Aim: Culturing *Drosophila* using standard methods

Objective: To set up *Drosophila* using standard methods.

Principle: Drosophila melanogaster is one of the most widely used model organism in research on genetics and genome evolution. Mass culture of D. melanogaster is important to produce enough amounts of flies for research purposes. Drosophila species have extensive literature on their genetics, development and ecology. Besides, the short generation time of Drosophila aided the studies on genetics especially studies of the laws of heredity. One of the most extensively used model organisms is Drosophila melanogaster, which is also known as the Cinderella of genetics. This species of fruit flies not only possesses of well-defined genetics information, they also have short generation time which one generation only requires about two weeks. In addition, one pair of parent flies is able to produce several hundreds offspring which ease the process of genetics. D. melanogaster are bred in more solid culture media with yeast added manually to ferment those culture media. One of the most common ingredients added to fruit culture media in order to solidify the culture media is agar. In addition, sugar content in Drosophila culture media must be sufficient too. This sugar content is not only vital for the growth of Drosophila, it is also required by yeast for fermentation. methyl parahydroxy benzoate and propionic acid are also used as antifungal and antibacterial agents respectively.

Materials: Jaggry, corn flour, Agar, Yeast, methyl parahydroxy benzoate and propionic acid, ether, culture bottles, nonabsorbent cotton, whatman no 1filter paper, brush, petriplate, BOD incubator 25^0C, microscope

Method:

Media preparation:

Ingredients: 27 g agar, 200 g cornmeal (organic, fine ground), 140 g sugar, 50 g yeast, 20 ml propionic acid (added to prevent bacterial growth), 20 ml of methyl parahydroxy benzoate.

Procedure:

1. Dissovle agar in 2L tap water by boiling
2. Dissolve cornmeal, sugar and yeast in 1L cold tap water so that it is free of lumps.
3. Once agar is dissovled add cornmeal mix and bring to boil. Stir constantly to aviod caking/burning on bottom.
4. Boil for 15 minutes, stirring constanty.
5. Take off stove and cool. Add Propionic acid and Nipagen. Ensure you are working in

a well ventalted area.

6. Distrubute fly food into vial/bottles as required. Only fill each container up to 1.5 cm.

7. Cover with paper towels and allow to cool and dry (over night) at room temperture.

8. Vials/bottles should be plugged before storing in a fridge.

9. Bring the vials at room temperature before use

10. Transfer required males and females in to the vial and keep vials in horizontal position until flies revive if etherized.

11. Once flies revive keep the bottles vertical in BOD incubator at 25^0C

Handling Flies: Once the flies have been knocked you can move the flies around using a clean paint brush. Using the paint brush carefully means you will not damage the flies when sorting and manourving. Transferring flies from one container to another involves tapping the bottle gently, the flies dislodge from the walls and fall to the bottom. Removing the lid from the bottle you want to transfer the flies to and placing the flies over this bottle, then tap and the flies will fall from one bottle to another, quickly put the lids on the bottles.

Anesthetizing flies: The problem with fruit flies is that they fly! Therefore a variety of methods have been developed to anesthetize flies. Include are ether, commercial brands such as Flynap, carbon dioxide, and cooling. Each has its strengths and weaknesses. Ether is flammable, has a strong odor and will kill flies if they are over-etherized (and can anesthetize younger students!). Set up a large test tube with a tube and stopper system. Anesthetizing flies by cooling is also simple method. In order to incapacitate the flies, place the culture vial in the freezer until the flies are not moving, generally 8-12 minutes.

Transferring flies from one vial to another: Flies should be transferred every 10 to 14 days. Students should maintain a backup culture of their flies and the instructor should maintain backup stock cultures of all fly strains. There are two basic ways to transfer flies when forming new cultures. One requires no anesthetizing but quick hands.

A) Place a funnel in the mouth of a fresh culture vial that already has media added. In the old vial (the one with flies in it), gently tap the flies down by softly tamping the vial on a soft surface, such as a mouse pad. The flies will fall to the bottom and remain there for a few seconds (no more than that!), enough time to quickly take the plug off the vial, invert it into the funnel, and gently tamp, together, the two vials to force flies down into the new vial.

B) An alternative way is to put the flies in the freezer for about 8 minutes. This will cause the flies to fall into a state of stupor. After placing a funnel on the new vial, invert the vial with motionless flies into the funnel. This is not as much fun but you won't have any flies flying around the classroom.

Sexing flies: It is quite easy to tell males from females and with a little practice students will become confident of their ability to do so. Notice that males are generally smaller and have a darker and more rounded abdomen. The coloration of the abdomen is the easiest to recognize. In addition, males have tarsal sex combs on their first pair of legs. These are black and very distinctive but can only be seen under relatively high magnification. With a little practice, by looking at the abdomen students will become proficient in accurately sexing flies. Sexing flies is critical when making crosses, so be sure student are confident in identifying the difference between the sexes. In order for students to feel comfortable sexing flies, give or have them obtain 25 or more mixed sex flies and allow them to sort the flies into two piles, male and female. Other students in the group and the instructor should verify the sorting. Each member of the group should be able to sex flies.

Collecting virgin females: While it's a simple matter of placing virgin females with males, it is important to recognize the time factor involved for obtaining virgins. Females remain virgins for only 8-10 hours after eclosure and must be collected within this time frame. NOTE: Females have the ability to store sperm after a single mating.

Removal method: Remove all flies 8-10 hours before collecting (generally this is done first thing in the morning). Visually inspect surface of food to ensure complete removal of flies. After 8-10 hours (usually before you leave work) collect all females that are present. All will be virgins. Since

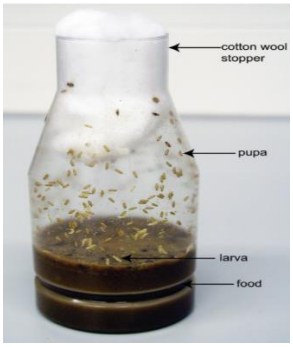
they are photoperiod- sensitive, females tend to eclose early in the morning. Therefore early collections will ensure the greatest number of virgins for experimentation.

Visual method: Note that virgin females are much larger than older females and do not have the dark coloration of mature females. In addition, in the early hours after eclosure, there will be visible a dark greenish spot (the meconium, the remains of their last meal before pupating) on the underside of the abdomen.

Temperature cycling: It is possible to maximize the number of virgins in a morning collection by using temperature cycling. When cultures are maintained at a temperature of 18°C, development is slowed so females will not mate until 16 hours after enclosure. By removing flies in the afternoon/evening and placing the vials in an 18°C incubator, 98% of flies obtained in the morning will be virgins.

Result:

Media poured in vials



Culure established

Culture Established