

DROSOPHILA CARE AND HANDLING

A. FEEDING:

- Obtain one vial and one foam stopper for the vial.
- Mix “Instant *Drosophila* Medium (Formula 4-24)” with water in a 1:1 ratio.
- Sprinkle 5-10 grains of yeast (found in refrigerator in genetics lab) on top of the initial food mixture.
 - DON’T** add too much yeast!! Yeast ferments and produces CO₂ and excess CO₂ will suffocate your flies.
- Allow the food mix to stand one minute to “set-up.”
 - Your flies can get stuck in food which is too wet.

B. ANESTHETIC:

- Fly-nap is less flammable, less harmful to humans and knocks the flies out longer than ether, which is commonly used as an anesthetic.
 - Keep Fly-nap in the fume hood at **ALL TIMES!!!**
- Dip the wand in the Fly-nap.
- Tap the vial of flies on the counter several times to get the flies to the bottom of the vial and ensure that they don’t fly out.
- Quickly move the foam cork aside and insert the wand into the vial.
 - Try not to get any Fly-nap on the foam cork.
 - If you do get Fly-nap on the cork, invert the cork when you replace it in the vial.
- Fly-nap takes about 2 minutes to work on small vials of flies and about 5 minutes on large vials.
 - While waiting for the anesthetic to work, lay the vial on its side and occasionally tap the vial to keep the flies out of the food.
 - It is normal for the flies to continue twitching while under the influence of Fly-nap.
- After the flies are knocked out, gently tap them out of the vial and onto an index card.

C. SEXING FLIES (Distinguishing males and females):

- Use a stereomicroscope to determine sex.
- Move the flies gently around the index card with a small paint brush or pin probe.

1. genitalia - ventral posterior

2. sex combs

- The front pair of legs in males contain a dark set of bristles called sex combs - these are not present in females.
- Once you know what you are looking for, this is probably the fastest and most accurate way to sex flies.

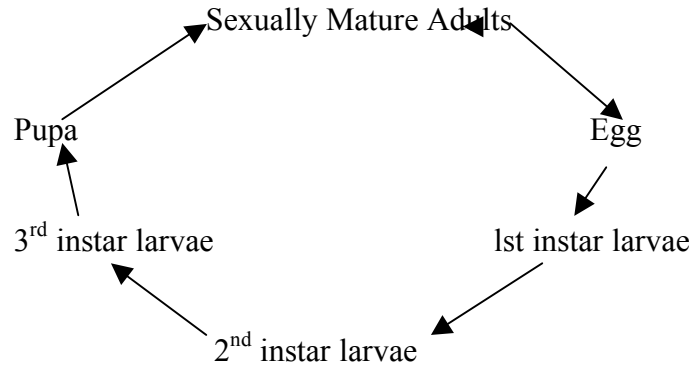
3. abdomen

- Females have a pointy, creamy abdomen, while males have a darker, more rounded abdomen.
- This is an unreliable method of sexing when setting up crosses but it's a good way to glance at your vial, without anesthetizing your flies, and see what's alive.

D. SET UP THE CROSS:

- Select 4-6 mutant virgin females.
 - Record their phenotype (appearance) with respect to eye color (white or red), body color (grey or yellow - thorax), wing length (miniature or long), and bristles (straight or forked).
 - The "mutant" vials contain only mutant virgin females so you need not sex these flies.
- Select 4-6 "wild-type" male flies.
 - Record their phenotype for the same characteristics listed above.
 - The male flies are mixed with females and so you will need to determine the sex of the flies in order to find males to use in your cross.
- Add the anesthetized flies to the vial of food you made up, cork the vial and put in the 21 degree C incubator. (If the vial is kept on it's side, the flies are less likely to fall into the food and get stuck and die.)
 - Any unused flies should be returned to the vial from which they were taken.
- During the next lab period, check on your flies to make sure that they have recovered from the anesthetic.
- During every lab period, check on your flies and note where they are in their life cycle.

E. LIFE CYCLE:



- Males flies are sexually mature as soon as they emerge from the pupal case.
- Females are sexually mature about 8 hours after they emerge from the pupal case.
 - Sexually mature females can mate once and store that sperm for a lifetime of use; thus, if a specific cross is desired, it is necessary to ensure that the female flies used in a cross are virgins.
 - Virgin female flies can be obtained in the following way:
 - Pupae darken during the last day before the adult flies emerge from the cuticle.
 - When some pupae have darkened, remove all adult flies from the vial and dispose of the flies in the morgue (called “clearing the vial”).

Method #1:

- Check the vial within the next 8 hours for the presence of any female flies. They must be virgins because they cannot yet be sexually mature.
- Anesthetize these flies, dispose of any males and preserve the virgin females in a separate vial.
- Repeat the process every 8 hours until sufficient flies are obtained.

Method #2:

- After clearing the vial, keep it at room temperature for 8 hours. As above, any females present at this point must be virgins.
- Move the flies to the 18°C incubator. At this temperature flies mature more slowly and females will be virgins for up to 16 hours.
- Shift between 18°C and room temperature as your schedule warrants.

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