

## Polytene Chromosomes in Fruit Flies

*Drosophila melanogaster* is the common fruit fly. When you aren't interested in them they breed indiscriminately around fruit in your home. The only dipteran more annoying is the fungus gnat that breeds in unsterilized potting soil. Thomas Morgan's work with the fruit fly in the early 1900's started it on its distinguished genetics career.

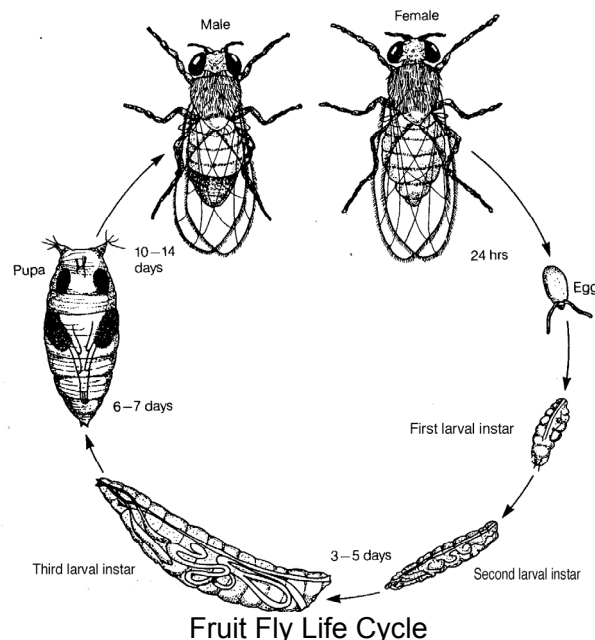
*Drosophila* is an excellent genetic research organism because fruit flies:

- have a short generation time (important for research spanning a number of generations)
- are small and easy to keep in a laboratory
- produce reasonably good numbers of offspring
- have a number of easy to see inheritable characteristics
- have a chromosome number of 8 (4 pairs of chromosomes)

And, if you are willing to hang out in the lab at all hours, fruit flies are pretty easy to selectively breed (which means you choose which hatching female will be bred with which hatching male rather than letting the emerging adults choose for themselves) so you can do a number of different inheritance tests without too much effort.

Insects typically have multiple life cycle stages, including egg, larva, pupa and adult. The fruit fly is typical of those insects whose larval stage is the assimilative stage. Larvae may pass through several stages before they pupate. After pupating for the requisite time, adults hatch out and find mates within hours (minutes?). Adults generally are short-lived. When doing inheritance tests it is critical to differentiate between male and female as well as determining the trait you want to examine. As an introduction to our fruit fly experience, you will practice identifying male and female adult flies.

Fruit flies also have genetically interesting salivary glands. As the free-living larva (or maggot, as some say) grows and feeds voraciously on "Fruit Fly Chow", its salivary gland chromosomes duplicate, but the DNA strands in these chromosomes stay attached to each other. This results in what are called **polytene chromosomes**, thousands of double-stranded DNA molecules that can be viewed under a light microscope. The unusual size and banding of these chromosomes makes them ideal for locating genes and analyzing structural changes that occur in chromosomes.



## Exercise I Identifying Male and Female Adult Flies

### Materials Needed

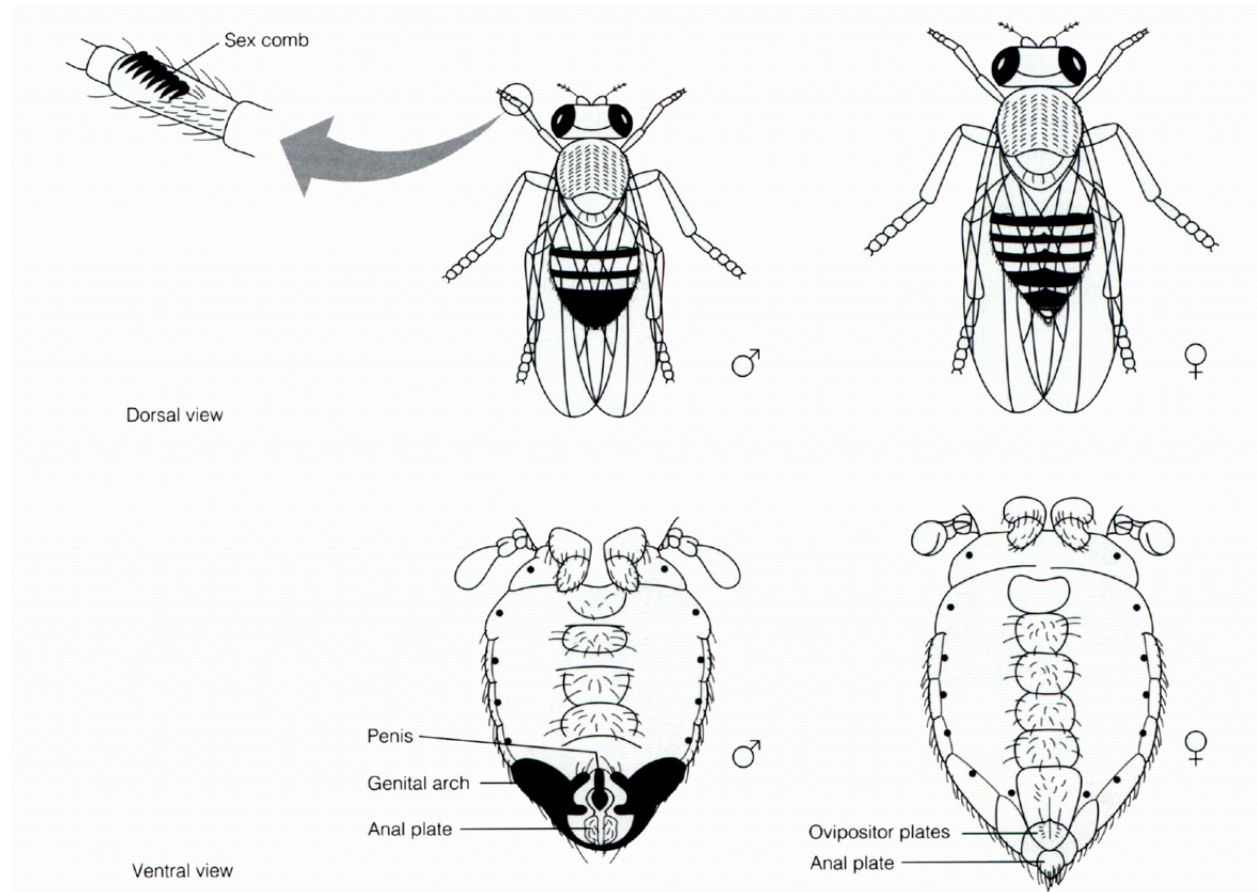
- Fruit Flies
- Fly nap
- Watch glass
- Stereoscopic (dissecting) microscope

### Procedure

Obtain a vial of Fruit Flies and some fly nap. Anesthetize the flies. Gently dump them onto the watch glass. You may want to use a dissecting microscope for your identification. Using the descriptions and diagrams below to guide you, separate the male and female flies. Do you notice any somatic genetic differences among your flies?

### Distinguishing Gender Features in Fruit Flies

- Females are generally larger than the males.
- The abdomen of the female is larger and more pointed than the abdomen of the male.
- 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.
- Male flies have sex combs, tiny brush like tufts of hairs on the front legs near the feet (the basal tarsal segment); females lack sex combs.
- Female flies have anal plates and very dark ovipositor plates on their ventral side. Males have anal plates, a dark colored genital arch and a penis.



## Exercise II Fruit Fly Salivary Gland Chromosomes

Each student will remove the salivary glands from *Drosophila melanogaster* and prepare a microscope slide presentation of the polytene chromosomes. Obtaining a good salivary gland chromosome squash requires skill, patience, perseverance and luck. You may repeat your attempts to remove the salivary glands and make a chromosome preparation until successful (or until there are no more larvae or class time remaining). Your instructor does not have to do this exercise but, as usual, reaps the benefit of the students' efforts and successes. Prior to doing this lab exercise you should watch the video presentation.

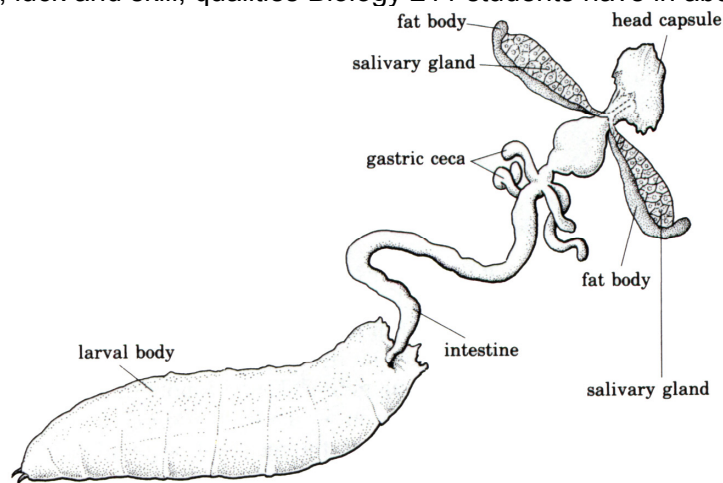
### Materials Needed

*Drosophila* video  
Living *Drosophila melanogaster* larvae  
Dropper bottles of  
0.7% saline  
aceto-orceine stain  
Probes  
Forceps  
Clean slides and cover slips  
Compound light microscope  
Stereoscopic (dissecting) microscope

### Procedure

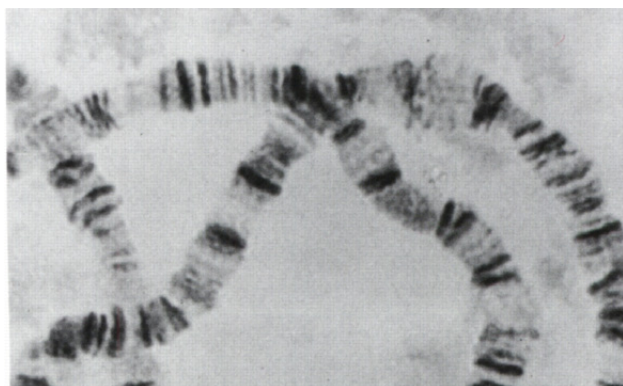
This procedure has been adapted from, Life Science: A Laboratory Manual, by Glenn Powell, Bellevue Community College

1. Prepare a clean slide with 2-3 drops of 0.7% NaCl (saline) and put the slide (without a cover slip) on a stereoscopic (dissecting) microscope.
2. Select a large *Drosophila melanogaster* larva and place it on the slide.
3. While looking through the microscope use probes or forceps to grasp the larva by its midsection just behind its jaws.
4. Gently stretch the larva by pulling on it until its head separates from the rest of its body.
5. Look for the salivary glands in the head section. The glands are very small, fairly transparent, usually paired and have dark fat particles attached. Remember, this takes patience, luck and skill; qualities Biology 211 students have in abundance!



6. When you have located the salivary glands, separate them from the rest of the fruit fly tissues. Once you are certain that you have successfully done this, you may dispose of the rest of the larva appropriately. **Keep the salivary glands moist with saline at all times.**

7. Add 2-3 drops of aceto-orcein stain to your *Drosophila* salivary glands. If the slide is too "messed up" from the separation of the salivary glands from the rest of the larva, you might transfer the glands to a clean slide, using a forceps or probe, prior to adding the aceto-orcein stain.
8. Without a coverslip, put the slide on the stage of the compound microscope. **Use the scanning objective lens.** Have your instructor verify that you have the salivary glands if you have not previously done so.
9. Remove the slide from the microscope and set it on the table. Allow the glands to stand in the stain for 10-15 minutes. Do not let the slide dry out. Add more stain if needed.
10. After the stain has set, get two paper towels and place your slide on one of them. Put a coverslip on the slide (on top of the salivary glands). Fold the second paper towel and place it on top of the coverslip.
11. Place your thumb on the paper towel over the coverslip and press down slowly and firmly, rocking your thumb back and forth a few times. Use sufficient pressure but do not allow the coverslip or slide to slip or move.
12. Examine your stained, squashed salivary glands using the medium power objective lens. Look for nuclei and chromosomes. After you have located chromosomes, use the high power objective lens to see details of the chromosomes. They will look like small thin ribbons with light and dark bands across them.



Polytene Chromosomes

### Laboratory Report

Write a complete lab report of this exercise. You should include outside references to prepare an informative and creative report. Your lab report will be evaluated on a number of criteria including:

- Introduction  
Topics that may be appropriate in the introduction include the life cycle and genome of *Drosophila melanogaster* and research on polytene chromosomes.
- Materials and Procedure  
This should have sufficient detail to permit your experiment to be repeated as you did it.
- Results  
Your results should include successful isolation of the salivary glands, successful preparation of a chromosome squash and the overall presentation of your results in the report.
- Discussion  
Interpretation of your results and evaluation of the exercise.

Materials for this lab were provided by BCC Life Science Instructors, Jim Ellinger and Melodye Gold. The demonstration video was done by Glenn Powell, former BCC Life Science Instructor.