This article reprinted from:

Bixler, A., and F. Schnee. 2005. Application of the Hardy-Weinberg model to a mixed population of *Bar* and wild-type *Drosophila*. Pages 175-191, *in* Tested Studies for Laboratory Teaching, Volume 26 (M.A. O'Donnell, Editor). Proceedings of the 26th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 452 pages.

ISBN 1-890444-08-1

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Chapter 9

Application of the Hardy-Weinberg model to a mixed population of *Bar* and wild-type *Drosophila*

Andrea Bixler[1] and Fred Schnee[2]

[1]Clarke College
1550 Clarke Drive
Dubuque, IA 52001
andrea.bixler@clarke.edu

[2]Loras College
1450 Alta Vista Drive
Dubuque, IA 52001
fred.schnee@loras.edu

Andrea Bixler earned her B.A. in biology at Swarthmore College and her Ph.D. in ethology at the University of Tennessee, Knoxville. She is an Assistant Professor of Biology, teaching Diversity and Ecology, Evolution, Environmental Biology, and related courses. Her research has focused on subjects as varied as habitat use by raccoons in a human-altered landscape and the importance of acoustic stimuli in *Drosophila busckii* courtship.

Fred Schnee earned his B.S. and M.A. in biology at Brooklyn College and his Ph.D. in genetics at the University of Oklahoma. He is an Associate Professor of Biology, teaching Introduction to Biology I, Genetics, Microbiology, and Ethics and Human Genetics. His research interests focus on the evolution of compensatory mating behaviors in *Drosophila* with wing mutations.

Contents

Introduction

This laboratory provides students with hands-on experience applying the Hardy-Weinberg model to a live population of *Drosophila melanogaster* (fruit flies). Our objective is to increase students' working knowledge of the Hardy-Weinberg model, which should in turn help them understand the importance of mathematical models in general. The activity requires problem-solving ability as students evaluate which of the Hardy-Weinberg assumptions has been violated.

While an understanding of sex-linked inheritance and incomplete dominance is required to complete the population genetics lab, it could be modified in a number of ways to make it suitable for students of different levels.

The simplest option is to use the population genetics lab alone as an introduction to the Hardy-Weinberg model with first- or second-year introductory biology students. The instructor could set up the population bottles and the students count the classes of phenotypes and analyze the data. All the data that the students don't collect, but need for Hardy-Weinberg analysis, is included in the lab handout. For a higher level class or for a genetics class, students could be introduced to flies and determine the inheritance pattern of *Bar* (Appendix A), further study the inheritance pattern and observe whether *Bar* has lethal or semi-lethal effects (Appendix B), and study courtship and mating of *Bar* flies (Appendix C). This way, the students collect, during a series of labs, all the data they need. For a class such as ecology or evolution, the population genetics experiment and courtship behavior lab might be an appropriate compromise.

The major drawback of this laboratory is the time required to collect virgin flies. For very large classes, this could be a logistical nightmare. If students collect flies for themselves, they need access to the fly stocks and equipment, and appropriate supervision. If instructors collect flies for students, they need fly stocks and "spare" time suitable to the number of bottles to be set up.

We usually perform the Hardy-Weinberg activity during a 3-hour period, but since only the counting of the flies requires actual laboratory time, a 2-hour slot with follow-up discussion in lecture would be sufficient. The activity detailed in Appendix A requires 2-3 hours, depending on how much time students are given to examine mutants. The activity in Appendix B may be performed in less than 1 hour, but if students need a substantial introduction to chi-square tests, that could take an additional hour. The experiment in Appendix C involves repeated 30-min tests, and so may take a total of 2-3 hours depending on how many test are performed.

Student outline

Application of the Hardy-Weinberg model to a mixed population of *Bar* and wild-type *Drosophila*

Populations can be characterized by the frequency of the alleles they carry for a given gene. The frequency of alleles can change from generation to generation. This change in gene frequency is the basis of evolutionary changes that occur in populations.

Hardy-Weinberg

The Hardy-Weinberg model is a valuable mathematical tool for studying how changes occur to a population's gene pool. The model makes five assumptions about the population it describes:

1) the population is large in size
2) mutations do not affect the population's gene frequency
3) there is no migration of individuals in or out of the population
4) selection does not affect the trait in question
5) mating within the population is random

If we make these five assumptions, we in effect assume that there are no forces acting on the population to cause the frequency of alleles in its gene pool to change. The model is useful because with these assumptions we can predict the genotypes we will find in such a population after one generation of random mating. The formula for this prediction is given as:

$$(p+q)^2$$

where p is the frequency of allele A and q is the frequency of allele a. If we expand this equation, we get the expression

$$p^2+2pq+q^2$$

where p^2, 2pq and q^2 are the frequencies of the AA, Aa and aa genotypes, respectively. After one generation of random mating, the genotype frequencies will be set and will remain unchanged as long as the five assumptions hold true. The population will be in *Hardy-Weinberg equilibrium*.

Procedures

The study organism and mutation

In this lab, we will examine the Hardy-Weinberg model and see how it can be used to study the factors that affect a population's evolution. The populations we will study are bottle cultures of *Drosophila melanogaster*. The frequencies we will examine will be for the alleles of the *Bar* gene. *Bar* is useful because the mutation is sex-linked and shows incomplete dominance. Females that are homozygous for *Bar* and hemizygous *Bar* males have eyes that are reduced to a slit or bar shape. Heterozygous females have a kidney- or heart-shaped eye (see Figure 1). As a result, we can determine the genotype of a fly by examining its phenotype.

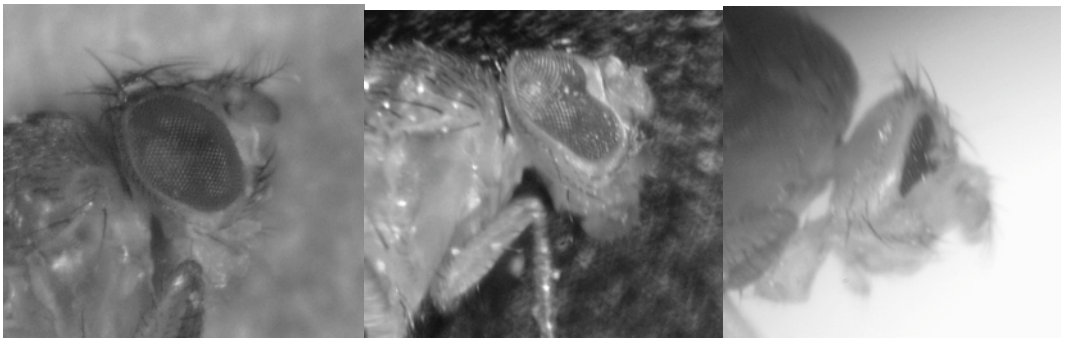

Figure 1. Comparison of three eye phenotypes/genotypes: left, wild-type round eye; middle, heterozygous kidney- or heart-shaped eye; right, *Bar* slit-shaped eye.

The lab

In lab today, you will

1) determine whether your populations of wild-type and *Bar* flies are in Hardy-Weinberg equilibrium and
2) if the populations are not in equilibrium, determine from other evidence provided which of the five Hardy-Weinberg assumptions has been violated.

1a) Count the number of each genotype of each sex in your population bottles. *Be very careful to distinguish males from females and X^BX^+ from X^+X^+*. Record the data below:

Table 1a. Numbers of flies of each genotype.

Phenotype	**Genotype**	**number**
Bar female	X^BX^B	
Heterozygous female (kidney eyes)	X^BX^+	
Wild-type female	X^+X^+	
Bar male	X^BY	
Wild-type male	X^+Y	

1b) From the information above, you can calculate the allele frequencies in your population bottles. This will be a little different from other Hardy-Weinberg calculations you've done because *Bar* is sex-linked. Use the equations below:

$$q = \frac{(\#\ Bar\ \text{females} \times 2) + (\#\ Bar\ \text{males}) + (\#\text{heterozygous females})}{(\#\text{females} \times 2) + (\#\ \text{males})}$$

$$p = \frac{(\#\ \text{wild-type females} \times 2) + (\#\ \text{wild-type males}) + (\#\ \text{heterozygous females})}{(\#\ \text{females} \times 2) + (\#\ \text{males})}$$

Table 1b. Observed allele frequency.

Allele	**Frequency**
B	
+	

1c) In (1a) and (1b), you were dealing with *observed* genotype and allele frequencies. You also need to calculate *expected* allele and genotype frequencies. What was the frequency of the *Bar* and wild-type alleles at the *start* of the experiment, given that each bottle population was started with 5 wild-type females, 5 wild-type males, 5 *Bar* females and 5 *Bar* males? (Remember, if the population is in Hardy-Weinberg equilibrium, the allele frequencies won't change from one generation to another. That means that the allele frequencies at the start of the experiment would be the expected frequencies at the end.)

Table 1c. Expected allele frequency.

Allele	**Frequency**
B	
+	

1d) Assuming the conditions of the Hardy-Weinberg Model are true of the populations we examined, what are the *expected* numbers of *Bar* and wild-type males, *Bar* females, wild-type females and heterozygous females? Remember that *Bar* is sex-linked, so the equations for calculating genotype frequencies from allele frequencies will be different than the usual: the frequencies of males will be calculated differently from the frequencies of females. How would you do this?

Table 1d. Expected genotype frequencies.

Genotype	Frequency	Number
X^BX^B		
X^BX^+		
X^+X^+		
X^BY		
X^+Y		

1e) Based on a chi-square test, do the data you collected fit the hypothesis of a Hardy-Weinberg equilibrium? Show your work, and write out a one-sentence summary of the statistical results. (A chi-square formula and table are attached to this handout.)

Now, we try to determine why you got the results you did.

2) The table below shows the results of three experiments that were done exactly as yours (numbers in parentheses indicate the frequency of genotype by sex). Fill in the data from the population bottles we observed so you can compare all the populations together.

Table 2. Observed genotype frequencies of several populations.

Geno-type	Pop. 1 # (freq.)	Pop. 2 # (freq.)	Pop. 3 # (freq.)				
X^BX^B	0 (0)	18 (0.09)	0 (0)				
X^BX^+	74 (0.46)	68 (0.35)	61 (0.53)				
X^+X^+	86 (0.54)	111 (0.56)	55 (0.47)				
X^BY	21 (0.12)	39 (0.32)	22 (0.26)				
X^+Y	150 (0.88)	83 (0.68)	62 (0.74)				

What trends do you see in the data? Based on this table, what role do you think genetic drift plays in determining the observed genotype frequencies?

3) The table below shows the results of experiments that tested the mating abilities of *Bar* and wild-type flies. Based on these data, do you think that mating would be random in the population studied? What effect does the relative mating ability of *Bar* flies have with respect to changes in the genetic make-up of the population?

Table 3. Courtship behavior of *Bar* and wild-type flies.

Phenotype of fly tested		**% time male spent courting**	**Copulation success**	
male	**female**		**# copulated/# tested**	**%**
Bar	**Bar**	24	5/54	9
Bar	+	22	5/48	10
+	**Bar**	59	17/52	33
+	+	68	23/50	46

4) In another experiment, heterozygous (X^BX^+) females were crossed to *Bar* (X^BY) males and the following results were obtained:

Table 4. Genotype frequencies resulting from an F2 cross.

Genotype	**Frequency (within each sex)**	**Number**
X^BX^B	0.47	43
X^BX^+	0.53	49
X^+Y	0.50	38
X^BY	0.50	38

Based upon these data, what is the importance of lethal or semi-lethal effects? Do you think selection on the *Bar* allele affects the genotype frequencies in the populations you have observed?

5) In numbers 2, 3 and 4 above, you discussed the effects of drift, non-random mating and selection on your populations. What are the other two Hardy-Weinberg assumptions? Can they be ruled out in your populations? Why or why not?

6) Which of the Hardy-Weinberg assumptions was/were violated in the populations we studied? Which factor(s) do you think were important in producing the gene frequencies found in your population, as well as in populations 1, 2 and 3 (in the table given in #2)?

The chi-square statistic is calculated by $\chi^2 = \sum(\text{observed-expected})^2/\text{expected}$
The degrees of freedom (df) equal the number of categories minus 1.

If your χ^2 value is greater than or equal to the value in the table below, your observed data are significantly different from expected at the probability value listed.

Table 5. Table of chi-square distribution.

df	**P = 0.05**	**P = 0.01**	**P = 0.001**
1	3.84	6.64	10.83
2	5.99	9.21	13.82
3	7.82	11.35	16.27
4	9.49	13.28	18.47

Materials

- Population bottles set up two weeks previously with 5 + males, 5 + females, 5 *Bar* males and 5 *Bar* females; ***female flies must be virgins***
- Anesthetic and anesthetizing apparatus (ether and etherizers; FlyNap™ and wands; or other methods)
- Dissecting microscopes
- White cards on which to place anesthetized flies
- Paintbrushes for moving anesthetized flies
- Fly morgues (containers of oil—any kind—or soapy water)

Notes for the Instructor

Hardy-Weinberg model

The Hardy-Weinberg model is described extensively in most introductory biology, genetics, ecology, and evolution texts. We do not wish to repeat a great deal of information from either the student handout or those sources here. However, we would like to recommend Lewis (2004) for the instructor's reference, since it provides background on Hardy-Weinberg theory, examples of worked problems, and numerous problems using human populations.

The study organism

We use the Canton-S strain of wild-type flies. They are available from the Bloomington *Drosophila* Stock Center (http://flystocks.bio.indiana.edu/; stock number 1). Carolina Biological sells the Oregon R strain of wild-type flies (order # ER-17-2100), which may or may not yield the same results (see the comment below under "Interpretation of Results—Natural selection").

Bar mutants are available from Carolina Biological (order # ER-17-2110). The *Bar* loci are necessary for normal development of the lens and certain pigment cells in the eye. They also remove extra cells from mature ommatidia (Brody, 1996). For more information on all aspects of *Drosophila* biology, we recommend Brody (1996) and FlyBase (2003), both on the web.

While we think this lab works best using *Bar* mutants, the experiment could be done using autosomal mutants. The advantage would be in simplification of the Hardy-Weinberg math; the disadvantage would be the loss of exact correspondence of phenotype and genotype (we know of no autosomal mutants that show incomplete dominance). Thus, students would be unable to distinguish p^2 from 2pq. They would then have two phenotype frequencies: dominant (equal to p^2+2pq) and recessive (q^2). One of us (FS) has run the experiment this way using the mutation *apterous*, with good results. If you would like to try the experiment with *apterous*, *scalloped*, or *vestigial*, FS has courtship data he could share with you to replace those in Table 3. These mutants are available from Carolina Biological (order #s ER-17-2320, ER-17-2180, and ER-17-2460, respectively). Any other mutant could be used if you and your students collect sufficient courtship data.

Setting up the population bottles

Bottles (we would recommend something similar to Carolina Biological # ER-17-3134 with plugs ER-17-3122) should be set up 2 weeks in advance of the lab, assuming that the flies will be kept at 25ºC. Bottles may be set up by the instructor or by the students, depending on how proficient

the instructor thinks the students will be at collecting virgins (see below). Set up one bottle for each lab group. We use Carolina 4-24 Instant *Drosophila* Medium (Carolina # ER-17-3200) but any standard medium is fine

Each bottle should contain 5 wild-type males, 5 wild-type females, 5 *Bar* males and 5 *Bar* females. The females must be virgins when they are placed in the bottle, or else they will lay eggs fertilized by unknown males and will probably not mate with males in the population bottle. The age of the flies does not matter for this experiment.

We have performed this experiment by leaving the flies sitting on a lab bench in a room with windows, so they receive ambient light, and by leaving the flies on a lab bench for up to one day, then placing them in an incubator that is lit only when the door is opened (which occurred irregularly). Both methods produced the same results. See "inquiry-based variations" below for further comments on the light regime.

There are two relatively easy ways to collect virgin females. The first is to clear the bottles completely (being absolutely certain no flies are left alive in the bottles) and then return in 6-8 hours. Any flies in the bottle will still be virgins. This is the better method for beginners. The second method is to examine all flies in a bottle, identifying the virgins by their physical appearance. Very young virgins (within the first few hours after eclosion) will not yet have unfolded their wings. Older but still usable virgins will have unfolded wings, but their exoskeletons will not be completely hardened so that their bodies will be elongated, and their pigmentation will be very pale (see Figure 2). After the flies have been collected but before they are placed in the population bottles, they should be kept in single-sex groups or individually in vials.

Figure 2. Comparison of virgin fly (left) with adult fly. Note the differences in body length and coloration. The virgin eclosed from the pupal case some hours before, so his wings are already unfolded.

Students may learn more and be more involved in the experiment if they set up the bottles. If the instructor is concerned about student reliability in collecting virgins, the instructor could compromise by collecting virgins and giving them to the students to set up the population bottles.

The adults put into the population bottle should be removed about a week after the crosses are set up (when larvae are visible). This prevents the parental generation from being counted with the offspring.

Counting bottles

During the population genetics lab, the students will count the offspring of the flies placed in the population bottles two weeks before. It will be necessary to anesthetize the flies with ether, FlyNap™, CO_2 or another method.

The most common problems for students counting the flies is inaccurately classifying the phenotypes. They must distinguish males from females, and then distinguish *Bar* males from wild-type males and *Bar* females, heterozygous females and wild-type females. *Bar* males and females are clearly different from wild-type because their eye shape is very narrow (like a bar or slit). Heterozygous females have a larger eye, but there is usually an irregularity in it, so that it is shaped like a kidney or heart (see Figure 1 in Student Handout). If students have worked with these phenotypes before (e.g., Appendices A & B), they will just need a refresher, but if they are unfamiliar with *Bar* mutants, they will need more help.

Interpretation of results

We have run this lab at least annually for more than 10 years and we almost always find that the number of *Bar* flies, especially the number of *Bar* females, is lower than expected from the Hardy-Weinberg model.

Once students have determined how their results compare to Hardy-Weinberg predictions and analyzed whether there is a statistically significant difference, they need to determine which assumption has been violated (assuming one has been).

Migration and mutation

Certain assumptions can be quickly eliminated from consideration. It is not possible for migration/gene flow to have affected the results because the populations are maintained in closed bottles. It is highly unlikely that mutation could have affected the results, because the experiment was only run for one generation, mutation rates at a single locus should be low, and forward/backward mutations are likely to cancel each other out. (Note that because students often think of mutations as being always detrimental, they may assume that the analysis of lethal and semi-lethal effects—Table 4 in the student handout—is related to whether mutation is the assumption that was violated. We use the lethal and semi-lethal data to examine the assumption of natural selection.)

Genetic drift

Since genetic drift is non-directional, its effects can be seen by repeating the experiment and looking for results in which genotype or allele frequencies vary between replicates. For example, looking at Table 2 of the student handout, Populations 1 and 3 had no *Bar* females while Population 2 had 18 *Bar* females. This method of comparing frequencies across populations is not always clear to students; they may jump to the conclusion that drift is the assumption that is violated simply because the population size is small, not because they see random effects across populations.

Because the founder population size is small, genetic drift will always have some effect on this experiment as far as how much the frequency of the *Bar* allele decreases and which specific ratios result. Nonetheless, some consistent trends emerge (*Bar* frequency is low and numbers of *Bar* females are very low), suggesting that the changes in allele frequencies are not *just* due to drift.

Random mating

The data in Table 3 of the student handout clearly show that mating is non-random in this population. However, while non-random mating can lead to significant deviations in genotype frequencies, it does not in and of itself disrupt allele frequencies (Freeman and Herron, 2001). You may have your students complete a chi-square analysis comparing the observed and expected numbers of wild-type and *Bar* alleles to determine whether there is a significant difference. While it may or may not be significant, we expect at least a trend toward lower allele frequency of *Bar*. Therefore, some other factor is likely to be at work in this population. Changes in allele frequency would be expected if non-random mating involves a *selective* advantage of *Bar* or wild-type flies in passing on their genes, and thus we turn to the last of the Hardy-Weinberg assumptions.

Natural selection

Survival and reproduction are the two important components of natural selection, but it is impossible to cover all factors that might affect them in this lab. As far as survival, there are no predators in this experiment, the temperature is appropriate, and there should be sufficient food and moisture. Thus, survival should be high unless *Bar* has some lethal or semi-lethal effect. To test this, use the results from your students' own F2 crosses (see Appendix B) or the data provided in Table 4 of the student handout. In this cross (heterozygous—X^BX^+—females crossed to *Bar*—X^BY—males), males should occur in equal frequencies unless *Bar* affects survival. There should also be equal frequencies of *Bar* and heterozygous females. You could also do the opposite backcross in a separate bottle (heterozygous—X^BX^+—females crossed to wild-type—X^+Y—males) to further examine this question. We don't find any semi-lethal effects, but this result could vary depending on the genetic background (strain) of the flies you use (the effects of lethal alleles varies depending on the rest of the organism's genome; Griffiths et al. 1999).

As far as the effects of natural selection on reproductive ability of *Bar*, we find that *Bar* males typically court less and copulate at a lower frequency than wild-type males (Table 3 of the student handout). This is apparently because their vision is impaired, and they have difficulty seeing and following females. *Bar* females are courted by males just as much as are wild-type females.

This difference in male behavior explains our typical results quite well. *Bar* male offspring can result from a cross between a wild-type male and a *Bar* female, which is likely to occur. But *Bar* females can only be produced from a cross of a *Bar* male with a *Bar* female. This cross is unlikely to be successful, because of the *Bar* male's disadvantage in courtship. While the *Bar* allele is clearly causing non-random mating, it is also producing a selective disadvantage in the ability of *Bar* males to pass on their genes. Thus, the mating deficiencies of *Bar* lead to changes in both the genotype and allele frequencies.

You may use one of two methods to illustrate the *Bar* male's courtship deficiencies to students. One possibility is to have them examine the data provided in Table 3 of the student handout. This method will be greatly enhanced by showing them a video of courtship by wild-type and *Bar* males. Contact either author for a copy of an amateur video showing the dramatic difference between courtships of these two genotypes. Alternatively, students may observe courtship and mating of the flies themselves (see Appendix C).

This experiment demonstrates the usefulness of the Hardy-Weinberg model as a framework for analysis of population genetics. Most students do not understand the value of a null model, but working through this example can greatly increase their understanding.

Possible Inquiry-based Variations on the lab

There are a number of possible continuations to this lab that we have never had time to explore. Students could be encouraged to study the mixed *Bar* and wild-type populations further, perhaps as independent, inquiry-based labs. Some ideas include: 1) If the changes in allele frequencies are explained ultimately by deficiencies in *Bar* eyesight, then population bottles placed in the dark should not show changes (or such drastic changes) in allele frequencies. This type of result was found by Burnet and Connolly (1973) in a more complicated eye pigment double-mutation study conducted over 10 generations. However, it is known that in wild-type *Drosophila,* pairs of flies reared under the same light conditions are more likely to mate than pairs consisting of individuals reared under differing circumstances, so experience, as well as visual acuity, plays a role in courtship (Barth et al., 1997; Hirsch et al., 1995). 2) Students could observe these mixed populations for several generations to study the long-term changes in allele frequencies. 3) The basic experiment could be repeated with different initial frequencies of *Bar* and wild-type flies. 4) Larger population sizes could be used to reduce drift effects. However, larger populations will increase crowding effects, if any.

Acknowledgements

We would like to thank Laurie Tompkins, in whose *Drosophila* behavior genetics lab we first met.

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Appendix A

This is a standard introductory lab for genetics courses, but is included here to illustrate how the use of *Bar* mutants, starting with the first lab of the semester, could tie together a number of labs. There are two main considerations for instructors with this lab and the lab in Appendix B. The first is once again whether to have students collect virgin flies for crosses or to collect virgins themselves (see Notes for Instructors above). The second consideration is the time involved, because each cross requires two weeks to produce offspring

Introduction to *Drosophila* and Genetics

The genus *Drosophila* (fruit flies) is found in all temperate, tropical and sub-tropical regions. There exist a very large number of genetic strains in the wild and many of these grow well in the laboratory. Since the genus has a small number of chromosomes (the haploid number is four), the genetic analysis of the organism is relatively easy. Further, *Drosophila* lends itself to genetic analysis because of its short generation time (about two weeks), its small size and space requirements, and because of the ease of breeding under laboratory conditions to produce a large number of progeny

The purposes of this lab are for you to:

1. become familiar with *Drosophila.*
2. learn how to handle, sex and recognize various characteristic structures of these flies.
3. experience one of the most powerful aspects of the scientific method—careful and detailed observation of the world around you.

A. Life Cycle

The egg of a fruit fly is fertilized internally and laid shortly thereafter. Fertilization occurs in the oviduct. Eggs are white, ovoid and about 0.5 mm long. About 21 hours from the time they are laid, eggs hatch and the larvae emerge.

The newly hatched larvae, known as the first instar, are voracious eaters. They are tiny and difficult to see with the naked eye; however, the tunnels made in the food as the larvae eat their way through it are usually visible. Within about 2 days, the first instar will undergo another molt and form the second instar. Again, these larvae will eat, grow and molt again to become the third instar.

Pupation occurs as the third instars crawl out of the food and onto a dry surface. The larval body shortens and the cuticle hardens and becomes pigmented, developing into the pupal case. Metamorphosis occurs within the pupal case; dormant localized tissues (imaginal discs) laid down during the embryonic stages develop to adult proportions while larval tissue is broken down to furnish both raw material and the energy needed for adult development. After 3 days, the adult emerges.

B. Directions for Handling Flies

1. To examine flies, it is necessary to etherize them first. A few drops of ether will anesthetize a batch of flies and they can be kept asleep for up to 30 minutes by re-etherizing when they start to wake. Care should be taken not to over-etherize flies. Flies that are over-etherized and dead will have their wings raised vertically over the thorax.

Note: When dumping flies into the etherizer, make sure the food is not too wet or dry! If it is, ask your instructor to help you.

2. Don't try to handle too many flies at once. Use an empty vial for some, so that when you are ready to observe them you can easily re-etherize the flies.
3. When examining the flies, move them into different categories and then count the number in each group. The camel-hair brush or dissecting needle may be used to move the flies about.

Caution!!!! You are handling an explosive compound—ether. Make sure you don't start a fire with cigarettes, matches or Bunsen burners.

C. Recognizing the Sex of the Adult Fly

Male and female flies can be distinguished from each other in several ways. The tip of the abdomen is elongated in the female, and somewhat more rounded in the male. In wild-type flies, the pattern of darker markings on the abdominal segments is sufficiently distinctive in the two sexes to permit their separation on this basis without using a microscope. The abdomen of the female has seven segments that are readily visible with low power magnification, while the male has five. Males also have a **sex comb**, a fringe of about ten stout black bristles on the distal surface of the basal (uppermost) tarsal joint of the foreleg. Such bristles are lacking in the female. In the genital region, females have **anal** and **ovipositor plates**, while the male has **anal plates**, **genital arch, claspers** and a **penis.**

Separate about five wild-type flies by sex. Have your lab instructor check that you have done it correctly.

D. Identification of some Mutant Traits

1. Etherize about five wild-type flies. Examining the illustrations provided, locate the following aspects of the wild-type morphology:

a. **Head**- find the **antennae** (consisting of 3 segments); **aristae** (arise near the base of the distal segment of antennae); **proboscis; compound eyes** (composed of a large number of **ommatidia;** make note of their shape and color), **ocelli** (simple eyes—three are located between the compound eyes)

b. **Thorax**- composed of three fused segments (prothorax, mesothorax, and metathorax). On the thorax find the following structures- **wings** (make note of shape, size and location of various veins); **halteres** (highly modified wings)

c. Examine flies and make note of the wild-type **body color** and examine the various **bristles** that cover the body of the fly. Examine the shape and number of bristles associated with the wild-type fly.

2. Examine about five flies from each of the two pure breeding stocks of mutant flies. Note those characters that differ from the wild-type. Carefully record and describe the mutant characteristics on your lab hand-in sheet.

E. Genetic Analysis of Mutant

For your mutant #1, how could you determine if it was sex-linked or autosomal? Dominant or recessive? For your genetic cross or crosses you will be given a stock of pure breeding wild-type flies and pure breeding mutant flies. Who would you cross (give the genotypes of the male and female parents of your crosses) and what results would you look for to tell you if the trait was dominant or recessive, sex linked or autosomal?

Get together as a group to design an experiment to try and answer these questions. Double check with your instructor that the crosses you set up will answer your question.

Note: One problem in setting up fly crosses is that the females store sperm from previous matings. Therefore, you will need to use virgin females for your crosses. Check with your instructor as to how to obtain virgin females.

You can prepare bottles for your crosses by mixing 2 units of fungicide with 2 units of *Drosophila* Instant Medium. After placing flies in appropriate bottles you will need to label them as to the cross, the date and the name of your group.

F. Identification of Mutants

If mutant flies are wild-type for a given trait, place a "+" in the appropriate spot. If they are different from wild-type, briefly describe (or make a drawing comparing wild-type and mutant) the mutant phenotype.

Describe the phenotype of mutant 1 and mutant 2:

Table 1. Phenotypic descriptions of mutants.

	wild-type	**mutant 1**	**mutant 2**
body color	+		
eye color	+		
eye shape	+		
wing phenotype	+		
bristles	+		

G. Questions

1. In a cross, male *Drosophila* with brown eyes are crossed to wild-type females. The F1 offspring of this cross are all flies with wild-type eyes. When these offspring are allowed to mate with each other, their F2 offspring are found to be wild-type and brown eyes.
    a. Is brown eyes a dominant or a recessive trait? Explain.
    b. What is the genotype of the F1 wild-type flies?
    c. What is the genotype of the F2 brown eyed flies?
2. A man with brown teeth marries a woman with normal teeth. They have 20 children. All ten of their daughters have brown teeth but all their sons have normal teeth. When sons marry girls with normal teeth, all their children have normal teeth, but when their daughters marry men with normal teeth, they find that half their children (regardless of sex) are normal and half have brown teeth.
    a. Is brown teeth autosomal or sex linked?
    b. Is brown teeth dominant or recessive?

3. For mutant 1, how did you determine if it was sex linked or autosomal? Dominant or recessive? State who you would cross (giving the genotypes of the males and female parents of your crosses) and what results you would look for to tell you if the trait was dominant or recessive, sex linked or autosomal.

Appendix B

This is really a continuation of the lab in Appendix A. See the note at the beginning of that Appendix for considerations about this lab.

Confirmation of the Inheritance Pattern of an Unknown Mutant: The F2 Generation

Two weeks ago you analyzed reciprocal crosses involving a mutant *Drosophila.* At this point, you should be able to hypothesize whether your mutant is due to an autosomal or sex-linked mutation and whether your mutant is recessive or shows some other interaction with the wild-type allele. These ideas can be tested by using them to predict the results of the F2 generation of the reciprocal cross.

Procedure

Count the flies in the bottles you set up two weeks ago. Remember to count them with respect to both their phenotype and their sex. Record the results below. Using the chi-square test, determine whether the data from your two crosses fits the predictions you made two weeks ago about the F2 generation.

1. In the space below, record the number of flies in each phenotypic class (remember to classify for sex as well as the trait affected by the mutation).

2. Write down the hypothesis you are testing and the predictions you made for the F2 generation based upon the hypothesis.

3. Using the chi-square test, determine whether the results you obtained fit your hypothesis. Show all calculations and state the p value you obtain for each cross.

Appendix C

This lab can be a fun addition to the other *Bar* labs, but there are some difficulties associated with its success. A "Notes to Instructors" is included at the end of this student handout.

Behavioral Genetics

Genetics can be used as a tool for the analysis of complex phenotypes. One of the most complex phenotypes in *Drosophila* is their courtship behavior. Males display a variety of behaviors when courting females. Courtship begins with the male orienting towards and then following the female. "Singing" follows, with the male extending one wing toward the female and vibrating it. This produces a courtship song, which helps induce the female to mate. After singing comes licking, in which the male makes contact with the female's genitalia by means of his proboscis. The final step is the male attempting to copulate with the female.

While it may appear that the female is relatively passive during the courtship ritual it is important to note that in *Drosophila* it is the female who decides whether copulation occurs. In addition, the female can display several behaviors that can act as rejection signals. For example, females that have already mated often signal this to males by extruding their ovipositor.

While one of the goals of genetics is to identify the genes involved in determining a behavior, it can also be used to analyze the components of the behavior. For example, the importance of the courtship song in the mating behavior can be studied via the use of genetic mutations such as *apterous*, a mutant with no wings that cannot produce a courtship song. In this lab we will examine the importance of vision on courtship by using the mutant *Bar*.

Bar is a mutation that reduces the size of the eyes in *Drosophila*. As a result, the vision of *Bar* flies is significantly lessened. In today's lab, we will examine the effect this reduction in vision has on the ability of male and female flies to mate.

To test courtship we will use virgin flies aged 3 to 5 days. Both the age of the fly and prior mating experience can affect the ability of the fly to mate. For example, females that have already mated will reject all attempts by the male to copulate.

Etherization can also affect the behavior of flies and we must therefore handle the flies that we wish to test without ether. You will need to dump a vial containing a single male fly into a vial containing a female.

You will be conducting four types of test

1. wild-type male x wild-type female
2. *Bar* male x *Bar* female
3. wild-type male x *Bar* female
4. *Bar* male x wild-type female

Once males have been introduced into the female vial, observe the behavior for 20 minutes or until copulation has occurred. Observe all courtship behaviors (orientation, singing, etc.) displayed by the flies. Using a stopwatch, record the amount of time the male is courting the female. Record whether the male is able to copulate with the female. For those males that do copulate, make sure you record the amount of time needed to induce the female to mate with them (total observation time). At the end of the experiment,

calculate the percent of time the male spent courting (% time male courted= time male courted/ total time of the experiment * 100).

1. Describe the mating behavior of wild-type males. How is the behavior of *Bar* males different from wild-type males? Using your observations and the class data, what effect does *Bar* have on the mating behavior of males?

2. Using the class data and your observations, does *Bar* have any effect on the courtship behavior of females?

3. In general what role do you think vision has on the courtship behavior of *Drosophila?*

Table C1. Courtship behavior of *Bar* and wild-type flies

Phenotype of fly tested		**% time male spent courting**	**Copulation success**	
male	**female**		**# copulated/# tested**	**%**
Bar	*Bar*	24	5/54	9
Bar	+	22	5/48	10
+	*Bar*	59	17/52	33
+	+	68	23/50	46

4. What other experiments could be done to study the importance of vision or the effect of the *Bar* mutation on the courtship of *Drosophila?* Based on what you already know about *Bar* and its effect on mating what would you predict to be the results of your new study?

Notes for the Instructor for the Behavioral Genetics Lab (Appendix C)

Preparation

We usually plan to have each lab group/individual observe only two pairs of flies during a lab period, because of the time associated with set-up and explanations. Try to insure that each group conducts one test with a wild-type male, or else they may be very bored watching flies do virtually nothing!

To insure two pairs of flies for each lab group, you (or the students) must collect many extras (at least double the number you want) because flies die or may be damaged between the time of collection and the lab, or may be lost or damaged during the lab. Damaged flies, e.g., those with crumpled wings or missing legs, should not be used in mating tests because they may be incapable of performing normal courtship.

For these tests, *both males and females should be virgins* (see Intructor's Notes for Hardy-Weinberg laboratory for information on collecting virgins). Each fly should be housed individually in a vial with food until it is 3-4 days old. Keeping the flies in a consistent light cycle seems to be very important for successful matings later on (see Instructor's Notes above, and references therein). We use a 12:12 L:D cycle, but other consistent cycles will probably work, too.

Testing

Some scientists (e.g., Drapeau and Long, 2000) conduct mating tests in small, specialized mating chambers, but we find more success using vials containing food (food increases female receptivity, unpubl. data). To standardize the tests, we always introduce the male into the female's vial.

Flies should *not* be anesthetized for transfer between vials. Simply upend one open vial onto the other or use an aspirator. (This can be made from a length of plastic tubing and a large pipet tip. Place a piece of gauze over the wide end of the pipet tip before inserting it into the tubing. This prevents you from aspirating the fly into your lungs! You may then suck the fly into the pipet tip, hold it there by continuing to inhale, and then release it where you want it by letting out your breath or tapping the tip into the receiving container.) During the first few minutes after the male is introduced into the vial, the flies are probably not reacting to each other but to the new environment or the disruptive stimulus of being transferred (especially if the students are inept at this process). Therefore, we recommend that they wait 2 min, or until the male begins courting, before starting to record data.

Many fly courtship behaviors are difficult to see, especially with the unaided eye. Typically, students will be able to observe only the following courtship behaviors: orientation and singing (wing vibration). Hall (1994:1702) reviews the various behaviors that make up *Drosophila* courtship, and discusses "the connections among flies, genes and romance". Burnet and Connolly (1974) provide clear drawings of the different behaviors.

Initially, students often seem confused by the directions. It is important to explain that they will be starting and stopping a stopwatch repeatedly as the male starts and stops courting. In other words, they cannot simultaneously observe two mating tests if they only have one stopwatch! Also, they should not "zero" the stopwatch every time they stop it during the test—they need an additive record of time spent courting.

Using these techniques, we have good success with this lab. Students usually see some matings. Even if none of their own flies mate, they will probably be able to observe copulation since it lasts for about 20 minutes (Hall, 1994) and they can thus catch another group's flies in the act. Because students are often inherently interested in sex, we usually find them joking about and cheering on their flies throughout this lab.

Literature Cited

Burnet, B. and K. Connolly. 1974. Activity and sexual behavior in *Drosophila melanogaster*. Frontiers of Biology, 38:201-258.

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Hall, J. C. 1994. The mating of a fly. Science, 264:1702-14.