

LABORATORY 7. GENETICS OF ORGANISMS

OVERVIEW

In this laboratory you will use living organisms to do genetic crosses. You will learn how to collect and manipulate the organisms, collect data from F_1 and F_2 generations, and analyze the results from a monohybrid, dihybrid, or sex-linked cross. The procedures that follow apply to fruit flies; your teacher may substitute other procedures using other organisms.

OBJECTIVES

Before doing this laboratory you should understand:

- chi-square analysis of data, and
- the life cycle of diploid organisms useful in genetics studies.

After doing this laboratory you should be able to:

- investigate the independent assortment of two genes and determine whether the two genes are autosomal or sex-linked using a multi-generation experiment, and
- analyze the data from your genetic crosses using chi-square analysis techniques.

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* over the last 50 years has resulted in a wealth of reference literature and a knowledge about hundreds of its genes.

The Life Cycle of *Drosophila*

It is important to realize that a number of factors determine the length of time of each stage in the life cycle. Of these factors, temperature is the most important. At room temperature (about 25°C), the complete cycle takes 10 to 12 days.

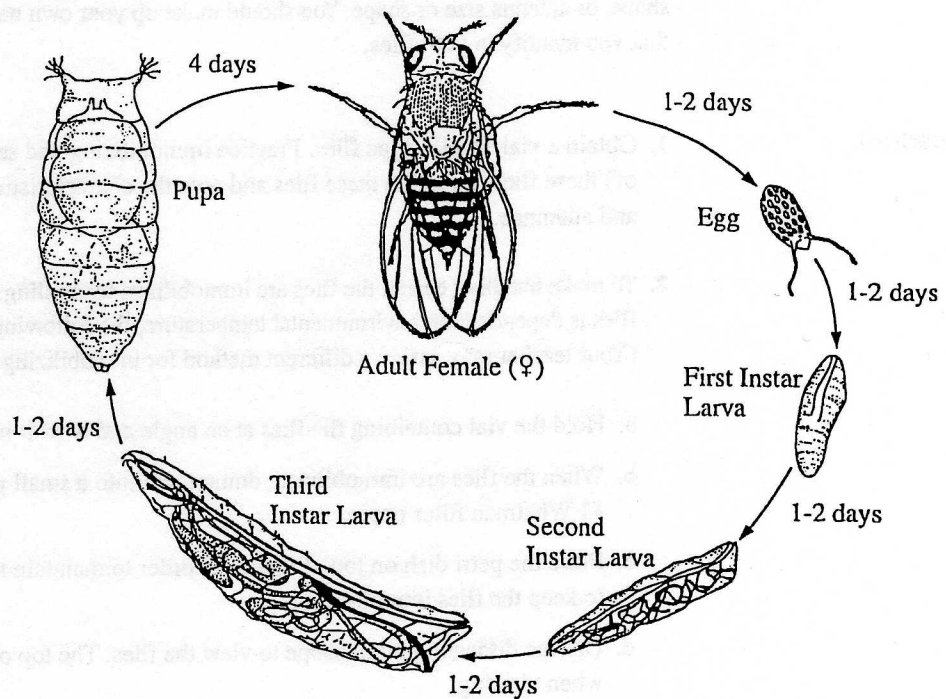
The eggs. The eggs are small, oval shaped, and have two filaments at one end. They are usually laid on the surface of the culture medium and, with practice, can be seen with the naked eye. The eggs hatch into larvae after about a day.

The larval stage. 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 may be seen readily under low-power magnification after proper staining.

The pupal stage. When a mature larva in a laboratory culture is about to become a pupa, it usually climbs up the side of the culture bottle or on to the paper 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.

The adult stage. 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 the adult fly. They live a month or more and then die. A female does not mate for about 10 to 12 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 (virgins).

Figure 7.1: The Life Cycle of *Drosophila melanogaster*



Design of the Exercise

This genetics experiment will be carried on for several weeks. *Drosophila* with well-defined mutant traits will be assigned to you by your teacher. You are responsible for making observations and keeping records concerning what happens as mutant traits are passed from one generation to the next.

You will be assigned to study a certain mode of inheritance using particular genetic crosses of flies having one or two mutations. The modes of inheritance most commonly used are:

1. *Monohybrid*. In these experiments, the mode of inheritance is determined when a single contrasting pair of characteristics is involved.
2. *Dihybrid*. In these experiments, the mode of inheritance is determined when two pairs of contrasting characteristics are considered simultaneously.
3. *Sex-linked*. In these experiments, the mode of inheritance is determined when the mutant characteristic is associated with the X chromosome.

To make these experiments interesting and challenging, you will not be told the mode of inheritance, nor the name for the particular mutation(s) you are studying. Study the wild-type flies (both male and female) until their phenotypic characteristics are familiar. Flies having one or two mutations can then be identified by making comparisons with the wild-type flies. The most commonly studied mutations are in eye color or shape, bristle number or shape, wing size or shape, or antenna size or shape. You should make up your own name for the particular mutation(s) that you identify in your flies.

Procedure

1. Obtain a vial of wild-type flies. Practice immobilizing and sexing (determining the gender of) these flies. Examine these flies and note the characteristics of their eyes, wings, bristles, and antennae.
2. To make handling easier, the flies are immobilized by chilling. Since the activity level of the flies is dependent on environmental temperature, the following steps immobilize the flies. (Your teacher may assign a different method for immobilizing the flies.)
 - a. Hold the vial containing the flies at an angle and twirl it in ice for several minutes.
 - b. When the flies are immobilized, dump them into a small plastic petri dish containing a #1 Whatman filter paper.
 - c. Place the petri dish on top of the ice in order to maintain the cool temperature necessary to keep the flies immobilized.
 - d. Use the dissecting microscope to view the flies. The top of the petri dish can be on or off when viewing.
3. Distinguish male flies from female flies by looking for the following characteristics:
 - a. Males are usually smaller than females.
 - b. Males have dark, blunt abdomens, and the females have lighter, pointed abdomens.
 - c. Only the males have sex combs, which are groups of black bristles on the uppermost joint of the forelegs.

