Lab 3. Inheritance in *Drosophila*

Notes:

- For the pre-lab exercise (see page 5 of this handout), first you will learn a little about the life cycle of *Drosophila melanogaster* and apply your understanding of life cycles to the fruit fly life cycle. Second, you will work through some problems on basic inheritance. Both of these activities will help you prepare to work with the fruit flies in lab *and* for the inheritance problems we'll tackle in Lab 4!
- This lab is based on Bixler, A. and F. Schnee (2005). Proceedings of the 26th Workshop/Conference of the Association for Biology Laboratory Education

Objectives:

1. Practice working with populations of flies (*Drosophila melanogaster*)
2. Demonstrate your understanding of mitosis and meiosis.
3. Be able to construct a Punnett square and predict offspring genotypes and genotype frequencies.

KEY WORDS: model organism; *Drosophila*; instar; mitosis and meiosis (and all their stages); cytokinesis; chromatid; sister chromatid; centromere; homologous chromosomes; synapsis; crossing over; chiasma; haploid; diploid; allele; genotype; phenotype; parental generation; F_1 generation

Contents

1 Background on *Drosophila* and Life Cycles

1A The model organism *Drosophila melanogaster*

Next week in lab, we will apply the Hardy-Weinberg Model to populations of *Drosophila melanogaster*, more commonly known as fruit flies. This week in lab, we will become better acquainted with *Drosophila* by practicing distinguishing male flies from female flies, and distinguishing flies with *Bar* eyes from flies with wild-type eyes.

Species of *Drosophila* occur in the wild all over the world, and they have been adopted for lab work in a variety of fields because they are easy to raise in large numbers in the lab, have a short generation time (about 2 weeks), and have only 4 pairs of chromosomes ($2n = 8$). Their wide use in laboratory work has made *D. melanogaster* a model organism: a species that is well-suited for lab work and has been studied extensively in order to provide insight into the biology of related organisms, including

humans. For example, in 1933, Thomas Hunt Morgan won the Nobel Prize in Medicine for his work using *Drosophila* to identify chromosomes as the unit through which genes are inherited. Closer to home, in 2012-13, biology honors student Darrin Shultz used *D. melanogaster* to study neuronal longevity—work which has implications for Alzheimer's research.

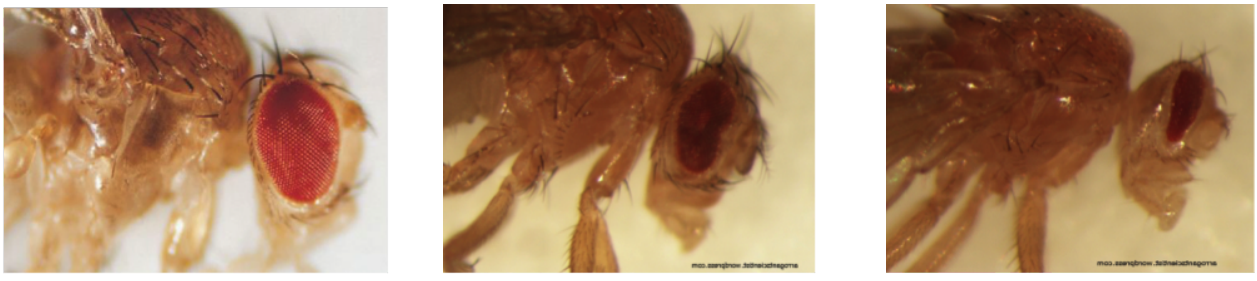
1B Life Cycle

A new *Drosophila* zygote is created when **sperm** fertilize **eggs** inside the female parent's oviduct. The sperm and eggs are haploid, the products of meiosis, while the new zygote is diploid. The female parent then lays the **fertilized egg (zygote)** on a suitable food source. Eggs are white, ovoid, and about 0.5 mm long. About 21 hours after they are laid, eggs hatch and the **larvae** emerge.

The larvae develop in stages known as **instars**, which are common to many insect species. The newly emerged 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. The **larvae grow rapidly**, and within about two days, the first instar will molt into the **second instar**. These larvae will eat, **grow**, and molt again to become the **third instar**. After the third instar crawls out of the food and onto the surface, the larvae begin to **pupate**. In the **pupal stage**, 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 that originated during the embryonic stages **develop** into their adult forms, while the remaining larval tissue is broken down to furnish both raw material and the energy needed for adult development. After three days, the **adult** emerges. The adult flies reach sexual maturity and begin producing new **gametes** about eight hours after emerging from the pupae, and then the cycle can begin again.

2 Phenotyping mutant and wild-type flies

In next week's lab about the Hardy-Weinberg Model, we will examine *Drosophila* for **alleles** of the *Bar* gene, which affects eye morphology. An **allele** is the name for any of the alternate forms or copies of a gene that an organism possesses. We will study the patterns of inheritance of the *Bar* gene by examining the **phenotype** of our flies, or their observable characteristics, in order to determine their **genotype**, or genetic characteristics.



(a) Wild-type round eye **(b)** Kidney- or heart-shaped eye **(c)** *Bar* slit-shaped eye

Figure 1: Comparison of three eye phenotypes. [Images: Bixler and Schnee 2005]

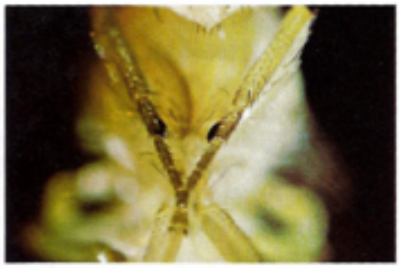
The *Bar* gene is useful to us because (a) the gene is sex-linked, located on the X chromosome; (b) there is a clear phenotypic effect of mutant alleles for this single gene; and (c) the mutant allele shows incomplete dominance (i.e., the heterozygotes are phenotypically different from either homozygote)—which allows us to make the jump from genotype to phenotype. Females that are homozygous for *Bar* (X^BX^B) and males

that are hemizygous for *Bar* (X^BY) have eyes that are reduced to a slit or bar shape (Figure 1, right). *Bar*-heterozygous females (X^BX^+) have a kidney- or heart-shaped eye (Figure 1, center). Wild-type females and males (X^+X^+, X^+Y) have round eyes (Figure 1, left).

We will also need to distinguish male flies from female flies using several visible anatomical features. Below (Figure 2) are images that can guide you in distinguishing male flies from female flies. One of the easiest methods for distinguishing flies is to look at the ventral side of the tip of the abdomen. In the genital region, the male has a dark pigmented genital disc with anal plates, genital arch, claspers and a penis (Figure 2a). In contrast, females have an unpigmented genital disk with anal and ovipositor plates (Figure 2b). Additionally, males have dark brown-black sex combs on their forelegs (Fig. 2c). The males use sex combs during courtship, and as such they are lacking in females.

(a) Ventral view of male abdomen.

(b) Ventral view of female abdomen.



(c) View of male sex combs on forelegs.

Figure 2: Comparison of *D. melanogaster* by sex. [Images: Carolina Biological Supply Company 2005]

During lab, your instructor will provide you with flies to sort by sex and *Bar* status and the materials necessary to do so. The flies that your group is counting today are the parents of the flies that you will be working with next week.

We will be sedating the flies using FlyNap, an anesthetic that immobilizes flies for about 50 minutes. To anesthetize your flies, first, you will need to get them into the empty vial provided. See your instructor's demonstration on how to accomplish this feat. Next, dip the pipe cleaner end of the black wand into the small bottle of FlyNap, being careful to get rid of excess FlyNap by briefly running the wand through the constriction at the neck of the bottle. If any flies are close to the top of the vial, knock them to the bottom by gently tapping the bottom of the vial against your lab notebook (or another padded surface). Then, quickly, put the foam stopper with wand, in place. Avoid letting the wand tip touch the sides of the vial. Leave the wand in the vial until all of the flies have stopped crawling. Finally, spill the flies onto the card provided and separate them by sex and *Bar* status. Use the table below to record the number of flies of each genotype observed in your group's population.

Population # _____

Phenotype	wild-type female	heart-eye female	Bar-eyed female	wild-type male	Bar-eyed male
Genotype	X^+X^+	X^BX^+	X^BX^B	X^+Y	X^BY
# of flies					

After you finish counting your flies, begin the Mitosis and Meiosis demonstration, after which you may begin the post-lab assignment.

3 Meiosis and mitosis demonstration

By this point in the semester, you've been thinking a good bit about meiosis and mitosis – weeks 2 and 3 of class and readings have covered these topics. **As the completion of this exercise, you must explain these processes to your instructor in order to verify that you understand them.**

Using the snap beads that your instructor will provide, work with your group and practice demonstrating the processes of mitosis and meiosis. Make sure you have the appropriate number of chromatids / sister chromatids / homologs at each step in each process. Remember the differences in how chromatids behave in mitosis, compared to meiosis, and be sure to use the correct terminology to describe the chromosomes and each stage of the process.

Next, think about how meiosis relates to Mendelian inheritance, which uses parental genotypes to predict the expected frequency of each genotype in offspring. Using a differently colored bead to represent the mutant *Bar* allele, demonstrate the inheritance pattern of the *Bar* gene in *Drosophila*.

Imagine that the cell undergoing meiosis is from a *Bar*-heterozygous mother. Incorporate the new beads into your demonstration of meiosis and answer the questions in the Post-lab Assignment below (due next week in lab; starts on page 6).

4 Pre-lab Exercise: Due at start of lab period

Read **1 Background on *Drosophila* and Life Cycles (page 1)** and **2 Phenotyping mutant and wild-type flies (page 2)** before completing the questions below.

If you could use a refresher on mitosis and meiosis, we suggest one of the following resources:

- The course content videos on Mitosis and Meiosis (from week 3)
- Chapters 10 and 11 of the online text Biology 2e
- Mitosis vs. Meiosis: Side by Side Comparison video by the Amoeba Sisters

1. For each of the following *Drosophila* life history stages, mark whether that stage is haploid or diploid:

Stage	sperm	egg	zygote	1st instar	2nd instar	3rd instar	pupa	adult
haploid								
diploid								

2. For each of the following *Drosophila* life history stages, mark whether meiosis or mitosis is occurring:

Stage	**mitosis**	**meiosis**
1st instar larvae grow rapidly		
2nd instar larvae grow		
3rd instar larvae pupate		
pupa develops into adult		
mature adult flies produce gametes		

3. When the cells of a *Bar*-heterozygous female undergo meiosis, what types of gametes are produced, with respect to the *Bar* gene? How many of each type are produced by meiosis of a single cell? What is the percentage of gametes with each allele?

4. How would the percentages of gamete types differ if...

   A. the female was *Bar*-homozygous?

   B. the female was wild-type?

5. Remember that a female parent only provides half of her offspring's genotype – the male parent contributes the other half. Predict all possible genotypes for offspring (male and female) of a cross between a *Bar*-heterozygous female and a wild-type male. What are the expected proportions of each genotype in their offspring?

5 Post-lab Assignment: Punnett squares and populations

Note that this assignment is due at the start of next week's lab.

One way you can simulate inheritance is through Punnett squares, which provide a graphical representation of the alleles each parent can contribute to their offspring. Punnett squares are an alternative way to carry out the same calculations that you did in the pre-lab exercise.

We're going to use a Punnett square to predict the genotypes that would result from a cross of our mutant flies, but first, let's work through a simple cross for a gene encoding a protein that affects body color in *Drosophila*.

For this example: each parent possesses two alleles for a body color gene, in which *B*, brown body color, is dominant and *b*, black body color, is recessive. Both the female and the male parent in this cross are heterozygous for the gene (this gene is not sex-linked).

1. Draw your Punnett square, following the steps below, and fill in the percentage of offspring with each phenotype.

   (1) Write down the genotypes of the parents, indicating which is male and which is female. Each parent will contribute one allele to each possible offspring genotype.

   (2) Draw a square with a cell for each possible offspring genotype. Since we are considering a single gene in a diploid organism, the number of possible offspring genotypes will be the number of alleles that the diploid female parent has times the number of alleles that the diploid male parent has. (NOTE: the two alleles possessed by a parent do not have to be different from each other to count as two alleles.)

   (3) List the alleles of one parent along the top of the square and those of the other parent down the left side of the square, matching up with the cells in your square. It doesn't matter which side has the female's alleles and which side has the male's alleles.

   (4) Copy each allele along the side into all the cells in its row or column. Alleles on top of the square get copied down the column, and alleles on the left get copied across the row.

   (5) Determine the phenotypes of the offspring based on the genotypes displayed in each cell in the Punnett square.

   A. Parental genotypes in this cross:

   B. Punnett Square:

   C. What percentage brown-bodied offspring do we expect? _____

   D. What percentage black-bodied offspring do we expect? _____

The inheritance of *Bar* works a little differently than the example of a gene encoding a protein affecting body color. As you'll remember from section **2 Phenotyping mutant and wild-type flies**, the *Bar* allele is inherited on the *X* chromosome, making it sex-linked in this species.

2. In the example about body color, each parent has two alleles for the gene that they could contribute to their offspring. Is this situation true for the *Bar* gene? If not, then how many alleles (at most) might the female parent have for the gene? The male parent? How would these numbers change if the gene were on the *Y*-chromosome (which is another way that a gene can be sex-linked)?

3. How would you set up a Punnett square to accommodate sex-linked alleles? Below, work through a Punnett square to determine the genotypes produced in a cross between a *Bar*-heterozygous female and a wild-type male.

4. For a population with those 3 alleles of this sex-linked *Bar* gene (X^+, X^B, and Y, which is like a "null" allele here):

   A. How many *genotypes* are possible (remembering that a genotype is determined by how many of which alleles are present in an individual)? List them.

   B. How many eye *phenotypes* are possible? List them.

   C. Which, if any, genotypes are found in both sexes?

   D. Which, if any, eye phenotypes are found in both sexes?

5. So, you've already completed a Punnett square for one possible cross that could occur in a population with *Bar* mutants! In the "Crosses" column below, write out the five remaining possible crosses. Draw out Punnett squares (in the space below) for each of the additional crosses, and then calculate expected genotype frequencies. Below the columns for each of the genotypes, write the proportion in decimal form (not percentage and not fraction) of offspring with that genotype that you expect to result from each cross. Be careful about calculating the frequencies - remember that *Bar* males and *Bar* females have the same *phenotype* but NOT the same *genotype*. The sum of all genotype frequencies for a cross will be 1 (with half of that from males and half from females).

	Expected genotype frequencies				
	Female genotypes			Male genotypes	
Cross	X^+X^+	X^BX^+	X^BX^B	X^+Y	X^BY
$X^+X^B \times X^+Y$					

6. Note that offspring have a 50% chance of inheriting each allele from each parent. How could you use this knowledge to write an equation to calculate the probability of a given offspring having a genotype without physically counting the frequency of each genotype in the offspring population? Below, write and clearly label an equation calculating the probability that a particular offspring from a cross between a *Bar*-heterozygous female and a *Bar*-hemizygous male will be a *Bar*-homozygous female. Explain how you created the equation.

7. Remember that since males have the chromosomes XY, a male has to get his only X chromosome from his female parent (in contrast to his sisters, which get X chromosomes from both parents). **Given this information, and what you know about how *Bar* is inherited, what general rule could you devise to predict the frequencies of the male genotypes of any particular cross?** Note that this should be one rule that applies regardless of the female's genotype (not a rule listing every specific possible outcome).

8. You've predicted the genotypes of the F_1 generation, or offspring, of a cross between 2 flies. However, in the coming lab, we're going to be working with populations of flies, not just one set of parents. Remember that genotypes result from the inheritance of alleles, and that when you have one set of parents with two alleles each, the offspring have a 50% chance of inheriting either allele from either parent.

   But when you're looking at a population of individuals instead of just one set of parents, the chance of one offspring chosen at random from the population inheriting a particular allele may not be 50%. Consider a population of *Drosophila* in which 80% of the alleles (not the phenotypes) for a non-sex-linked gene are wild type, and there is only one other allele for that gene.

   Using a Punnett square, how would you use this information to calculate the frequency of each genotype in the F_1 generation of the above population? Write out your plan, and then show and clearly label all of your calculations.