LABORATORY # 1: INTRODUCTION TO GENETICS AND MONOHYBRID CROSSES

LEARNING OUTCOMES

After completing this lab, students should be able to:

-Describe the different stages of a model organism (*Drosophila melanogaster*)

-Identify the different phenotypic traits and recognize male and female flies.

-Use the *Drosophila* genetics notation to represent different genotypes

-Determine de mode of inheritance of a trait based on the analysis of offspring number.

INTRODUCTION

One of the main aims of genetics is to understand how traits are transferred from parents to offspring (heredity). To do so, many genetics analyses rely on the study of parental generations (P_1-P_2), their offspring (F_1), and their further generations (F_2, F_3, F_4, etc.). If these types of studies are performed in organisms with relatively long-life cycles (humans, cattle, elephants, etc.), data collection, analysis and drafting conclusions may require an extensive amount of time (several decades). One way to overcome this potential issue is to use **model organisms** with shorter life cycles that are easy to breed under laboratory conditions. Several model organisms are available to perform genetics analyses in microorganisms, plants, amphibians, fishes, birds, and mammals.

An important and widely used model organism is ***Drosophila melanogaster***. It is commonly named "fruit fly" because fermented fruit is its main source of food in. nature (bananas, oranges, apples, etc.). There are several aspects that make fruit flies a model organism for genetics teaching and research Some of those include:

1) The fly is small and can be **easily raised** in bottles on a simple culture medium.

2) ***Drosophila* flies breed rapidly**. One complete life cycle requires about 10 – 12 days at 25 ^{0}C (short life cycle).

3) Only one set of parents can produce **several hundred** offspring flies.

4) There are only **four pairs of chromosomes**, which facilitates genetic studies.

5) **Availability** of many well-characterized and easy to score **morphological mutants**.

Please consider performing a web search for *Drosophila* mutants. There is plenty of multimedia and graphic content available that illustrates the morphological variation in fruit flies

THE DIFFERENT STAGES OF *Drosophila melanogaster*

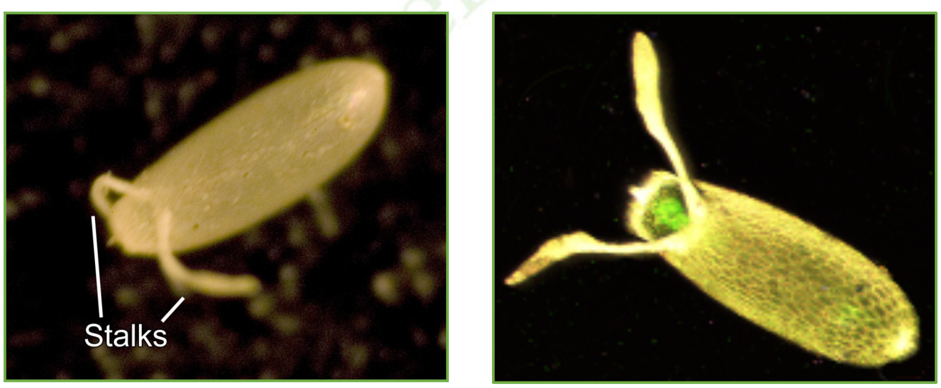
THE EGG

The egg is fertilized as it passes from the ovary through the uterus prior to being laid. Usually, the mature unfertilized egg is arrested at metaphase I and the rest of the meiotic division continue after the entrance of the sperm. Virgin females may lay unfertilized eggs, although they are relatively few in comparison with those laid by an inseminated female. The eggs are white, oval, and about 0.5 mm in length.

A pair of stalks extends from the egg and prevents it from sinking into the soft medium (attachment to surface). One day after the egg is laid a small larva hatches out of the egg case (at 25^{0}C).

THE LARVAE

The larva is white and segmented with black mouth parts visible at the anterior end. Testes are already present in male larvae at the time of hatching with each testis containing 36 to 38 germ cells. In female larvae of similar age, each ovary is smaller than the testis, and 8 to 12 germ cells are present. Larvae eat their way through the medium, making tunnels as they go. The larvae grow very rapidly and pass through **three** stages called **instars**. The first and second larval instars occur at about 1-day intervals, but the third instar lasts 2 days. At the start of the third instar, the first spermatocytes are found in the testis and by the end of this stage, spermatozoa are present in the tips of the testis, with spermatocytes filling the remainder. The ovaries develop; however, they still contain only oogonia. The eye-antenna disc can be seen to be divided into its two parts at the beginning of the third instar. The cells in the eye portion are arranged in clusters corresponding to the number of ommatidia in the eye of the adult. By this time, the segmentation of the antenna portion has been completed. The mature third instar larva is the stage used for cytological examination of **salivary gland chromosomes**.



Eggs

Larvae

THE PUPA

A few hours after the mature third instar larvae crawl out of the soft medium and onto a dry surface, the cuticle begins to thicken, forming what it is known as pupa.

The pupa is a stationary brown structure that can be seen on the sides of the culture bottle. Inside the hard case, tremendous changes are taking place as the larval tissues undergo histolysis and the new tissues and organs of the adult are formed. About halfway through the pupal period, pigmentation begins to be deposited in the compound eyes and the testes. The ovaries become attached to the oviduct and the testes to the *vasa efferentia*. The wing becomes fully formed and folded. Only during the last half of the pupal period, the oocyte appears in the ovary for the first time. There are still no mature eggs when the female adult is about to emerge from the pupa.

THE ADULT

About 9 to 11 days after the egg is laid, metamorphosis is completed, and an adult fly emerges from the pupa. Within half a day, the adults are sexually mature and able to mate, thus completing the life cycle.

Pupa

Adult (yellow body, white eyes, miniature wing mutant)

Illustration of male (left) and female (right) *Drosophila* flies.

MORPHOLOGY OF A WILD-TYPE *Drosophila*

HEAD

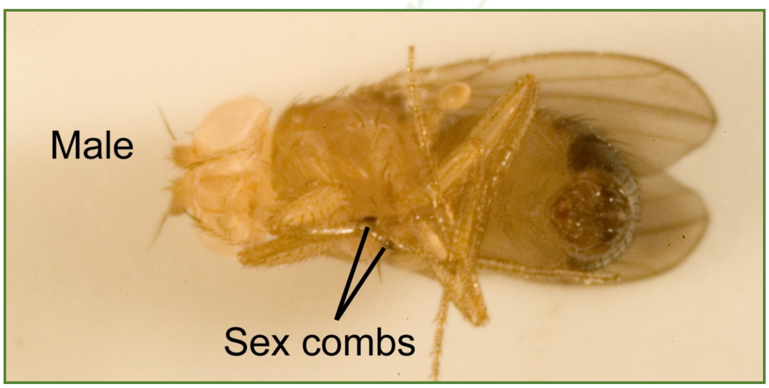
It has a pair of antennae and a pair of eyes. The eyes are of two types: simple and compound. The compound eyes are large and are made up of many small units called the ommatidia. These ommatidia form a honeycomb type of arrangement. In **wild type** flies, the color of the eye is **dull red**. This is due to different kind of pigments: a red-type and a brown one. If only one of them is present, the eye would either be **brown (bw) or bright red** (**scarlet** **(s)** red). Genetic mutations that affect the distribution of one or both of these pigments result in many different possible mutant eye color phenotypes (i.e., **sepia (se)**), while the complete absence of pigmentation will produce **white**-eyed flies (**w**).

THORAX (BODY)

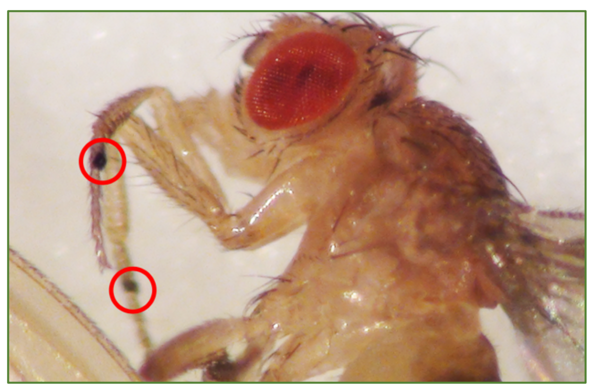
The thorax is composed of three fused segments, each of them carrying a pair of walking legs. The first pair of legs in **male flies** contain a clump of black bristles called the **sex comb**. Because it is found only in adult males (not female flies), it is an **important** feature to **determine the sex** of an adult fly. The abdominal-terminal part is **darker** in coloration in **males** than in females because the abdominal rings are very close together in male flies. **Wild-type** flies show a **brownish** body color when compared to other mutants (i.e., **yellow (y)**).

WING

The wing contains veins from the base to the margin to provide support for flying movements. Although there are two pairs of wings, only one pair is well developed and is used for flying (Order Diptera). The other pair is reduced as a small bulbous structure called the halteres, probably used for balance to stabilize flight. Several mutations affect the wing size and shape. In **wild-type** flies, the wing is elongated, and its length is about **twice the size of the body length**. Some mutations, for example the **miniature mutation (m)**, produces flies with wings **slightly** larger than the body size but never as long as the wild-type wing size.



Adult male (yellow body, white eyes, miniature wing mutant)



Adult male (wild-type eye color)

GENETICS NOTATION USED IN *Drosophila* RESEARCH

In many introductory genetics courses, you may have used an upper-case letter (e.g., **A**) to represent a dominant allele and a lower-case letter (e.g., **a**) to represent a recessive allele. However, genetic nomenclature varies among organisms, and different systems are used in different fields of genetics. In this course, we will use the standard *Drosophila* genetic nomenclature. It is important that you understand and apply this notation correctly, as marks may be deducted for incorrect usage in assignments and laboratory reports.

In *Drosophila* genetics, the most common phenotype observed in a natural population is referred to as the **wild-type** phenotype. Alternative, less common phenotypes are called **mutant** phenotypes. Gene symbols are assigned according to the mutant phenotype rather than the wild-type phenotype.

For mutations in which the mutant allele is **recessive** (the most common situation), the mutant allele is represented by one or more lower-case letters, usually derived from the name of the mutation. For example, **w** represents the recessive allele for white eyes, and **sn** represents the recessive allele for singed bristles. The corresponding wild-type allele is indicated by adding a superscript plus sign (**+**) to the same symbol (e.g., **w^+**, **sn^+**).

If the mutant allele is **dominant**, the mutant allele is represented by an upper-case letter. For example, **B** represents the dominant Bar-eye mutation. The corresponding wild-type allele is written using the same symbol with a superscript plus sign (e.g., **B^+**).

Therefore, in *Drosophila* notation:

- The gene symbol is based on the **mutant phenotype**.
- Lower-case symbols indicate that the **mutant allele is recessive**.
- Upper-case symbols indicate that the **mutant allele is dominant**.
- The superscript **+** always designates the **wild-type allele**.

Examples:

- **w/w** → white-eyed female
- **w^+/w** → wild-type female (red eyes)
- **B^+/B** → Bar-eyed fly
- **B^+/B^+** → wild-type fly with normal eyes

Quick guide for *Drosophila* notation

	Wild Type	**Mutant**
If the **mutation** is **Dominant**	**A^+**	**A**
If the **mutation** is **Recessive**	**a^+**	**a**

LOCATION OF GENES ON CHROMOSOMES

In addition to indicating whether an allele is wild type or mutant, *Drosophila* notation can also provide information about the chromosomal location of genes and whether genes are linked.

The slash symbol (**/**) separates homologous chromosomes. Because *Drosophila* is diploid, each genotype contains two homologous chromosomes, represented on either side of the slash.

Genes Located on Different Chromosomes (Unlinked Genes)

Genes located on different chromosome pairs are written separately because they assort independently. For example, if genes **a** and **b** are located on different autosomes, the genotype of an individual homozygous for both mutations would be written as:

$$a/a\ ;\ b/b$$

The semicolon (**;**) indicates that the genes are located on different chromosome pairs.

Genes Located on the Same Chromosome (Linked Genes)

When two or more genes are located on the same chromosome, they are written together on each homolog. For example, if genes **a** and **b** are located on the same chromosome, a fly homozygous for both mutant alleles would be written as:

$$ab\,/\,ab$$

Likewise, a heterozygous fly carrying both mutant alleles on one chromosome and both wild-type alleles on the homologous chromosome would be written as:

$$a^+b^+/\,ab$$

Sex-Linked Genes

Genes located on the X chromosome are written using standard chromosome notation. Female flies possess two X chromosomes (**XX**), whereas males possess one X chromosome and one Y chromosome (**XY**).

For a gene **w** (white eyes) located on the X chromosome:

- Female homozygous mutant: $Xw\,/Xw$
- Female heterozygous: $Xw{+}\,/\,Xw$
- Male mutant: $Xw\,/\,Y$
- Male wild type: $Xw{+}\,/\,Y$

When more than one gene is located on the X chromosome, the alleles present on each X chromosome are written together. For example:

- Female heterozygote: $Xa^+b^+/\,Xab$
- Male mutant: $Xab\,/\,Y$

Activity: Complete the Notation trainer available at www.ampossot.com/trainer
Export the PDF report from the app and upload it to Canvas (Assignment # 0)

HANDS ON: IN-PERSON PRACTICAL COMPONENT
Drosophila BREEDING EXPERIMENT (MAIN CROSS)

IMPORTANT INFORMATION:

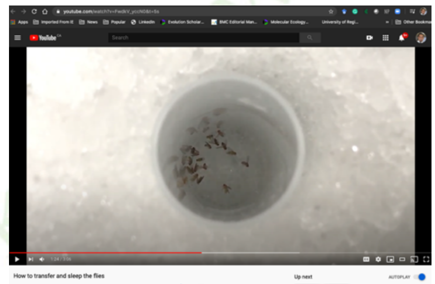
The objective of the main *Drosophila* cross (living flies) is to unravel the genetics basis of **four different genes (phenotypes).**

Similar to the work of the genetics' pioneers (Gregor Mendel, Thomas Morgan, and many others), you will collect phenotypic data during the next couple weeks. Then, you will analyze this information to determine the genetics basis of the genes controlling those traits (dominant vs. recessive alleles; autosomal vs. sex-linked genes).

TECHNIQUE FOR HANDLING FLIES

It is very important to be an expert on the procedure for **handling living flies**. Before starting a *Drosophila* cross, students must be familiar with **anesthetizing the flies** with ice and observing them under the dissecting microscope. Then, as the flies are sleeping, you can observe and identify the different phenotypes and distinguish between male and female individuals.

Before you continue, please check the video ***"how to transfer and sleep the flies"*** available in Canvas or the following link:
https://youtu.be/FwdkV_yccN0



MAIN MATERIALS:

- A dissection microscope
- A frozen cool-pack and a container with ice
- A couple of paint brushes
- A vial containing parental flies (**main cross**)
  -Females: **yellow** body, **white** eyes, **miniature** wings
  -Males: **sepia** eyes

P1 Female: yellow body, white eyes, miniature wing

P2 Male: Sepia eyes (wild type for the other traits)

PROCEDURE FOR LABORATORY WORK

1. Work in **pairs** of students. Collect one vial containing flies. They are in the cart at the front of the lab room. We already set up your main cross **one week ago**.

Remember the main cross:

P_1= virgin **females**, **yellow** body, **white** eyes, **miniature** wings x P_2= **males, sepia** eyes.

2. Label your tube as P_1 **X** P_2 cross. Add your names.

3. Transfer the flies from the food vial to a clean vial. Use ice to anesthetize the P_1 **and** P_2 flies. Observe them using the **dissecting scope**.

If you are not sure how to use the dissecting scope, please check the video!

https://youtu.be/ssxfsgI81Zs

4. Using a brush, move the flies around and observe the phenotypes of wild type and mutants. Identify male and female flies. Make sure that you have the **right parental flies**.

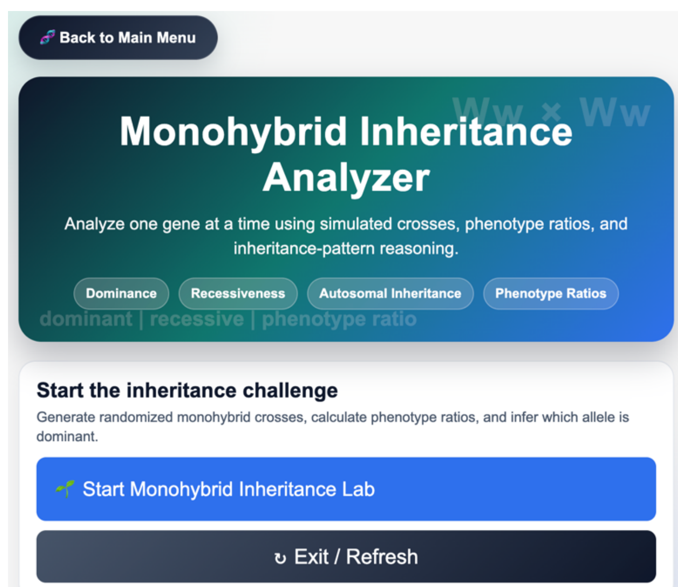
5. Score the flies and put them in the **morgue**. Keep this information safe, as you need this data to complete your assignment.

6. Return the tube to the rack. You will need the flies that will start emerging next week. These will be the **first generation or F_1 flies**.

Although this cross involves **four (4) genes** (mutations) you will not determine the mode of inheritance of all of them at once. Instead, you will consider **one or two** genes at a time for the first labs. Then, you will consider the mode of inheritance of **three** genes (**y w m**) to test if they can be mapped in relation to each other.

PROCEDURE FOR THE VIRTUAL GENETICS LAB

1. Launch the Virtual Genetics Lab. For this lab, you will use the "Monohybrid Inheritance Analyzer" tool. It is available on Canvas (lab # 1 tab) or here:



www.ampossot.com/mono

2. Click on the "Start" button. Read the information presented and start performing the suggested crosses. This part of the lab is individual, and every student will have a different set of data.

3. Perform the number of crosses described in your assignment. Record the number of offspring phenotypes and **ratios** in the provided templates. An easy way to obtain phenotypic ratios is dividing the **higher-class number** by the **lowest class number**. Example: If you get 60 wild-type flies and 19 vestigial flies, the ratio will be 60/19 wild-type: 19/19 vestigial flies. That is 3.15 wild-type: 1 vestigial flies. **If only one phenotypic class is present, the ratio would be 1:0**.

PRACTICE YOUR PHENOTYPING

Left: A yellow body, white eyes, and miniature wing fly. **Right:** A wild-type fly for wing size, body, and eye color. Can you identify the sex of the flies (male vs. female)?

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