



## An Introduction to( fruit or vinegar fly)

## Drosophila Melanogaster

**Drosophila melanogaster** is a small (about 3mm long), common fly found near unripe and rotted fruit so that it called fruit or vinegar fly. It has been in use for over a century to study genetics and lends itself well to behavioral studies. Thomas Hunt Morgan was the preeminent biologist studying Drosophila early in the 1900's. Morgan was the first to discover sex-linkage and genetic recombination, which placed the small fly in the forefront of genetic research. Due to its small size, ease of culture, short generation time, and is cheap and easy to keep large numbers geneticists have been using Drosophila ever since.. Mutant flies, with defects in any of several thousand genes are available. It is one of the few organisms whose entire genome is known and many genes have been identified.

Drosophila genome consists of 165 million base pairs in contrast to the human's 3,000 million base pairs. The sequencing of the fly's DNA and gene manipulation have aided biologists in perfecting skills in gene manipulation and has given insight into how genes function in living organisms.

Fruit flies are easily obtained from the wild and most biological science companies carry a variety of different mutations. In addition these companies sell any equipment needed to culture the flies. Costs are relatively low and most equipment can be used year after year. There are a variety of laboratory exercises one could purchase, although the necessity to do so is questionable.

## Why use Drosophila?

- 1. They are small, easily handled and easy to keep in a laboratory
- 2. Have a short generation time (important for research spanning a number of generations).
- 3. Have a number of easy to see inheritable characteristics and many mutations to study.
- 4. Have a chromosome number of 8 (4 pairs of chromosomes).

5. You can anesthetize them easily and manipulated individuals with very unsophisticated equipment.

6. Drosophila are sexually dimorphic (males and females are different), making it is quite easy to differentiate the sexes.

7. It is easy to obtain virgin males and females, as virgins are physically distinctive from mature adults.

8. Flies have a short generation time (10-12 days) and do well at room temperature.

9. The care and culture requires little equipment, is low in cost and uses little space even for large cultures.

10. It have giant polytene chromosomes( unique to insects) in their larval stage, That chromosomes may be visualized with staining technique for study and can be used for genetic mapping.

# **Classification**:

Domain: Eukarya Kingdom: Animalia Phylum: Arthropoda Class: Insecta Order: Diptera Family: Drosophilidae Genus: Drosophila ("dew lover") Species: melanogaster ("dark gut")



**Life cycle of** *D. melanogaster:* It exhibits complete metamorphism, meaning the life cycle includes an egg, larval (worm-like) form, pupa and finally emergence as a flying adult. This is the same as the well-known metamorphosis of butterflies and many other insects. The larval stage has three instars, or molts.

## Life cycle. Drosophila go through 4 stages in their lives.

1. Egg. Eggs are laid by the mother on the food and take about 1 day to hatch. They are small, oblong and translucent, with two "ears" sticking out.



2. Larva. Larvae are maggots which crawl through the food in a jerky motion, eating as they go. The larvae go through 3 molts: they hatch from the egg as small, first instar larvae. Then after a day they molt to become larger, second instar larvae. After another day they molt again to become even larger third instar larvae. After two days in the third instar, the larvae climb up on the sides of the vial, glue themselves to the glass, evert their spiracles (breathing tubes), and settle down as pupae.



**3. Pupa.** Pupae are the cocoons in which the larvae metamorphose into adults. The larval cuticle becomes a shell, their muscles melt away, and a new adult exoskeleton and musculature forms inside. The pupal stage lasts five days. During the last day, you can see the red eyes and the dark wings forming inside.



**4.** Adult. The adult emerges from the pupal case as a white, elongated thing whose wings are still folded up. After about an hour, the wings will expand the the body will take on its normal shape and coloration. The adult become sexually mature after 8-10 hours. After this time, the males chase the females about in an endless quest for mating. Flies can live for up to 3 months, but they are pretty decrepit after 6 weeks or so.



## Life cycle by day

Day 0: Female lays eggs

Day 1: Eggs hatch

Day 2: First instar (one day in length)

Day 3: Second instar (one day in length)

Day 5: Third and final instar (two days in length)

Day 7: Larvae begin roaming stage. Pupariation (pupal formation) occurs 120 hours after egg laying

Day 11-12: Eclosion (adults emerge from the pupa). Females become sexually mature 8-10 hours after eclosion.

The time from egg to adult is **temperature- dependent**. The above cycle is for a temperature range of 21-23 degrees C. **The higher the temperature, the faster the generation time**, whereas a lower (to 18 degrees C) temperature causes a longer generation time. Females can lay up to 100 eggs/day.

After the eggs hatch, small larvae should be visible in the growing medium. If your media is white, look for the black area at the head of the larvae, larvae are easily seen. In addition, as the larvae feed they disrupt the smooth surface of the media and so by looking only at the surface one can tell if larvae are present. After the third instar, larvae will begin to migrate up the culture vial in order to pupate.



## **Distinguishing Gender Features in Fruit Flies (Sexing flies):**

## SEX DIFFERENCE

Several criteria may be used to distinguish male and female flies.

#### 1. Size of adult

The female is generally larger than the male, as shown.

#### 2. Shape of abdomen

The female abdomen curves to a point (seven segments); the male abdomen is round and much shorter (only five segments).

#### 3. Markings on the abdomen

Light and dark bands are easily visible on the dorsal surface of the female. The last few segments of the male's dorsal surface have a fairly uniform dark pigmentation (segments of the male are fused).

#### 4. Appearance of sex comb

On males there is a tiny tuft of hairs on the basal tarsal segment of the front leg. This is the only sure method of distinguishing young male and female flies (less than 2 hours old), since the other adult traits are not always immediately recognizable. Sexing via the sex comb can also be

done successfully in the pupal stage, females lack sex combs.

#### 5. External genitalia on abdomen

Located at the tip of the abdomen, the ovipositor of the female is pointed. The claspers of the male are darkly pigmented, arranged in circular form, and located just ventral to the tip.

#### 6. Sex organs during larval stage

During the late larval stage males can be distinguished by the presence of a large, white

mass of testicular tissue. This tissue is located at the beginning of the posterior third of the

larva in the lateral fat bodies and can be seen through the integument. The corresponding ovarian tissue of the female constitutes a much smaller mass.



## The chromosomes of *Drosophila melanogaster*

The individual *Drosophila* has four pairs of chromosomes. A female has two each of chromosomes 1 (more commonly called the X chromosome), 2, 3, and 4. A male has one X chromosome, one Y chromosome, and two each of chromosomes 2, 3, and 4. The Y chromosome and chromosome 4 are both very small, and carry few genes. The majority of the fly's genes are carried on chromosomes X, 2, and 3. The X and Y chromosomes are involved in sex determination, and are thus called the sex chromosomes. Chromosomes 2, 3, and 4 are called the autosomes.



**HEREDITARY TRAITS:** Before one observes their mutants, one needs to be familiar with the appearance of the wild type Drosophila, the type found most often in natural populations of the organisms. Although thousands of mutations in Drosophila are known.

## Terminology

**Wild-type -** flies that have the "normal" characteristics, red eyes, normal length wing and tan or gray body (brown) bodies, brick red eyes, large compound eyes, and long winds.

+/+ = wild type fly for any phenotype or +/-

**Mutant flies** - any variation from the wild type. Mutant alleles can be carried on autosomes or sex chromosomes. Most mutations are recessive they are indicated with one or two letters in lower case

## 1. Eyes

Wild type: red, oval in shape, and many-faceted.

Mutants: white, black, apricot, scarlet red, pink, or brown; changes in shape and number of facets.

Eye color: rosy- ry( pink) ,sepia- se( dark brown),scarlet- st, brown- bw , white eyes- w

Eve shape: Lobe- L( dominant gene) - reduced eye size, Bar- B - dominant gene - reduced eye size

## Lozenge- lz- narrowing of eye, eyeless- ey - rounded eye - reduced number of ommatidia.

## 2. Wings

Wild type: long, smooth edges, uniform venation, extend beyond the abdomen

**Mutants:** changes in size and shape; absence of specific veins; changes in position in whichwings are held when at rest.

<u>Wings:</u> ap - apterous -no wings- they look like ants, scalloped - sc - cut edges on the wings, vestigial - vg- shortened wings- stubby in nature.

## 3. Bristles

Wild type: fairly long and smooth (note distribution on head and thorax)

Mutants: shortened, thickened, or deformed bristles changes in patterns of distribution.

## **<u>Bristles:</u>** singed – sn.

## 4. Body color

Wild type: basically gray, with pattern of light and dark areas

**Mutants:** black (in varying degrees), yellow, in doubtful cases, color can often be determined most clearly on wing veins and legs. Unless otherwise stated by the instructor, assume that all mutant traits are recessive to the wild type.

## Body color: yellow- y, ebony- eb, black - b



## **CULTURING DROSOPHILA**

We grow flies in vials with about 2 cm of food on the bottom and a foam or cotton plug in the top. At the beginning of the semester, the food will be made by the lab preparator. The foam plugs are necessary to keep foreign flies out!

**Fruit Fly Culture Media(Sugar media):** 30 gm flour, 50gm yeast, 100gm sugar, 15 gm agar, 5 ml proprionate acid (for preventing molds from growing), and 1 liter distal water.

**Procedure**: Mix all ingredients then heat until boiling. Quickly pour mixture into clean culture jars. Cap and let cool to room temperature. This mixture can be stored in the refridgerator with a tightly capped lid until ready for use. When ready to use add fruit flys. Proprionate acid is a mold inhibitor used in bread.

## **Examination of wild-type fruit flies:**

To examine the flies, you need to anesthetize them--otherwise they'll fly away!. The anesthetizer is a plastic gadget with a funnel-shaped opening in one end--this goes over the vial of flies. On the other end is a foam

reservoir with a cap. Put several drops of FlyNap on the foam, then close the cover.

## Putting the flies to sleep (anesthetizing the flies) :

**You will be provided with:** A vial containing wild-type fruit flies, a fly anesthetizer, fly anesthetic chemical(Fly nap or Ether), a white paper card, a paint brush, empty fly culture vials.

Caution : Ether is *dangerously* explosive, so there must be *no* flames in the room.





## Procedure:

1) Remove the bottom cap of your fly anesthetizer, and take out the foam rubber pad.

2) "Charge" your fly anesthetizer by putting about 10 drops of Fly Nap anesthetic on the foam rubber pad, and placing the pad back inside the apparatus. Put the bottom cap back on.

### BEWARE: too much ether will KILL your flies.

3) Remove the top cap from your fly anesthetizer. Tap the bottom of the vial of flies lightly and rapidly on a pad on your bench, then remove the plug from the vial. Quickly invert the fly vial over the top of the open anesthetizer, and tap the whole thing lightly and rapidly on a pad, so that the flies fall into the anesthetizer.

4) Quickly cap the anesthetizer, and keep the flies in the anesthesia chamber until they all stop moving (this should take a couple of minutes).

5) Dump the anesthetized flies out of the anesthetizer onto a white paper card, and view them using a dissecting microscope.

# CAUTION! If your flies start waking up, place them in the sleep box, or put the sleep box over them briefly again.

6) Practice moving the flies around on the white paper card with a fine paint brush. Notice the wild-type, dark red color of the eyes.

7) Separate the males from the females.

8) Once you feel comfortable working with flies and telling males apart from females, it is time to begin screening for mutant flies!



**Appendix:** This is an abbreviated genetic map of Drosophila melanogaster, showing some of the genes that have been mapped to date. Dominant mutations are shown with capitalized symbols. Lethal mutations are marked with an \*.

<b>an</b> -apterous : no wings	<b>D</b> - Dichaete : wings divergent
<b>b</b> - black body.	<b>cn</b> -cinnabar eyes : eyes bright red.
<b>bw-</b> brown eyes	e - ebony body : shining black body.
<b>st</b> - scarlet eyes	<b>vg</b> - vestigial wings : wings and halters reduced.
<b>w</b> -white eyes	y- yellow body
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**m**- miniature wings : wings just slightly longer than abdomen and proportionately narrower.

- se sepia eyes : eyes going from brownish red to black with aging.
- **B** Bar eyes : narrow eye in male and homozygous female, kidneyshaped in heterozygous female.