Studying Mechanisms of Inheritance using *Drosophila*

Learning Objectives:

1. To explain why the fruit fly is an ideal model organism for inheritance studies.
2. To describe the features that differentiates male flies from female.
3. To describe sex-linked inheritance and use correct terminology and letters to represent the genotypes of female and male flies for sex-linked traits.
4. To perform crosses and use Punnett squares to determine genotype and/or phenotype percentages or ratios in offspring and to predict genotype and phenotype of offspring for sex-linked traits.

Why is Drosophila used as a model organism for genetic studies?

When choosing a model organism for biological studies, there are a certain characteristics which make a model organism ideal: (1) *Drosophila* are small; you can house 1000's of flies in a small area; (2) they have a short life cycle (on the order of days); (3) they are easy to maintain for multiple generations; (4) they produce many, many offspring in a short period of time; and (5) they have a number of different heritable characteristics that are easily distinguishable.

The purpose of this experiment is to determine if the inheritance pattern of the alleles that determine the eye colour trait are ***autosomal dominant***, ***autosomal recessive***, ***sex-linked dominant*** or ***sex-linked recessive***. This can be determined by setting up a series of crosses between male and female flies which are red eyed (normal) or white eyed (mutant). Looking at the traits of the offspring will allow us to determine the pattern of inheritance of the eye colour trait (Figure 1).

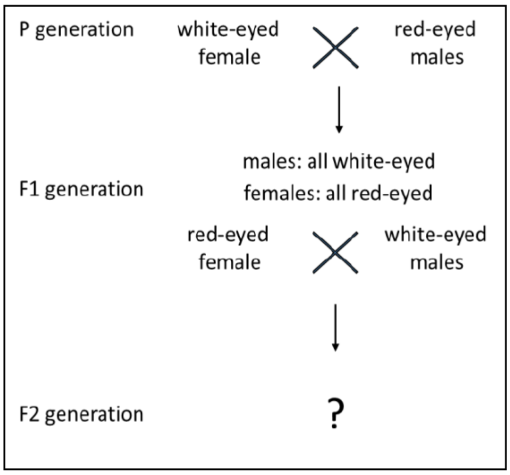


Figure 1: P, F1 and F2 crosses used to determine the inheritance pattern of eye color trait

Part 1: Setting up our F1 cross:

The parental cross (white eyed female X red eye male) was completed. All male offspring were white eyed and all female offspring were red eyed. This information will help to predict the inheritance pattern of eye colour. Set up the F1 cross. The data from this cross will help to confirm your prediction.

1. Obtain a culture vial containing flies of this F1 generation.
2. Anesthetize the flies. Because *Drosophila* can fly, they must be immobilized in order to set up your crosses and analyse progeny. If anesthetizing flies in their original culture vial, use the following procedure (your instructor may do this for you):

a) Obtain an anesthetizing wand. Dip the absorbent end of the wand into the Fly Nap and remove excess liquid by running the wand across the rim of the bottle.

b) Tap the vial of flies against a hard surface so that the flies are *gently* knocked to the bottom of the vial.

c) Use one finger to push the vial plug slightly to the side and quickly stick the anaesthetic end of the wand into the vial. (Do this quickly or the flies could escape!).

d) Lay the culture vessel *on its side* while the flies are anesthetized. (DO NOT leave the vial upright while the flies are unconscious or you risk drowning them in the media!)

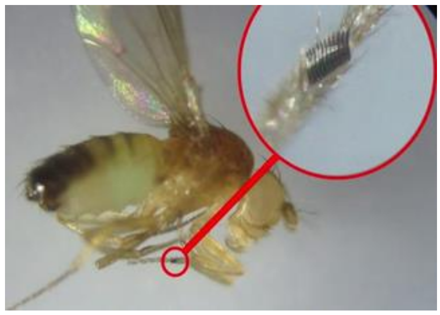
e) Wait until the flies have stopped moving and then empty the flies out onto an index card or piece of white paper.

3. You need to be able to select both male and female flies in order to have success with your crosses. Being able to determine the sex of the offspring will also allow you to see if any of the traits you are observing are sex-linked. Physical characteristics that are useful for distinguishing males and females are as follows:

Body size – males tend to be slightly smaller than females

Tip of abdomen – dark in males; lighter, striped & more pointed in females

Sex combs – males have sex combs on the uppermost joint of their forelegs; females do not.

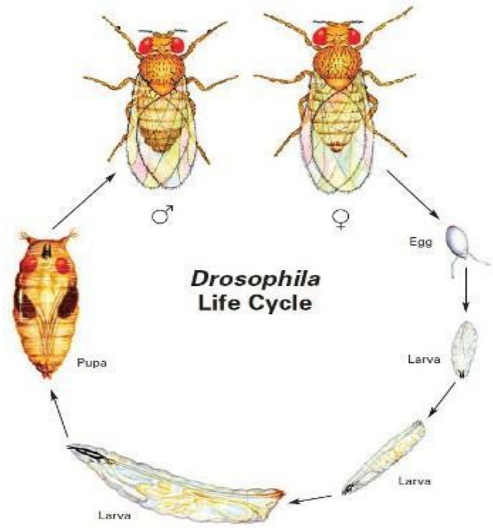


Using these markers separate the males from the females using the brush. Make sure you can easily identify the eye colour as this is the trait you will be observing. It can be difficult to make these observations using the naked eye, to aid in viewing specimens in more detail we can use a dissecting microscope.

4. Transfer 5 female flies and 5 male flies to a newly prepared culture vial (vial plus food). Leave the vial on its side until the flies wake up. Label your vial with your group name, date and lab section.

Part 2: Remove F1 generation flies

Fruit Fly Life Cycle: The life cycle of *Drosophila* can take place in plastic culture vials that contain a source of food. The life cycle has four stages: egg, larva, pupa, and adult. You should be able to see all four stages present in the cultures you will use today. The eggs are small and white (usually found on the surface of the food). The larva hatch within 24 to 36 hours, and burrow into the food at the bottom of the vial; they look like little wriggling grains of rice. After about 5 days, they crawl out of the food onto the sides of the vial and transform into brown, oblong pupae. During the pupation stage, the larvae metamorphose into adult flies. After about 4 days, the adult flies emerge from the pupae and begin the life cycle anew. The entire cycle takes approximately 10 to 12 days to complete. Observe your culture vials for larvae and pupae. Anesthetize and remove the F1 generation from the vials; discard them in the morgue.



Part 3: Collecting data about F2 generation

Look at the F_2 generation for the eye colour trait. Collecting this data will allow to determine the pattern of inheritance of the eye colour trait. Before collecting data write out hypothesis regarding the inheritance pattern of the alleles that determine the eye colour trait (is the pattern *autosomal dominant*, *autosomal recessive*, *sex-linked dominant* or *sex-linked recessive?)*. Pre-lab activity will help to determine hypothesis using the parental cross and outcome (F_1 data) along with the completed Punnett squares as part of the pre-lab activity.

Use prediction of pattern of inheritance to calculate the expected ratio of phenotypes in the F2 generation. To do this show a Punnett square of the F1 cross and calculate the expected percentages for each phenotype in the F2 generation.

What are the genotypes of the F1 generation? ♂________________× ♀_________________

Punnett square (be sure to correctly represent the female and male genotypes)

Female F1

Male F1

This Punnett square gives the expected ratios of the phenotypes in the F2 generation
What % of the F2 generation is expected to be red-eyed male? _________________
What % of the F2 generation is expected to be white-eyed male? _______________
What % of the F2 generation is expected to be red-eyed female? _______________
What % of the F2 generation is expected to be white-eyed female? _____________
What % of the F2 generation is expected to be male? __________________ female? _____________

To determine the actual % or ratios of the different phenotypes in the F2 generation we need to count the flies with each phenotype and complete the table below with class data.

1. Anesthetize the adult flies in the F_2 generation.
2. Identify the sex and eye colour of each individual. Record your data (along with the data from the other six lab groups) in the table below. Discard the counted flies in the morgue after determining their phenotypes

Table 1: Data for phenotypes of the F_2 generation from the F1 (red eyed X white eye male) cross:

Lab Group	Males		Females	
	Red-eyed	White-eyed	Red-eyed	White-eyed
1.				
2.				
3.				
4.				
5.				
6.				
7.				
Class Total				
% (total # with phenotype / total # of flies)				

Analysis of the Results:

What are the genotypes of the individuals who were in the P generation?

♂________________× ♀___________________

What are the genotypes of the F_1 generation?

♂________________× ♀___________________

Compare the ratios that you expected (from the Punnett square) to the actual numbers that were counted:

What % of the F2 generation is expected to be red-eyed male? _________________

What % of the F2 generation did you count as red-eyed male? _________________

What % of the F2 generation is expected to be white-eyed male? _______________

What % of the F2 generation did you count as white-eyed male? _______________

What % of the F2 generation is expected to be red-eyed female? _______________

What % of the F2 generation did you count as red-eyed female? _______________

What % of the F2 generation is expected to be white-eyed female? _____________

What % of the F2 generation did you count as white-eyed female? _____________

What % of the F2 generation is expected to be male? __________________ female? _____________

What % of the F2 generation was male? __________________ female? _____________

How do the ***actual*** ratios compare to the ***expected*** ratios?

If there is a difference, propose an explanation for this difference?

Does the collected data support hypothesis and prediction for the inheritance pattern of the alleles that determine the eye colour trait?