Experiment 1

Aim of the Experiment: Application of probability in the law of segregation and fertilization with coin tossing.

Background Information: Genetics is the study of the passing of traits from parents to offspring. Each trait is associated with certain genes we get from our parents. Genes come in two forms, ***dominant*** and ***recessive***. The **dominant** of the two genes masks or "covers up" the weaker recessive gene. These genes determine what the offspring looks like and even how it behaves. In heredity, we are concerned with the occurrence, every time an egg is fertilized, of the probability that a particular gene or chromosome will be passed on through the egg, or through the sperm, to the offspring. As you know, genes and chromosomes are present in pairs in each individual, and segregate as they go into the gametes (egg and sperm). There are two possible genes that the egg or sperm might obtain from each pair, but it actually receives only one of them. If the probability of getting either one is equal, this probability can be expressed as 1/2, like the probability of getting heads or tails when you flip a penny. But one cannot examine the genes in a sperm or egg. One must wait until fertilization has occurred and a new individual has been produced, and some characteristic controlled by the genes has had time to develop. Thus we are faced with the probability that it will go in to the sperm, together with the probability that these will combine at fertilization.

Materials Required: Two coins, the heads side of the coin will represent the DOMINANT trait **(T)** and the tails side of the coin will represent the RECESSIVE trait **(t)**.

Part I: Probability and Mendel's Law of Segregation

Now we will simulate the process of meiosis and its effects by using a coin to represent an individuals genotype. Heads on the coin will represent the dominant allele (T) and tails will represent the recessive allele (t). Flipping the penny will simulate the segregation of alleles into gametes. For a heterozygous individual, the probability of producing gametes with the dominant allele (T) is equal to the probability of flipping heads on a penny and the probability of producing gametes with the recessive allele (t) is equal to the probability of flipping tails on a penny. Flip the penny and tally the results in the table below. Count the tallies and record this number in the last two columns of the table.

	Tally		Total	
	T allele (heads)	t allele (tails)	T allele (heads)	t allele (tails)
50 tosses				

Part II: Probability and Fertilization

Flipping a single coin and the sorting of individual alleles for one trait into gametes during meiosis are both examples of independent events. Predicting the outcome of these events is pretty straightforward. But, how does probability operate with two independent events occurring simultaneously, such as two coins being flipped at once? Or, in a genetics related example, what are the chances of the same allele (T or t) ending up in the egg and sperm produced by two heterozygous parents? Geneticists use Punnett squares to predict the chances of this happening based on the parents genotypes. Complete the Punnett Square to predict the possible genotypes of a zygote resulting from fertilization between two gametes from heterozygous parents. (Tt x Tt)

	T	t
T		
t		

Now you will determine the ratios by using pennies to represent the crosses. You will need two pennies. The "heads" side of the penny represents the dominant allele (T) and "tails" represents the recessive allele (t). This entire penny represents a parent that has the genotype Tt. A second penny represents the other parent. One partner is going to play the role of female parent, the other will play the role of male parent. When the coin is flipped, you are determining what allele the sperm or egg is receiving during meiosis. When you put the alleles from the two flipped coins together, you are simulating fertilization.

To determine actual probabilities, flip your coins 100 times, recording in the table below how often each combination came up. (Use tally marks to record your data then summarize as a number)

Gene Combination	Tally Marks	Total
Homozygous Dominant (2 heads)		
Heterozygous (1 head, 1 tail)		
Homozygous Recessive (2 tails)		

Comparing Actual and Predicted Results		
	Predicted % Probability (from Punnett square)	Actual Result (from flipping pennies)
Homozygous Dominant		
Heterozygous		
Homozygous Recessive		

Chance of TT zygote: _____ in 4 or _____ %

Chance of Tt zygote: _____ in 4 or _____ %

Chance of tt zygote: _____ in 4 or _____ %

Comparing the results from the Punnett square and the coin flipping should reveal similar results. This is because Punnett squares apply two rules of probability to accurately make predictions about the probability of certain genotypes resulting from parents of known genotypes. The first rule is the rule of multiplication and the second is the rule of addition.

Experiment 2

Aim: Frequency of the following genetic traits in human: widow‟s peak, attached ear lobe, dimple in chin, hypertrichosis, colour blindness, PTC tasting

Introduction

The chromosomal theory of inheritance outlines how the movement and thereby transmission of chromosomes from parent to child, results in the patterns of inheritance described by Gregor Mendel. While the law of segregation stipulates the diploid set of each parent genome will separate into the haploid gametes, segregation does not ensure the chromosome will be identically inherited.

Having a complete set of genetic information is critical to organismal health and function. A regular human cell has 46 chromosomes, including 44 autosomes, which come in pairs, and two sex chromosomes, which specify gender (XX for female and XY for male).

The pairs of autosomes are called homologous chromosomes. One of each pair came from mom, and the other came from dad. Homologous chromosomes have all the same genes (arranged in the same order), but often exhibit slight differences in the DNA sequence of the genes.

Crossover is a unique meiotic event that occurs between homologous chromosomes aligned at the metaphase plate in meiosis I. Here, the diploid set of parental chromosomes may transpose homologous (regions coding for the same traits) sections of a given chromosome, contributing a new variation to the gene pool Crossover, which increases genetic diversity is considered beneficial.

In contrast, a single chromatid can be subject to anomalies, such as duplications, translocations, inversions, and deletions. These variations can be the result of incorrect distribution into gametes (the egg or sperm cells) during meiosis. The failure to segregate correctly is called nondisjunction. A gamete with too many or too few chromosomes, upon fertilization, may produce a zygote with too many (trisomy = three copies) or too few (monosomy = one copy) chromosomes. Most autosomal (non-sex chromosome) trisomies and monosomies are lethal. A few trisomies will result in viable offspring. However, these individuals exhibit severe genetic disorders.

Even within a chromosome, alternate versions of a trait may be coded for by the genetic content. Each trait is coded by the two distinct forms (alleles), one from each parent. Genetic conditions caused by a mutation in a single gene follow predictable patterns of inheritance within families. Inheritance patterns are described, depending on the type affected chromosome, and the relationship between genotype (genetic composition) and phenotype (physical expression).

Autosomal dominant conditions are expressed in individuals who have at least one copy of the mutant allele. Affected individuals, regardless of gender, have an equal probability of passing on the trait to their offspring. Affected individuals may retain one normal copy of the gene, providing each offspring a 50% chance of inheriting the normal allele.

Autosomal recessive conditions only manifest when an individual has two copies of the mutant allele. If an individual is heterozygous (contains one normal and one altered allele), they will be unaffected, but a carrier. Both genders are equally affected, and should two carriers mate; there's a 25% chance of the child being a homozygous mutant (affected) and a 50% chance of being a carrier.

Like the autosomal recessive trait, X-linked recessive traits do not typically manifest if there is a normal copy of the gene. However, because males have only one X-chromosome, any altered allele will be exhibited. Women, on the other hand, are rarely affected as they must possess two copies of the mutant allele. Because the gene is on the X-chromosome, there is no father to son transmission, but all daughters will become carriers. Both genders have a 50% chance of inherited the altered allele from the mother.

X-linked dominant transmission can result from an affected woman passing on the mutant allele (50% possibility if she is heterozygous) to her children or a father to his daughters (100% certainty). A single mutant allele will result in clinical manifestation.

Procedure:

Determine your phenotype and possible genotypes for each of the following characteristics.

1. Tongue Rolling: Attempt to roll your tongue into a “U” shape. Tongue rollers carry the dominant allele for the gene “R”.

2. Widow's Peak: a dominant allele of the gene "W" causes the hairline to form a distinct point in the center of the forehead. Baldness will mask the expression of this gene.

3. Earlobe attachment: The inheritance of the dominant allele of the gene "E" results in the free, or unattached earlobe. Other genes affect the size and shape of the earlobe.

4. PTC Tasting: Phenylthiocarbamide (PTC) is a harmless chemical in small quantities. To individuals with the dominant allele of the gene "T", it tastes undesirably bitter.

5. Hitchhiker's Thumb: Some individuals can bend the last joint of their thumb backward at about a 45-degree angle. These individuals are homozygous recessive for the gene "H". Note there is considerable variation in this trait.

6. Bent Little Finger: The dominant alleles of the gene "B" causes the terminal bone of the little finger to angle towards the fourth (ring) finger. Check for the characteristic by laying your hands flat on the table and relaxing them.

7. Mid-Digital Hair: The presence of hair on the middle segment of the fingers is caused by the dominant allele of the gene "M".

8. Facial Dimples: the inheritance of cheek dimples is controlled by the dominant alleles of the gene "D".

9. Hallux length: The length of the big toe (Hallux) is governed by the gene "Ha", individuals whose hallux is shorter in comparison to the second toe possess the dominant allele.

10. Index finger length: Relax your hand on top of the table. Compare the length of your second finger (index finger) to that of your fourth (ring finger). The allele for the short index finger "S^S" is sex influenced in its expression (like baldness) and is dominant in males and recessive in females.

The allele for a long second finger is "S^L". See the table below to determine your possible genotypes:

Gender	**Phenotypes and Possible Genotypes**	
	Short Second Finger	**Long Second Finger**

Male	S^SS^S or S^SS^L	S^LS^L
Female	S^SS^S	S^LS^L or S^SS^L

11. Write your name and identify your Dominant or Recessive form for the above-mentioned traits. Compile the class data and calculate the frequency of a given trait.

Trait	**Phenotype**	**Genotype**	**Class Occurrence**	**Frequency of Expression**
Tongue Rolling				
Widow's Peak				
Attached Earlobe				
PTC Tasting				
Hitchhiker's Thumb				
Bent Little finger				
Mid-digital Hair				
Facial Dimples				
Hallux Length				
Index Finger Length				

Experiment 3

Aim: **Study of mode of inheritance of the genetic traits in humans by pedigree charts**

Background:

A pedigree chart is a flowchart or a diagrammatic representation prepared to exhibit the occurrence and appearance or phenotypes of a particular gene or organism along with its ancestors from one generation to the next generation.

In the pedigree chart, males are represented by a square and a circle represents the females.

Requirements

Information about traits in a family for more than one generation.

Procedure

1. Select a family with anyone of the monogenic traits like rolling of tongue, blood groups, ear lobes, widow's peak and colour blindness.
2. Ask the person exhibiting the trait as to who in his/ her family has the trait in question.
3. Prepare a pedigree chart on the basis of the information collected, using appropriate symbols.
4. Examine the pedigree chart carefully to find out whether the disease is autosomal recessive, autosomal dominant, X-linked dominant or recessive and Y-linked dominant or recessive.

Explanation

Autosomal Dominant Trait-

Blood Groups, Free hanging earlobes, Widow's Peak, Rolling of tongue.

The encoding gene for these genes is present on any of the autosomes. In these traits, the mutant allele is dominant.

Such type of traits exhibit the following features:

1. The traits get transmitted from the parents to either gender.
2. It affects males and females equally.
3. The trait is present in each of the generations, i.e., the pedigree is vertical.

4. Some common traits of this type include blood groups, polydactyly, brachydactyly, the dimple in cheeks, etc.

Autosomal Recessive Trait

The mutant allele of such traits is recessive. Salient features of such type of traits include:

1. It is found equally in multiple male and female siblings whose parents are carriers.
2. Homozygous siblings for defective alleles, but parents are heterozygous.
3. If men and women who are genetically related are married to each other, they might exhibit this trait.

X-Linked Dominant Traits

The encoding gene for such traits is located on the X chromosome. The mutant allele is dominant in this trait.

The features of such type of traits are:

1. Inheritance is vertical and is found in all the generations.
2. If the female is affected, half of her sons are also affected.
3. If the male is affected, all the daughters will be affected but no sons will be affected, i.e., there is no male-to-male transmission.

X-Linked Recessive Traits- Colour Blindness

In such type of traits, the mutant allele is recessive to the wild type allele. The features of X-linked recessive traits include:

1. This is expressed only by homozygous females but homozygous and hemizygous males.
2. If the female is the carrier, about half the sons are affected. If the female is homozygous, 50% of the daughters and 100% of the sons can be affected. That is why the male population is the most affected.

Y-chromosome Linked Traits

The gene for such traits is present on the Y-chromosome. Any trait linked to Y-chromosome is found only in males and not in females because the Y-chromosome is present only in males. All the sons of the affected male exhibit the trait, whereas, none of the daughters exhibits the trait.

Experiment 4

Aim – To study the methods of handling and making cultures of *Drosophila* and identifying its genders and mutations.

Background - *Drosophila* is a genus of small flies, commonly known as fruit flies, belonging to the family Drosophilidae. These flies are often used as model organisms in scientific research, particularly in the fields of genetics, developmental biology, and evolutionary biology. The species *Drosophila melanogaster*, in particular, is widely used in genetic studies.

Drosophila melanogaster has a relatively short life cycle, allowing researchers to study multiple generations in a relatively short period. They are easy to rear in the laboratory, have a well-understood and sequenced genome, and share many genetic similarities with higher organisms, including humans. As a result, *Drosophila* has played a crucial role in advancing our understanding of genetics, development, and various biological processes.

Methods for handling - Handling *Drosophila* in a laboratory setting involves a combination of specific techniques and tools to maintain and manipulate the flies for various experiments. Here are some common handling techniques for *Drosophila*:

1. Fly Stocks and Media:

   Fly Stocks: Maintain different strains of flies, each with specific genetic characteristics. Keep track of the genotypes and use them for experiments.

   Media: Provide a suitable medium for fly culture. Standard fly food typically includes ingredients like agar, cornmeal, molasses, and yeast.

2. Setting up Flies:

   Fly Vials or Bottles: Place flies in vials or bottles with the appropriate food medium. These containers often have cotton plugs to allow for air exchange.

   Density Control: Avoid overcrowding to prevent competition for resources and maintain a consistent environment.

3. Fly Transfers:

Anesthetization: Anesthetize flies using carbon dioxide (CO_2) or fly anesthetics. CO_2 is commonly used by placing the vials in a CO_2 chamber.

Transfer Tools: Use brushes, aspirators, or other tools to transfer flies between vials without causing harm.

4. Crosses and Mating:

Mating Chambers: Set up mating chambers to facilitate controlled crosses between different fly strains.

Virgin Collection: Collect virgin females or males by separating them shortly after eclosion (emergence).

5. Aging and Sorting:

Fly Sorting: Sort flies based on age or sex using specific sorting tools.

Aging: Age flies for specific experiments by transferring them to new vials at regular intervals.

6. Genetic Markers:

Genetic Marking Techniques: Use genetic markers, such as visible mutations or fluorescent proteins, to identify specific genotypes.

7. Microinjection (for some advanced studies):

Microinjection Tools: Perform microinjections for genetic manipulation. This is more advanced and is not commonly used for routine handling.

8. Recording and Data Collection:

Lab Notebooks: Keep detailed records of the fly stocks, experimental conditions, and observations.

Data Collection: Record data on traits, behaviors, or other characteristics being studied.

It's important to maintain a clean and organized laboratory environment to prevent contamination and ensure the success of experiments. Additionally, ethical considerations and

proper care of the flies are essential aspects of *Drosophila* handling. Always follow institutional guidelines and ethical standards when working with laboratory animals, including fruit flies.

Identification of genders – Distinguishing between male and female *Drosophila* (fruit flies) is generally straightforward. Here are some common characteristics that can help you identify the sex of *Drosophila*:

1. Abdominal Shape:
   Females: The abdomen of female fruit flies has a pointed or tapered shape. This is because the female reproductive system extends towards the posterior end of the abdomen.
   Males: The abdomen of male fruit flies is more rounded and blunter compared to females.
2. Genital Structures:
   Females: The posterior end of the abdomen in females has a dark, rounded structure called the ovipositor, which is used for laying eggs.
   Males: The posterior end of the abdomen in males has a pair of dark, rounded structures called claspers, which are used during copulation.
3. Pigmentation Differences:
   Females: In some *Drosophila* species, females may have a lighter pigmentation on the abdomen, especially in the area surrounding the genitalia.
   Males: Males may have a darker pigmentation on the abdomen.
4. Size: In some species, females are slightly larger than males, although this difference may not be very pronounced.
5. Behavioral Differences:
   Females: Females may be observed laying eggs and tend to be more stationary.
   Males: Male fruit flies often exhibit courtship behaviors, including following and wing vibration, as they try to attract females.
6. External Genitalia (under a microscope):
   Females: Under a microscope, the external genitalia of females show the ovipositor, a tube-like structure.
   Males: The external genitalia of males exhibit the aedeagus, a more complex structure involved in copulation.

For routine identification in a laboratory setting, observing the shape of the abdomen and the presence of characteristic structures is usually sufficient. If needed, you can also use a light microscope for a closer examination of the external genitalia. It's worth noting that specific characteristics may vary among different *Drosophila* species, so it's essential to familiarize yourself with the features of the particular species you are working with. Additionally, experience and practice in handling and observing fruit flies will improve your ability to quickly and accurately identify their sex.

Identification of wild type and mutated flies – The identification of wild-type and mutated *Drosophila* based on morphology, especially characteristics like eyes and wings, is a common practice in genetic research. Here are some key morphological features to consider:

1. Eyes:
    i. Wild-Type Eyes: Typically, wild-type *Drosophila* have red eyes. The eyes are normally pigmented, giving them a dark red or maroon appearance.
    ii. Eye Color Mutations: Many mutations affect eye color. For example - White Eyes: Mutations in the white gene can result in white or light-colored eyes. Sepia Eyes: Mutations in the sepia gene can lead to brown eyes.
    iii. Fluorescent Markers: Some mutant strains may have fluorescent markers, resulting in eyes that emit fluorescence under specific light conditions.
    iv. Eye Size and Shape: Certain mutations may affect the size or shape of the eyes. Compare the size and morphology of eyes between wild-type and mutant flies.
2. Wings:
    i. Wild-Type Wings: Wild-type *Drosophila* typically have well-formed, normal wings. The veins and wing structure should be regular and symmetrical.
    ii. Wing Mutations: Vestigial Wings - Mutations in the vestigial gene can lead to smaller, vestigial wings. Curly Wings - Mutations in the apterous gene can result in curly or deformed wings. Wingless - Some mutations may cause complete absence of wings.
    iii. Wing Vein Patterns: Mutations can alter the pattern of wing veins. Compare the vein pattern of mutant flies to that of wild-type flies.
3. Additional Considerations:
    i. Bristle Patterns: Mutations can affect the number and arrangement of bristles on the body and wings. Compare the bristle patterns between wild-type and mutant flies.

ii. Thoracic and Abdominal Segmentation: Certain mutations may affect the segmentation of the thorax and abdomen. Observe the number and arrangement of segments.
iii. Body Color: Some mutations may influence the overall body color of *Drosophila*. Compare the body color of wild-type and mutant flies.
iv. Sexual Dimorphism: In some cases, mutations may cause differences in morphology between male and female flies. Be aware of sexual dimorphism when making comparisons.
v. Crosses and Controls: Perform controlled crosses between wild-type and mutant flies to ensure accurate comparisons. Include appropriate genetic controls in your experiments.

Always refer to available genetic information and stock records to understand the expected phenotypes of your *Drosophila* strains. It's also essential to confirm your observations with genetic analyses, such as PCR or sequencing, to ensure accurate identification of wild-type and mutant flies based on morphology. Additionally, consult with experienced researchers or genetic stock centers for guidance on specific morphological characteristics associated with known mutations in *Drosophila*.

Setting up cultures – Setting up *Drosophila* cultures in a laboratory involves creating and maintaining conditions suitable for the growth and reproduction of these fruit flies. Here's a step-by-step guide on how to set up *Drosophila* cultures:

Materials and Equipment:

1. Fly Vials or Bottles: Use vials or bottles containing a suitable medium for fly culture. Standard fly food typically includes ingredients like agar, cornmeal, molasses, and yeast.
2. Culture Medium Ingredients: Prepare or purchase the appropriate culture medium ingredients. Some labs use pre-made fly food while others prepare their own according to established recipes.
3. Yeast: Include yeast in the medium as a protein source. Autoclave or heat-treat the yeast to prevent contamination.
4. Fly Anesthetics: Obtain a source of anesthetic (such as carbon dioxide) for anesthetizing flies during transfers.
5. Fly Brushes or Aspirators: Use brushes or aspirators for transferring flies between vials.
6. Incubators: Set up incubators to maintain the desired temperature for Drosophila. The standard temperature for *Drosophila melanogaster* is around 25°C (77°F).

7. Fly Rearing Space: Allocate a dedicated space in the lab for fly rearing to avoid contamination.

Procedure:

1. Prepare Culture Medium: Prepare the fly food according to the selected recipe. Pour the medium into vials or bottles, leaving enough space at the top for easy access.
2. Autoclave or Heat-Treat Medium: Sterilize the culture medium using autoclaving or heat treatment to eliminate any potential contaminants.
3. Fill Vials or Bottles: Dispense the sterilized medium into the vials or bottles. Allow the medium to solidify if it's in an agar form.
4. Inoculate with Yeast: Add a small amount of yeast to the surface of the medium to provide a food source for the flies.
5. Allow to Cool: Allow the medium to cool and solidify if necessary before introducing the flies.
6. Introduce Flies: Transfer a small number of flies (either wild-type or mutant strains) into each vial or bottle. Use brushes or aspirators for gentle handling.
7. Anesthetize Flies: Anesthetize the flies using carbon dioxide or another suitable method before transferring them between vials, especially during the initial setup.
8. Maintain Conditions: Place the vials or bottles in incubators set to the desired temperature. Maintain a 12-hour light/dark cycle, mimicking natural conditions.
9. Monitor and Record: Regularly monitor the cultures for signs of contamination, check for fly health, and record important information in a lab notebook.
10. Subculturing: Periodically, transfer a portion of the flies to fresh vials with new food medium to prevent overcrowding and maintain a healthy population.
11. Genetic Record Keeping: 1Keep detailed records of the genetic characteristics of the fly strains, including their genotypes and any observed phenotypic traits.
12. Quality Control: Implement quality control measures to ensure the health and genetic integrity of the fly strains. Perform regular genetic checks to confirm the presence of desired mutations.

Setting up and maintaining *Drosophila* cultures requires attention to detail and adherence to sterile techniques to avoid contamination. Regular monitoring, proper record-keeping, and careful handling are essential for successful *Drosophila* culture management in the laboratory.

Experiment 5

Aim – To demonstrate the law of segregation/ sex-linked inheritance in *Drosophila*.

Theory – The law of segregation, also known as Mendel's first law, is a fundamental principle of inheritance discovered by Gregor Mendel in the 19th century. This law describes the behavior of alleles during the formation of gametes and the inheritance of traits from one generation to the next.

The law of segregation applies to the inheritance of genes located on sex chromosomes, which are the chromosomes that determine an individual's sex. In organisms with a sex chromosome system like humans (XX/XY) or Drosophila (XX/XY or ZZ/ZW), the inheritance of genes on the sex chromosomes follows the principles of the law of segregation. However, there are some unique aspects to consider in the context of sex-linked inheritance.

Sex-Linked Inheritance:

1. Alleles on Sex Chromosomes:
    - In sex-linked inheritance, genes are located on the sex chromosomes (X and Y in mammals or Z and W in some birds and insects). Typically, X-linked genes are more commonly studied due to their presence in both male and female individuals.
2. Inheritance in Mammals (X-Linked Genes):
    - Females have two X chromosomes (XX), while males have one X and one Y chromosome (XY). X-linked genes follow the law of segregation during gamete formation, and the inheritance pattern is analogous to autosomal genes.
3. Inheritance in *Drosophila* (X-Linked Genes):
    - In *Drosophila*, which has an XX/XY sex determination system, X-linked genes are present on both X chromosomes in females and on the single X chromosome in males

Method – To demonstrate the law of segregation in *Drosophila*, especially in the context of sex-linked inheritance, you can perform a cross between flies with specific traits. Let's

consider a cross between white-eyed (w) and wild-type (w+) flies with dumpy wings (dp) and wild-type (dp+).

Cross 1: Monohybrid Cross for Eye Color (White Eye - w and Wild Type - w+):

1. Parental Generation (P generation):
    - Cross white-eyed male (wY) with wild-type red-eyed female (w+X).
    - The genotype of the parental flies is:
        - Male (wY) x Female (w+X)
2. F1 Generation (First Filial Generation):
    - All F1 offspring will have red eyes because the wild-type allele (w+) is dominant over the white-eye allele (w).
    - Genotype of F1 offspring:
        - All F1 offspring will be heterozygous for eye color (w+w).

Cross 2: Test Cross for Dumpy Wings (Dumpy Wing - dp and Wild Type - dp+):

1. F1 Generation (from Cross 1):
    - Take F1 white-eyed, wild-type red-eyed flies (w+w) and cross them with dumpy-winged (dp) flies.
    - Genotype of F1 fly (female): w+w dpY
    - Genotype of the F1 fly (male): w+w dp+
2. Test Cross:
    - Cross the F1 white-eyed, wild-type red-eyed flies (w+w) with dumpy-winged (dp) flies. This is a test cross to determine the genotype of the F1 white-eyed, wild-type red-eyed flies.
    - Genotype of F1 offspring:
        - Half of the offspring will be white-eyed with dumpy wings (w dpY).
        - The other half will be white-eyed with wild-type wings (w dp+).

Interpretation:

1. Law of Segregation (Monohybrid Cross):

- The monohybrid cross for eye color demonstrates the law of segregation, where the F1 generation shows a 1:1 ratio of white-eyed to red-eyed individuals.

2. Criss-Cross Inheritance (Test Cross for Dumpy Wings):
    - The test cross for dumpy wings demonstrates criss-cross inheritance because the F1 white-eyed, wild-type red-eyed females carry the dumpy wing allele on the Y chromosome.
    - The ratio of the F2 generation reflects the criss-cross pattern:
        - 1/4 will be white-eyed with dumpy wings (w dpY),
        - 1/4 will be white-eyed with wild-type wings (w dp+),
        - 1/2 will be red-eyed with wild-type wings (w+ dp+).

This experiment illustrates both the law of segregation and the criss-cross inheritance pattern associated with sex-linked traits in *Drosophila*. The demonstration involves eye color as a monohybrid trait and wing shape as a sex-linked trait.

Experiment 6

Aim – To demonstrate lethal alleles using Curly (Cy) mutant gene in *Drosophila*.

Theory - Lethal genes are genes that, when present in certain combinations or in homozygous form, lead to the death of the organism. These genes often interfere with essential biological processes or functions, resulting in non-viability or severe developmental abnormalities. Lethal genes can be classified into a few different categories based on their effects:

1. Dominant Lethal Genes:
    - In some cases, a single copy of a dominant lethal allele can be enough to cause the death of the organism. This means that individuals carrying just one copy of the lethal allele (heterozygotes) will display the lethal phenotype.
2. Recessive Lethal Genes:
    - Recessive lethal genes cause lethality only when present in a homozygous state. Heterozygotes for a recessive lethal allele may appear normal and are viable.
3. Semi-lethal Genes:
    - Semi-lethal genes cause some individuals carrying the allele to survive to adulthood, but a portion of them will still exhibit lethality or severe defects. The lethality might be influenced by environmental factors or genetic modifiers.
4. Conditional Lethal Genes:
    - These genes cause lethality under certain conditions, such as specific environmental factors or during particular developmental stages. Under other conditions, the organism may survive.

Lethal genes are often studied in the context of genetics and inheritance patterns. Geneticists use them to understand the roles of specific genes in development and to study the interactions between different genes. Additionally, lethal genes can be manipulated in model organisms, such as fruit flies or mice, to investigate the molecular mechanisms underlying their lethality.

In *Drosophila melanogaster* (fruit flies), the Curly (Cy) mutant gene is often used as an example to demonstrate the concept of lethal alleles. Lethal alleles are variations of genes that, when present in certain combinations, result in the death of the organism. The Curly gene in fruit flies affects wing morphology, causing a curly-wing phenotype.

Understanding lethal genes is crucial for breeding programs, genetic research, and medical genetics. In some cases, lethal genes may be associated with human genetic disorders, highlighting the importance of studying them to gain insights into health and disease.

Method – The lethality associated with the Curly gene is typically observed when flies are homozygous for the mutant allele. In other words, individuals that inherit two copies of the Curly allele (CyCy) exhibit a lethal phenotype, leading to their death before reaching maturity. The heterozygous condition (Cy+Cy) results in the expression of the curly-winged phenotype without lethality. Here's an explanation of how the Curly gene can demonstrate lethality:

- Normal Wing Phenotype (Wild-Type): The wild-type (normal) wing phenotype in Drosophila is characterized by straight wings. Flies with a normal wing genotype can be represented as Cy+Cy+.
- Curly-Wing Phenotype: The Curly (Cy) mutant allele causes a curly-wing phenotype. Flies with one copy of the Curly allele (Cy+Cy) have curly wings but are viable and can survive.
- Lethal Allele Combination: Let's assume that having two copies of the Curly allele (CyCy) is lethal. Flies that are homozygous for the Curly gene experience a severe disruption in wing development or another essential process, leading to death.
- Genetic Cross: To demonstrate the lethality associated with the Curly gene, you can set up a genetic cross between flies heterozygous for the Curly gene (Cy+Cy) in the following way:

  Cross a Curly-winged fly (Cy+Cy) with a normal-winged fly (Cy+Cy+). The Punnett square for this cross might look like this:

	Cy+	**Cy**
Cy +	Cy+Cy+	Cy+Cy
Cy	Cy+Cy	CyCy (lethal)

  In this cross, 50% of the offspring inherit one copy of the Curly allele (Cy+Cy), resulting in the curly-wing phenotype. However, 25% of the offspring inherit two copies of the Curly allele (CyCy), which is lethal.

- Phenotypic Ratios: The phenotypic ratio of the offspring would be 2:1, with 2/3 of the flies having curly wings and 1/3 dying due to the lethal combination of Curly alleles.

This demonstration illustrates how the presence of a lethal allele (CyCy) can affect the phenotypic ratios in a genetic cross involving the Curly gene. The lethal genotype results in non-viability and death, emphasizing the impact of certain gene combinations on the survival of organisms.

Experiment 7

Aim – To demonstrate multiple allelism by showing mutants of white eye series in *Drosophila*.

Theory – Multiple allelism refers to the presence of more than two alleles (alternative forms of a gene) at a specific gene locus within a population. In most diploid organisms, individuals inherit two alleles for each gene, one from each parent. However, in cases of multiple allelism, there are more than two possible alleles for a particular gene. Here are some key points to understand about multiple allelism:

- Alleles and Gene Loci: Alleles are different versions of a gene that occupy the same position, or locus, on homologous chromosomes. Multiple allelism occurs when there are more than two different alleles at a specific gene locus.
- Co-Dominance and Incomplete Dominance: Multiple allelism can lead to co-dominance or incomplete dominance. In co-dominance, both alleles are expressed fully in the heterozygous condition. In incomplete dominance, the heterozygote shows an intermediate phenotype.
- Population Variation: The presence of multiple alleles at a gene locus contributes to the genetic diversity within a population. Different individuals may carry different combinations of alleles, leading to a variety of phenotypes.
- Genetic Crosses: Genetic crosses involving multiple alleles can result in complex patterns of inheritance. The interactions between different alleles may lead to a variety of phenotypic outcomes.

Understanding multiple allelism is crucial in genetics as it reflects the complexity and diversity of genetic variation within populations. It provides insights into the inheritance patterns of traits and the ways in which different alleles interact with each other. Multiple allelism contributes to the genetic richness that allows populations to adapt to changing environments over time.

Method – In *Drosophila melanogaster*, the common fruit fly, eye color is a well-studied trait that is controlled by the white (w) gene. The white gene is located on the X chromosome. The wild-type allele for this gene is denoted as w+ and is associated with red eye color. However, there are several mutant alleles of the white gene that result in different eye colors. Here are some key eye color phenotypes in Drosophila and the corresponding alleles:

- Wild-Type (Red Eyes): Genotype: w+w+ ; Phenotype: Normal, red eyes
- White Eyes: Genotype: w ; Phenotype: Mutant allele resulting in white eyes
- Apricot Eyes: Genotype: wa ; Phenotype: Mutant allele resulting in apricot-colored eyes
- Cream Eyes: Genotype: wc ; Phenotype: Mutant allele resulting in cream-colored eyes
- Buff Eyes: Genotype: wb ; Phenotype: Mutant allele resulting in buff-colored eyes
- Ruby Eyes: Genotype: w^r ; Phenotype: Mutant allele resulting in ruby-colored eyes
- Brown Eyes: Genotype: bw ; Phenotype: Mutant allele resulting in brown eyes

These are just a few examples of the many alleles that can affect eye color in *Drosophila*. The different eye color phenotypes arise due to variations in the production and transportation of pigments in the eye cells. The white gene is involved in the synthesis of pigments in the eye, and mutations in this gene can lead to the loss or alteration of pigmentation, resulting in different eye colors. The study of eye color in *Drosophila* has been crucial in understanding basic principles of genetics, including the concepts of gene mutations, dominance, and epistasis. The white gene is often used as a model in genetic experiments, and researchers have identified numerous alleles with distinct eye color phenotypes.

Experiment 8

Aim – To study structural chromosome aberrations from prepared slides/photographs.

Theory - Polytene chromosomes are giant chromosomes that are formed by repeated rounds of DNA replication without cell division. They are commonly found in certain tissues of *Drosophila* larvae, particularly in the salivary glands. Polytene chromosomes are useful in genetics research because they allow for the visualization of chromosomal banding patterns and structural aberrations. Structural aberrations in polytene chromosomes can include various types of chromosomal rearrangements.

1. Dicentric aberrations - A dicentric aberration is a structural chromosomal abnormality where a chromosome possesses two centromeres instead of the normal one. This can occur through different mechanisms, such as chromosomal breakage and fusion or the abnormal replication and segregation of chromosomes. The presence of a dicentric chromosome in a polytene chromosome can lead to distinctive structural features. Here's how dicentric aberrations might be visualized in polytene chromosomes:

- Bridge Formation: Dicentric chromosomes can result in a bridge-like structure between the two centromeres during the polytene chromosome replication process. This bridge may be visible as a connection between two regions that would normally be separate.
- Loop Formation: The two arms of the dicentric chromosome may form a loop, representing the connection between the two centromeres. This loop structure can be observed within the polytene chromosome bands.
- Bridging Bands: The bands on the polytene chromosome may appear abnormal, with extra material connecting regions that would normally be distinct. This bridging can be a consequence of the dicentric aberration.
- Breakage and Fusion Points: Breakage and fusion points on the polytene chromosome may be visible, indicating where the dicentric chromosome has experienced structural changes.

2. Ring chromosomes - Ring chromosomes are structural abnormalities that can occur when the ends of a broken chromosome fuse together, forming a circular or ring-like structure. These ring chromosomes can be associated with various genetic disorders and can have significant consequences on gene expression and cellular function. Ring chromosomes may arise spontaneously or result from chromosomal breakage and

rearrangement. Ring chromosomes in Drosophila polytene chromosomes can be visualized during microscopic examination. Here's how ring chromosomes might be observed in polytene chromosomes:

- Circular Structures: Ring chromosomes in polytene chromosomes appear as circular structures or loops during microscopic examination. These structures may be visible within the characteristic banding pattern of polytene chromosomes.
- Bridge Formation: A ring chromosome in a polytene chromosome can lead to the formation of a bridge-like structure between the two ends of the ring. This bridge may be observable during the replication process of polytene chromosomes.
- Band Disturbances: The presence of a ring chromosome can cause disturbances in the normal banding pattern of polytene chromosomes. This may result in gaps or unusual structures within the bands.
- Visualization Techniques: Ring chromosomes in polytene chromosomes can be visualized using staining techniques that highlight the banding patterns. Techniques such as Giemsa staining or aceto-orcein staining can be used to enhance the visibility of chromosomal structures.
- Analysis of Genetic Consequences: The presence of a ring chromosome in polytene chromosomes allows researchers to study the genetic consequences of chromosomal abnormalities at the microscopic level. This analysis includes understanding how the circular structure influences gene expression, chromosomal stability, and cellular function.

3. Inversions - Inversions are chromosomal rearrangements where a segment of a chromosome is flipped in orientation, so that the order of genes within that segment is reversed. In *Drosophila melanogaster*, the fruit fly, inversions are commonly observed in polytene chromosomes. Here's how inversions can be visualized and studied in polytene chromosomes:

- Loop Structures: In polytene chromosomes, inversions often result in loop structures. These loops occur when the inverted segment forms a loop within the chromosome. The loops are visible during microscopic examination.
- Bridges and Connections: Inversions can create bridges or connections between different parts of the polytene chromosome that would normally be separate. These connections are formed by the inverted segment.

- Altered Banding Patterns: The presence of inversions can lead to alterations in the normal banding pattern of polytene chromosomes. The rearranged segment may appear as a distinct band or disrupt the continuity of existing bands.
- Giant Balloon Structures: Some inversions result in the formation of giant balloon structures. These balloons are swollen regions that represent the inverted segment of the chromosome.

Experiment 9

Aim – To study human karyotypes and numerical alterations.

Theory – A karyotype is the number and appearance of chromosomes in the nucleus of a eukaryotic cell. In humans, the typical karyotype consists of 23 pairs of chromosomes, for a total of 46 chromosomes. These chromosomes can be categorized into two types: autosomes (22 pairs) and sex chromosomes (1 pair). The sex chromosomes determine an individual's biological sex, and they are denoted as X and Y.

Numerical alterations in the human karyotype involve changes in the number of chromosomes and can result in conditions known as aneuploidies. Aneuploidies are associated with various genetic disorders and developmental abnormalities. Some examples of numerical alterations in the human karyotype are:

- Trisomy: Trisomy occurs when an individual has three copies of a particular chromosome instead of the normal two. The most well-known example is trisomy 21, which results in Down syndrome. Individuals with **Down syndrome** have three copies of chromosome 21 (47 chromosomes in total).
- Monosomy: Monosomy occurs when an individual has only one copy of a particular chromosome instead of the normal two. **Turner syndrome** is an example of monosomy, where affected individuals have only one X chromosome (45 chromosomes in total) instead of the usual XX or XY.
- Polyploidy: Polyploidy involves having more than the diploid number of chromosomes. While polyploidy is common in plants, it is not typically observed in humans. However, triploidy (69 chromosomes) and tetraploidy (92 chromosomes) have been reported in some cases, usually associated with developmental abnormalities.
- Sex Chromosome Aneuploidies: Conditions such as **Klinefelter syndrome** (47, XXY) and Triple X syndrome (47, XXX) involve aneuploidies of the sex chromosomes. In Klinefelter syndrome, individuals have an extra X chromosome (XXY), while in Triple X syndrome, females have an extra X chromosome (XXX).
- Mosaicism: Mosaicism involves the presence of two or more genetically distinct cell populations within an individual. It can result from mitotic errors during early development. For example, a person with mosaic Down syndrome may have a mixture of cells with trisomy 21 and normal cells.

Numerical alterations in the karyotype are often identified through cytogenetic techniques, such as karyotyping or fluorescence in situ hybridization (FISH). These techniques allow for the visualization and analysis of chromosomes under a microscope. Understanding numerical alterations in the human karyotype is crucial for diagnosing genetic disorders, providing genetic counseling, and gaining insights into the relationships between chromosomal abnormalities and various health conditions.

Experiment 10

Aim – To extract genomic DNA from bacteria.

Theory – The genomic DNA of bacteria is a circular, double-stranded molecule located in the bacterial cell's nucleoid region. Unlike eukaryotic cells, bacteria lack a true nucleus, and their DNA is not enclosed within a membrane-bound organelle. Instead, the bacterial genomic DNA exists as a single, continuous loop in the cytoplasm. Key features of bacterial genomic DNA include:

- Circular Structure: Bacterial DNA is typically circular, forming a closed loop. This circular structure simplifies processes such as replication, transcription, and recombination.
- Double-Stranded DNA: The DNA in bacteria, like all cellular organisms, is double-stranded, with complementary base pairing between adenine (A) and thymine (T) and between guanine (G) and cytosine (C).
- Nucleoid Region: Bacterial DNA is found in the nucleoid region of the bacterial cell. The nucleoid is not surrounded by a membrane, and the DNA is in close proximity to the cytoplasm.
- Supercoiling: Bacterial DNA can exist in a supercoiled state. Supercoiling helps in the packaging of the DNA into the limited space of the bacterial cell and is essential for various cellular processes.
- Plasmids: In addition to the genomic DNA, bacteria may carry extra-chromosomal DNA in the form of plasmids. Plasmids are smaller, circular DNA molecules that often contain genes providing additional functions, such as antibiotic resistance or the ability to metabolize specific compounds.
- Replication: Bacterial DNA undergoes semi-conservative replication, where each strand of the original DNA molecule serves as a template for the synthesis of a new complementary strand. Replication begins at the origin of replication and proceeds bidirectionally around the circular DNA.
- Genomic Size: The size of bacterial genomes varies widely among different bacterial species. Bacterial genomes are generally much smaller than eukaryotic genomes. Some bacteria have relatively small genomes, while others have larger genomes with complex regulatory networks.

- Coding and Noncoding Regions: Bacterial genomes consist of both coding and noncoding regions. Coding regions contain genes that encode proteins or functional RNA molecules, while noncoding regions may include regulatory elements, intergenic regions, and sequences involved in genome maintenance.

Procedure – The extraction of genomic DNA from bacteria involves breaking open the bacterial cells to release their genetic material and then purifying the genomic DNA from other cellular components. Here's a general procedure for extracting genomic DNA from bacteria:

Materials and Reagents - Bacterial culture, Tris-EDTA (TE) buffer (pH 8.0), Lysozyme, Proteinase K, Sodium dodecyl sulfate (SDS), Phenol-chloroform-isoamyl alcohol (25:24:1, pH 8.0-8.2), Chloroform, Isopropanol, Ethanol (95% or 100%), RNase, DNA precipitation solution (e.g., sodium acetate), Microcentrifuge tubes, Centrifuge, Vortex mixer, Heating block or water bath, Pipettes and tips, Microcentrifuge.

Steps –

1. Prepare Bacterial Culture: Grow a bacterial culture in an appropriate medium overnight or until it reaches the desired cell density.
2. Harvest Cells: Pellet the bacterial cells by centrifugation at 5,000 x g for 5 minutes. Discard the supernatant.
3. Resuspend Cells: Resuspend the bacterial pellet in TE buffer (pH 8.0) to wash the cells.
4. Lysozyme Treatment: Add lysozyme to the resuspended cells. Incubate the mixture at 37°C for 30 minutes to digest the cell wall.
5. Proteinase K and SDS Treatment: Add proteinase K and SDS to the lysozyme-treated cells. Incubate the mixture at 37°C for 1-2 hours to digest proteins and release genomic DNA.
6. Phenol-Chloroform Extraction: Add an equal volume of phenol-chloroform-isoamyl alcohol to the cell lysate. Mix thoroughly and centrifuge at maximum speed for 5-10 minutes. Transfer the aqueous phase (containing DNA) to a new tube.
7. Chloroform Extraction: Add chloroform to the aqueous phase, mix, and centrifuge again. Transfer the aqueous phase to a new tube.

8. Precipitate DNA: Add isopropanol to the aqueous phase to precipitate the genomic DNA. Mix gently and incubate at -20°C for at least 1 hour or overnight.
9. Centrifugation: Centrifuge the DNA precipitation at maximum speed for 10-15 minutes. Discard the supernatant.
10. Wash DNA Pellet: Wash the DNA pellet with 70% ethanol to remove residual salts. Centrifuge again and remove the ethanol.
11. Air-Dry and Resuspend: Air-dry the DNA pellet briefly and then resuspend it in TE buffer or water.
12. RNase Treatment: Treat the DNA with RNase to remove residual RNA contamination. Incubate at 37°C for 1 hour.
13. Quantification: Measure the concentration and purity of the extracted genomic DNA using a spectrophotometer or fluorometer.