

**Biology 423L**

**Oct. 20/21, Nov. 3/4 and Nov. 17/18**

**Drosophila Lab Three-point cross**

Report due Dec. 1/2.

**Reading:** Drosophila: Hartwell Chapter 20 pp. 732-749.  
Sex-linked inheritance: Hartwell Chapter 4 pp.105-112.  
Mapping: Hartwell Chapter 5 pp. 123-141.

**Objective:** The objective of this lab is to give you experience working with *Drosophila*. We think you will appreciate the ease of working with this animal model. You will be able to do controlled crosses and count large numbers of offspring. In this lab, you will set up a parental cross of two pure breeding lines to obtain females that are heterozygotes for three different traits. You will then cross the heterozygous females with triple mutant males in a test cross. You will examine the offspring from the test cross to determine the recombination frequencies between the three genes. You will be able to tell if the genes are linked, if they are on sex chromosomes or autosomes and you will be able to calculate the linkage distances between the genes.

**Introduction:** You will mate flies with mutations affecting three different traits. You will carry out the mating yourselves and analyze the offspring. The traits are yellow body color, forked bristles and presence or absence of cross veins in the wings. You will learn to distinguish these mutations from the wild type and how to distinguish female and male flies.

You will start with mutant females crossed to wild type males. This will result in heterozygote females with wild type phenotype and mutant males. These will be mated for the three-point test cross.

Genotypes: Wild type: Oregon R

Mutant: *crossveinless (cv)*, *yellow body (y)*, *forked bristles (fb)*

**Start with** collecting virgin females from the mutant line. When the flies come out of their pupae (eclose) they will not mate for about the first day of adult life. This allows you to collect virgins. You will cross them to wild type males. As a control, also cross some with males from the same mutant stock. The flies will lay eggs and you will then clear the vials of adult flies. Two weeks after setting up the matings, the adult flies will emerge from their pupae. You will score their phenotypes and determine their genotypes. You will take females from this cross if they are wild type in appearance and mate them with mutant males. This will allow you to determine where the mutant and wild type chromosomes have recombined in the heterozygous females. Two weeks later you will count the offspring and carefully score their phenotypes for all three traits. This will allow you to calculate the linkage distances of the mutant genes.

**Materials:**

Fly vials

Triple mutant females, triple mutant males, wild type males

CO<sub>2</sub> stations

Dissecting microscopes

Fly Morgue (a flask with water and dishwashing detergent)

Feather brushes

**Method:****First week of the experiment.**

Collect virgin females from the mutant stock you have been given. Bottles containing fly larvae will be placed in the lab room. You will be working in groups as usual. You or your partner will come in each day during the week to collect virgin females. We will show you how to remove flies from bottles and transfer them to new vials using the CO<sub>2</sub> stations and a dissecting microscope.

Protocol to collect flies:

Turn on the CO<sub>2</sub> using the regulator on the tank. Be sure the lines are hooked to your side-arm flask and your stage and that the valves to your lines are open. The CO<sub>2</sub> will knock your flies out without harming them. Keep the CO<sub>2</sub> on at a low level and work quickly. The flies cannot stand being knocked out for more than 15 - 20 minutes. Remove the stopper from the vial and tip the flies into the side-arm flask through the funnel. Tip them gently to avoid fly food falling out. The flies will be caught in the tube inside the flask. Working quickly, deposit the flies onto the CO<sub>2</sub> stage. Using the dissecting microscope, examine the flies. Look at the body shape to tell male from female. Look at the wings. They have veins extending from the base to the tips like a fan. There are also some veins going between the major veins at right angles. In the cv mutant there are no veins between the major veins (no cross veins) eye color. Look at the bristles on the wing. Are they straight or forked. Is the body yellow or dark? Use a feather to separate the flies. Gently, tip the females into a collecting vial so that they land on the glass side of the vial and not in the food. Close the vial with a cotton stopper and place it on its side until the flies wake up and begin flying around. Then you can turn the vial upright and store at room temperature.

Collecting virgins:

You will get a bottle in which flies have laid their eggs. You can see the larvae in the food and the pupae cling to the sides of the bottle above the food. Every day some of the pupae will eclose. You will check the bottle each day and remove the adult flies onto the CO<sub>2</sub> stage. The virgin females will be collected into a separate vial for mating. If the females eclosed on the same day, you separate them from the males, they should be virgins. Virgins also have a translucent spot on the side of the abdomen. We will show you how to identify them. Using a paint brush or a feather, transfer no more than 5 virgins into each mating vial. Be careful not to get any mutant males in the vials. Label the vials mutant X wt and add the date and your initials.

Matings

As in plant crosses, female type is always written first. Then add the same number of males from the wild type strain.

Also set up at least one vial with mutant females and mutant males as a control to ensure that the mutant strain is "true breeding".

Continue to collect virgins and set up matings as necessary

You can continue to collect virgins from the vials containing the triple mutant strain. However, it is essential that all the flies are removed each day to be sure that the newly emerged females have not already mated with a male in the first bottle. When you are finished with collecting virgins, discard all other adult flies from the vial. Kill unwanted flies by dropping them into the Morgue.

The TA will open the lab in the morning and remove adult flies from the bottles containing the pure strains. You can come into the lab in the afternoon to collect more virgins and set up additional matings. Each group should set up 4-5 vials of mutant female X wild type male and at least one vial of mutant female X mutant male. The TA will put all mating vials into the 18 degree incubator at 5 pm each day.

### **Second week** Clear adults

The females will have laid their eggs. You will remove the adult flies and kill them in the morgue. Open the vial over the morgue and shake the adult flies out. Be careful not to dump the food and the larvae into the morgue. Reclose the vials and store at room temperature.

### **Third week** Backcross F1 females to mutant males

Collect female virgins and set up matings with males of the mutant pure line.

We will collect virgins from among the new flies. Look at the males and female adults from the first cross. Is the mutant type dependent on the sex of the flies? If so the mutations could be sex-linked. Be sure the female flies all look wild type. This means they are the progeny of mutant females mated to wild type males. Set up new matings by transferring 2 or 3 F1 females to a new vial. Add 2 or 3 males from the triple mutant stock. Label the vials with your initials, the date and the cross. List the female first. If the males are wild type do not use the flies from the vial. If the males are mutant but some of the females are also mutant, discard the mutant females and only use wild type appearing females to set up new crosses. While you are setting up the crosses, count the adults and score their phenotype. Are the mutant traits autosomal or sex-linked?

### **Fourth week.** Clear adult flies

Remove the adult flies from the vials

### **Fifth week.** Analyze the phenotypes of the progeny

We will knock out the flies in the vials with CO<sub>2</sub> and you will drop them on the CO<sub>2</sub> stage. Count as many flies as you can. Ideally, we would like to count 500 progeny. Score the flies for sex, wing veins, body color and bristle type. If there are enough female flies (over 100) only score the females. This makes body color easier to score. Enter your results on the table at the end of the protocol. We will also have a table posted in the lab. Enter your results so everyone's data can be pooled.

### **Report:**

Enter the number of each phenotype group you counted in your table. In the discussion, take the data from the entire class.

**Questions:**

1. Are any of the genes sex linked? Explain the relevant observations
2. Some of the genes are linked. Determine the gene order on the chromosome  
Calculate the distance between the loci. Show your work

Table for pooled class data. Enter counts for each phenotype in column for your group number.

Cross: wild type (heterozygous) female by triple mutant male

Phenotypes	2	3	Groups	1	2	3
Female	dark body	forked bristle	No cross veins			
Female	dark body	forked bristle	Cross veins+			
Female	dark body	straight bristle	No cross veins			
Female	dark body	straight bristle	Cross veins+			
Female	yellow body	forked bristle	No cross veins			
Female	yellow body	forked bristle	Cross veins+			
Female	yellow body	straight bristle	No cross veins			
Female	yellow body	straight bristle	Cross veins+			
Male	dark body	forked bristle	No cross veins			
Male	dark body	forked bristle	Cross veins+			
Male	dark body	straight bristle	No cross veins			
Male	dark body	straight bristle	Cross veins+			
Male	yellow body	forked bristle	No cross veins			
Male	yellow body	forked bristle	Cross veins+			
Male	yellow body	straight bristle	No cross veins			
Male	yellow body	straight bristle	Cross veins+			

**Discussion:** Summarize the results. And answer the questions above. Find the linkage map for *Drosophila*. Cite the reference you found. Include a picture of the relevant chromosome. What are the recorded map distances and how do they compare to the distances you found in your experiment? What is the function of each of the genes studied here, *fb+*, *cv+*, *y+*