

Drosophila melanogaster Culture Techniques (1/2002)

The basic principles of genetics are equally applicable to most plants and animals, not to mention other organisms. The general principles of gene transmission, linkage, sex determination, genetic interactions; molecular, biochemical and developmental genetics, chromosomal aberrations, penetrance and expressivity, and evolutionary change may all be admirably demonstrated by using the fruit fly, Drosophila melanogaster. D. melanogaster and its hundreds of related species, have been extensively studied for decades, and there is a extensive literature available.

This fly is a favorite laboratory animal for a number of reasons: (1) It is easy to culture at low expense. (2) It has a short life cycle (10 days at 25°C). (3) Its high reproductive potential allows the investigator to easily obtain sufficiently large samples for any amount of statistical accuracy. (4) Large populations may be maintained for generations in relatively small population cages. (5) A large number of genetically defined mutants are available which define most aspects of the fly's biology. Its large salivary gland chromosomes provide for fairly precise cytogenetic analyses.

Drosophila melanogaster life cycle - The principle stages of the life cycle are demonstrated on the back of the Carolina Drosophila Manual, and discussed on pp. 4-5. These include the egg, larval, pupal and adult stages. The adults are shown on the back cover. There are several differences which may be used to distinguish the sex of the fly. The female is normally slightly larger than the male, her abdomen curves to a point posteriorly, and has separate black bands along the dorsal surface right to the tip. The male is slightly smaller, his abdomen is more rounded and the dark bands of the last few segments are fused together, forming a solid black posterior. These differences are good for wild type adults, and most mutants, which have been adults for a day or more. They are not as reliable for flies which have recently emerged from their pupal case. Two more reliable criteria are the presence of sex combs on the foreleg of the male (see p.13), and the genitalia of both sexes (see p. 12). For accurate sexing, such as must be done when you are collecting virgin females for crosses, the only reliable method is by use of the genitalia. The color differences are not as obvious in newly emerged flies and some mutants. The sex combs are not always obvious, but with practice may be used to help identify males while still in the pupal case. Sexual mosaics, while rare, may also be misleading for these traits. **Note the large genital arch on the posterior ventral surface of the male. This arch is replaced by the vaginal plate in the female. The anal plates also differ.** The genital arch may be used to sex the flies prior to emergence.

The flies mate shortly after emergence from the pupal case, and the eggs are fertilized internally. Early embryonic development takes place within the egg, which is laid on the food medium. The egg then hatches into a larva, which is a rapidly growing and feeding form. This larva grows and undergoes two molts, shedding its skin each time. When it molts the third time, it stays within the old skin and undergoes metamorphosis into an adult. The metamorphosing fly is called a pupa. The pupa is usually found on the sides of the culture vial and is a quiescent stage. The average

adult life span is 37 days at 25°C. A female will lay up to 3,000 eggs, of which 95% may hatch. One female may deposit 50-75 eggs/day for the first two days, and then less per day after the second day. It is possible to obtain up to 30 generations per year!

Preparing vials of media - A vial of medium is prepared by adding one cup of dry Carolina Drosophila medium to a new plastic vial. (Use the small plastic cup which comes in the bag of medium.) Add some baker's yeast, so that approximately $\frac{1}{4}$ of the surface of the media is covered. Add one cup of water, filled to 1/8" from the top. Place a plug in the vial. The vial should be used the same day that it is prepared, and may be temporarily stored in the refrigerator. Close the bag of media with a tie and place the bag in the refrigerator when not in use.

Handling of flies - Anesthetization. While investigators have used many methods to immobilize fruit flies for examination, the best method for our purposes is the use of diethyl ether as an anesthetic. To use the Carolina Anesthetizer (etherizer) (p. 11), remove the hollow stopper from the top and remove the cap from the bottom. Fill the hollow stopper $\frac{1}{2}$ full with ether. 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.** Do not use petroleum ether, which kills flies. Do not use the Carolina Biological Fly Nap, which also kill and sterilizes flies under tropical conditions.

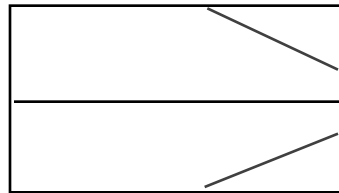
When you are ready to etherize flies, obtain an empty (no media) culture vial and a plug. Take the culture vial of flies and rapidly tap it several times against the table top, or the palm of your hand. This shakes down the flies which are near the mouth of the vial. While tapping, remove the plug of the culture. Place an inverted empty vial over the culture vial, so that their mouths perfectly meet each other. Invert the two vials together. Tap the flies down from the culture vial into the empty vial. Rapidly place the plugs into the two vials before any flies can escape. Do not tap the culture vial too hard, as this may loosen the culture medium which will then slide down on top of the flies and drown them. If the flies will not be immediately etherized, mark the identifying information on the side of the vial with a wax marker.

When you are ready to etherize the flies, remove the stopper from the mouth of the etherizer. Tap the flies down, as before. Remove the plug from the culture. Invert the vial over the etherizer and tap the flies into the chamber. After the flies have been tapped into the chamber, quickly remove the vial and place the stopper on the etherizer. Continue to tap the etherizer against the table top as you do this. 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 the dissecting microscope. Do not over-etherize the flies! This will delay reproduction at best, and may kill them. If they are holding their wings out perpendicular to their body, they are probably dead. Pale-colored flies with incompletely expanded wings have just emerged from the pupal case.

Usually the flies remain etherized for 5-10 minutes. With the stopper removed, the etherizer can be inverted over the flies to re-etherize them if necessary. The flies are killed or sterilized if etherized too often. As flies of this age may be sterilized by ether, over-etherization should be avoided in selecting flies for a cross. Flies may also

be re-etherized with a special etherizer made by taping a small piece of cotton to the inside of a petri dish. Add a few drops of ether to the cotton. Place the dish over the flies.

The anesthetized flies should be placed on a white index card, which has been folded down the middle. This card should be cut to a V-shape on one side. This card is then able to fit into the mouth of a vial when transferring etherized flies to a new culture vial. The flies may then be examined under the dissecting microscope, and



Cut on dashed lines.

Fold on center line to form a V.

sorted into different piles using a fine paint brush. You may want to trim the bristles of the brush to make a finer working tip. If the flies are to be scored and counted according to their phenotypes, this is the time to do it. When classifying flies, you usually have to wait until they are several hours old in order to let them darken up. Newly emerged flies often look rather abnormal; their wings may still be partly curled up, and they may be pale in color. In addition, some mutant stocks such as black, ebony, and sepia don't fully develop their color until they are a few days old. Flies that are to be discarded are to be dropped into a morgue - a jar of alcohol or oil, or detergent and water. You don't want discarded flies of a different genotype flying into your vials while you work!

Isolating virgins - Female flies mate several times and may store the sperm from these matings for use throughout their life. It is therefore necessary to use virgin females in all genetic crosses except some of those in which the females are to be mated to their brothers. In order to obtain virgin females, you need to get to them before the males do! This is the most important single source of possible error in your experiments! If there is any doubt as to whether or not a female is virgin, don't use her. The technique for gathering virgin females is based on the lucky fact that newly emerged male flies don't mate for at least 6 hours (depending upon the temperature). The method is as follows.

(1) Clear the culture vial of all adult flies. Make sure that none are hiding somewhere. If you can't get one out, kill it!

(2) Wait 6 hours and then remove all of the newly emerged flies. Select those that you are sure are females, and place them by themselves in a fresh vial with media. They can now be used in a cross to males of a specific genotype. If you goof and add one male to the vial by mistake, start over! One male can mate with over a dozen females in less than one hour. (You can keep the vial of virgin females for a few days in order to verify virginity. If they produce larvae, they aren't all virgins.)

(3) Since most flies emerge early in the morning, you need to do step #1 early in the morning. By waiting until later, you lose most of the virgin females. **We may be able to manipulate emergence time by changing the light/dark cycle in the incubator.

Before placing etherized flies in a fresh culture vial, warm the vial to room temperature. Wipe any moisture off the inside walls of the vial. Place the tip of the white card with the flies in the vial. Gently tap on the card so that the flies slide onto the side of the vial. Replace the plug in the vial. Leave the vial on its side until after the flies are fully awake and moving around. (Label the vial with a wax pencil or ink marker before adding the flies.) Never place etherized flies directly on the media, as there is enough water present to drown them.

Practice handling and sexing the wild type flies. After you have done this, then examine each of the mutant strains. Write a description of each strain in your note book.

Transfer all of adults from the original vials to new vials. The old cleared vials should be able to give you some virgins to use in your crosses.

Record Keeping - Keep a journal of all work pertaining to the Drosophila experiments. This is for your own benefit and you will not have to hand it in. In it you will enter the date, and what you did that day, plus any observations which you made. Thus on Sept. 22 you might have an entry indicating that you cleared all of the flies from a particular cross. In addition, you might have scored freshly emerging F₂ flies in another cross.

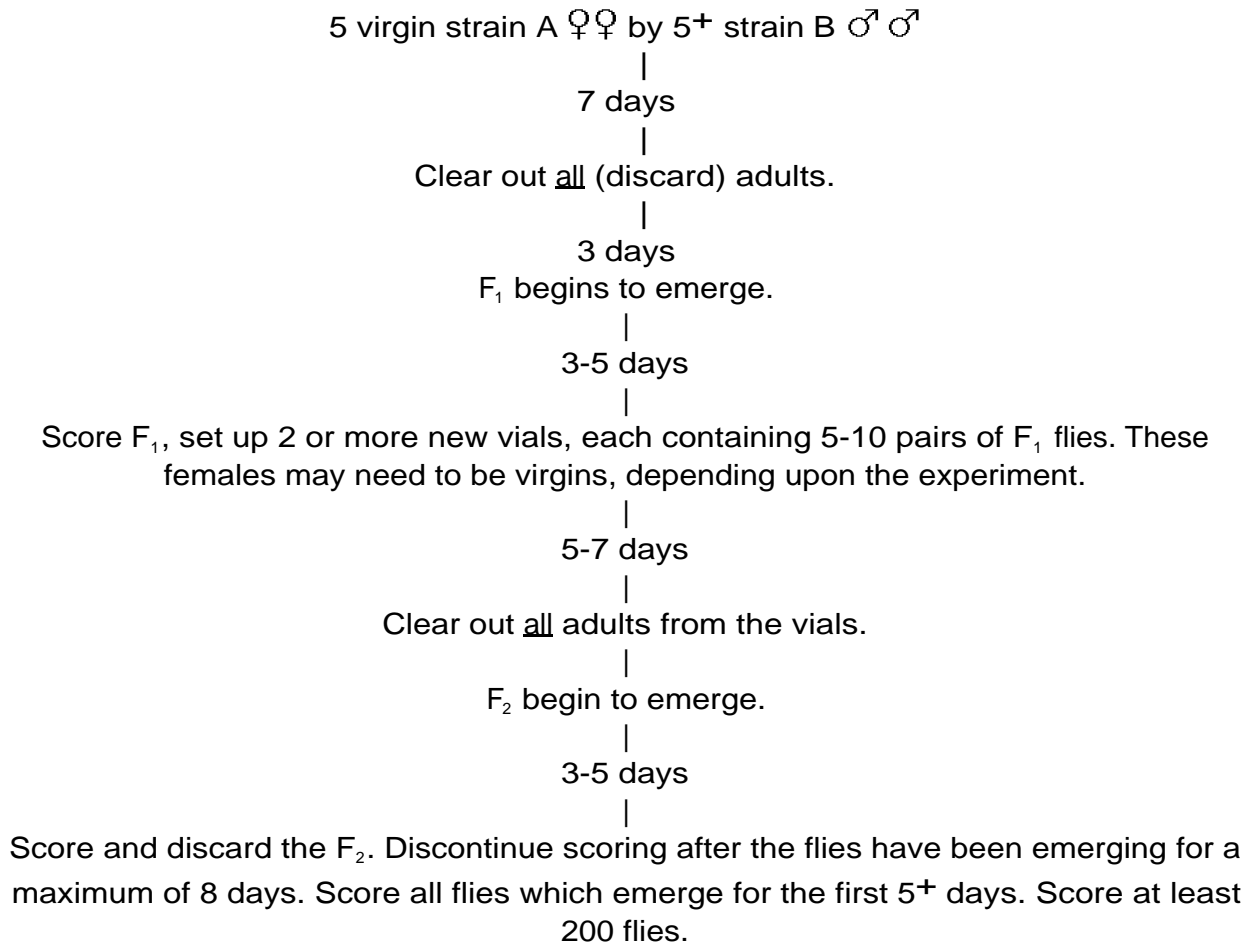
When ever you score flies of any generation, always record the exact number of flies in each category. Thus you might have a data sheet set up like this:

	date	3/6	3/7	3/8	3/9	3/10	3/11	3/12	3/13	Total
trait	day	1	2	3	4	5	6	7	8	
wild type	V									
	M									
white	V									
	M									

Make a note of any unusual flies which do not agree with your expectations. Such flies may lead to new discoveries. Obviously, you may want to keep such flies for special crosses.

Each vial must be correctly labeled. Each label should identify the adults which were placed in the vial, and the date the vial was set up.

Typical Flow Chart for a Single Cross



1. Collect virgin strain A females and add strain B males. Use at least 5 flies of each sex. It is often wise to add a few extra males, since some strains have a slight degree of male sterility.
2. In 7 days, remove the parents. You should be able to see larvae crawling through the media. Check daily for emerging F₁ flies. You may wish to place the P₁ in new vials in order to have a back-up in case you loose the F₁.
3. After the F₁ has been emerging for 3-5 days, score them and set up 2 new vials, each containing 5-10 pairs of F₁ flies (and perhaps a few extra males). Continue to score the F₁ as they emerge, discarding the rest. Try to score at least 100 of each sex. Do not score flies after they have been emerging for 8 days; you may start to confuse F₂ with F₁. (Count 8 days from the earliest possible day of emergence.)
4. In 5-7 days, remove the F₁ (P₂) flies from the vials you set up.
5. Check daily for the emergence of F₂ flies.
6. After the flies have been emerging for 3-5 days, examine them and record the phenotypes of at least 200 F₂ flies. Keep a separate record for each F₂ vial!
7. Discard the F₂ flies after they have been scored. (Place them in the morgue.) Do not score the F₂'s later than 8 days after the earliest possible day of emergence.