. It helps to drive away air bubbles and also dries the slides.

8. Clearing:

In many plant materials, various cell inclusions often interfere with staining and makes the stained preparation difficult to study. Such sections can be cleared by transferring them from 50% alcohol to a dish of water with a fine forceps or a brush.

Then, using a needle or forceps they are placed in a watch glass containing some sodium hypo-chlorate, household bleach or para-zone and kept for not more than 5 minutes. The whole section may dissolve if left long enough. Wash the sections thoroughly in water after bleaching, don't immerse the brush in the bleach, the bristles will dissolve.

Staining Schedules:

Two main types of stains are in use:

1. Temporary Stains – whose colour fades or which gradually damages the sections, and

2. Permanent Stains – whose colour lasts for many years.

1. Temporary Stains:

a. Methylene blue:

1% aqueous solution is used. The stain is usually mixed with glycerine, 10 ml of 1% aqueous stain is added to 90 ml of 50% glycerine. Sections are directly mounted into this medium. Macerated tissues can also be stained with this mixture.

A drop of washed macerate in water is mixed with a drop of the mixture on a slide and the cover glass is put on. All cell walls turn blue, except cutinized walls which remain unstained. The intensity of the blue colour depends upon the chemical and physical nature of the cell wall. Various wall layers stain differently.

b. Chlor-zinc-iodine solution (Schult's solution):

This solution consists of — Zinc chloride 30 g, potassium iodide 5 g, iodine crystals 1 g and distilled water 140 ml. Sections are placed on the slide and 1 or 2 drops of this solution are added. This is blotted off after 2-4 minutes and a drop of 50% glycerine is added. Alternatively, 50% glycerine is added directly and sections are mounted in the mixture.

Cellulose wall turns blue, starch turns blue-black, lignin and suberin turn yellow and moderately lignified wall turns greenish-blue. This stain swells the cell walls and eventually dissolves them. So, observations must be made immediately.

c. Chlorazol black:

A saturated solution of the dye in 70% alcohol is used. Cell walls turn black or grey. It is particularly good for showing pitting.

d. Phloroglucin and conc. HCl:

Add a drop of saturated aqueous solution of phloroglucin to the section taken on a slide and then the HCl. Some prefer to use 20% HCl. Lignin turns red.

e. Sudan IV:

Stain in a saturated alcoholic solution for about 10 minutes and wash rapidly in alcohol. Mount in 50% glycerine. As fat is soluble in alcohol, keep in alcohol no more than necessary. Sudan IV is a specific stain for fat which turns orange. Lecithin, latex, wax, cutin and resins also stain. Chloroplasts are stained a dull red.

f. Iodine with potassium iodide:

An aqueous solution of 1 % iodine and 1 % potassium iodide is used. The sections are stained for a few minutes and observed directly or after mounting in 50% glycerine. An aqueous 1% iodine solution may also be used. Starch turns blue, cellulose, inulin deposits, proteins and alkaloids turn brown and pectin, cutin, callose and cork turn yellow.

g. Eosin:

An 1% aqueous solution of eosin is sometimes used. It gives general yellowish colour to the tissue.

2. Permanent Stains:

Permanent staining is always accompanied by complete dehydration and clearing followed by mounting in Canada balsam or euparal etc. (For details see Cytology section).

Depending upon the number of stains used, it is called:

(a) Single staining,

(b) Double staining, etc.

(a) Single Staining:

Single staining is done generally in case of thallophytes and bryophytes where no lignified tissue is present and the cell wall material is mostly cellulose:

I. Heidenhain's Iron Hematoxylin:

1. Transfer the sections from water to a mordant (a mordant is any substance that combines with and fines an dye-stuff in material that cannot be dyed direct) containing iron, aluminium or copper, because hematoxylin cannot stain tissues without mordanting with one of these three metals.

Ordinarily, ferric ammonium sulphate (iron alum) is used. The strength of the solution is usually 3% but varies from 2% to 4%; weaker solutions being more suitable for softer tissues such as algae. The solution should be freshly prepared before use.

A permanent mordanting mixture can be prepared by mixing 500 ml. distilled water, 5 ml. glacial acetic acid, 0.6 ml. C.P. sulphuric acid and 15 g of clear violet coloured alum crystals. The time required for mordanting should not exceed 2 hours and, with thin sections, 1 hour is sufficient.

2. Wash thoroughly in running water for 5 minutes and then rinse in distilled water to remove salts present in tap-water, which interfere with staining.

3. Stain in 0.5% aqueous Heidenhain's hematoxylin solution. As a rule, the sections are left in the stain at least as long as they were left in the mordant; but, in most cases, 24 hours is the optimum time.

4. Wash off excess stain with water.

5. Destain in 2% ferric ammonium sulphate (or, ferric chloride). The time required for de-staining varies with the nature of the material, the thickness of sections, etc. Differentiate under a microscope. When the sections appear grayish-black, de-staining is complete.

6. Wash in running water for 30 minutes to 1 hour to remove all traces of the de-staining solution, which, if left, will gradually fade the stain.

7. Dehydrate in 50%, 70% and 95% alcohol, keeping at least 5 minutes in each. Give two changes in absolute alcohol at 5 minutes' interval.

8. Keep in absolute alcohol-xylem mixture (1: 1) for 5 minutes.

9. Give two changes in xylol at 5 minutes' interval.

10. Mount in Canada balsam and dry on a hot plate.

II. Bismarck Brown Staining:

This is a suitable stain for soft and non-lignified plant tissues such as bryophytes and thallophytes.

The schedule is:

1. Take the sections from water and pass through 30% and 50% alcohol keeping 5 minutes in each. 30% alcohol may as well be omitted.

2. Stain the sections in Bismarck brown (1% solution in 70% ethanol) for 5 to 15 minutes. The duration of staining depends upon the concentration of the stain and the thickness of sections. If the stain appears to be relatively more concentrated, it may be diluted with a few drops of 70% alcohol. If the sections are rather thick, stain for a shorter period so that the stain remains light.

3. Pass through 80% (or 90%) and 95% alcohol keeping 5 minutes in each. Give two changes in absolute ethyl alcohol keeping at least 5 minutes in each.

4. Clear in xylol, preferably giving 2 changes of at least 5 minutes' duration.

5. Mount in Canada balsam, Euparol or DPX mountant. All cell walls turn brown.

III. Light Green Staining:

Single staining can also be done using light green instead of Bismarck brown. Staining is done after dehydration in 90% alcohol as light green is dissolved in 95% alcohol (1% solution in 95% alcohol). The rest of the staining schedule is the same as in Bismarck brown staining.

IV. Ehrlieh's Hematoxylin Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain in matured Ehrlieh's hematoxylin solution for 5 to 30 minutes.

3. Wash out excess stain with 50% alcohol.

4. Dehydrate, clear and mount in Canada balsam.

This is an excellent stain for algae, fungi and small bryophytes.

(b) Double Staining:

Double staining is resorted to in case of all sections having both lignified and non-lignified tissues, i.e., in case of pteridophytes, gymnosperms and angiosperms. One of the two stains is specific for lignified tissues and the other stains the non-lignified tissues, i.e., mainly cellulose. The sections with two different stains show more contrast. There are numerous double staining schedules.

Some of these, which are usually followed in the class, are discussed:

I. Safranin and Delafield's Hematoxylin Staining:

1. Freshly mix safranin (1% in 50% alcohol) and matured Delafield's hematoxylin in the proportion of 1: 4. Filter the mixture. This stock mixture can be used up to a week, but should be filtered each time before use.

2. Stain the sections in this mixture for 2 to 6 hours. Some sections need less time. If it is more convenient to stain overnight, use safranin-hematoxylin mixture in the proportion of 94: 6.

3. Transfer the sections to 50% alcohol containing 2 to 3 drops of conc. HCl. This solution removes the stain, acting first on safranin. The duration of de-staining has to be learnt by experience. Generally, sections should be removed when they still appear slightly dark or over-stained under microscope.

4. Transfer the sections to 95% alcohol and keep for 5 minutes. This is followed by 5 minutes in absolute alcohol.

5. Keep the sections in alcohol-xylol mixture (1: 1) for 5 minutes.

6. Transfer to xylol and keep for 10 minutes.

7. Mount in Canada balsam and dry on a hot plate.

Lignin turns red, cellulose turns dark blue and cellulose walls with some lignin become purple.

II. Safranin and Fast Green Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain in a 1% solution of safranin in methyl cello solve 50% alcohol for 2 to 24 hours, or even 48 hours (Prepare safranin solution by dissolving 4 g of safranin in 100 ml each of 95% alcohol and distilled water followed by 4 g of sodium acetate and 8 ml of formalin. The acetate intensifies the stain and formalin acts as a mordant.).

3. Wash off the excess stain with running water for a few moments.

4. Differentiate and dehydrate with 95% alcohol to which 0.5% picric acid has been added. Ordinarily, about 10 seconds of treatment is sufficient for differentiation.

5. Stop action of the acid by immersing the slide in 95% alcohol to which 4 to 5 drops of ammonia per 100 ml of alcohol has been added. This treatment should not be continued for more than 2 minutes, as alcohol extracts stain.

6. Dehydrate in absolute alcohol for 10 seconds.

7. Counterstain in fast green for not more than 15 seconds (Prepare a nearly saturated solution of fast green in equal parts of methyl cello solve and absolute alcohol and 75 parts clove oil.). This stain can be used repeatedly.

8. Pour the fast green stain back into dropping bottle and rinse off the excess stain with clove oil. Used clove oil may be used.

9. Clear in a mixture of 50 parts clove oil, 25 parts absolute alcohol and 25 parts xylol — for a few seconds (Actually this reagent mixture, after use, is collected in a bottle and used in step 8.).

10. Remove the clearing mixture by treating for a few seconds in xylol (Add 3 to 4 drops of absolute alcohol to this xylol to take care of any moisture that may be inadvertently brought over.).

11. Give two changes in pure xylol for at least 10 minutes' interval and then mount in Canada balsam.

The safranin appears a brilliant red in chromosomes, nuclei and ii} lignified and cutinized cell walls; while the fast green gives a bright green colour to cellulose cell walls and cytoplasm. Both the colours are permanent and they persist for many years.

III. Safranin and Light Green Staining:

1. Keep the sections in 30% alcohol for 5 minutes.

2. Stain the sections in 1% solution of safranin in 50% alcohol for 30 minutes. The exact duration of staining depends upon the intensity of the dye solution. Some anatomists (Johansen etc.) prefer to make a 1% solution of the dye in 95% alcohol. A part of this stock solution is diluted with an equal volume of distilled water when needed for use. If this proves to be a strong solution, it may be further diluted with 50% alcohol.

3. Dehydrate in 70%, 80% and 90% alcohol, keeping 5 minutes in each.

4. Counterstain in 1% light green in 95% alcohol, for 15 seconds to 1 minute. The exact duration of staining is to be determined by experience. It depends on the intensity of the dye solution. If required, the staining solution may be further diluted by adding more 95% alcohol.

5. Dehydrate in absolute alcohol for 5 minutes.

6. Pass through alcohol xylol (1: 1) mixture keeping the sections in it for 5 minutes.

7. Clear in xylol for at least 10 minutes.

8. Mount in Canada balsam and dry on a hot plate.

Lignified walls turn red and cellulose walls green. Often the entire section turns green indicating over-staining in light green and under-staining in safranin. In such cases, the duration of safranin staining should be increased, while reducing the duration of light green staining.

The light green solution may also be further diluted. Sometimes, both the stains appear to be rather fade. To counteract this, the duration of treatment in different alcohol grades should be reduced. Although both the stains are permanent, light green appears to fade rather quickly.

Exercise:

Prepare the following Fixatives and Stains

Identify Fixatives and Stains

Exercise no. 4 **Date: _____**

PREPARATION OF SLIDES SHOWING VARIOUS STAGES OF MITOSIS AND MEIOSIS.

Objective:

Study of stages of Mitosis and meiosis using permanent slides

Somatic growth in plants and animals takes place by the increase in the number of cells. A cell divides mitotically to form two daughter cells wherein the number of chromosomes remains the same (i.e., unchanged) as in the mother cell. In plants, such divisions rapidly take place in meristematic tissues of root and shoot apices, where the stages of mitosis can be easily observed. In animals, mitotically dividing cells can be easily viewed in the bone marrow tissue of a vertebrate, epithelial cells from gills in fishes and the tail of growing tadpole larvae of frog.

Meiosis is a type of cell division in which the number of chromosomes is halved (from diploid to haploid) in the daughter cells, i.e., the gametes. The division is completed in two phases, meiosis I and meiosis II. Meiosis I is a reductional division in which the chromosomes of homologous pairs separate from each other. Meiosis II is equational division resulting in the formation of four daughter cells. Stages of meiosis can be observed in a cytological preparation of the cells of testis tubules or in the pollen mother cells of the anthers of flower buds. Requirement: Permanent slides of meiosis and compound microscope.

Requirement: Onion bulbs, wide mouth glass tubes/jar/bottle, glacial acetic acid, ethanol 2-4% acetocarmine/acetoorcein stain, N/10 HCl, spirit lamp/hot plate, slide, cover slips, blotting paper, molten wax/nail polish and compound microscope
Permanent slides of meiosis and compound microscope.

Procedure:

Exercise no. 5 Date: _____

TESTING THE VIABILITY AND GERMINATION OF POLLEN GRAINS

Pollen grains:

Pollen grains are microgametophytes and are haploid. They have male reproductive gametes in plants. The tube cell of the pollen grain forms a pollen tube and the generative cell discharges the sperm nuclei in the interior region of the pollen grain, which contains cytoplasm.

Pollen Germination:

During pollen germination, the tube cell expands into a pollen tube. The pollen tube then grows towards the flower's ovule, where it releases the sperm generated in the pollen grain for fertilization. The germinated pollen grain with its two sperm cells in the adult male microgametophyte of these plants.

Pollen viability:

Pollen viability is the capability of pollen to get mature and then fertilize and after fertilization, it's the ability to develop into seed and fruit.

Male gametophytes are pollen grains. They're made within microsporangia in anthers and discharged when the anther dehisces. Microsporogenesis is the process of producing microspores from a pollen mother cell. To form haploid microspore tetrads, each pollen mother cell divides meiotically. Pollen grains develop from microspores because each cell of sporogenous tissue can produce microspores, each microsporangium can contain thousands of pollen grains in comparison to a single embryo sac in an ovule.

Procedure:

Pollen Germination

In vitro pollen germination It can be assessed with hanging drop method. Pollen germination and pollen tube growth can be determined by placing a small drop of germination medium on a cover glass; pollen grains are speckled on that drop with a clean brush, and the cover glass are then inverted and rested on the cavity slide. Pollen are incubated under dark conditions at 25°C in a culture medium containing 5% sucrose, 5 ppm boric acid (H_3BO_3), and 1% agar for 24, 48, and 72 hours of time. For each incubation period, germination is recorded in three drops by counting three fields of microscope.

Pollen Viability

A pollen grain is considered germinated when pollen tube length is at least equal to or greater than the grain diameter. Germination percentage (%) is determined by dividing the number of germinated pollen grains per field of view by the total number of pollens per field of view. Measurements of pollen tube length (μm) are recorded directly by an ocular micrometer fitted to the eyepiece of the microscope. Mean pollen tube length is calculated as the average length of 5 pollen tubes measured from each drop. Pollen viability test Pollen viability can be estimated using two colorimetric tests, using 2, 3, 5 triphenyl tetrazolium chloride (TTC) or acetocarmine. Both methods allow the addition of colorant on pollens, followed by observation under a compound microscope. The pollen viability can be scored according to staining level (pollen with bold red colour as viable and colourless pollen as nonviable). The percentage of pollen viability can be determined as the ratio of the number of viable pollen grains to the total number of pollen grains.

Pollen viability = (Number of viable pollen grains / Total number of pollen grains) x 100

Exercise:
Attach Photograph and neat diagram of observed Pollen grain in the microscope.

Exercise No. 6 **Date: _____**

DETERMINATION OF GAMETES

Gametes Definition

Also referred to as sex cells, gametes are reproductive cells of an entity. These are haploid cells wherein each of it carries one copy of chromosome. Male gametes are known as sperms while female gametes are known as ova or eggs. The reproductive cells are an outcome of the process of meiosis.

During this type of cell division, the diploid parent cell having two copies of each chromosome experiences one round of DNA replication followed by two distinct cycles of nuclear division to generate four diploid cells. In turn, these cells go on to form the ova or the sperm.

Gametes Example

Ova and sperms are the most common gametes. These differ in size and are haploid in nature. They may experience external or internal fertilization. There are some entities, however, that produce both of these cells in the same entities. Such organisms are known as hermaphrodites. Majority of entities who reproduce sexually produce one type of gamete.

Formation of Gametes

Meiosis, a two-step process gives rise to gametes. The outcome of this process is 4 haploid daughter cells, each of which contains only one set of chromosomes. Through the process of fertilization (which can either be external or internal), they unite forming the zygote. The zygote is the future foetus which is diploid in nature containing two sets of chromosomes, each from both the parents.

Modes of Sexual Reproduction

The shape and size of gametes largely decide the mode of sexual reproduction. While some male and female gametes are almost of the same size, some others vary by a large margin. In a few species of fungi and algae, both male and female gametes are almost of the same size and are motile. When two similar gametes unite, it is isogamy. While the formation of gametes having dissimilar shapes and sizes is known as heterogamy or anisogamy. There is a particular form of anisogamy called oogamy observed in higher species of animals and plants (also in some fungi and algae). In this, the female gamete is much larger and is non-motile compared to its male counterpart. This is the reproduction observed in humans.

Gametes and Chromosomes

The gametes in humans are haploid cells which comprise 23 pairs of chromosomes. Haploid cell are cells containing one set of chromosomes. The haploid condition can refer to the chromosomal number in sperm or egg cells having a single set of chromosomes. Number of chromosomes in one set is indicated as “n” which is also referred to as haploid number. n is 23 in humans.

Gametes have half the chromosomes present in normal diploid cells of the body which are called somatic cells. It is during the process of meiosis, wherein chromosomes are reduced in a parent diploid cell by half, that haploid gametes are produced.

Exercise:
Determine the different types of gametes:

1. AABbCc genotype forms how many types of gametes.

2. How many types of gametes will be produced by an individual having genotype AaBbcc?

3. How many types of gametes are expected from the organism with genotype AABBCC?

4. How many types of gametes are formed by pea plant having VVRRtt genotype?

5. How many type of gametes are found in F1 progeny of cross between AABBCC and aabbcc?

Exercise No. 7 **Date:_____**

Objective: Solving the problems on monohybrid, dihybrid and test cross ratio using chi-square test, Gene interaction

The Chi-Square Test:

Chi-squared tests are a statistical measure that are used to determine whether the difference between an observed and expected frequency distribution is statistically significant

If observed frequencies do **not** conform to those expected for an unlinked dihybrid cross, this suggests that either:

1. Genes are linked and hence not independently assorted
2. The inheritance of the traits are not random, but are potentially being affected by natural selection.

A chi-squared test can be applied to data generated from a dihybrid cross to determine if there is a statistical correlation between observed and expected frequencies

A chi-squared test can be completed by following five simple steps:

1. Identify hypotheses (null versus alternative)
2. Construct a table of frequencies (observed versus expected)
3. Apply the chi-squared formula
4. Determine the degree of freedom (df)
5. Identify the p value (should be <0.05)

An important question to answer in any genetic experiment is how can we decide if our data fits any of the Mendelian ratios we have discussed. A statistical test that can test out ratios is the Chi-Square or **Goodness of Fit test**.

Chi-Square Formula

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

Degrees of freedom (*df*) = n-1 where n is the number of classes

Let's test the following data to determine if it fits a 9:3:3:1 ratio.

Example No. 1

The trait for smooth peas (R) is dominant over wrinkled peas (r) and yellow pea colour (Y) is dominant to green (y)

A dihybrid cross between two heterozygous pea plants is performed (RrYy × RrYy)

The following phenotypic frequencies are observed:

- 701 smooth yellow peas ; 204 smooth green peas ; 243 wrinkled yellow peas ; 68 wrinkled green peas

Step 1: Identify hypotheses

A chi-squared test seeks to distinguish between two distinct possibilities and hence requires two contrasting hypotheses:

- Null hypothesis (**H_0**): There is **no** significant difference between observed and expected frequencies (i.e. genes are unlinked)
- Alternative hypothesis (**H_1**): There **is** a significant difference between observed and expected frequencies (i.e. genes are linked)

Step 2: Construct a table of frequencies

A table must be constructed that compares observed and expected frequencies for each possible phenotype

- Expected frequencies are calculated by first determining the expected ratios and then multiplying against the observed total

	Smooth yellow	Smooth green	Wrinkled yellow	Wrinkled green	Total
Observed (*O*)	701	204	243	68	1216
Expected (*E*)	684 *1216 × (9/16)*	228 *1216 × (3/16)*	228 *1216 × (3/16)*	76 *1216 × (1/16)*	1216

Step 3: Apply the chi-squared formula

The formula used to calculate a statistical value for the chi-squared test is as follows:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

Where: $\sum$ = Sum ; O = Observed frequency ; E = Expected frequency

These calculations can be broken down for each phenotype and added to the table to make the final summation easier

	Smooth yellow	Smooth green	Wrinkled yellow	Wrinkled green
Observed (*O*)	701	204	243	68
Expected (*E*)	684	228	228	76
$(O - E)$	17	−24	15	−8
$\frac{(O-E)^2}{E}$	0.42	2.53	0.99	0.84

Based on these results the statistical value calculated by the chi-squared test is as follows:

- ^{2} = (0.42 + 2.53 + 0.99 + 0.84) = **4.76**

Step 4: Determine the degree of freedom (df)

In order to determine if the chi-squared value is statistically significant a degree of freedom must first be identified

- The degree of freedom is a mathematical restriction that designates what range of values fall within each significance level

The degree of freedom is calculated from the table of frequencies according to the following formula:

df = (m – 1) (n – 1)

Where: m = number of rows ; n = number of columns

For all dihybrid crosses, the degree of freedom should be: (number of phenotypes – 1)

- In this particular instance, the degree of freedom is **3**

Step 5: Identify the p value

The final step is to apply the value generated to a chi-squared distribution table to determine if results are statistically significant

- A value is considered significant if there is less than a 5% probability ($p < 0.05$) the results are attributable to chance

df	p values for Chi-Square (χ^2) distribution							
	0.90	**0.75**	**0.50**	**0.25**	**0.10**	**0.05**	**0.025**	**0.01**
3	0.584	1.212	2.366	4.110	6.251	7.815	9.348	11.345

statistically significant

When df = 3, a value of greater than 7.815 is required for results to be considered statistically significant ($p < 0.05$)

- A value of 4.76 lies between p values of 0.25 and 0.1, meaning there is a 10 – 25% probability results are caused by chance
- Hence, the difference between observed and expected frequencies are **not** statistically significant

As results are not statistically significant, the alternative hypothesis is rejected and the null hypothesis accepted:

- Null hypothesis (**H_0**): There is **no** significant difference between observed and expected frequencies (genes **are** unlinked)

Exercise on problems:

1. In corn, purple kernels (D) are dominant over yellow (d), and smooth kernels (G) are dominant over shrunken (g). An ear of corn has 381 kernels, illustrated at right: A: purple, smooth = 216 B: purple, shrunken = 79 C: yellow, smooth = 65 D: yellow, shrunken = 21 Does the data fit your predicted phenotypic ratio? (Your prediction would be the kernels are the result of a double heterozygous cross with a ratio of 9:3:3:1)

2. Color blindness is a sex-linked trait in Bombats. A female who is a carrier of the color blind allele mates with a male who is color blind. The phenotypes of their offspring are: Normal female = 132 Color blind female = 124 Normal male = 126 Color blind male = 136 Does the data fit your predicted phenotypic ratio?

3. In cats, fur color is determined by the codominant, sex-linked alleles: black (B) and orange (O). A calico female (XBXO) is bred (many times) with a black male (XBY). They produce the following offspring: Black female = 78 Calico female = 65 Black male = 81 Orange male = 45 Does the data fit your predicted phenotypic ratio?

Exercise No. 8 **Date: _____**

ESTIMATION OF LINKAGES USING THREE POINT TEST CROSS FROM F_2 DATA AND CONSTRUCTION OF LINKAGE MAP

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

These eight classes are identified in two different ways, viz:

(1) By phenotypic frequencies, and

(2) By alteration of gene sequence in the genotype as a result of single crossing over or double crossing over between three linked genes.

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

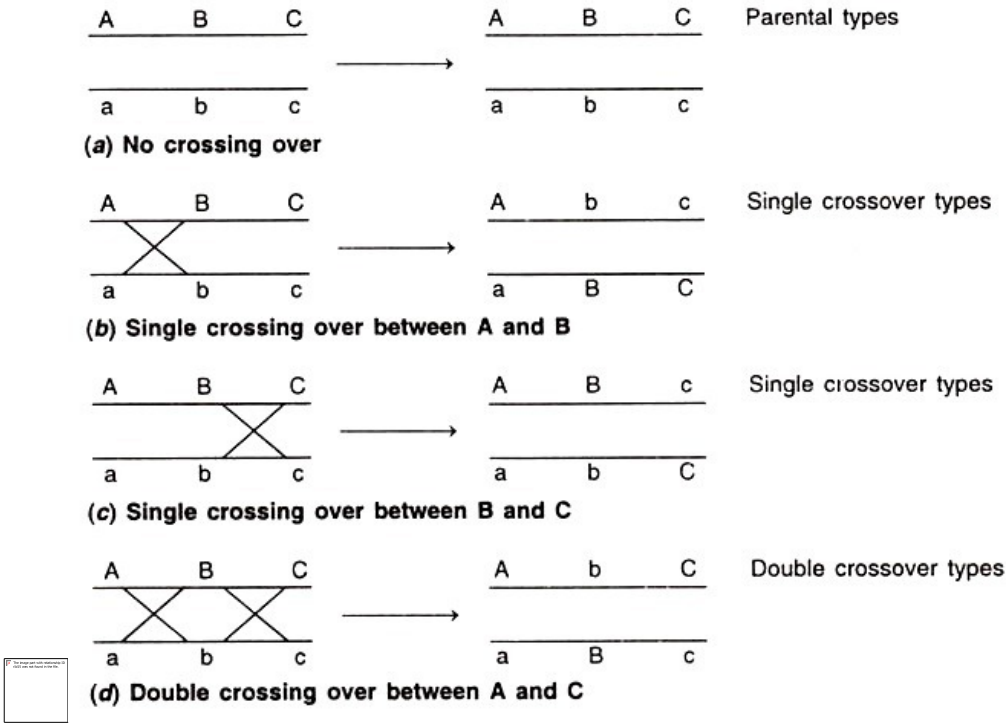


Figure: Single and Double crossing over between three linked genes.

1. Single crossover between A and B will alter the position of two genes, viz., B and C (Fig.).

2. Single crossover between B and C will alter the position of only one gene, i.e., C (Fig.).

3. Double crossover between A and C will alter the position of only middle gene, i.e., B (Fig.).

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).

TABLE 9.3. Summary of the results obtained from a three point test cross between *ABC/abc* × *abc/abc*

Genotypic classes	*Phenotypic classes*	*Assumed frequencies*	*Remarks*
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. Calculation of Three Point Test Cross:

The recombination percentage or unit distance between genes is worked out by calculating the crossing over percentage between different genes. Suppose number of crossover progeny between genes A and B is P, between genes B and C is Q, between genes A and C is R, and total progeny is T. Then,

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

1. Between genes A and B = P + R/T x 100 = 230 + 9/1200 x 100 = 19.92

P = 114 + 116 = 230

R = 5 + 4 = 9

2. Between genes B and C = Q + R/T x 100 = 252 + 9/1200x 100 = 21.75

Q = 128 + 124 = 252

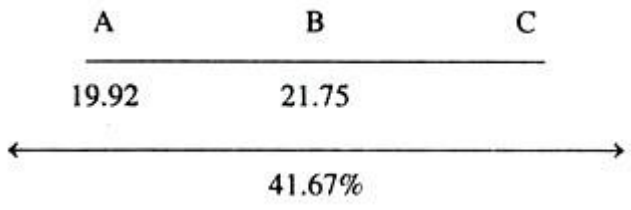
R = 5 + 4 = 9

3. Between genes A and C = P + Q/T x 100 = 230 + 253/1200 x 100 = 40.30

4. Gene Sequence of Three Point Test Cross:

The gene sequence is determined with the help of crossing over percentage between two genes. Greater the recombination percentage between two genes, more is the distance between them and vice versa. In this case, the maximum crossing over % is between gene A and C (40.3%).

This indicates that B is located between A and C as given below:



5. Coefficient of Coincidence in Three point Test Cross:

Coefficient of coincidence = Observed double crossovers/Expected double crossovers x 100

1. Observed double crossovers = 9/1200 x 100 = 0.75%

2. Expected double crossovers = Product of two single recombination values = 19.92 x21.75/100 = 4.33 %

Coefficient of coincidence = 0.75/4.33 x 100 = 17.32%

Coefficient of interference = 1 – 0.1732 = 0.8268 or 82.68%.

Exercise:

1. Three corn seed trait loci again and use data from one cross to map these three loci. The first step would be to obtain a trihybrid individual that is heterozygous at all three loci and then perform a testcross with this trihybrid.

Test cross offspring

Seed trait	**Gamete from trihybrid**	**Number**
Red, shrunken, normal	CsW	2777
White, plump, waxy	cSw	2708
Red, plump, waxy	CSw	116
White, shrunken, normal	csW	123
Red, shrunken, waxy	Csw	643
White, plump, normal	cSW	626
Red, plump, normal	CSW	4
White, shrunken, waxy	csw	3
Total number of progeny:		**7000**

Contruct linkage map.

2. The F_2 progeny flies from the inter-cross comprised eight phenotypically distinct classes, two of them are parental and six recombinant.

Class	**Phenotypes**	**Characters**	**Genotypes**	**Counts**
1.	Scute, echinus, crossveinless	Parental	Sc, ec, cv	1158
2.	Wildtype (non scute, non echinus, crossvein)	Parental	Sc+, ec+, cv+	1455
3.	scute	Recombinant	Sc, ec+, cv+	163
4.	Echinus, crossveinless	Recombinant	Sc+, ec, cv+	130
5.	Scute, echinus	Recombinant	Sc, ec, cv+	192

6.	Crossveinless	Recombinant	Sc+, ec+, cv	148
7.	Scute, crossveinless	Recombinant	Sc, ec+, cv	1
8.	echinus	Recombinant	Sc+, ec, cv+	1
Total				**3248**

Construct Linkage map.

Exercise no. 9 **Date:_____**

STUDY ON GENETICS WITH PEAS

Grow out test on Two varieties of peas and record their seven characters.

Procedure:

Click photos of these contrasting characters and find out the dominant and recessive characters.

Exercise:

Trait	Dominant Expression	Recessive Expression
Form of ripe seed (R)		
Color of seed albumen (Y)		
Color of flower (P)		
Form of ripe pods (I)		
Color of unripe pods (G)		
Position of flowers (A)		
Length of stem (T)		