

LAB 11 – *Drosophila* Genetics

Introduction:

Drosophila melanogaster, the fruit fly, is an excellent organism for genetics studies because it has simple food requirements, occupies little space, is hardy, completes its life cycle in about 12 days at room temperature, produces large numbers of offspring, can be immobilized readily for examination and sorting, and has many types of hereditary variations that can be observed with low-power magnification. *Drosophila* has a small number of chromosomes (four pairs). These chromosomes are easily located in the large salivary gland cells. *Drosophila* exists in stock cultures that can be readily obtained from several sources. Much research about the genetics of *Drosophila* during the last 50 years has resulted in a wealth of reference literature and knowledge about hundreds of its genes.

The Life Cycle of *Drosophila melanogaster* (Figure 1)

The eggs are small, oval shaped, and have two filaments at one end. They are usually laid on the surface of the culture medium and, with practice, can be seen with the naked eye. The eggs hatch into larvae after about a day.

The wormlike larva eats almost continuously, and its black mouth parts can easily be seen moving back and forth even when the larva itself is less distinct. Larvae tunnel through the culture medium while eating; thus, channels are a good indication of the successful growth of a culture. The larva sheds its skin twice as it increases in size. In the last of the three larval stages, the cells of the salivary glands contain giant chromosomes, which many be seen readily under low-power magnification after proper staining.

When a mature larva in a lab culture is about to become a pupa, it usually climbs up the side of the culture bottle or on to the strip provided in the culture bottle. The last larval covering then becomes harder and darker, forming the pupal case. Through this case the later stages of metamorphosis to an adult fly can be observed. In particular, the eyes, the wings, and the legs become readily visible.

When metamorphosis is complete, the adult flies emerge from the pupal case. They are fragile and light in color and their wings are not fully expanded. These flies darken in a few hours and take on the normal appearance of an adult fly. They live a month or more and then die. A female does not mate for about ten to twelve hours after emerging from the pupa. Once she has mated, she stores a considerable quantity of sperm in receptacles and fertilizes her eggs as she lays them. To ensure a controlled mating, it is necessary to use females that have not mated before.

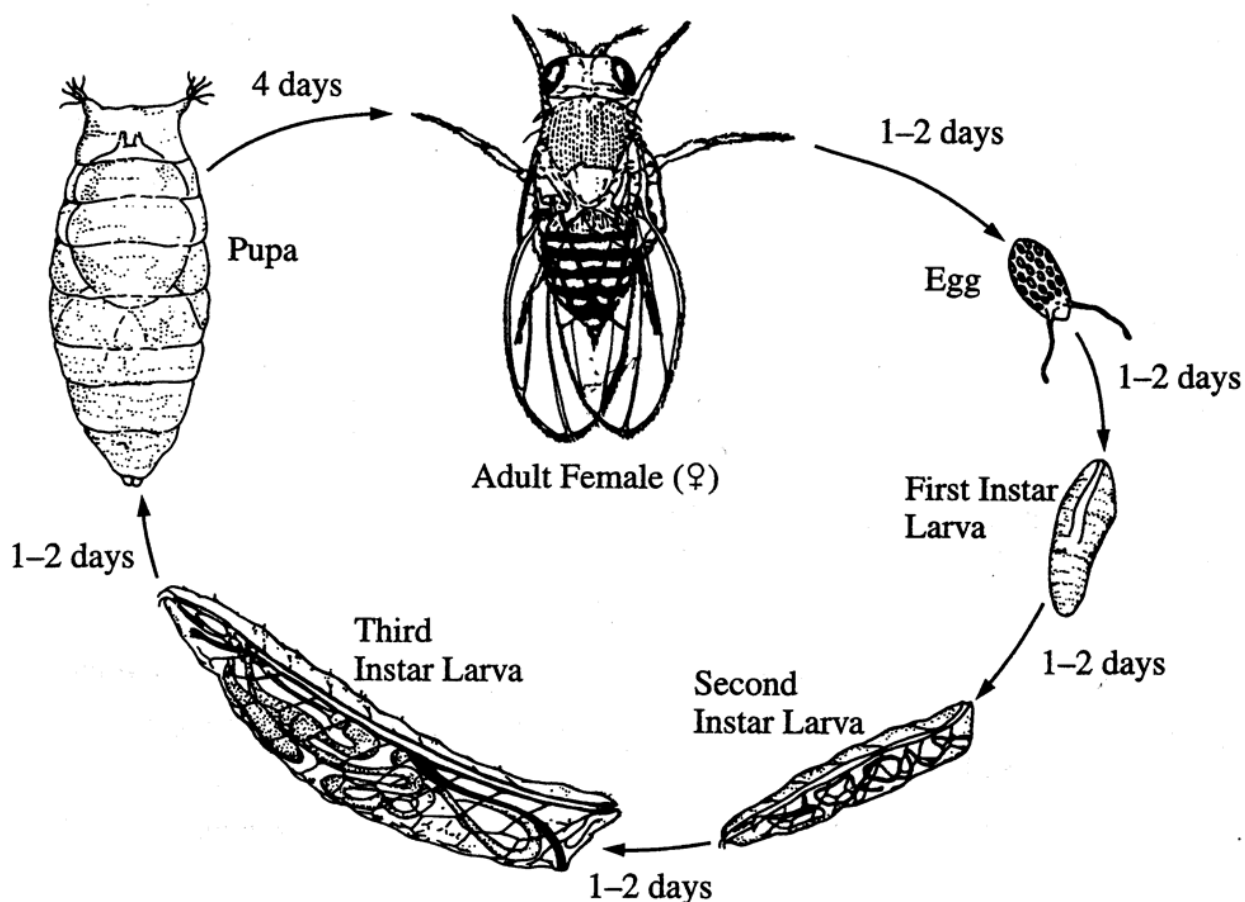


Figure 1: The Life Cycle of *Drosophila melanogaster*

It is important to realize that a number of factors determine the length of time of each stage in the lifecycle. Of these factors, temperature is the most important. At room temperature (about 22 – 25°C), the complete cycle takes ten to twelve days.

The genetics experiment will be carried on for several weeks. *Drosophila* with well-defined mutant traits will be assigned to you and your group. Common inheritance patterns used for these experiments are simple autosomal and sex-linked patterns. To make these experiments interesting and challenging, you will not be told the inheritance pattern you are studying. You are responsible for making observations and accurate record keeping concerning what happens as mutant traits are passed from one generation to the next.

SAFETY CONCERNS

Flies are anaesthetized with a material called Fly Nap. Do not inhale Fly Nap. Although safe to work with, continued exposure can be irritating to your eyes and nasal passages. This can be avoided by maintaining a 'reading' distance between you and the flies. Do not work too closely with the flies.

Procedure:

1. **First Week:** Obtain a vial of wild type flies. Practice immobilizing and sexing these flies. Examine these flies with regard to eye color and wing shape.
2. Distinguish male flies from female flies by looking of the following characteristics with a dissecting microscope. Males are usually smaller than females. Males have dark, blunt, posteriors, whereas the females have lighter pointed posteriors. The males have sex combs, which are groups of black bristles on the uppermost joint of the forelegs, whereas the females do not. See **Figure 2**.

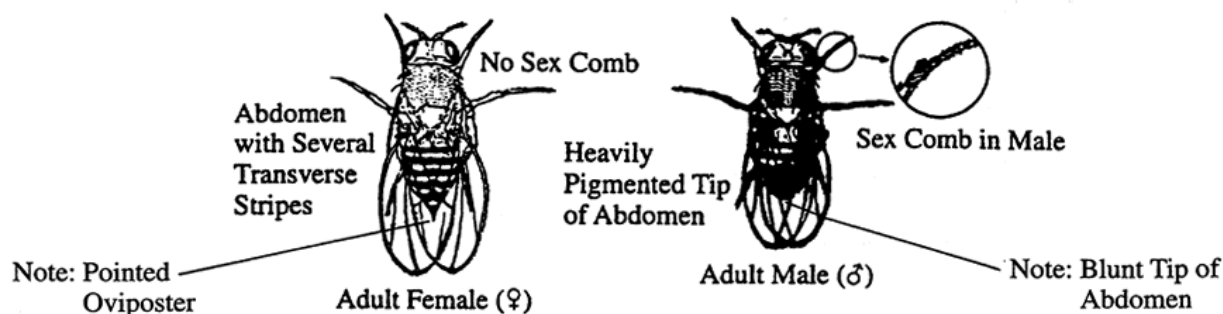


Figure 2: Female and male characteristics of *Drosophila*

3. Obtain a vial containing pairs of experimental (mutant) flies. Record the number/phenotype of the vial. This number will serve as a record as to which flies you have obtained. Note whether the mutation(s) is/are associated with the males or the females. Identify the mutation(s) and give it/them a made-up name and gene symbol. Confirm your findings with the teacher. These flies are the parental generation (P) and have already mated. The females should have already laid eggs on the surface of the culture medium. Follow the instructor's instructions on how to set up and label a culture tube. Take 3 – 5 flies of each gender and place in your sub-culture vial. Do the same for the wild-type flies. An example of the label is as follows: if a curly-winged female is crossed with a straight-winged male, the label could read: "curly ♀ X straight ♂". Also be sure to label the vial with your group name, date, and vial number. Place the vial in the 'nursery'.
4. **Second—Third Week:** By this time, you should have eggs or larvae on the side of your subculture vial. Immobilize and remove the adult parent flies. Place the parents in the morgue (an alcohol filled jar).
5. **Third Week:** As soon as you start seeing adults (ideally on the *morning* that they hatch), you must isolate the females before they start to mate. For example, when you notice a few, new adults for the first time, isolate 3 – 4 females and place in a new, separate vial. Keep your original subculture in the nursery as you might need them later on.
6. Check your new vial every day to see if larvae are crawling around. If so, then your females were very promiscuous in their youth and you have to start over. If, after 5 days or so, you have no larvae, then you can use them as one half of your parental (P) generation.
7. Take your virgin female vial, immobilize them, and place 3 – 5 immobilized males (of the appropriate stock – depending on which mutation you are studying) into the vial. This is your parental (P) generation. Record appropriate information on the label and also on the ***Drosophila* Record Sheet.**
8. **Fourth Week:** Observe your parental generation vial. You should start to see larvae crawling around.

9. **Fourth—Fifth Week:** Now, you should have eggs and larvae on the side of your subculture vial. Immobilize and remove the adult parent flies before the next generation hatches. Place the parents in the morgue (an alcohol filled jar).
10. **Fifth Week:** Begin observing the F_1 flies. Immobilize and examine all the flies. Record their sex and the presence or absence of the mutation(s) (as observed in the parental flies) in the ***Drosophila* Record Sheet**. You now can make your hypothesis about the inheritance pattern for this mutation after observing these flies! Consider the conclusions that can be drawn from these data. Place 5 or 6 mating pairs of F_1 flies in a fresh culture bottle and the rest of the flies in the morgue. For this cross the females need not be virgin flies. Label the new vial " $F_1 \times F_1$ ". Also, label the vial with symbols denoting the cross, the date, and your name.
11. **Sixth—Seventh Week:** Observe your F_1 generation vial. You should start to see larvae crawling around. Remove the F_1 adult flies from the vials and place them in the morgue. The F_2 generation flies are the eggs and/or larvae in the vial. Place the vial back into the nursery.
12. **Seventh Week:** Once you start to see adults hatching, begin removing the F_2 flies. Record their sex and the presence or absence of the mutant phenotypes (as observed in the parental flies) in your ***Drosophila* Record Sheet**. After they have been counted, place the flies in the morgue. The more F_2 flies collected, the more reliable the data will be. You will have to collect F_2 flies over a 3 or 4 day period. Try to collect at least 100 flies.

Hints For Your Lab Report:

The Introduction section of your lab should have (but not be limited to) your hypothesis on the inheritance pattern expressed by your cross of experimental flies determined after examining the F₁ generation. **Use a Punnett square** to demonstrate how you came up with this hypothesis (P x P for both mutations). Also, **have Punnett squares** showing the expected ratios of each F₁ cross (F₁ x F₁ for both mutations). This will establish the expected numbers of offspring for each phenotype of the F₁ and F₂ generation for your Chi-Square analysis.

The Results section of your lab report will include:

- A written summary of all data. For each cross, you should mention total numbers observed. You should **state** the chi-square value obtained and the p-value that corresponds with it **without support or defense of your** hypothesis. That comes later. As for all data on the Record Sheets and the Chi-square Calculations, see the last section below...

The Discussion section of your lab report will include your discussion of the significance of your Chi-Square analysis results. ***This is essential! Do not forget to properly explain what your calculations reveal!*** **Now you must do your hypothesis defense!**

SPECTIAL: You will have an Appendix A and an Appendix B section in this lab. Appendix A should be all of the ***Drosophila Record Sheets*** – one for each mutant studied. These can be referenced when you summarize these in the Results section. Appendix B should be the chi-square calculations/tables of your F₂ results (as shown below). You should complete a Chi-square analysis for each mutation that you studied...

Chi-Square Analysis Chart – You should be using this for EACH of your genetic crosses based on your Punnett Square hypothesis!

Phenotype	# Observed (o)	# Expected (e)	(o-e)	(o-e) ²	$\frac{(o-e)^2}{e}$
					$\chi^2 =$
					p-value =

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Group #: _____ Group Members: _____

Drosophila melanogaster Data					
wild X <hr/>		P female phenotype	P male phenotype	P generation pairing date	P generation removal date
		F ₁ female phenotype	F ₁ male phenotype	F ₁ generation pairing date(s)	F ₁ generation removal date(s)
		F ₂ generation phenotype data*			
vial #	date counted	wild female	wild male	_____ female	_____ male
	Total				
	Inferred Ratio**				

* It is a good idea to list out the phenotypes for both females and males - you never know if your inheritance pattern will show sex linkage – you always can remove the male – female option and just count wild-type vs. mutant if you are sure that there is no sex linkage.

** This will be compared to your Punnett square hypothesis - modified for distribution between the sexes too...

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[illegible]

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		F ₁ female phenotype	F ₁ male phenotype	F ₁ generation pairing date(s)	F ₁ generation removal date(s)
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