

Introduction to *Drosophila* and Conducting Crosses

INTRODUCTION

The fruit fly, *Drosophila melanogaster*, is a common pest organism. However, the U.S. government and numerous research institutions expend millions of dollars each year to raise *Drosophila*. This is because *Drosophila* is one of the most important model organisms for studying the genetics of eukaryotic organisms. Since the early 1900s, when Thomas Hunt Morgan first began to study *Drosophila*, researchers have accumulated an amazing amount of information on the inheritance of various traits in this organism. Thousands of mutations have been characterized, and in 2000, *Drosophila* became the third eukaryotic organism for which the entire genome had been sequenced.

Although *Drosophila* is a relatively simple organism, it exhibits many physical and biochemical characteristics that exhibit variations (i.e., mutant phenotypes). This abundance of variety is one characteristic that has made this organism so fascinating to geneticists. Many of the other reasons it has become such a popular subject of study are related to the characteristics of its life cycle. First, it is relatively easy to distinguish males from females and to set up controlled crosses. Second, it is a very small organism and can be easily grown in large numbers in small spaces. Finally, it proceeds through its life cycle very rapidly, making it possible to conduct genetics experiments relatively quickly.

In this laboratory activity, you will become acquainted with the basic characteristics of *Drosophila* that make it useful for genetic studies. You will anesthetize flies and learn how to tell females from males, and you will learn to identify some of the mutant phenotypes. You will also learn the basic techniques needed to conduct crosses with this organism, and you will set up a simple cross. These procedures may be conducted during two or more scheduled lab periods, or your instructor may instruct you to come back to the lab on your own later to complete some of the steps (i.e., setting up a cross often requires several brief visits to the lab to obtain sufficient numbers of flies at appropriate stages).

PROCEDURES

Anesthetizing Flies

It is very important to learn to anesthetize flies properly. First, good anesthetization practices prevent the escape of flies. Second, good techniques are necessary to prevent flies from dying or becoming reproductively inviable, both of which are significant impediments to fly breeding.

The instructor will demonstrate this important technique during the lab. The following outline of the procedure assumes that you will be using ether for anesthetization. However, several other alternatives are available (CO₂, cold treatment, commercial anesthetics, etc.). If ether is used, ensure that

proper ventilation is used, and avoid breathing the fumes. Excessive exposure to ether is hazardous to humans, as well as to flies! Also, do not use open flames when ether is in use, because ether is highly flammable.

To anesthetize flies with ether, you will need the following materials: cultures of flies, a funnel, ether, a transfer bottle (with a stopper), an etherizing bottle (with a stopper with cotton attached), and a reetherizer (a Petri plate lid with cotton attached to the inside).

To view the anesthetized flies, you will need a dissecting microscope, a white 3 x 5-inch card (to hold the flies), and a fine-tipped paintbrush, used for moving individual flies.

The following is an outline of the steps used to anesthetize flies with ether:

Step 1: Organize the necessary materials.

Obtain a culture with lots of adult flies and identify a working area with a soft surface (several paper towels, a soft-cover book, etc.) on which to “bang” the fly culture. It is necessary to “knock” the flies down inside the culture before transferring them (discussed later), and the soft surface deadens the banging sound and helps to prevent culture bottles from breaking.

Dampen the cotton in your etherizing bottle and reetherizer with ether; place the stopper for the etherizer into the bottle (this allows ether vapor to saturate the inside atmosphere of the etherizing bottle).

Place the funnel into the transfer bottle and ensure that the stopper for this bottle is within easy reach.

Step 2: Knock the adult flies down within the culture. By lightly banging the culture bottle on the soft surface, flying and crawling flies are knocked down from the top of the bottle. This is necessary so that they don't escape during transfer. If glass culture bottles are used, *be very careful not to break the bottles*. Check culture bottles for hairline fractures prior to this step; do not use bottles if such fractures are evident. If intact bottles are banged softly, they should not break.

Step 3: Transfer flies to the transfer bottle. The transfer bottle is used to hold adult flies just prior to anesthetization. Adult flies will be shaken into this bottle and then later transferred into the etherizing bottle. It is possible to shake adult flies directly into the etherizing bottle from the culture; however, the ether fumes are more damaging to eggs and larvae than to adults, so a transfer bottle is used if it is desirable to obtain additional healthy adults from the culture at a later time.

To move the flies from the culture bottle into the transfer bottle, continue lightly banging the culture bottle on the soft surface. While continuing to bang the culture bottle (don't forget to keep banging!), remove the stopper from the culture vessel. If you continue banging the culture, the flies will remain at the bottom — if you stop, they will escape! Once the stopper is removed from the culture vessel (keep banging), use your free hand to steady the transfer bottle containing the funnel. Next, use one quick, fluid motion to invert the culture vessel into the transfer bottle, and immediately begin banging the combined transfer bottle-funnel-culture bottle. This will knock flies from the culture bottle into the funnel and into the transfer bottle.

Once the flies are inside the transfer bottle, they will usually not be able to escape by flying up into the narrow end of the funnel. At this point, replace the stopper into the culture bottle and proceed with the next step.

Step 4: Transfer flies from the transfer bottle to the etherizer. Use essentially the same technique described in Step 3 to move flies between these two bottles. Begin by knocking the flies into the bottom of the transfer bottle. While the flies are being knocked into the bottom of the transfer bottle (keep knocking!), remove the stopper with the ether-soaked cotton from the anesthetizing bottle, and transfer the funnel to the etherizing bottle. Keep knocking. Use the technique described earlier to shake the flies into the etherizing bottle. As quickly as possible (while knocking the flies down inside the etherizing bottle), replace the funnel with the stopper. Do not allow the flies to remain in the etherizer for too long.

Step 5: Remove and observe flies. It is important to find the correct balance between too little and too much anesthetization. The flies should be sufficiently immobilized so that they will not stand up when the etherizing bottle is rotated and/or slightly knocked. However, too much anesthetization will reduce fertility or even kill the flies. It is better to underanesthetize the flies if you are uncertain, and then use the reetherizer to reanesthetize them later, if necessary. The reetherizer is used by ensuring that the cotton is dampened with ether and then using it to completely cover flies that are awakening.

Handle anesthetized flies on a white 3 x 5-inch card, and move them while viewing with a dissecting microscope by pushing them with a fine-tipped paintbrush.

Distinguishing Differences in the Sexes

To conduct specific crosses with *Drosophila*, it is very important to be able to distinguish the two sexes. Crosses are generally made by placing several males of one strain and several virgin females from a different strain together in a fresh culture vessel. If the gender of any of the flies used in such a cross is incorrectly assessed, the entire cross will be invalid. To ensure that you can correctly identify male and female flies, follow the instructions below carefully.

Obtain and anesthetize several wild-type flies, and find at least one male and one female. The following are the most obvious sexual characteristics that help to distinguish the two sexes of *Drosophila* (also see Figure 3.1):

1. The posterior of the male body is dark; the female abdomen appears striped.
2. The tip of the male abdomen is rounded, whereas the female abdomen is elongated.
3. The male abdomen has five segments, while the female abdomen has seven segments.
4. The male has sex combs on the distal surface of the basal tarsal joint of the first leg; the female does not.
5. The male sex organ is enclosed by a genital arch, while the female sex organ is covered by a vaginal plate.
6. On average, the male body is smaller than the female body (however, this is not an extremely reliable difference).

Verify for yourself that you can actually see the differences in the distinguishing characteristics. As you continue your work and study various non-wild-type phenotypes, check to be sure you can distinguish males from females when these phenotypic variations exist.

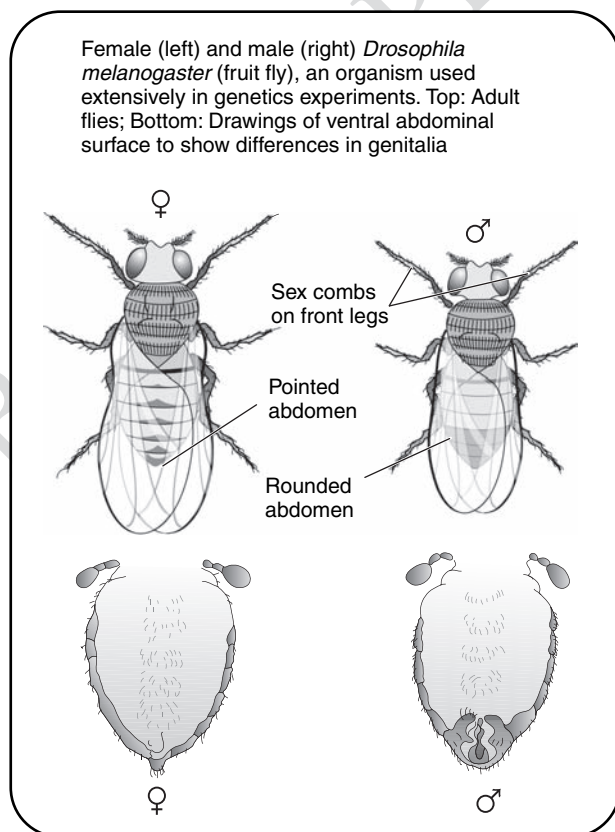


Figure 3.1 – The sexual characteristics of *Drosophila melanogaster*. See the text for descriptions of the distinguishing characteristics.

Distinguishing Different Mutant Phenotypes

In this part of the lab activity, you will become familiar with several mutant phenotypes of *Drosophila*, which your instructor has chosen for you to use. These flies are from the strains that you will be instructed to use for crosses at the end of this activity.

For this portion of the activity, you should work in small groups of several students. Obtain a collection of different coded vials from the instructor. Each vial contains flies that either are wild-type or exhibit one particular mutant phenotype. A list of the possible mutant phenotypes will be available in the lab. Your group should determine which phenotypes are present in each coded vial. Each member of the group should verify the conclusions made in this portion of the assignment. You can check unknown phenotypes against the flies available in stock cultures for verification. Be careful not to mix known flies with unknown flies! The instructor will have a list of codes and corresponding phenotypes; however, this list will not be accessed during the lab period.

Q2 List the codes for each of the vials that you worked with, and indicate the official designation for the phenotype of the strain present in each vial. Also provide a brief written description of the appearance of each phenotype you observed.

Q1 Which traits are likely to be the most reliable for distinguishing males from females and which are likely least reliable? Justify your choices by indicating how easy or how difficult it may be to observe the various traits in wild-type adult flies. Also consider and describe how the traits might vary at different growth stages and for various phenotypes. (Note: You should probably wait to finalize your answer to this question until after you have viewed several flies with different mutant phenotypes.)

Conducting Crosses

In this part of the lab activity, you will be conducting a cross and analyzing the results. In each case, you will be working with strains that differ by only one gene (a monohybrid cross). Your goal will be to characterize the allele associated with the mutant phenotype (i.e., to determine whether it is dominant, recessive, etc.) and to determine whether it is associated with an autosomal gene or a sex-linked gene.

For this portion of the lab activity, you will work in small groups, and each group will be assigned to conduct a specific cross, using the wild-type and a particular mutant strain. Some steps of conducting the cross will have to be completed outside normal laboratory meeting times. Be sure that your group carefully coordinates the various aspects of conducting the cross.

Each group will be instructed to set up one or more replicates of the assigned cross in a reciprocal fashion. Doing the cross in reciprocal fashion means that each group will actually be conducting their cross in two ways. In one version of the cross, the females will be homozygous for the mutant allele and the males will be wild-type; in the other version, the phenotypes will be associated with flies of the opposite sex.

- Q3** Conducting crosses in reciprocal fashion is a typical aspect of genetic analysis. Why is it important to conduct reciprocal crosses — that is, under what circumstances might different results occur with reciprocal crosses? Explain your answer.

The *Drosophila* Life Cycle

Before you can conduct crosses, you must be familiar with the basic aspects of the fruit fly life cycle (see Figure 3.2). Several manipulations you will conduct must occur at specific stages of development.

The timing of the life cycle will vary somewhat at different temperatures. The life cycle speeds up slightly at higher temperatures; however, higher temperatures also promote fungal and/or bacterial growth (which can ruin cultures) and may cause variations in some phenotypes. If cultures are maintained at 21°C, mature flies can grow from eggs in about 14 days. All stages of development should be present in a healthy, mature culture. As you read the following description, you should try to identify the major, visible stages in such a culture.

The first several stages are not easily visible with the unaided eye or even a dissecting microscope. The tiny egg laid by the female usually hatches within 24 hours. The larva that emerges from the egg passes through three “instar” stages (which are separated by larval molts). The instar stages last from less than 24 hours to several days. At 21°C, the fly spends about 6 days in the egg/larval stages. The second and third instars are relatively large and can be seen with a dissecting microscope. The third instar stage is clearly visible with the unaided eye, especially when it begins the “wandering” stage, at which time it usually crawls up onto the culture vessel wall. The fly spends approximately the next 6 days in the pupal stage. It is while the fly exists as an immobile pupa that the incredible, internal changes of metamorphosis occur. At the end of the pupal stage, the adult fly (also called the *imago*) ecloses from the pupal case, and very quickly becomes capable of reproducing.

Drosophila Life Cycle

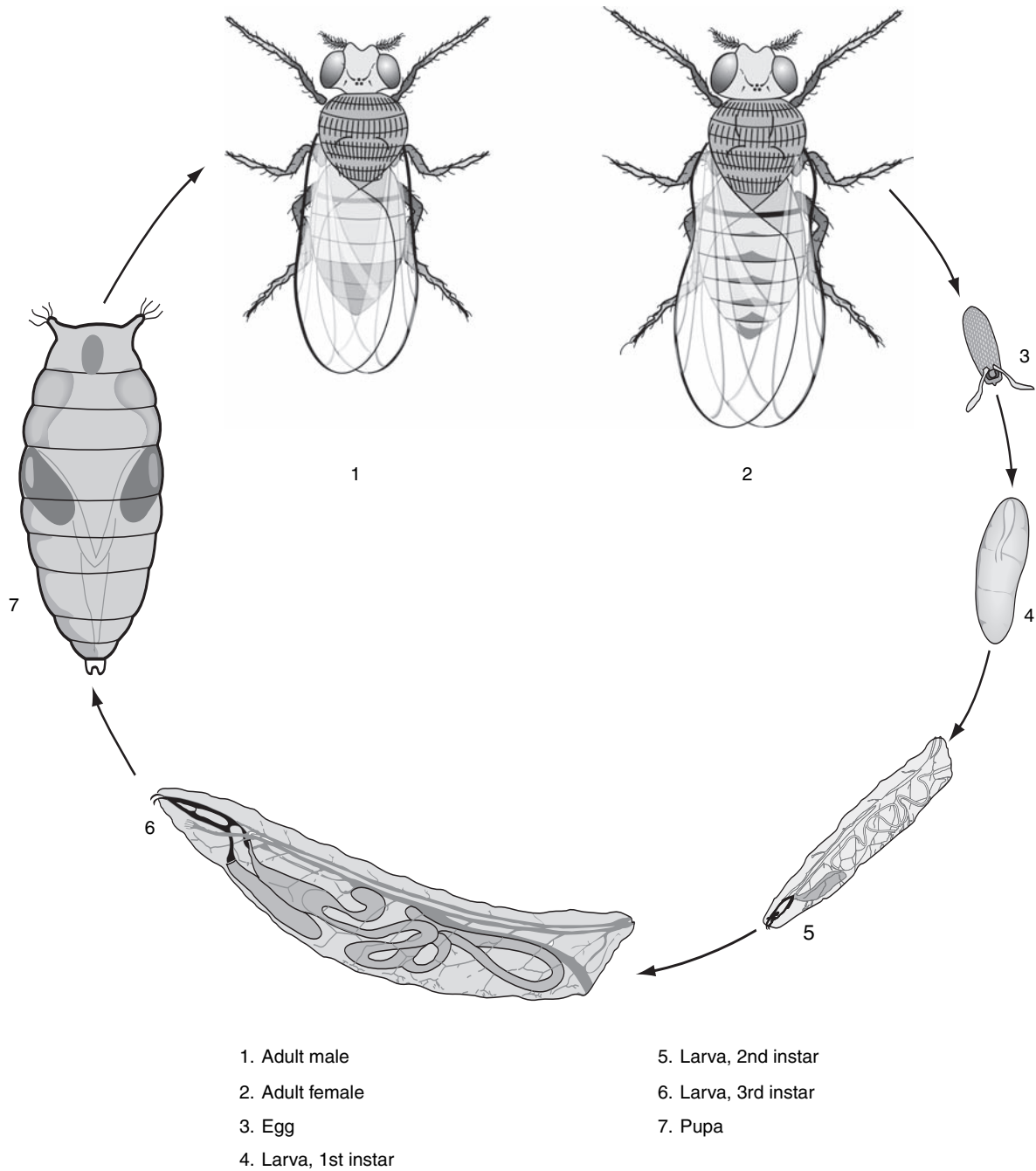


Figure 3.2 – The life cycle of *Drosophila melanogaster*. See text for a detailed description.

Preparing Culture Vials

You will need fresh culture vials containing fly medium for crossing your P1 and F1 flies. Different kinds of culture media are available for this purpose. Your instructor may choose to provide premade culture media or have you prepare your own media (mixes with very simple preparation instructions are available). In either case, be sure to label your culture vessels with specific information about each cross you prepare (including your name, information about the cross, and date).

Preparing the P1 Crosses: Anesthetizing Flies and Selecting Virgin Females

The parental strains (P1 strains) are the homozygous flies that are initially crossed to obtain the “hybrid” offspring (called the first filial, or F1 generation). As indicated earlier, you will be conducting your cross in reciprocal fashion. Great care must be taken to correctly determine the sex of each fly used in the P1 generation. Furthermore, all of the females used must be virgins (discussed below).

The general technique for anesthetizing flies is the same as described earlier. However, you should be especially careful not to overanesthetize flies, since this can reduce fertility. When anesthetized flies are placed into culture vessels, care should be taken to ensure that they don't become stuck in wet culture medium. It is wise to place anesthetized flies onto the culture vessel wall in a stoppered vessel that is oriented on its side. This allows flies to revive on the dry wall surface, rather than the surface of the medium, which may be sticky.

Selecting flies to serve as the P1 generation must be done in several steps. Males can be isolated and added to a cross at any time (they can also be “saved” in a separate culture vessel for later use). However, when isolating females for a cross, steps must be taken to ensure that they are virgin females. Nonvirgin females that have been inseminated by males of the same strain will lay eggs for a long period of time, and these eggs will be homozygous for the genotype of the mother (i.e., not the result of the cross that you are trying to set up).

Since female flies cannot be inseminated during the first 8 to 12 hours of their adult life, virgins can be obtained by selecting females that have recently eclosed (i.e., “emerged”) from their pupae (it is recommended that females be isolated as virgins no later than 8 hours after eclosure). To accomplish this, a stock culture with many late-stage pupae is used. All of the adult flies are first removed (the males can be used for the reciprocal cross), and newly eclosed flies are removed continuously (or at a period of time no later than 8 hours following clearing of adults). Newly eclosed females removed in this way can be used as virgins. Flies eclose most actively during the morning hours, so whenever possible, remove adults early in the day.

When you set up each cross (and each replicate), use about 5 to 10 males and about 5 to 10 virgin female flies for each culture. You can allow the P1 flies to remain in the culture and continue to mate for about 1 week to 10 days. However, you should watch your cultures closely, and *always remove P1 flies at or before the time when pupae first become visible* in the cultures. When the F1 flies eclose, remove a large number of these, and count and categorize them according to their phenotypes (including male vs. female as a phenotypic trait). Maintain a record of your results for future analysis.

Q4 Answer this question for your particular cross. If necessary, include separate responses for the two reciprocal crosses. Your instructor may ask you to hand in the answers to Parts A through E within a week or so (along with answers to the other questions in this lab) and then hand in an answer to Part F after you have collected the data from the cross.

- A. What mutant phenotype are you using in this cross?
- B. If the mutant allele in this cross is a dominant, autosomal allele, what phenotype(s) should be present in the F1 of your cross, and what fraction of the F1 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- C. If the mutant allele in this cross is a recessive, autosomal allele, what phenotype(s) should be present in the F1 of your cross, and what fraction of the F1 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- D. If the mutant allele in this cross is one that exhibits “lack of dominance” (i.e., codominance, or incomplete dominance) and is an autosomal allele, what phenotype(s) might be present in the F1 of your cross, and what fraction of the F1 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)

- E. If the mutant allele in this cross is a sex-linked, recessive allele, what phenotype(s) should be present in the F1 of your cross, and what fraction of the F1 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- F. What actual phenotype(s) were present in the F1, and what fraction of the F1 did each phenotype comprise? Based on these results, what type of allele (dominant, recessive, autosomal, etc.) does the mutant phenotype you studied seem to be associated with?

Preparing the F1 Crosses

Establishing crosses with F1s as the parents is simple compared to establishing P1 crosses. As indicated earlier, you must have removed the P1s at a time prior to when the F1 adults were first present (so that F1s were not able to mate with P1s). After you remove the P1s, you can collect and use F1s for the next cross without ensuring that the F1 females are virgins.

Q5 Why is it unnecessary that the F1 females be virgins prior to establishing the cross?

To set up the F1 crosses (remember to continue with reciprocal crosses), simply remove about 5 to 10 male F1s and 5 to 10 female F1s from the culture that previously contained the P1s, and transfer them to a fresh, labeled culture. You can make several replicates of the F1 crosses by conducting this manipulation for several days over the course of about 1 week after the F1s first eclose. Do not use flies from this culture that develop later than 1 week after the F1s eclose, because some of these flies could be the offspring of the F1s.

You can allow the F1 flies to remain in the culture and continue to mate for about 1 week to 10 days. However, you should watch your cultures closely and always remove F1 flies at or before the time when F2 pupae first become visible in the cultures. When the F2 flies eclose, remove a large number of these and count and categorize them according to their phenotypes (including male vs. female as a phenotypic trait). Maintain a record of your results for future analysis.

- Q6** Answer this question for your particular cross. Include separate information for the two reciprocal crosses. Your instructor may ask you to hand in the answers to Parts A through E within a week or so and then hand in an answer to Part F after you have collected the data from the cross.
- A. What phenotype(s) was present in the F1 flies in this cross?
- B. If the mutant allele in this cross is a dominant, autosomal allele, what phenotype(s) should be present in the F2 of your cross, and what fraction of the F2 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- C. If the mutant allele in this cross is a recessive, autosomal allele, what phenotype(s) should be present in the F2 of your cross, and what fraction of the F2 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- D. If the mutant allele in this cross is one that exhibits “lack of dominance” (i.e., codominance, or incomplete dominance) and is an autosomal allele, what phenotype(s) might be present in the F2 of your cross, and what fraction of the F2 should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype only if it is necessary to do so.)

- E. If the mutant allele in this cross is a sex-linked, recessive allele, what phenotype(s) should be present in the F₂ of your cross, and what fraction of the F₂ should each phenotype represent? (Include “male” and “female” as part of your description for a given phenotype, only if it is necessary to do so.)
- F. What actual phenotype(s) were present in the F₂, and what fraction of the F₂ did each phenotype comprise? Based on these results, what type of allele (dominant, recessive, autosomal, etc.) does the mutant phenotype you studied seem to be associated with?

Q7 Compare the frequencies of phenotypes in your data (for each reciprocal cross) to the frequencies that would be predicted from a model based on your response to question 6F. Use chi-square analyses to compare the data to the ideal ratio(s). What are the chi-square values you obtained from these comparisons? What are the approximate p values associated with the chi-square values? Do these analyses support your assessment of the mode of inheritance you suggested in response to question 6F? (Show your calculations.)

The crosses you and your classmates conducted for this lab activity represent the “classical,” Mendelian, approach to genetics analysis. The kind of knowledge gained using this approach is extremely important. It often forms a foundation for many types of genetic analyses that are more technically sophisticated. Another laboratory activity in this manual build on this foundational understanding of *Drosophila* genetics by introducing molecular techniques to study a genetic marker in this organism.

© Bent Tree Press

© Bent Tree Press

© Bent Tree Press

© Bent Tree Press

© Bent Tree Press

© Bent Tree Press