

CULTURING AND HANDLING *Drosophila melanogaster*

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http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Drosophila_chromosomes/Drosophila_Culture.htm

We have recently eliminated the classic *Drosophila* crosses from our genetics labs, but still use the larvae to demonstrate chromosomes. Here is how we get the larvae.

EQUIPMENT:

sterile 125 mL flasks	petri dish bottom for sorting
foam rubber plugs	petri dish lid with ½ in. cotton ball taped inside
etherizer: funnel with cloth strip or string wrapped around neck	small, fine paint brush
250 mL wide-mouthed flask	dissecting scope and illuminator
	two dissecting needles

SUPPLIES:

dehydrated <i>Drosophila</i> medium	selected mutants of <i>Drosophila melanogaster</i>
dry granulated baker's yeast	ethyl ether (caution , <i>extremely</i> flammable)

PREPARATION OF MEDIA:

1. Into a sterilized 125 mL flask plugged with a foam rubber closure, measure 30 mL of powdered *Drosophila* medium (2 tablespoons).
2. Add 20 mL tap water, swirl very briefly to thoroughly moisten. (Don't splash up the sides.)
3. Sprinkle a small pinch of granulated yeast across the surface. Let the medium set for 15 minutes before adding etherized flies. (They get stuck in un-gelled medium.)

ETHERIZING THE FLIES:

1. Place several drops of ether on a strip of cloth wrapped around the end of a powder funnel. Place in a 250 mL wide-mouthed flask and let the atmosphere become saturated with ether.
2. Tap the sides of the culture flask to knock flies away from the mouth. Then remove the plug, quickly invert and place into the mouth of the funnel.
3. Set up a bright light to shine on the beaker below; tap the sides of the culture flask to get the flies into the etherizing chamber. (Don't jar the medium loose.)
4. Allow flies to remain in the etherizing chamber until they stop walking around. Do not over-etherize, or you will kill them. (If their wings *stick up*, they are dead...)
5. Pour anaesthetized flies out into a petri dish for sorting and counting. If they begin to wake up, re-etherize them by covering with a petri dish top into which a small piece of cotton has been taped and a few drops of ether has been added.

PRACTICE SEXING FLIES:

1. Before the first cross is started, sort and sex flies from the clearing of a culture flasks. This is facilitated by sweeping the flies into a row, and working your way along the row, moving males to one side, females to the other.
2. If you have more than a single set of phenotypes, sort the sexed groups.
3. Count the number in each set, record the data, enter in the class table. Exchange flies with your bench mate, count each other's flies and compare your results.

TO ISOLATE VIRGIN FEMALES:

1. As early in the morning as possible, clear 100% of the flies from a culture flask.
2. As close to 8 hours later as you can manage, clear all of the *newly hatched* flies, separate males from females. These will all be virgins. Females may be kept in an all-female culture flask until needed if not used immediately.