The Genetics of *Drosophila* Project
A Fruit Fly "Crash Course" Review

Directions:
After reading your packet of Fruit Fly information, please answer the following review questions.

1. Explain the 5 learning objectives that you will master after completing the Fruit Fly Project.

2. Draw a picture of the Fruit Fly Life Cycle and briefly explain each stage.

3. Discuss 4 clues used to determine the gender of a Fruit Fly?
   MALES FEMALES

4. Discuss 5 things that might jeopardize the health of your Fruit Fly cultures.

5. Explain the technique to anesthetize a Fruit fly culture using FlyNap.

6. Explain the 3 types of breeding crosses you will be exploring in this project.

7. What do Fruit Flies eat?

8. How long do adult Fruit flies typically live?

9. How many eggs will some females lay over 10 days?

10. How long is a typical life cycle and what is the main factor that influences the timing?

11. Explain how to distinguish an adult Fruit Fly from one that has recently emerged from the pupa stage?

12. What signs would you look for indicating that your Fruit Fly cultures are successfully growing?

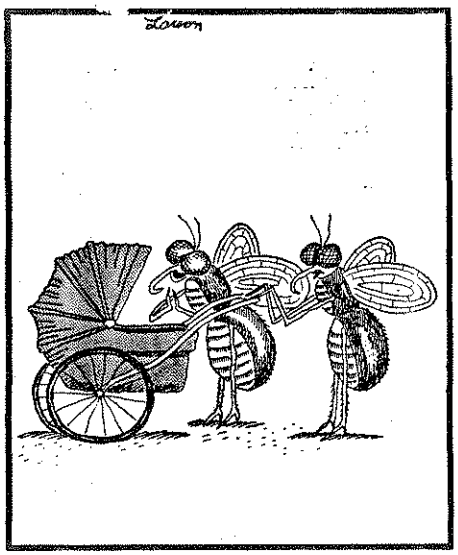
13. Explain 1 NONharmful side effect you may witness using FlyNap?

14. How long should you anesthetize a Fruit Fly vial and how long do they sleep?

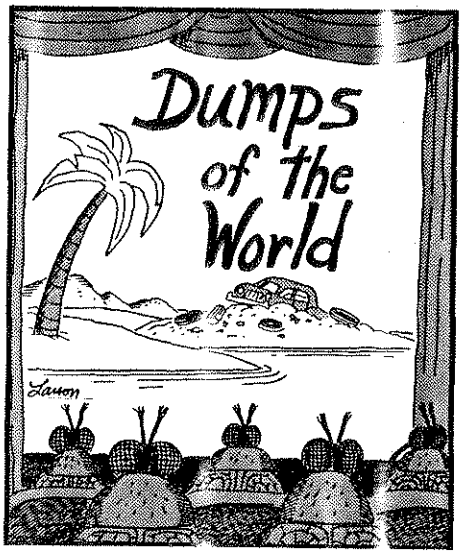
15. What is the haploid chromosome number for Fruit Flies (how many different kinds or pairs)?

16. Explain how you would control mating to be sure that a female would only mate with a Fruit fly with a different allele for a trait and not with other Fruit flies that carry the same allele trait?

17. What kinds of Fruit fly traits or features may show mutations that you might look for in this project?



"Oh my!...What a *cute* little maggot!"



Fly travelogues

"Yeah, yeah, buddy, I've heard it all before: You've just metamorphosed and you've got 24 hours to find a mate and breed before you die....Well, get lost!"

LABORATORY 7. GENETICS OF *DROSOPHILA*

OVERVIEW

In this laboratory you will use fruit flies to do genetic crosses. You will learn how to collect and manipulate fruit flies, collect data from F_1 and F_2 generations, and analyze the results from a monohybrid, dihybrid, or sex-linked cross.

OBJECTIVES

At the completion of this laboratory you should be able to

- conduct a genetics experiment for a number of generations
- compare predicted results with actual results
- explain the importance of chi-square analysis
- design genetic crosses in an experiment to illustrate independent assortment and sex-linkage
- discuss the life cycle of the fruit fly, recognize the sex of fruit flies, and recognize several types of classic mutations

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, may be immobilized readily for examination and sorting, and has many types of hereditary variations that can be recognized 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.

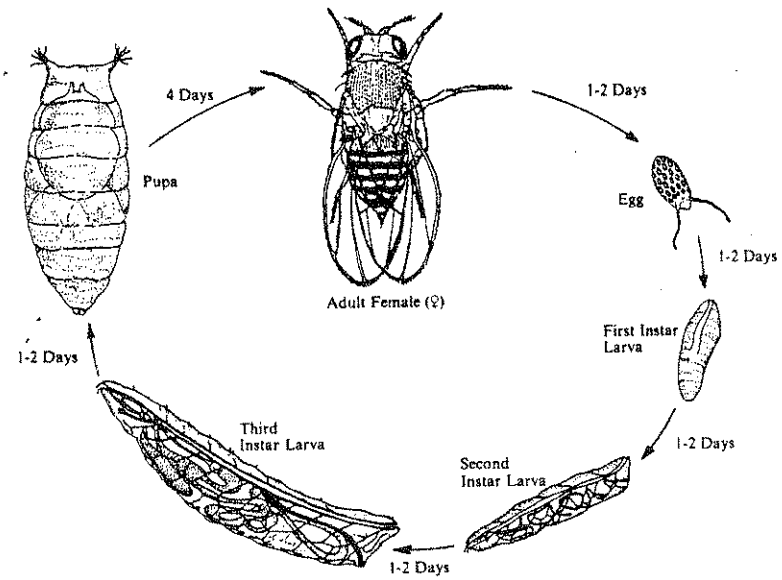
The eggs. The eggs are small and ellipsoid and have two filaments at one end. They are usually laid on the surface of the culture medium and, with practice, can be seen with the naked eye. The eggs hatch into larvae after about a day.

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 channel 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 onto the paper strip provided in the culture. The last larval covering then becomes harder and darker, forming the pupa 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 pupa 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. Hence, to ensure a controlled mating, it is necessary to use females that have not mated before.

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.

Assignments will be made for particular genetic crosses of flies having one or two mutations. The types of crosses available are:

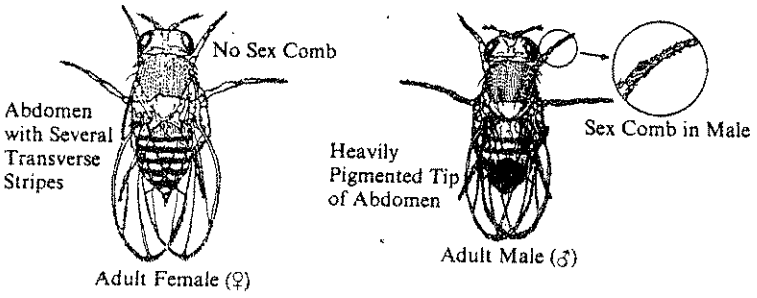
1. *Monohybrid*. In these experiments, the method of inheritance is determined when a single contrasting pair of characteristics is involved.
2. *Dihybrid*. In these experiments, the method of inheritance is determined when two pairs of contrasting characteristics are considered simultaneously.
3. *Sex-linked*. In these experiments, the method 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 given any information about the types of crosses in the experiments, and the name for the particular mutation(s) assigned will not be revealed. Study the wild-type flies (both male and female) until their phenotype characteristics are familiar. Flies having one or two mutations can then be identified by making comparisons with the wild-type flies. The mutation(s) most likely will be in eye color or shape, bristle number or shape, wing size or shape, or antennae 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 these flies. Examine these flies with regard to eye color and wing shape.
2. To make handling easier, the flies are immobilized by chilling. The activity of the flies is dependent on environmental temperature. The following procedures can be used to 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 the ice (in the bucket) 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 a bowl of 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. 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.

Figure 7.2: Female and Male *Drosophila*



4. Obtain a vial containing pairs of experimental flies. Record the cross number of the vial. This number will serve as a record as to which cross you have obtained. These flies are the parental generation and have already mated. The females should have already laid eggs on the surface of the culture medium. The eggs (or maybe larvae now) represent the first filial, F_1, generation and will be emerging from their pupa cases in a week.

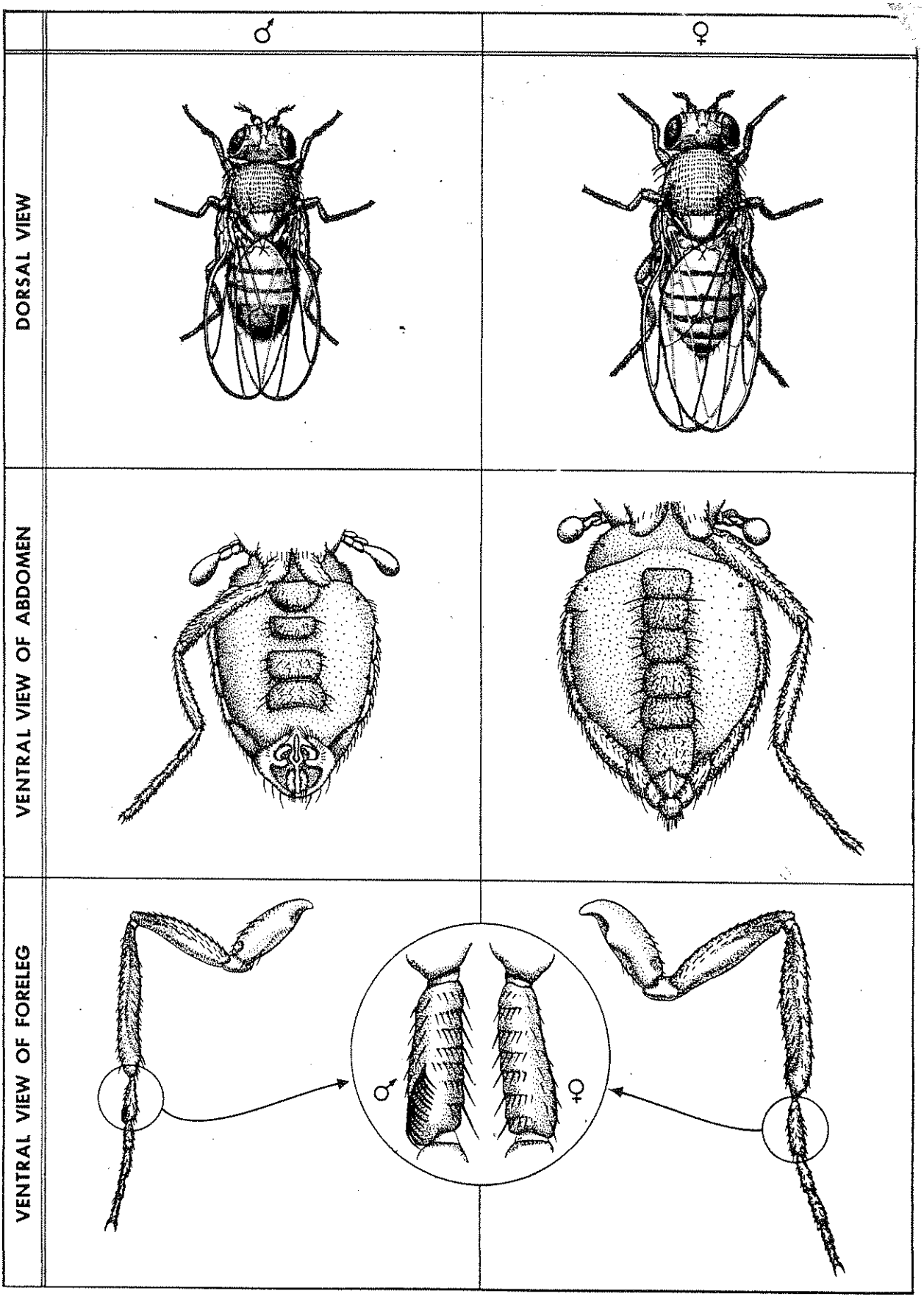


Figure IV–4. Male and female *Drosophila*. Left, male. Right, female.

Introduction

Basic genetic mechanisms arose early enough in primitive organisms or were so superior to alternatives that they are shared by most organisms. It is possible to study the principles of genetics in one or a few organisms, and to gain understanding of the mode of inheritance in many.

The animal most widely used for genetic studies is the common fruit fly, *Drosophila melanogaster*. The fly is easily cultured, and its generation time is only two weeks at 21° C. One female may lay as many as 500 eggs in 10 days. Because *Drosophila* is small, cultures occupy little space; however, the fly is large enough for rapid notation of mutant characters.

The fruit fly has been the subject of genetic studies since about 1909. Myriad spontaneous mutations have been found and many others have been induced with radiation. *D. melanogaster* has a tremendous number of genes for study, but practicality dictates the selection of a few readily identifiable phenotypes for use in instruction.

Culturing

When cultures arrive, remove the caps but leave the plugs in place. Put the cultures in a clean location not exposed to direct sunlight. Cultures should be kept at 20° to 25° C. While the life cycle of *Drosophila* may be shorter at higher temperatures, it is generally better to carry the cultures at 20° to 21° C. Higher temperatures are conducive to the growth of bacteria, fungi, and mites; lower temperatures greatly slow development of the flies.

The minimal equipment (Fig. 1) for raising fruit flies and for making crosses includes culture vessels, plugs, and medium; marking pencil or labels; anesthetic; white background (card) for examining flies; fine brush; bright, cool light; and magnifying glass, or (preferably) a widefield stereomicroscope.

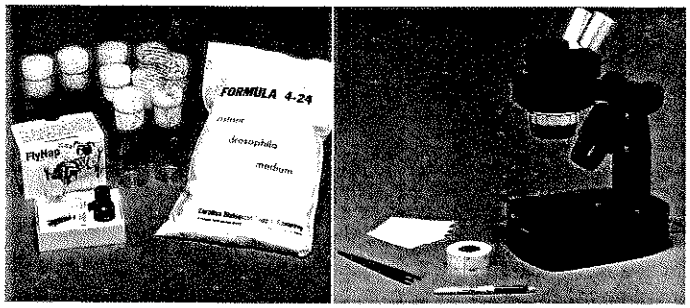


Figure 1 Minimal equipment for studying fruit flies.

Culture Vessels

Figure 2 Formula 4-24® Instant Drosophila Medium is easily prepared.

Transparent vials or bottles of glass or plastic can be used as culture vessels for *Drosophila*. For most classrooms and research, the optimal size of the vessels is 50 to 100 cm^3. Vessels should be clean, but they need not be sterilized when the medium is properly prepared. Plastic vials should not be autoclaved, and may be used as they come from our shipping carton.

Plastic (polyurethane) foam or nonabsorbent cotton is used for plugs. Plastic plugs are neater, easier to handle, and last longer than cotton plugs. Plastic plugs may be used directly from the shipping package. Both cotton and plastic plugs should be autoclaved before reuse.

Continued use and pressure may compress the plastic plugs. Compressed plugs will expand when wetted with isopropyl alcohol. Reexpanded plugs should be free of fumes before they are used with cultures.

Plastic plugs can be held in place with plastic breathing caps. The caps are useful for controlling moisture and for securing cultures against accidental opening.

Media

Fruit flies can be raised on a variety of fermenting plant materials. The first cultures were raised on grapes or ripe banana with yeast added, but excess moisture and molding were serious problems. Cooked preparations with agar added resulted in media with greater firmness. Later, the use of mold inhibitors in *Drosophila* media greatly simplified the culture of fruit flies.

INSTANT DROSOPHILA MEDIUM: The ultimate development in fruit fly culture is Formula 4-24® Instant Drosophila Medium which needs neither cooking nor sterilizing. Each unit of about 1 liter is suffiecient for preparing 50 to 70 cultures. Equal volumes of Instant Drosophila Medium and cool tap water are dumped into a vial and a few grains of dry viable yeast are sprinkled on top (Fig. 2). The metabolic byproducts of yeast include CO_2. Large amounts of yeast in a culture can produce enough CO_2 to render Drosophila sterile or even cause death. 6 to 10 grains per culture are sufficient. After one minute, flies can be introduced and the vial plugged. In half-pint bottles and larger vessels it is generally advisable to use less water than the volume of Instant Drosophila Medium.

Early in the history of *Drosophila* culture, it became a practice to place a paper strip in the culture medium to hold anesthetized flies so they would not stick in soft or wet medium. As better media were developed, those in the habit of using the paper inserts continued to do so with the idea that paper served other uses, such as a surface for pupation. The larvae pupate on the sides of the culture vessel just as well as on paper, and often the paper collapses into the medium, drowning the pupae. If paper is used in *Drosophila* cultures, potential problems with molding can be reduced by soaking the paper in 0.1% Tegosept in isopropanol and allowing it to dry before use. It is not necessary to use paper inserts in *Drosophila* cultures with Formula 4-24®. The cultures of fruit flies that we ship contain plastic inserts to hold the medium in place during shipment.

COOKING MEDIUM: Mold inhibitors (ours is methyl p-hydroxybenzoate, Tegosept-M) are used in *Drosophila* culture media to reduce the growth of undesirable fungi that may contaminate cultures and can retard the development of the flies. It is important not to use more mold inhibitor than necessary as it also inhibits the growth of yeast and flies.

Various published formulae call for concentrations of Tegosept ranging from 0.07% to 0.2%. Although most references call for dissolving the mold inhibitor in alcohol, we have found it simpler to add the mold inhibitor powder to boiling water.

Dissolve 15 g of agar and 1 to 2 g of Mold Inhibitor (87-6161) in 500 ml of boiling water. Add 130 ml of sulfur-free molasses and again bring to boiling. Mix 100 g of dry yellow cornmeal (fine grain) with 250 ml of cold water; pour this mixture into the boiling solution and cook for a few minutes. While the medium is still thin enough to pour easily, pour it 2 to 3 cm deep in the culture vessels. Sterilizing is not necessary.

Cooked Drosophila Media (Five alternate formulae)

Ingredient	Amount				
Water (tap)	750 ml	750 ml	750 ml	750 ml	500 ml
Tegosept	1 g	1 g	1 g	1 g	1 g
Agar	15 g				15 g
Molasses (sulfur-free)	130 ml	100 ml	100 ml	30 ml	
Cornmeal (yellow)	100 g		150 g	60 g	
Cream of wheat		100 g			
Oatmeal (not instant)			16 g	60 g	
Brewer's yeast				10 g	
Banana pulp					500 ml

Bacterial Infection

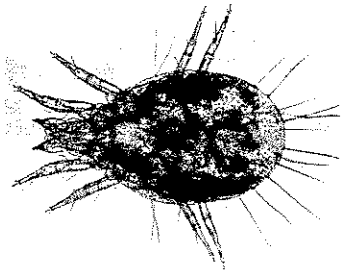


Figure 3 A typical long-haired mite.

Bacterial contaminants sometimes infect *Drosophila* cultures, causing reduction in fly hardiness, sterility, and often death. The infections appear as a gray or drab yellow-green viscous sheen on the medium surface. Periodic cleansing of laboratory work space and utensils and the use of filtered or distilled water will aid in preventing accidental introduction of bacteria into the cultures. If infected cultures occur, daily applications of an aqueous solution of 0.5 percent penicillin G or 0.1 percent tetracycline should irradicate the infection. If transferred, fruit flies from an infected vial will carry the bacteria to other cultures; therefore, isolate infected cultures while treating them. If the infection persists or kills all immature and mature flies, discard or sterilize the culture vessel and plug.

Controlling Mites

Drosophila cultures sometimes become infested with mites (Fig. 3). Any infested culture should be removed immediately from the laboratory and sterilized. The best prevention against an infestation of mites is cleanliness. All utensils and working areas should be kept clean. A culture should not be kept longer than one month.

Lindane-treated shelf paper will kill mites which walk across it. If culture vessels of *Drosophila* are set on such paper, mites cannot cross the paper and the fruit flies in the culture vials are in no way injured. Mites can also be controlled by treating work surfaces with 1 part benzyl benzoate in 5 parts isopropanol.

Figure 4 Fruit fly egg.

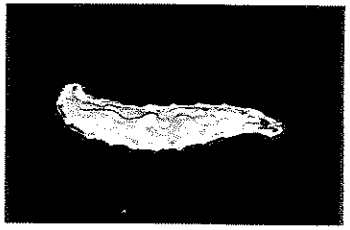


Figure 5 Fruit fly larva.

Life Cycle

Figure 6
Fruit fly pupa.

Figure 7
Fruit fly adult.

There are four distinct stages in the life of the fruit fly: egg (Fig. 4), larva (Fig. 5), pupa (Fig. 6) and adult (Fig. 7). At 21° C a fresh culture of *D. melanogaster* will produce new adults in two weeks; eight days in the egg and larval stages, and six days in the pupal stage. The adult fruit flies may live for several weeks.

The day after the egg is laid, the larva hatches. The larva molts twice; that is, it sheds the cuticle, mouth hooks, and spiracles. During the periods of growth before and after molting, the larva is called an instar. The fruit fly has three instars. The cuticle of the third instar hardens and darkens to become the puparium.

Metamorphosis occurs within the puparium. The pupa begins to darken just prior to the emergence of an adult fly. About one day before emergence, the folded wings appear as two dark elliptical bodies, and the pigment in the eyes is visible through the puparium.

When metamorphosis is complete, the adult emerges (ecloses) by forcing its way through the anterior end (operculum) of the puparium. At first the fly is light in color, the wings are unexpanded, and the abdomen is long. In a few hours the wings expand, the abdomen becomes more rotund, and the color gradually darkens.

Two days after emerging, a female can start laying eggs. After maturity, fruit flies are fertile as long as they live.

Virgin Flies

A female *Drosophila* can store and use the sperm from a single insemination for the major portion of her reproduction. Thus, it is necessary to select virgin females for genetic crosses. The males need not be virgin.

Older males will mate with newly emerged females. Therefore, it is extremely important that all adult flies be removed (cleared) from a culture 8 to 12 hours before it is used for the selection of virgin females.

When pupae appear to be ready for emergence (Fig. 6), clear all adult flies from the culture vessel as late in the evening or as early in the morning as practical. The flies tend to emerge in greater numbers during the early part of the day.

To insure virginity, females should be selected before they are 12 hours old. The virginity of the flies can be tested by keeping the females by themselves in a culture vial for 3 to 4 days before transferring them to another vial with the males. If larvae appear in the vial that contained only the females, then the females were not all virgin and the cross will not be meaningful.

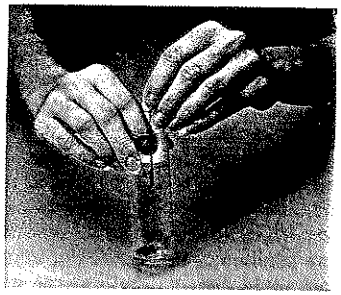


Figure 8 Young fruit flies can be anesthetized in an empty culture vial with FlyNap®.

Figure 9 FlyNap® safely anesthetizes *D. melanogaster* for 50 minutes and longer.

Anesthetizing

Investigators have used many means to immobilize fruit flies for examination. They have crowded them into constricted glass tubes. They have chilled them. They have knocked them out with ether and methoxyflurane, and they have kept them in a continuous flow of carbon dioxide. When bulky or expensive apparatus is not available, the novice kills or loses many flies with these antiquated approaches to immobilizing fruit flies. Now, even elementary-grade students can easily, safely, and inexpensively anesthetize fruit flies with our FlyNap Kit (patent No. 4,224,898).

FlyNap®

With FlyNap (Fig. 8) fruit flies can be anesthetized in an empty vial (Fig. 8 & 9), in an anesthetizer, or while they are still in their culture vial. FlyNap is safer than ether for both the students and the flies. It is not explosive like ether and a single exposure to FlyNap safely anesthetizes **young** *D. melanogaster* for 50 minutes to several hours.

The anesthetic wand is a primary component of the 17-3010 FlyNap Kit. Dip the absorbent end of the wand into the FlyNap. Tap the bottom of the culture vial on the tabletop to knock the flies to the bottom of the vial. With one finger, push the plug slightly to one side. Remove the wand from the FlyNap and quickly stick the anesthetic end into the culture vial beside the plug so the anesthetic tip is below the plug. Keep the culture vial upright with the wand in place. Watch the flies closely and remove the plug and wand when the flies are anesthetized (2 minutes in an empty vial, 4 minutes in a vial with medium). Immediately spill the flies onto a card for study. When replugging the culture vial, be careful not to insert a part of the plug that may have FlyNap residue on it.

The length of time the flies remain anesthetized depends on the amount of FlyNap on the wand, and on the number and age of the flies in the culture vial. The FlyNap wand meters the correct amount of anesthetic for our standard Drosophila Culture Vial. Less anesthetic is needed for smaller vessels; more anesthetic is needed for larger ones. Use two wands with FlyNap for 250-ml vessels. Flies can be anesthetized in 2 minutes in a Carolina Anesthetizer charged with 1 ml of FlyNap.

In the amount transferred on the wand, FlyNap has no ill effect on *Drosophila* eggs, larvae, pupae, or **young** adults. Under moist culture conditions anesthetized flies might stick to the culture medium or become sticky from contact with the medium. If you think that could happen, the flies can be transferred to an empty culture vial and then anesthetized within 2 minutes. Some flies may exhibit light trembling of the legs and/or wings immediately after being anesthetized with FlyNap; this is acceptable and will stop after 1 or 2 minutes.

Ether

To use the Carolina Anesthetizer (Fig. 10), remove the hollow stopper from the top and remove the cap from the bottom. Fill the hollow stopper one-third full with ether for anesthesia. Pour the ether on the foam pad in the bottom of the anesthetizer. Replace the cap and put the stopper back in the top of the anesthetizer. *Caution: Ether is highly flammable.*

If you have not etherized flies before, read this paragraph and use an empty culture vial to practice transferring the flies. When ready to etherize flies, remove the stopper from the top of the anesthetizer. Tap the bottom of the culture against the palm of your hand to knock the flies down. Remove the plug from the culture. Invert the culture over the anesthetizer and tap the flies into the chamber. After the adults have been tapped into the chamber, quickly right the culture vessel so its base covers the top of the anesthetizer. Plug the culture vessel. Tap the base of the anesthetizer on the table, remove the culture vessel, and plug the anesthetizer with the stopper. Watch the behavior of the flies in the chamber. About 20 seconds after the flies stop moving, they can be dumped onto a white card for examination with a hand lens or stereomicroscope.

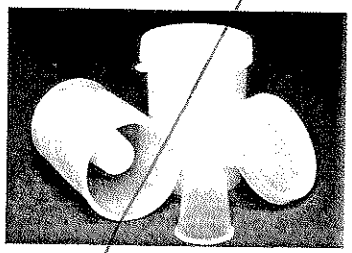


Figure 10 *Drosophila* Anesthetizer.

When the novice etherizes fruit flies, he often has a tendency to over-etherize them; therefore, the Carolina Anesthetizer is made to release the ether slowly.

Individuals experienced in handling fruit flies may wish to etherize them rapidly. It is a simple matter to speed up the release of ether into the inner etherizing chamber by adding holes with the end of a red-hot teasing needle (with no ether in the anesthetizer).

Usually the flies remain etherized for 5 to 10 minutes. With the stopper removed, the anesthetizer can be inverted over the flies to re-etherize them if necessary. The flies are killed or sterilized if they are re-etherized too many times in a short period.

Flies that extend their wings and legs at right angles to their bodies are over-etherized and should be considered dead. Pale-colored flies with incompletely expanded wings have just emerged from the pupal case. As flies of this age may be sterilized by ether, they should be avoided in selecting for a cross.

Sorting and Selecting

The anesthetized flies should be placed in a row on a white card. The flies are moved about with a teasing needle, a fine brush, or any suitable tool. The flies should be examined with a stereomicroscope at a magnification of at least 12X to 15X unless the strains carry special sex markers. With the flies strung out along the card, one type can be sorted to one side and a second kind to the other side.

Flies that are to be discarded are dropped into a morgue—a jar of alcohol or oil, or a jar of water and detergent.

Sexing

In selecting flies for genetic mating, it is absolutely essential that the sex of each fly be properly identified. The sex of *Drosophila* is most reliably

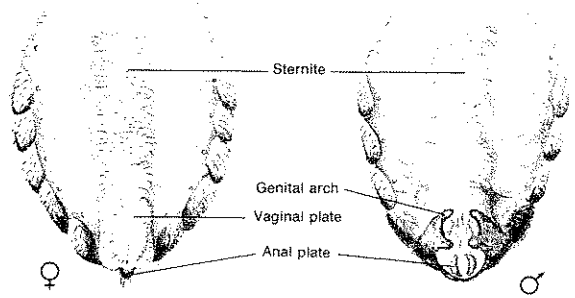


Figure 11 Ventral posterior view of female and male fruit flies.

distinguished through examination of the genital organs with magnification (Fig. 11). The male genitalia are surrounded by heavy dark bristles which do not occur on the female. This characteristic is quite distinct even in a fly that has just emerged from the pupal case (puparium).

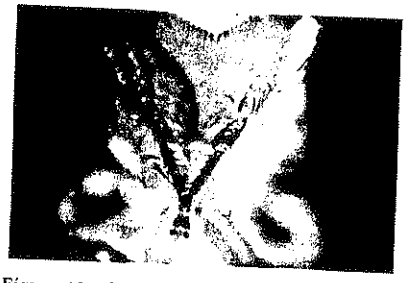


Figure 12 Sex combs on front legs of male fly.

In older flies the posterior part of the abdomen is quite dark in males and considerably lighter in females. The tip of the abdomen is more rounded in males than in females, and the female has more sternites. In general, male fruit flies are smaller than females of the same strain, but size is not a reliable character for sorting the sexes.

With care the sexes can be distinguished by examination of the front legs. There are sex combs (Fig. 12) on the front legs of the male but not on those of the female. This characteristic can even be used to identify the sex of the individual while it is still within the pupal case.

Mating and Counting

Every vial should be clearly labeled with the characteristics of each parent. The first cross between strains is called the parental generation, the P_1. The progeny of the first cross is the first filial generation, the F_1. The next generation is the F_2 and so on.

The virgins selected for a cross should be swept onto a card that has been folded down the center. About six virgins are tapped into a culture vial with about the same number of males of another strain. For reciprocal crosses, additional cultures are set up with the sex of each strain reversed.

About 7 to 10 days after a cross is started, the parents should be removed. This is to preclude breeding between generations and to avoid confusion when counts are made.

About six pairs of flies, which need not be virgin, are chosen from the F_1 and placed in a fresh culture vial to produce an F_2. After 7 to 10 days the F_1 flies should be removed from the culture.

A count of the F_2 progeny should start the day after emergence of the new generation. Generally on the first day of emergence, a culture will produce more females than males. On successive days the proportion of males tends to increase so that the sex ratio balances.

The progeny should be anesthetized and counted every other day for about 10 days. Counts made during less than 10 days may omit individuals with slow developmental rates because of their sex or some mutation. Counts beyond 10 days run the risk of including flies of the next generation. Once counted, the flies should not be returned to the same culture vessel.