Working with Drosophila

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Introduction

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Drosophila melanogaster is an excellent organism for use in the study of genetics. Its small size, relative ease of maintenance, short life cycle, and its ability to produce many offspring have lent it to the demonstration of Mendelian inheritance in every location from the elementary school to the university laboratory. From the thousands of available strains, we have selected an assortment of forty types, which serve most purposes.

The basic requirements for growing and working with Drosophila are as follows:

I. Media

Many different types of media have been used for the culture of Drosophila. The simplest is just a slice of banana. However, each medium has its drawbacks. In order for a medium to be useful, it must be solid and dry enough so the adult flies do not drown, and it must inhibit the growth of molds, which are almost always present on the flies and in the air.

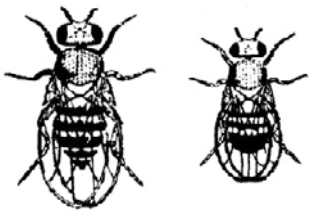
II. Handling Flies

Materials needed for working with flies are: a Petri dish, an ice pack or cryolizer, a magnifying device (binocular dissecting microscope, large magnifying glass, etc.), a light (goose-neck desk lamp, a microscope lamp, etc.), a camel's hair brush, a white viewing surface, and a "morgue" bottle (any bottle with a cap, partially filled with alcohol, mineral oil, etc. into which dead flies and those to be killed can be dropped).

Put the culture of flies in a refrigerator on its side so that the flies will not be trapped in the media. After 20 minutes or so check the cultures to make sure the flies are unconscious. Then tap the flies into a Petri dish. Using the camel's hair brush, separate the flies into different groups (e.g., males and females; red eye and white eye, etc.) In preparing a new culture, five to ten pairs of flies should be used.

It is not difficult to distinguish between the male and female Drosophila. With a little practice, the difference can be seen without magnification (***refer to Figure 1***). The female is usually slightly larger than the male. The abdomen of the male appears to have a distinct black tip and a blunt posterior, while the female has a somewhat lighter and pointed posterior with bands visible almost to the tip. The male also has sex combs on the front legs and the female does not. As the female becomes older, her abdomen becomes distended with eggs, making identification even more simple.

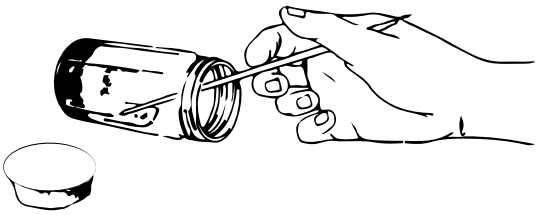
Figure 1



Female **Male**

To inoculate the culture jar with the flies, sweep the flies onto the end of a piece of filter paper. Lay the culture jar on its side and remove the plug. (Do not allow the flies to fall off the paper.) With the handle of the camel's hair brush, push the end of the filter paper down into the medium (***See Figure 2***). Plug the jar. Allow the jar to remain on its side until the flies awaken. This will help to avoid the possibility of the unconscious Drosophila falling into any moisture and drowning.

Figure 2



It is good practice to check the characteristics of each strain under magnification when subculturing. There is always the possibility that a stray fly may enter the culture and cause loss of the strain's purity.

Stocks should be subcultured every 2–4 weeks depending upon the temperature. Old cultures should be discarded after the new cultures become established, in order to avoid contamination. At least two cultures of each strain should be kept in case one should be unsuccessful.

It is important to make sure that each culture jar is labeled with the type of fly and the date the jar was inoculated with the flies. The best way to do this is to use the shorthand notation for the particular strain of fruit fly in the jar. An example of a few shorthand notations would be: + = wild type (red eye), vg = vestigial wing, b p c = black purple curved. Small letters refer to recessive genes, capital letters refer to dominant genes (e.g., B = Bar eye). Letters separated by spaces refer to genes on the same chromosome (e.g., wem = eosin miniature). Letters separated by a semicolon (e.g., w;se – white sepia) refer to genes located on different chromosomes.

Therefore, a culture jar with a heldout strain of *D. melanogaster* on February 14 would have the following label:

ho
2/14

There are so many other notations and additional symbols that it would be impossible to list them here. All cultures are shipped with a label giving the name of the strain, the shorthand notation, and the chromosomes on which the genes occur.

Cultures of Drosophila should be kept at a relatively constant temperature closely approximating room temperature, no lower than 20°C (68°F) and no higher than 25°C (77°F). They should not be exposed to direct sunlight and may be kept entirely in the dark.

Embryonic development (following fertilization) takes place within the egg membrane. The egg hatches and produces a larva which feeds by burrowing through the medium. As the larva grows, it undergoes two molts so that the larval period consists of three stages (instars), the first instars being the newly hatched larva. The final larval stage or third instar may attain a length of 4.5 mm. The third instar stage, toward the end of the larval period, will crawl up the sides of the culture jar, attach itself to a dry surface (the jar, the filter paper, etc.) and form the pupa. After a period of time the adult or imago will emerge.

The duration of the above stages will vary with the temperature; at 20°C (68°F) the average length of the egg-larval period is eight days, while at 25°C (77°F) it is reduced to five days. Thus at 25°C (77°F) the life cycle may be completed in about ten days, while 20°C (68°F), fifteen days are required.

The sperm received by a female fly during mating is retained, serving to fertilize a number of eggs. Therefore, in an experimental cross between two different strains, virgin females must be used. This can be done quite simply by taking advantage of the fact that females do not mate before twelve hours from the time of emergence. If all the adult flies are removed from a culture with many pupae, all of the females collected within the next twelve hours will probably be virgin.

III. Experimental Crosses

Perhaps the best way to explain the technique used in making experimental crosses would be to trace the step-by-step procedure, using an example. Careful records should be kept of all experiments, giving dates of crosses, results of crosses, comments about technique, and results of the experiments.

For our experiment, we will use the wild type (+) strain and the vestigial wing (vg) strain.

1. Select virgin vestigial (vg) females and place six in each of three culture bottles. Into each bottle place six wild type (+) males. Mark the date and the type of mating on each bottle.

   (vg ♀) + (+ ♂)
   2/14

2. After seven days remove the parent flies from the mating bottle and discard. There should be many larvae in the bottle.

3. When the flies begin to emerge, examine them and record the characteristics. This is the F1 generation. In this case, since the (vg) is recessive, all of the flies should exhibit (+) characteristics.

(**Note:** *If any (vg) flies appear in a bottle, one of the parent females was NOT a virgin and the culture should not be used in the rest of the experiment.*)

4. Place six males and six females of the F1 generation in each of three culture bottles. Mark each bottle with the date, the type of original mating, and the generation.

   (vg ♀) + (+ ♂)
   F1
   2/28

5. Remove the F1 flies from the culture bottles after seven days and discard them.

6. When the offspring of this cross (the F2 generation) begin to emerge, they should be killed (by over-anesthetizing), removed, and the sex and characteristics determined and recorded. Counting should go on until all flies have emerged and have been counted (usually about eight days, depending on the temperature). Counts should be made daily.

7. The results should look like this:

wild type (+)	**439 males**
	452 females
	891 total
vestigial (vg)	**159 males**
	142 females
	301 total
total flies counted =	**1192**

The wild type flies outnumber the vestigial wing flies by about three to one. The sex of the flies does not appear to affect the ratio (in this case). The crosses can be diagrammed to predict this in a very simple manner.

Since the F1 generation is the result of two homozygous strains of flies, the wild type fly has only one possible characteristic in the gametes, wild (+). The vestigial fly also has only one possible type of gamete, vestigial (vg). The F1 generation can therefore be diagrammed:

	vg	**vg**
+	**+ vg**	**+ vg**
+	**+ vg**	**+ vg**

–

The zygotes (fertilized eggs) all have both the wild and the vestigial genes. Since the wild type is the dominant factor, all of the F1 flies will appear to be wild type, even though they are not homozygous strains.

The F1 has TWO possible gametes, wild (+) or vestigial (vg). Therefore, the F2 would look like this in a diagram:

	+	vg
+	+ +	+ vg
vg	+ vg	vg vg

For every F2 fly with the vestigial characteristic, there will be three flies with the wild characteristic (one homozygous for wild and two heterozygous for wild).

As an additional experiment, you could try crossing F1 generation virgin females with pure wild or vestigial strains and try to predict the results.

A brief survey of methods for working with Drosophila is all that has been possible within the scope of this leaflet. The reader is urged to take advantage of some of the fine works available which deal with Drosophila and genetics in general for more detailed information.

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