Drosophila-
Study the developmental stages and the life cycle from fruit fly stock culture
WHY Drosophila?

- first used in genetic experiments in 1907 by Thomas Hunt Morgan, and has been a staple of genetic research ever since and is the genetically best-known of all eukaryotic organisms

- has high fecundity, produce large numbers of offspring and complete their entire life cycle in approximately 12 days.

- relatively easy to maintain, as they are hardy and have simple food requirements.

- a great deal has been learned about its genome (sequenced in 2000).

- similarities between the Drosophila genome and those of other species is helpful in determining patterns of evolution and species divergence.

- About 75% of known human disease genes have a recognizable match in the genetic code of D. melanogaster and 50% of fly protein sequences have mammalian analogs. Thus, it is an excellent animal model for studying a variety of human diseases.
Physical Description of

*Drosophila* or sometimes called vinegar flies or gnats
• Small-about ⅛ inch in length

• Three body parts: the head, thorax and abdomen, two prominent red eyes, a pair of barely perceptible antennae and the mouth parts

• Three pairs of legs and a single pair of wings attached to the thorax

• Generally yellowish in color with several black bands across the abdomen

• Females and males can be readily distinguished by examining their abdomens
FIGURE 2. Male-female Identification

Females tend to be larger than the males. They have longer and more pointed abdomens with several small rows of black lines across the top of it. Males have more rounded abdomens with a thick black zone near the posterior end of the dorsal surface. Males may also be identified by a black sex comb near the first joint of their front legs.
THE NUTRITIONAL REQUIREMENTS OF DROSOPHILA MELANOGASTER
• As the name implies, the fruit flies lives primarily on plant material.

• The adults thrive on rotting plants, and fruits; while eggs are usually laid on unripened/slightly ripened fruit, so by the time the larva develop the fruit will have just started to rot, and they can use the fruit that the egg was laid on as their primary source of nutrition.

• *Drosophila* are considered major pests in some area of the world for this reason.
Experimental Conditions
Preparing Fly Larvae Food

Fly food is a gel at room temperature. When heated to 65 degrees C., it becomes a liquid with a consistency of thin oatmeal that can then be dispensed into tubes or other vessels.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>Distilled water</td>
<td>350 ml</td>
</tr>
<tr>
<td>Agar Agar</td>
<td>3.01 g</td>
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<tr>
<td>Corn flour</td>
<td>20.5 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>44 g</td>
</tr>
<tr>
<td>Yeast</td>
<td>11 g</td>
</tr>
<tr>
<td>10 % Nipagine (mould inhibitor)</td>
<td>3.5 ml</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>3.5 ml</td>
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</tbody>
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The Cross

Female fruit flies are capable of storing sperm in their bodies for a considerable time after mating. Before you make your cross, you must be sure that you are using virgin females, or else they will lay eggs that were fertilized with sperm from a male in their stock culture, and not by the male in the cross. An experienced worker can usually separate the virgin females from others by sight, but this skill is not quickly learned.

The easiest way to obtain virgin females is to remove all adult fruit flies from your stock culture about 10 hours before making your cross. Females usually do not mate until 12 hours after hatching. Therefore, after a 10-hour wait, all females in the culture should be virgin.
Life Cycle
• After a short courtship in which the male circles the female while vibrating his wings, the female spreads her wings laterally and insemination takes place. The sperm are received in seminal receptacles within the female and will be used to fertilize eggs laid by the female for her entire life.

• Undergo complete metamorphosis, four distinct stages in their life cycle: egg, larva, pupa and adult

• Length of the life cycle and of each stage is directly related to the temperature, with warmer temperatures tending to increase the rate of development (68°F, 15 days, but at 76°F, 10 days)
• Drosophila embryos develop in the egg membrane. The egg hatches and produces a larva which feeds by burrowing through the medium.

• The **larval period** consists of three stages, or **instars**, the end of each stage marked by a molt.

• The first instar is the newly hatched larva; the third instar is the final larval stage, where the larva may attain a length of 4.5mm.

• Near the end of the larval period, the third instar larva will crawl up the sides of the culture vial, attach themselves to a dry surface (the jar, the filter paper, etc.) and form **pupae**.

• After a period of time the **adults** emerge from pupae (Eclosion). Females will become sexually mature after 8-10 h of **Eclosion**.
• Fertilization is internal

• Oviposition takes place on the surface of medium

• Small ovoid shaped, shiny white

• Two distinct phases in development: embryonic phase & post-embryonic phase
Larva (maggot)

- Anterior end can be recognized by a pair of dark projections. Also, it will be moving, first being extended and then retracted.

- Worm-like segmented creature with black jaws.

- Voracious feeder.

- Pair of spiracles (openings) to exchange gases.

- Three instar stages; first instar = hatching to 1st molt; second instar = first molt to second molt; third instar = second molt to pupal stage.

- 10 pairs of imaginal discs present.
PUPA

• Larvae requires dry location to metamorphose into an adult

• "prepupal" stage: cuticle hardens and takes on a darker pigmentation

• Imaginal discs (which have remained dormant since the embryonic stage), develop rapidly into the tissues of the adult fly

• New tissues completely replace larval tissues
• Soft wrinkled adult fly emerges from the pupal case (eclosion)

• Takes several hours for the fly to unfold its wings, dry out, and harden

• Female flies are sexually unreceptive during this post-eclosion period, and for several hours afterwards, but mating may take place as early as 8 to 12 hours after eclosion
Fruit fly maggots pupate before becoming actual "flies."
New fruit fly starter culture on left. Ten days later on right.

**Eggs:** Adults lay their eggs in the culture medium. When they hatch, the tiny fruit fly larvae burrow thru the gooey stuff while stuffing their guts.
Larvae: As they reach their $\frac{1}{4}$+ inch size, the fruit fly larvae (maggots) crawl up the sides of their container.
**Pupae:** Once they crawl out of their medium, fruit fly maggots begin drying into cocoons. Cocoons develop into adults in a few days. Warmer temps speed up the process.

**Adults:** Pupae change into adults in their cocoons. The egg-laying adults emerge about 10 days after they started life as eggs. Females have larger bellies than the males (in case you want to know). They start breeding within 24 hours. Female fruit flies easily produce 100+ eggs.