



## Winning Experiment Procedures from the NIH LAB Challenge

### Exploring Genetic Inheritance in *Drosophila*

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**Suggested grade level:** High School

#### Background

Our module aligns with what students are learning in the science classroom from K–12 through undergraduate teaching laboratories. Adriana Méndez, an undergraduate research assistant, helped develop the procedure and collaborated with field testing in after-school programs at local schools. Our innovative, economical approach seeks to lower the financial barriers that prevent schools from accessing and using research-oriented activities at school.

#### Objectives

After completing this experiment, students will be familiar with

- *Drosophila* as a suitable genetic tool in biomedical research;
- the developmental stages, anatomical structures, and sex differences in *Drosophila*;
- basic Mendelian genetics, terms, and applications; and
- how to determine the genotypes of unknown parental strains by looking at the ratio of phenotypes in the resulting offspring.

By making genetic crosses between a wild-type and a mutant fly strain, students create offspring, known as the first filial generation. Students can then determine the genetic makeup of the parental flies.

#### Hypothesis

Students decide whether their hypothesis is that the mutation is on an autosomal or a sex chromosome.

#### Duration

Total actual in-class time (not to exceed 90 minutes): 30 minutes

Set-up time: 20 minutes

Experiment's run time: 2 weeks

Take-down time: 10 minutes

#### Materials

##### *If Purchasing Drosophila*

- Vial of *Drosophila* wild type (1; \$7)
  - <http://www.carolina.com/product/living+drosophila+wild+type.do?keyword=Drosophila>
- Vial of *Drosophila* mutants (1 or 2; \$7 each)

- Sex-linked mutants:
  - <http://www.carolina.com/product/drosophila%2C+bar.do?keyword=Drosophila>
- Autosomal mutants:
  - <http://www.carolina.com/product/drosophila%2C+living%2C+eyeless%2C+chromosome+4+mutant.do?keyword=Drosophila>
- 2 Alka-Seltzer tablets (\$3)
- Beaker or plastic cups (\$1)
- Cotton balls (\$1)
- Tweezers (\$1)
- Ziploc bags (sandwich size) (\$3)

### *If Collecting Wild-Type Drosophila*

- Vial of *Drosophila* mutants (1 or 2; \$7 each)
  - Sex-linked mutants:
    - <http://www.carolina.com/product/drosophila%2C+bar.do?keyword=Drosophila>
  - Autosomal mutants:
    - <http://www.carolina.com/product/drosophila%2C+living%2C+eyeless%2C+chromosome+4+mutant.do?keyword=Drosophila>
- 2 Alka-Seltzer tablets (\$3)
- Mesh (mosquito net) (1 sq. ft.; \$1)
- Handsaw blade (\$2)
- Polypropylene tube (\$10)
- Super glue (\$2)
- Aluminum foil (\$1)
- Small Petri dish (\$1)
- Fruit (\$1)

## Procedure

### *Preparation*

1. Acquire *Drosophila*. You may purchase mutant and wild-type flies online at a nearby laboratory, or you may purchase just the mutants and collect the wild-type ones yourself.

1a. To collect wild-type flies, start by building a collection chamber. See **Figures 1–3**. Next, select any available fruit (you can use mangoes, bananas, etc.). Cut a small piece of fruit and place in a 35-mm Petri dish and leave exposed for two to three days, or until fruit flies are attracted to the decaying fruit. When flies are on the fruit, close the chamber, as shown in **Figure 3**. Make sure it is not tightly sealed!

Wait a couple of days for flies to eclose (that is, for adults to emerge from larvae). Transfer to a “fly collection chamber.”

1b. To obtain flies from selected online sources or nearby *Drosophila* research laboratories, select from available, suitable wild-type and/or mutant fly lines.

2. Two weeks before the planned classroom activity, set up the genetic cross.

2a. Anesthetize wild-type flies and select 10 virgin adult female flies:

- Place a cup or beaker containing water inside a Ziploc bag.
- Leave a small opening, enough so a vial can be inserted and flies can be poured inside without falling into the cup with water.
- Rapidly insert an Alka-Seltzer tablet inside the cup of water, and seal the Ziploc bag. Leave until no movement is seen.

2b. Anesthetize mutant flies, and select five adult male flies following the procedure above.

2c. Sexing flies: Male and female fruit flies can be distinguished using the following criteria: (1) males have sex combs on the forelegs (**Figure 4**) and (2) the tip of the abdomen in females is slightly more elongated and “pointy” than in males (**Figure 5**).

2d. Add the wild-type female flies and the mutant male flies together into a vial. Add a cotton ball big enough to fit tightly. After a week, you will see larvae. Anesthetize the flies, discard them, and allow larvae to grow for one more week. Repeat the above procedure for every mutant line you have.

### *Experiment*

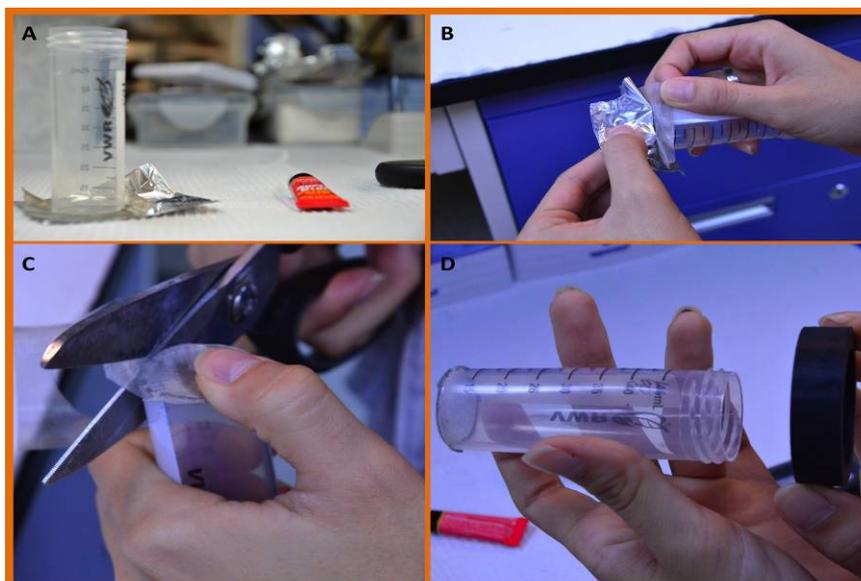
3. Your teacher will give you two *Drosophila* fly vials. Both vials contain the F<sub>1</sub> (first filial) generation of a cross set up two weeks ago.

3a. Using a magnifying glass or a microscope, observe the different stages of flies in your vial. Draw your observations in **Table 1** (on the Student Worksheet), paying particular attention to eyes, wing shape, and body pigmentation. Use **Figure 6** to help you identify the different stages.

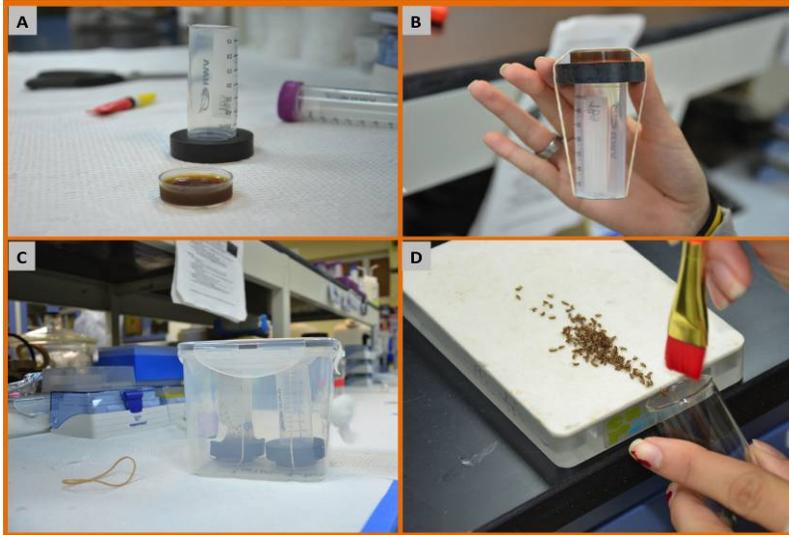
3b. Anesthetize flies. Place the cup containing water, inside the Ziploc bag. Leave a small opening, enough so a vial can be inserted and flies can be poured inside without falling into the cup with water. Rapidly, insert an Alka-Seltzer inside the cup of water and seal the Ziploc bag. Leave until no movement is seen. Observe under the microscope or magnifying glass. Classify flies according to sex and mutation. Write your observations in **Table 2** (on the Student Worksheet).



**Figure 1. Constructing a fly-handling and -collection chamber.** (A) Materials required for the construction of the chamber, *from left to right*: fine mesh, handsaw blade, scissors, 50-ml polypropylene tube, aluminum foil, custom-made plastic base, super glue. (B) Using the handsaw, cut the upper portion of the 50-ml polypropylene tube and clean the newly exposed edges. (C) Cut a 1-inch-by-1-inch fine mesh square. (D) Using super glue, attach the mesh and place it upside down in the aluminum foil. Aluminum foil is used to prevent damage to bench-top surfaces.



**Figure 2. Finalizing the fly-handling and -collection chamber.** (A) Once the glue is dried and the fine mesh firmly attached, (B) carefully remove the aluminum foil and (C) cut any excess mesh to conform the shape of the tube. (D) Here we used a custom-made plastic base on the tube, but you can use a small Petri dish bottom. Make sure the upper mesh is secure and that no holes are left where flies could escape.



**Figure 3. Preparing the fly-handling and -collection chamber for collecting wild-type flies.** (A) Use 35-mm Petri dishes to hold the piece of fruit used to attract wild-type flies. Leave exposed for a couple of days and (B) secure to the custom-made plastic base with a rubber band. (C) Store the collection chamber in a plastic container for safe handling and storage. Make a circular hole in the plastic container and place a cotton ball in it. This allows oxygen into the container for the survival of the flies. Also, make sure a wet napkin is placed at the bottom to keep a humid environment appropriate for survival of the flies (D).



**Figure 4: Sex determination by sex combs.**



**Figure 5. Sex determination using the abdomen as the criterion.**

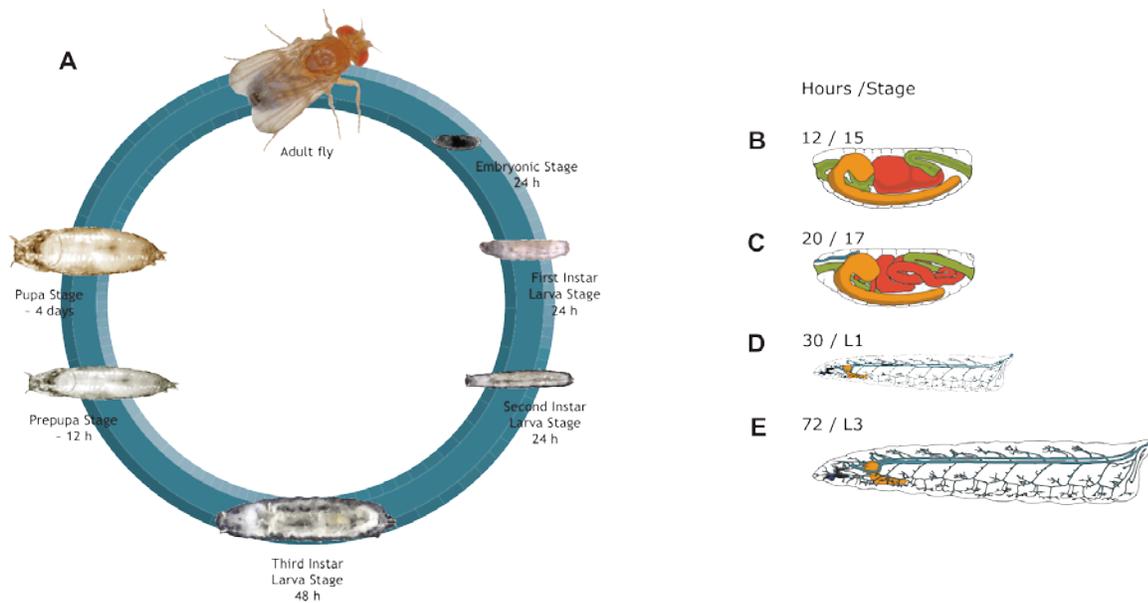


Figure 6: ***Drosophila* life cycle.** After egg-laying (AEL), it takes about 12 hours for embryos to reach stage 15, which we use throughout the present study. Shortly thereafter, all motor neurons find their correct postsynaptic partner muscle cells and establish a functional synapse. Once synaptogenesis is completed, muscle contractions begin and embryos hatch into a first-instar larva (L1). After about a day, it molts into a second instar (L2), and two days after hatching, the L2 larva molts again to become a third-instar (L3) larva. During these wandering stages, the larva feeds and eventually crawls out of the food and becomes encapsulated in the pupal case and undergoes metamorphosis. The *Drosophila* life cycle is completed in about eight days under ideal temperature (25°C) conditions (A). Diagram modified from Fly Move [<http://flymove.uni-muenster.de>]. (B-C) Embryonic developmental stages highlighting central nervous system and gut development events shown. (C) Stage 15 embryos are selected for the present study with corresponding light-micrograph stage 15 embryos. Once the embryo hatches at about 30 hours AEL, the first-instar larva (L1) develops mouth hooks and a tracheal system, is able to freely wander in search of food (D), and triples its size (E) in the next 32 to 48 hours after hatching to become a third-instar larva (L3). *Figure: Vega and Carrero-Martinez, 2011.*

### Experiment Take Down

Discard flies outdoors or down a drain. If a drain is used, drown flies with water, pour down the drain, and let the water run for a few seconds afterwards.

### Data Analysis

The idea is to work back to determine the parental genotypes from the ratio of  $F_1$ . Using your observations from **Table 1** (on the Student Worksheet), identify the sex and type of fly (wild type or mutant) in your cross in **Table 2**. In genetics, normal flies are called “wild type,” and any fly with a mutation is called a “mutant.” Mutant flies receive their names from the type of mutation. Each mutation is given a letter code. There are two types of mutations we could consider today: (1) **autosomal mutations** describe defects on a gene located in a non-sex-determining chromosome and (2) **sex-linked mutations**, which describe sex-linked genes, located on one of the sex chromosomes.

- Can you establish a relationship between the proportions of progeny with certain characteristics?
- With the proportions established, can you predict the genetic makeup of the parental flies?
- Is the mutation on an autosomal or sex chromosome? Explain how you reached that conclusion.

### Conclusion

Through our experiment in the lab and in-the-field trials with local public high schools, we were able to establish the feasibility of our proposed implementation. The *Drosophila* genetic crosses laboratory is a well-established laboratory that has remained accessible mostly at the undergraduate college levels.

### Relevance to NIH Mission

This activity is aligned with NIH's mission in that it seeks "fundamental knowledge about the nature and behavior of living systems [in this case, *Drosophila* and genetics] and the application of that knowledge to enhance health and reduce the burdens of diseases."

[Updated 06/08/12]

### Supplementary Information

The study of inheritance began with Gregor Johann Mendel, who published his work in 1865. Then, during the early 1900s, Thomas Hunt Morgan started studying mutations in *Drosophila* flies. Based on research by Morgan and his students, the chromosome theory of inheritance was proposed. This theory simply states that chromosomes are the basis for all genetic inheritance. It also states that chromosomes are linear structures composed of genes. In 1915, Morgan and his colleagues wrote the book *The Mechanism of Mendelian Heredity*, which became the fundamental textbook for students of this new field. In 1933, Morgan was awarded the Nobel Prize in Physiology or Medicine for his work in genetics. To learn more about Morgan's story, please visit:

[http://www.nobelprize.org/nobel\\_prizes/medicine/laureates/1933/morgan-bio.html](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1933/morgan-bio.html)

*Drosophila* are used extensively as a model organism in genetics, cell biology, biochemistry, developmental biology, and many other fields. In 1995, the Nobel Prize in Physiology or Medicine recognized *Drosophila*'s importance in human health. The recognition was awarded to Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric F. Wieschaus "for their discoveries concerning the genetic control of early embryonic development." Learn more at:

[http://www.nobelprize.org/nobel\\_prizes/medicine/laureates/1995/](http://www.nobelprize.org/nobel_prizes/medicine/laureates/1995/)

The **scientific classification** of the fruit flies you will be using today is the following:

**Domain:** Eukarya  
**Kingdom:** Animalia  
**Phylum:** Arthropoda

**Class:** Insecta  
**Order:** Diptera  
**Family:** Drosophilidae  
**Genus:** *Drosophila* (“dew lover”)  
**Species:** *melanogaster* (“dark gut”)

The *Drosophila* **life cycle** exhibits complete metamorphosis (see **Figure 1**). This means that the life cycle includes egg, larva (worm-like), pupa, and finally emergence (eclosion) as an adult. The larval stage has three instars, or molts. Below is a rough developmental timeline.

**Day 0:** Female lays eggs.

**Day 1:** Eggs hatch.

**Day 2:** First instar (one day in length).

**Day 3:** Second instar (one day).

**Day 5:** Third and final instar (two days).

**Day 7:** Larvae begin roaming stage.

Pupariation (pupal formation) occurs 120 hours (about five days) after egg-laying.

**Days 11, 12:** Eclosion (adults emerge from the pupa case). Females become sexually mature 8 to 10 hours after eclosion.

The time from egg to adult is temperature-dependent. The developmental timeline presented here occurs at 25°C. If the temperature is increased, the generation time becomes shorter. If temperature decreases, generation times become longer.

Organisms are composed of traits, which are determined by the genes individuals carry in their chromosomes. These traits that are expressed are called the phenotype (observable traits) and are determined by the genotype (genetic makeup) of an organism. In this experiment, we study how the expression of these genes affects traits using the fruit fly, *Drosophila*.

Genetic information is passed from parental figures to their offspring. Sexual reproduction starts by the formation of a zygote, which is a cell formed by gametes from the mother and father. Each gamete has genetic information from each of the individuals involved in the reproduction. In the zygote, the genetic information is stored in the chromosomes, which will be carrying the genes. Each gene expresses a certain characteristic due to the DNA it stores, but it can have two variants. It can express the characteristic passed down by the mother or the father. This is called an allele, an alternative form of a gene.

Genes inside chromosomes can be homozygous or heterozygous. The term homozygous refers to having two identical alleles for a certain trait, meaning the organism can have only one possible characteristic expressed because it inherited the same one from both parents. Unlike homozygous, heterozygous refers to having two different alleles for a particular trait. This means the organism will randomly express one of two possible characteristics passed down from each of the parents. In heterozygosis, one of these alleles will be determined as dominant,

which will be the one who is being expressed phenotypically. A recessive allele will also be present but will not be expressing its genetic information.

In this experiment, we use *Drosophila*, a fruit fly that is a common model of study in science fields due to its size, quick reproducibility, simple maintenance, and short life cycle. This fruit fly is available in its natural form (wild type) or it can be genetically altered to express characteristics different to that of its normal state (mutant). These alternate characteristics are determined by the flies' genotype. Usually, the wild-type flies' genetic makeup cannot be determined by its phenotype because it comes from a natural habitat. Mutants are genetically altered and already predetermined with a certain genotypic inheritance pattern.

For scientist to determine the genotype of a wild-type fly, they must cross it with a fly that has the recessive phenotype of the trait that is being studied. Phenotypes of the progeny will explain the genotype of the wild-type parent. If traits remain the same in progeny, the wild-type fly also has a recessive genotype. If a difference is observable, the wild-type fly will probably have a heterozygous pattern of inheritance or a homozygous-dominant pattern of inheritance. Observe the following example crosses for recessive eyeless characteristics.

Here, we have two example crosses for the characteristic of eye appearance. The eyeless characteristic is recessive; therefore we can use this line to determine the genotype of the wild-type fly. If the wild-type fly is homozygous dominant, we will see that all the progeny will express the eye characteristic. If the wild-type fly is heterozygous, we will see that half the progeny will express the eye characteristic, and the other half will not.

Wild type: +/+ or +/-

Eyeless: ey/ey (homozygous recessive)

	ey	ey
+	+/ey	+/ey
+	+/ey	+/ey

	ey	ey
+	+/ey	+/ey
-	-/ey	-/ey

## Student Worksheet: Exploring Genetic Inheritance in *Drosophila*

Name: \_\_\_\_\_

Date: \_\_\_\_\_

**Table 1.** Using a magnifying glass or a microscope, observe the flies at different stages in your vial. Draw your observations in Table 1, paying particular attention to eyes, wing shape, and body pigmentation. Use Figure 6 to help you identify the different stages.

Eyes	Wing shape	Body	Notes

**Table 2.** The idea is to work back to determine the parental genotypes from the ratio of  $F_1$ . Using your observations from Table 1, identify the sex and type of fly (wild type or mutant) in your cross in Table 2.

Fly type	Females	Males
Wild type		
Mutant		

- Can you establish a relationship between the proportions of progeny with characteristics?
- With the proportions established, can you predict the genetic makeup of the parental flies?
- Is the mutation on an autosomal or sex chromosome? Explain how you were able to reach that conclusion.