

DID YOU KNOW?

A female drosophila once fertilized, may lay 30-50 eggs per day throughout her lifetime.

OBJECTIVES

- Learn basic handling and culture techniques for working with *Drosophila*
- Apply concepts and principles of Mendelian inheritance patterns
- Diagram monohybrid, dihybrid, and sex-linked crosses
- Gain experience sorting, sexing, and crossing *Drosophila* through two generations
- Perform a chi-square statistical analysis of experimental results

MATERIALS

MATERIALS NEEDED PER GROUP

- 1 Culture vial of wild-type *Drosophila*
- 1 Culture vial A
or
- 1 Culture vial B
or
- 1 Culture vial C
- 1 Isopropyl alcohol 10%, 100 ml
- 1 Camel's hair brush
- 1 Thermo-anesthetizer
- 1 Petri dish
- 2 *Drosophila* vials and labels
- Drosophila* medium
- 1 Fly morgue
- Forceps

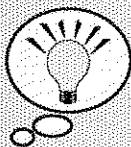
PROCEDURE

Part A: Working with *Drosophila*

*You will need to observe wild type *Drosophila* to familiarize yourself with the wild type phenotype. You will eventually be assigned a cross without being told what strain, genotype, or type of experimental cross that you have received. You will examine the flies in the parental generation of your cross, noting any phenotype variations from the wild type, and name the mutations.*



1. Thermally immobilize a vial of wild-type *Drosophila*. Your instructor will demonstrate the proper immobilization technique.



DID YOU KNOW?

The longest sperm ever recorded is 10,000 times longer than a human spermatozoan, and belongs to *Drosophila bifurca*. The typical male *D. bifurca* sperm is approximately 60 mm long, 20 times the length of the fly itself.



Your instructor may have immobilized the flies in advance. If this is the case, begin with step 2.

2. Observe the flies' traits, particularly body features that distinguish males and females, eye color, and wing size and shape. Record your observations in Table 1 in the Analysis section. If, at any time during your observations, the flies begin to become active, re-immobilize them according to your instructor's directions.



Use the camel's hair brush to move the flies when making observations.

Part B: Performing a *Drosophila* Cross

1. Obtain a vial of a prepared *Drosophila* cross.



These flies have been mated and may exhibit one or more mutations. They are the parental generation for your experiment. The offspring of this generation, which should already exist as eggs or larvae in the vial, are the F₁ generation.

2. Record the letter written on your vial in Table 2 in the Analysis section to help you keep track of which cross you have received. This will aid in determining expected results, as well as allow your instructor to identify any problems you may be having and to help correct them.
3. Immobilize the parental generation of your cross and observe the flies under a stereomicroscope. If, at any time during your observations, the flies begin to become active, re-immobilize them according to your instructor's directions.
4. Separate the males from the females. Note any mutations from the wild-type phenotype, as well as whether the mutation is apparent in the male or female flies. Record your observations in Table 2.



You may be sharing the parental generation with another group for observation. If this is the case, do not perform the next step until all groups have had a chance to make their observations.

5. Place the parental generation in the morgue.
6. Place the vial (with the parental generation removed) in a warm (28°C) place to incubate to allow the F₁ generation to mature. Observe the vial occasionally and record your observations.



Do not allow the temperature to exceed 30°C.

7. When the adult flies emerge, collect, immobilize, and examine them. Note the sex of each one, as well as the presence of any mutations. Record your observations in Table 3. Be sure every group assigned to that cross has a chance to observe and count the F₁ progeny.
8. Prepare a fresh culture vial: Place approximately one tablespoon of medium in the bottom of a vial. Add an equal amount of water and let it absorb. You may want to add a piece of plastic mesh to give the flies something to crawl on, but it is not essential. Insert a foam plug in the vial.
9. Place five or more mating pairs from the F₁ generation into the fresh culture vial (It is not necessary that these females be virgins). Label the vial with your name(s), date, and letter of cross. Place the culture vial in a warm place to incubate to allow the F₁ generation to mature.
10. Transfer the remaining non-mating F₁ flies to the morgue.
11. Leave the F₁ adults in the vial for about one week to mate and lay their eggs. Once they have laid their eggs and you can see larva on the sides of the culture vial (7- 10 days), remove the adults, place them in the morgue, and wait for the F₂ generation adults to emerge.

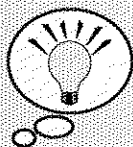


Do not allow the temperature to exceed 30°C.

12. The F₂ adults will gradually emerge from the pupae over several days. As the F₂ generation flies begin to emerge as adults, immobilize and examine them. Record the number of males and females, noting any mutations which may be present. Record your findings in Table 4.



Try to collect as many adults as possible.



DID YOU KNOW?

In 1913, American geneticist Alfred Henry Sturtevant developed a technique for mapping the location of specific genes of the chromosomes in *Drosophila*.

OPTIONAL EXERCISE

Polytene Chromosome Investigation

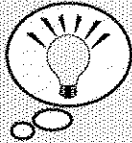


Students will investigate chromosomes and their activity by examining the salivary glands of Drosophila larvae, which contain giant polytene chromosomes as a result of the repeated replication of the DNA. They make excellent organisms for chromosome observation, and for investigations into chromosome function and structure. They are most evident in D. virilis larvae, but can be observed in any species of Drosophila.

MATERIALS

MATERIALS NEEDED PER LAB GROUP

- HCl, 8%
- Aceto-orcein stain, 30 ml
- Piccolyte II, 15 ml
- 1 Teasing needle
- 1 Pipet
- 1 Forceps
- 1 Microscope slide
- 1 Coverslip
- Drosophila larva
- Gloves
- Goggles
- Lab aprons
- Stereomicroscope
- Distilled water
- Paper towels

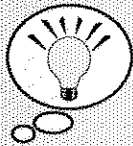


DID YOU KNOW?

Drosophila salivary glands consist of two major cell types secretory cells and duct cells.

PROCEDURE

1. Place a microscope slide on a flat surface. Add two or three drops of distilled water to the center of the slide.
2. With forceps, grasp a fully-grown Drosophila larva from the wall of the wild-type Drosophila culture vial. Place the larva in the drop of water on the microscope slide.
3. Under a stereomicroscope, locate the head of the larva. It will appear darker than the rest of the body, and the mouthparts should be evident. The larva will also be moving in that direction.
4. Grasp the larva in the midsection with the forceps. Squeeze it gently to force the head out.



DID YOU KNOW?

Measuring 2 mm in length, the polytene chromosomes found in the salivary glands of Diptera are much longer than metaphase chromosomes.

- Pierce the head with the teasing needle and pull the head away from the body. The salivary glands, and likely the digestive tract also, will detach from the body. The salivary glands will appear clear and cellular, but may be confused with the darker, opaque fat bodies. The intestine may be present as a tubular, branched structure.
- Remove all the excess materials from the slide.
- Touch a corner of a paper towel to the slide to blot off only the excess water.



Do not let the salivary glands dry out.

- Add two or three drops of 8% HCl to hydrolyze the genetic material for better penetration by the stain. Let it sit for three minutes.



Avoid skin or eye contact with HCl. Wear safety goggles, gloves, and a lab apron.

- Touch a corner of a paper towel to the slide to blot off the excess HCl.
- Add two or three drops of aceto-orcein stain to the salivary glands and let them to sit for four to five minutes. Do not let the stain dry out. Add more stain if necessary.



Aceto-orcein stain is an irritant. Avoid skin or eye contact. Wear safety goggles, gloves, and a lab apron.

- Blot the excess stain from the slide; leave a small amount of stain on the glands.
- Place the slide on a smooth, flat surface and cover with a coverslip. Tuck the slide into the fold of a paper towel and press down firmly on the coverslip with your thumb or a pencil eraser.
- Observe the preparation under a compound microscope. Note the bands in the polytene chromosomes, the "puff" regions, and the chromocenter of the chromosome. Draw what you see in the space provided in the Analysis section.

Name: _____
 Group: _____
 Date: _____

ANALYSIS

Table 1
Phenotypes of Wild Type Drosophila

	Eye Color	Wing Size and Shape
Male		
Female		

Table 2
Phenotypes of the Parental Generation

Cross Letter: _____

Phenotype	No. of Males	No. of Females

Table 3
Phenotypes of the F₁ Generation

Cross Letter: _____

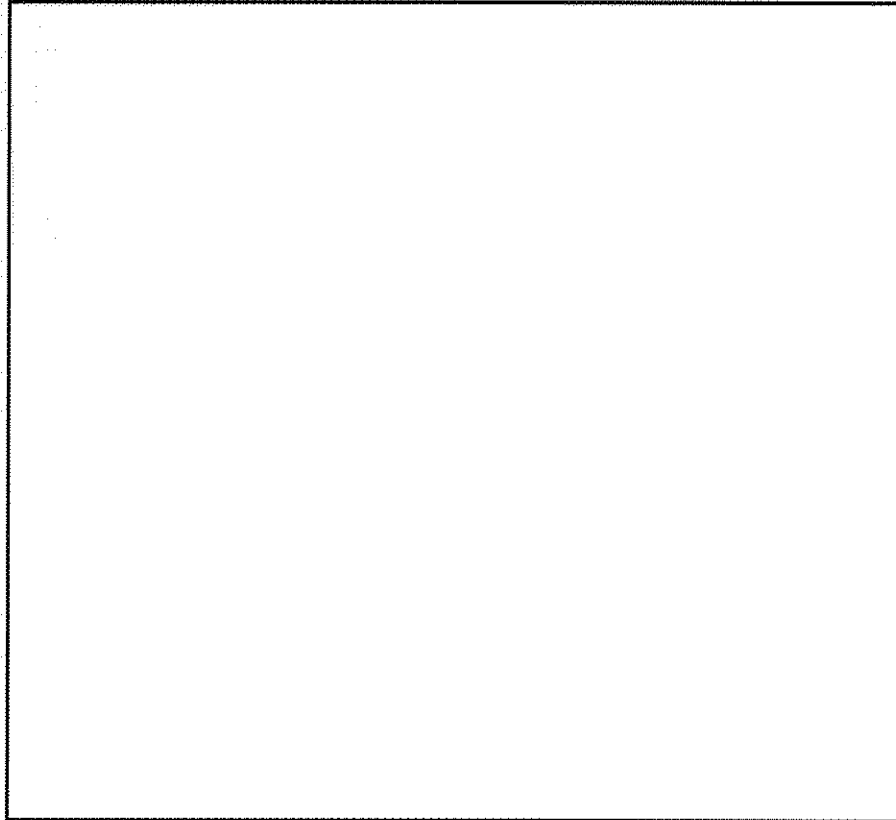
Phenotype	No. of Males	No. of Females

Table 4
Phenotypes of the F₂ Generation

Cross Letter: _____

Phenotype	No. of Males	No. of Females

Polytene Chromosome Investigation



WARD'S
AP Biology Lab 7
Genetics of Drosophila
Lab Activity

Name: _____
Group: _____
Date: _____

ASSESSMENT

1. Describe the parental cross you received; use genetic symbols.

Example: A cross between vestigial and wild-type flies would be expressed as $vv \times VV$.

Draw a Punnett square to show the possible allelic combinations for this gene in the F_1 generation.

2. Identify the genotype the F_1 flies should exhibit. Identify the phenotype. Compare your experiment results by counting the members of the F_1 generation.

3. Describe the F_1 cross you performed, and draw a Punnett square to show allelic combinations possible in the F_2 generation.

4. Identify the genotype ratio the F₂ flies should exhibit. Identify the phenotype ratio. Compare your experiment results by counting the members of the F₂ generation.

5. Identify the type of cross you received: monohybrid or dihybrid, autosomal or sex linked, mutations dominant or recessive.

6. Using a chi-square test, determine whether or not the variation between the observed and expected number of individuals of each phenotype can adequately be explained by chance alone. Use the following formula, and apply it to the chi-square table (on the following page) to determine the confidence level that states the variation is due solely to chance.

$$\chi^2 = \sum (O-E)^2/E$$

O = observed number of offspring for the phenotypic category

E = expected number of offspring for the phenotypic category

$$\chi^2 = \underline{\hspace{10em}}$$

Confidence level that variability is due entirely to chance = %

