



Development of Higher-Level Cognitive Skills In a Learner-Centered Lab on Extensions of Mendelian Inheritance Using *Drosophila*

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Abstract

Students can have difficulty comprehending complex concepts in science. They can memorize the definition but do not understand the underlying biological principles. In the Fundamentals of Genetics course at Arizona State University at the West campus, students grapple with the topic of “extensions of Mendelism.” Additionally, in lab, students are challenged by scoring phenotypes that are not binary. Both of these concepts require that students understand not only inheritance but also the principles of protein structure and function. A genetics laboratory exercise was developed that combines study of some extensions of Mendelian inheritance with practice in manipulating *Drosophila melanogaster* and scoring subtle and variable phenotypes. Students analyze *Drosophila* with mutations that demonstrate some extensions of Mendelian inheritance: temperature sensitivity, variable expressivity, incomplete penetrance, multiple alleles, dosage compensation, and gene dosage effect. The phenotypes in some of these mutants differ from individual to individual and are difficult to discern; thus, students also gain experience in investigating challenging phenotypes. Pre- and postlab assessments indicate that performing this exercise increased students’ mastery of the molecular basis of extensions of Mendelian inheritance and their abilities in scoring and manipulating flies. This is a discovery-based lab exercise in which students examine some extensions of Mendelian inheritance and gain experience in analyzing complex traits in *Drosophila*.

Article

There are many topics in a general or fundamentals of genetics class for which the definitions are easy for students to memorize but hard for them to understand. Students can learn the meaning of genetics material without understanding the context or the theory. The challenge for the instructor is to develop pedagogical techniques that help students understand both the concepts and the underlying molecular mechanisms of inheritance in genetics.

One of the broad topics in a general or fundamentals of genetics lecture course that is difficult for students to comprehend is “extensions of Mendelian inheritance” (1). In studying extensions of Mendelian inheritance, students are often unclear on topics that include: multiple alleles, variable expressivity, incomplete penetrance, dosage compensation, gene dosage effect, and conditional mutants. Students are confused by these topics because understanding of these modes of inheritance requires knowledge of underlying protein function as well as Mendelian inheritance itself. These advanced concepts require students not only to memorize but also to apply, analyze, and synthesize information learned in lecture (7).

The more examples that are covered in lecture, the more likely the students are to comprehend the topics. However, even covering copious examples in lecture is still a passive learning process. To provide for a more active exploration of the topics, some examples of extensions of Mendelian inheritance can be taught in the laboratory setting. Active learning has been shown to be superior to passive lecturing alone in teaching concepts (5, 8, 11). Active learning can include many forms; in this example students in a concurrently enrolled lab study and lecture course analyze *Drosophila melanogaster* mutants that demonstrate some of the extensions of Mendelian inheritance; students not only examine the phenotypes of the flies but also consider the protein function and protein level in the affected mutants.

In the Fundamentals of Genetics classes at Arizona State University at the West campus, students also grapple with concepts that are not “black and white.” Many students are stuck in the first stage of “Perry’s Stages of Undergraduate Cognitive Development” (7) in which students believe instructors have all the correct answers. As an instructor of a sophomore-level, Fundamentals of Genetics lecture, students try to confirm with me, before they turn in an assignment, that the homework answer they have calculated or the essay answer they have written is correct. Any data in lab that needs to be analyzed often will confound students as they look for instructor verification that they, indeed, have the one correct answer. Students want to make sure that all of their work is correct before turning it in; this is, of course, often in opposition to the purpose of the particular assignment, which is that the students should be learning through the process or procedure (7), not just magically coming up with or copying the correct answer. Informal discussions with colleagues in the department indicate that this is a general phenomenon for students in courses such as cell biology, biochemistry, and molecular genetics (C. Deutch and P. Jurutka, ASU at the West campus, personal communication).

Many students in the Fundamentals of Genetics classes are not progressing to upper levels of Bloom’s taxonomy of cognitive ability such as analysis and synthesis (7) using current class activities and lab work. Thus, a laboratory exercise was devised in which students examine *Drosophila* mutants and then are expected to analyze and synthesize the observation data to integrate concepts learned in lecture with experiments performed in lab. In this lab exercise, students evaluate *Drosophila* mutants with phenotypes that demonstrate some of the extensions of Mendelian inheritance to further their understanding of the molecular basis of phenotypes. In a discovery-learning format (9, 12), students are required to explore the phenotypes of *Drosophila* in order to understand the theory and molecular mechanisms underlying some extensions of Mendelian inheritance. They also are asked a series of questions relating to protein function in the mutants. Students examine fly phenotypes to understand better the concepts of conditional alleles, variable expressivity, incomplete penetrance, multiple alleles, dosage compensation, and gene dosage effect. Several of these phenotypes are variable and subtle, reinforcing the concepts that phenotypes are not always binary in nature but also may be ambiguous. Additionally, since analyzing these phenotypes requires students to use their own observational skills and judgment, this lab exercise helps students to become more confident in their knowledge and abilities (Table 1). In performing this exercise, students should mature and move to a higher level of “Perry’s Stages of Cognitive Development,” hopefully to the stage where they recognize that uncertainty exists in knowledge but that by using certain objective criteria, a decision can be made as to the validity of the data and the interpretations they make (7).

This hands-on exercise allows students to examine and work with mutant *Drosophila* strains or crosses that demonstrate some concepts of extensions of Mendelian inheritance. This exercise also provides students with practice in manipulating and sexing flies. One of the objectives of this lab exercise is to demonstrate to students that science can be seen in “shades of gray.” This lab also demonstrates that in science people are the data analyzers and that there may be some inherent uncertainties in the experiments. Several of the phenotypes in this lab are variable and subtle; thus reinforcing the concept that analysis requires time and thought, as opposed to rote memorization or quickly calculating through to the one “correct” answer. Through this lab exercise, students are encouraged to move forward in their cognitive development, to acquire knowledge and analyze information and data for themselves, and to become independent thinkers (7).

This activity was used in a 300-level Fundamentals of Genetics lab in which more than 95% of the students were concurrently enrolled in the lecture class, Fundamentals of Genetics. Post-lab questionnaires indicated that students felt that they had learned from the exercise, that the lab was an active process, and that the lab caused them to ask questions. Pre- and postexercise assessment indicated that students felt that the activity enhanced their understanding of each of the concepts and

their ability to work with and manipulate flies. Pre- and postexercise testing indicated that for the majority of the test questions, there was a statistically significant gain in test scores from pre- to posttest indicating that the exercise increased the students' understanding of extensions of Mendelian inheritance.

Learning Objectives of Lab

1. Students will become more comfortable with manipulating and sexing *Drosophila*.
2. Students will be able to score traits, including those that are subtle and those that do not conform to the expected norm of binary phenotypes.
3. Students will analyze extensions of Mendelian inheritance using *Drosophila* mutants as their model.
4. Students will relate phenotypes seen in *Drosophila* to the cellular and molecular biology of the flies.
5. Students will synthesize observations from lab with lecture material to understand the molecular basis of phenotypes.

MATERIALS AND METHODS

True breeding strains. The *Drosophila* mutants *shibire* (stock #2248) and *Lobe* (stock #324) were obtained from the Bloomington *Drosophila* Stock Center at Indiana University, Bloomington. Additionally, *white* (87 W 6553), *eyeless* (87 W 6592), *apricot* (87 W 6562), wild type (87 W 6621), and *Bar* (87 W 6551) flies were obtained from Ward's Biology and Chemistry (Rochester, NY). The *eosin* mutants (*white-eosin* 17-2240, no longer available; obtain *eosin* mutants from the Bloomington Stock Center) were obtained from Carolina Biological Supply (Burlington, NC). All flies were cultured in Ward's instant *Drosophila* medium or Carolina formula 4-24, kept at 25°C, and transferred to new vials at 1- to 2-week intervals to be kept as true breeding strains.

***Drosophila* crosses.** For the lab activity, heterozygous *apricot-white* and *eosin-white* female *Drosophila* were also needed. Four weeks before the activity, a vial of true breeding *eosin* flies was mixed with a vial of true breeding *white* flies and allowed to mate indiscriminately. Every week, all adults that were in the vials were transferred to a new food vial. After 4 weeks, the flies that were present in the vials represented a mixture of true breeding *white*, true breeding *eosin*, and heterozygous *eosin* females. Four weeks before the activity, a vial of true breeding *apricot* flies was mixed with a vial of true breeding *white* flies and allowed to mate indiscriminately. Every week, all the adults that were present in the vial were transferred to a new food vial. After 4 weeks, the flies that were present in the vials represented a mixture of true breeding *white*, true breeding *apricot*, and heterozygous *apricot* female flies.

Fly manipulation. Flies were manipulated as described in the Carolina *Drosophila* manual (4). Approximately 30 adult wild type flies and the *shibire* *Drosophila* true breeding adult flies were transferred separately to clean vials free of media. These flies were used for the conditional demonstration.

For all other *Drosophila* analysis, flies of the appropriate true breeding strain or cross were transferred to labeled clean vials, free of food and netting. Flies were distributed to students, and the students anesthetized them using Carolina Flynep and Flynep wands until the flies were no longer moving. These flies were used for the remainder of the examinations.

All anesthetized flies were analyzed under a dissecting microscope on white cards, using soft paint brushes to move the flies for sexing and phenotype examination.

Analysis of student learning. Exemption from human subjects approval was granted by the Institutional Review Board as pursuant to Federal regulation 45 CFR Part 46.101(b)(1). Willing students filled out anonymous pre- and postlab questionnaires (3, 6). Four total lab groups were analyzed (two in fall 2006 and two in spring 2007) and the data were combined such that the questionnaires could not be assigned

to a lab section. The mean for each question and the standard deviation were calculated using Excel. Identical pre- and postlab questions on the student self-assessment of learning were compared using Excel and a one-tailed homoscedastic student's *t* test to test for significance (Table 1). Additional survey questions (Table 2) and the opportunity to provide open-ended comments (Table 3) were included on the postlab survey. Pre- and postlab testing was also employed in the spring of 2007 to determine the extent of actual student learning; a quiz (included in Supplemental Material) was given directly before the exercise and then the same quiz was given the following week in lab. The data from the pre- and postlab quiz were analyzed for the percentage of correct answers using all of the student's results (Table 4).

TABLE 1. Pre- and postlab student self-assessment of knowledge and skills^a

Statement	Prelab student self-assessment mean ± SD (n = 71)	Postlab student self-assessment mean ± SD (n = 67)	<i>P</i> value
I understand well the concept of simple Mendelian inheritance.	4.63 ± 0.57	4.69 ± 0.50	0.23
I understand well the concept of multiple alleles.	4.00 ± 0.92	4.29 ± 0.81	0.024 ^b
I understand well the concept of gene dosage effect.	4.14 ± 0.86	4.50 ± 0.68	0.0034 ^b
I understand well the concept of expressivity.	4.18 ± 0.66	4.44 ± 0.65	0.010 ^b
I understand well the concept of incomplete penetrance.	3.79 ± 0.87	4.07 ± 0.85	0.028 ^b
I understand well the concept of protein level affecting phenotype and enzyme activity.	3.81 ± 1.06	4.22 ± 0.79	0.038 ^b
I understand well the concept of temperature sensitivity.	4.24 ± 0.85	4.47 ± 0.82	0.049 ^b
I feel comfortable manipulating and sexing flies.	3.47 ± 1.01	4.15 ± 1.05	8.25 x 10 ^{-5b}
I feel comfortable scoring phenotypes in <i>Drosophila</i> .	3.33 ± 0.93	3.91 ± 1.06	4.02 x 10 ^{-4b}

^a Students answered a series of questions before and one week after the lab activity. The answers were: agree strongly; 5, agree slightly; 4, neither agree nor disagree; 3, disagree slightly; 2, and disagree strongly; 1. The results were analyzed in Excel to determine the mean and standard deviation for each question. Excel was used to perform a one-tailed homoscedastic student's *t* test to determine the significance of the change between pre- and postexercise surveys.

^b *P* < 0.05.

TABLE 2. Postlab assessment of laboratory exercise ^a

Statement	Postlab student response mean ± SD (n = 67)
I enjoyed this lab.	3.82 ± 1.08
I would recommend that this lab exercise be kept in the genetics curriculum.	4.01 ± 1.06
This lab exercise made me think.	4.08 ± 0.97
This lab exercise fit in well with the curriculum of the lecture.	4.26 ± 0.92
I hate this particular lab.	2.39 ± 1.21
This lab was an active process for me.	4.12 ± 0.94
I learned something from this lab.	4.33 ± 0.73
This lab made me ask questions, such as "why does this fly look this way?"	4.26 ± 0.86

^a Students were asked a series of questions one week after the lab activity. The answers were: agree strongly; 5, agree slightly; 4, neither agree nor disagree; 3, disagree slightly; 2, and disagree strongly; 1. Excel was used to determine mean and standard deviation.

TABLE 3. All student comments written on the postexercise survey

Responses that indicate the learning objectives were achieved
"This lab is really interesting and fun! Definitely keep it for years to come!"
"Good lab."
"This was great practice for sexing and manipulating flies."
"This lab was very helpful in distinguishing the different phenotypes. Did not enjoy last weeks [sic] lab [setting up <i>Drosophila</i> parental crosses, vestigial ebony x wild type and sepia ebony x wild type, to obtain and analyze F ₁ and F ₂ generation]. It was kind of confusing but now that we know what it was that we were looking at it makes much more sense."
"I enjoy lab's [sic] like this that are not "cookbook" lab's [sic], but isn't something better than flie's [sic] we could study?"
"Differences in shades of color was [sic] much more frustrating than I thought."
Responses that indicate the learning objectives were not achieved
"Lobe eye was confusing."
"Counting the flies was confusing."
"Its [sic] a lab where we didn't do anything but observe; thoes [sic] are not my favorite labs."
"I believe specific instructions on what we needed to do will be better. We were not sure which ones to count, and why it was necessary to count."

TABLE 4. Pre- and postlab exercise quiz results ^a

Question number and topic	Prelab quiz percentage of correct answers	Postlab quiz percentage of correct answers	Change in percentage of correct answers from pre- to postlab	P value
1. conditional allele	83.3	97.2	13.9	0.0736
2. gene dosage effect	80.5	88.9	8.4	0.6171
3. gene dosage effect	52.8	94.4	41.6	0.0012 ^b
4. relate gene dosage effect to protein levels	50	52.8	2.8	0.7728
5. conditional allele	86.1	91.7	5.6	0.4497
6. multiple alleles	27.8	77.8	50	<0.0001 ^b
7. multiple alleles	72.2	83.3	11.1	0.4497
8. expressivity	72.2	100	27.8	0.0077 ^b
9. incomplete penetrance	69.4	86.1	16.7	0.0704
10. dosage compensation	8.3	44.4	36.1	0.0019 ^b
11. dominant gain of function mutation	38.9	66.7	27.8	0.0055 ^b
12. gene dosage effect	36.1	86.1	50	0.0002 ^b
13. dosage compensation	2.8	47.2	44.4	0.0002 ^b
14. multiple alleles	41.7	80.6	38.9	0.0055 ^b
Mean	51.6	78.4	26.8	

^aStudents (n = 36) were given the same quiz on the laboratory concepts directly before (pre-) and 1 week after (post-) the lab exercise to assess the extent of student learning from the exercise. Statistical significance was determined using a McNemar's test for paired enumerated data, two tails.

^bP < 0.05.

The data in Table 4 were further analyzed for a statistically significant difference between a pre- and postlab exercise quiz using a McNemar's test for paired enumeration data using Graphpad software (<http://www.graphpad.com/quickcalcs/McNemar1.cfm>) (Table 4), removing from the analysis those students who did not take both quizzes (one did not take the posttest and a different student did not take the pretest). The McNemar's test is used to determine if there is a statistically significant difference in paired dichotomous data. This statistical test is best used for data in which the results are in a yes-no format. Each student's quiz results are described as either correct on both pretest and posttest, correct on only pretest, correct on only posttest, or correct on neither pre- nor posttest. The null hypothesis is thus that those who did not know the answer before the exercise will not know the answer on the posttest.

Student prelab preparation. Prior to lab, students were given a reading assignment of the relevant chapters in the textbook and the laboratory procedures (included Supplemental Material). Then students were required to take an online prelab quiz through the BlackBoard course shell to ensure that they had read and understood the assignment. Students were also given a prelab self-assessment of their knowledge and abilities (Table 1) and a prelab quiz (spring 2007 only). This assessment was given during the lab time but before the activity commenced.

In lab exercises. (i) Conditional allele demonstration. Conditional alleles are those that have a wild type phenotype in one environmental conditional but show a mutant or variant phenotype in another environmental condition (1). Students analyzed an obvious and reversible temperature sensitive mutant of *Drosophila*, speculating about the protein function, activity, and structure at permissive and nonpermissive temperatures. *shibire* is a mutation of dynamin in which, at the restrictive temperature, the

flies are paralyzed because they cannot complete synaptic transmission (10). This mutation is fully reversible, and the flies regain movement once they are returned to the permissive temperature. The conditional allele demonstration of the *shibire* mutation is a variation of a demonstration by A. Bejsovec (<http://flystocks.bio.indiana.edu/Browse/misc-browse/Bejsovec.htm>). True breeding homozygous *shibire* and wild type flies were transferred to clean vials and stored at room temperature. Students were allowed to watch the fly activity in the vials to determine that both strains were alive and capable of movement. The instructor then grasped each vial tightly in her hand as the students used a watch with a second hand to determine how long it took for the *shibire* mutants to cease movement and fall to the bottom of the vial. Once the *shibire* mutants were no longer moving, both vials were placed on the counter at room temperature and the students measured how long it took for the *shibire* mutant flies to regain movement. After the demonstration, students were asked questions about the function of the dynamin protein and about the structure and activity of the dynamin protein in the *shibire* mutant at the restrictive and nonrestrictive temperatures in order to stimulate their thinking about the molecular process and protein function of temperature sensitive mutants.

(ii) Phenotype demonstrations for other extensions of Mendelian inheritance. For this exercise, separate dissecting microscope stations were set up for each true breeding strain or cross. Each lab group was given one or two labeled vials with at least 40 flies in each vial, free of food and netting, to anesthetize. Students used Flynap to anesthetize the flies, placed them under a dissecting microscope, and clearly labeled the station as to the true breeding strain or cross. Each student looked at all of the different true breeding strains and crosses in "round robin" style. Students compared all mutant flies to the wild type *Drosophila* strain.

(a) Variable expressivity and incomplete penetrance. Phenotypes in mutants can vary from individual to individual such that all members of a group will have the same genotype but will vary in their phenotype. Variable expressivity is the phenomenon in which all individuals of a given genotype will show differing degrees of a phenotype. Some dominant phenotypes can even skip generations, in an inheritance pattern known as incomplete penetrance (1). To illustrate variable expressivity and incomplete penetrance, students analyzed eye size phenotypes. Students examined true breeding *Drosophila* mutants of *Lobe* and *eyeless* and compared the eye phenotype of shape, size, and facet number to a wild type *Drosophila* strain. The recessive mutation, *eyeless*, shows variable expressivity. Homozygous *eyeless* flies have eyes that range from almost wild type in appearance to almost completely absent. The most striking observation was that *eyeless* flies can have one eye that has an almost wild type phenotype, while the other eye is almost completely absent; thus showing variable expressivity even within the same organism. The *Lobe* phenotype is more subtle and also demonstrates incomplete penetrance. The most common *Lobe* phenotype is a loss of the ventral side of the eye (2); however, the phenotype can be more or less severe as the allele also demonstrates variable expressivity. Upon first inspection of the *Lobe* mutants, most students indicated that all flies examined had a wild type phenotype. Only after explanation by the instructor and reexamination, did the students see the eye size phenotype of *Lobe* mutants. Students were asked questions about the protein function in the mutant fly strains. In order to reinforce the idea that traits are governed by protein function, students were asked in postlab exercise questions to describe how they thought protein function could be affecting the phenotype in terms of variable expressivity and incomplete penetrance.

(b) Multiple alleles. Many genes have more than the binary two alleles; this inheritance is termed multiple alleles (1). Students studied eye color mutants in order to understand multiple alleles. The X-linked *white* gene exhibits alleles of *eosin*, *apricot*, and *white*. Students examined eye color in these three mutants as compared to wild type and were asked in postlab questions to deduce a possible explanation of protein function for each allele.

(c) Dosage compensation. Dosage compensation is the phenomenon in which the gene expression of most X-linked genes does not vary between hemizygous males and homozygous females (1). In *Drosophila*, the expression of most X-linked genes is doubled in the male fly to equal that in the homozygous female fly. Students examined wild type eye color and the *apricot* eye color allele to demonstrate that both the hemizygous male and the homozygous female have the same eye color. In postlab questions, students were asked about the phenomenon of dosage compensation and how it occurs at the molecular and protein expression levels.

(d) *Gene dosage effect.* Gene dosage effect is an inheritance pattern in which the number of copies of an allele affects the intensity or severity of the phenotype (1). For understanding of gene dosage effect, students examined eye mutants, including two eye color mutants and one eye shape mutant. For analysis of all gene dosage effect mutants, students were asked a series of postlab questions about the phenotype of each mutant and the relationship of the protein function and levels to the phenotype. In postlab questions, students were encouraged to explore how protein levels in gene dosage effect mutants would lead to different phenotypic consequences.

The mutant alleles *eosin* and *apricot* are both X-linked genes that demonstrate gene dosage effect. For *eosin* eye color in *Drosophila*, the true breeding wild type females have twice as much pigment and a darker eye color than the hemizygous males do. Heterozygous females (*eosin-white* heterozygotes) have an eye color as intense as the males but less intense than the homozygous females (1). The phenotype of apricot eye color in *Drosophila* is more complicated. The *apricot* allele demonstrates dosage compensation as well as gene dosage effect. In males with the *apricot* allele, the eyes are peach in color. In the homozygous females, the eye color is the same color as the males. The heterozygous female (*apricot-white* heterozygote) has a lighter eye color than the homozygous female or the male. Students analyzed true breeding *white*, *eosin*, and *apricot Drosophila* to inspect eye color. Students also were required to sex these flies. Once students were familiar with the eye colors of the true breeding flies, they examined the eye color phenotype in the *Drosophila* crosses, which were a mixture of males and homozygous and heterozygous females. Students noted eye color and sex of flies in each cross for a minimum of 25 flies, looking for the heterozygous females in each case. For both the *eosin* and *apricot* true breeding strains, students were asked to describe in postlab questions the eye color in males and females in terms of the relationship to protein level and function. Students also had to describe why in true breeding *apricot* flies the males and females had similar eye colors, but in *eosin* the males had lighter eyes than the females.

The *Bar* allele is another example of an allele that is governed by gene dosage effect. This allele is also an example of an X-linked dominant gain-of-function allele. In true breeding bar eye flies, the facet number is reduced as compared to wild type, with the females having fewer facets than the males. Students analyzed a true breeding stock of bar eye flies to study the gene dosage effect of this allele. Students described the protein levels as they related to the differing eye phenotypes in males and females. In postlab questions, students also were asked about how they thought the *Bar* allele was working as a gain-of-function mutation, in order to help students link the allele with protein function. Students were also questioned about the formation of wild type revertants, males with typical female *Bar* phenotype and *Ultrabar* females (1) and what the protein function and levels would be in these cases.

Lab Handout

Analysis of Variable Phenotypes in *Drosophila* Mutants

OBJECTIVES

1. To investigate alleles that demonstrate extensions of Mendelian inheritance using *Drosophila* mutants as our model.
2. To practice scoring traits, including those that are subtle and those that do not conform to the expected norm of binary phenotypes.
3. To gain more experience manipulating and sexing flies.

REFERENCE

Please note that all page numbers, figures, and tables referred to in this lab handout come from the following source.

Brooker, R. J. 2005. *Genetics: analysis and principles*, 2nd ed. McGraw Hill Higher Education, New York, NY.

BACKGROUND

Some mutants of *Drosophila melanogaster* are easily identifiable. However, many mutant alleles are not so easily scorable. In some cases, it can be difficult to identify if the organism in question has a mutant phenotype, or to what degree the mutation is causing a phenotype. In this lab exercise, we will study some mutant *Drosophila* with difficult-to-score phenotypes, while reinforcing the concepts of five extensions of Mendelian inheritance: **variable expressivity**, **incomplete penetrance**, **multiple alleles**, **dosage compensation**, **gene dosage effect**, and **temperature sensitivity**.

Expressivity is the degree to which a trait is expressed (p. 86). Some mutant alleles show **variable expressivity**, in which the individuals have the same genotype but differ in their phenotype. An example in humans is the dominant myotonic dystrophy (<http://www.mda.org.au/specific/mdamyt.html>). Each individual who has the dominant M phenotype develops the disease, characterized by muscle wasting and other symptoms such as hair loss, gastrointestinal problems, and mental retardation, but each individual develops the disease at a different age and some become more seriously affected than others (they may only have very slight muscle wasting, for example, or may show all of the above symptoms and more). That variation in the degree (severity) of the disorder is due to variable expressivity. We will look at two fly mutants, *eyeless* and *Lobe*, whose phenotypes demonstrate variable expressivity.

Incomplete penetrance (p. 86) is a phenomenon in which the phenotype does not always match the genotype. A dominant allele or alleles are present, but the phenotype may still be wild type. This causes the phenotype to skip a generation even though the dominant allele is present. *Lobe* also exhibits incomplete penetrance.

Some genes are found in three or more alleles; these genes thus exist in **multiple alleles** that differ from each other in genotype and phenotype (p. 79–80). These alleles can exhibit different relationships of dominance and recessiveness. In lab today we will look at four X-linked alleles of a *Drosophila* eye color gene (*white*): wild type, eosin, apricot, and white. Wild type is dominant over all of these alleles. White is totally recessive. Eosin is dominant over white and recessive to wild type. Apricot is dominant to white and recessive to wild type.

Most of the time, X-linked alleles demonstrate **dosage compensation**. Dosage compensation refers to the phenomenon that in males the protein expression levels from the hemizygous genes on the X chromosome are the same as those from the XX female (p. 168). In *Drosophila*, expression of the genes on the one X chromosome of the male is “turbo charged,” so that the protein level of most of the X-linked genes in the male is the same as the XX female. We will look at apricot eye color and wild type eye color that demonstrate dosage compensation—the hemizygous XY male and the XX female have the same phenotype, even though the male only has one copy of the X-linked gene.

In a few cases, the **gene dosage effect**, in which the number of copies of a certain X-linked allele affects the phenotype of the allele, is seen. Generally the more copies of the allele that are present, the more intense the phenotype (p. 82). This occurs because each allele influences the amount of protein made; so zero alleles will have no protein made, one allele will have 50% of the protein made, two alleles will have 100% of the protein made, etc. (see Fig. 4.3, p. 79). In an X-linked trait, male flies will always have one copy of the gene (hemizygous genes only) and female flies will always have two. Thus, with genes that demonstrate the gene dosage effect, a male with the dominant allele (one copy, since it is X-linked) has a different phenotype than the female with two copies of the dominant allele. An example of this is the eosin fly eye color (p. 82–84), in which the female fly’s eye color (two eosin alleles) is darker than the male’s eye color (one eosin allele) or the heterozygous female’s (one eosin allele). We will look at eosin eye color mutant flies whose phenotype demonstrates gene dosage effect. We will also look at bar eye fly mutants

(p. 198–201) that demonstrate gene dosage effect. Interestingly, the apricot allele also demonstrates gene dosage effect, as the heterozygous female (apricot-white heterozygote) has a lighter eye color than the homozygous female or the hemizygous male. We will also look at the gene dosage effect in the apricot allele.

Conditional alleles only show the mutant (variant) phenotype under certain conditions. For example, in humans there is a loss-of-function mutation in the phenylalanine hydroxylase gene that, in the homozygous form, leads to the inability to breakdown phenylalanine from dietary protein; this disorder is called phenylketonuria or PKU (p. 8–9, 77). A diet high in phenylalanine leads to a toxic buildup of this amino acid and metabolic breakdown byproducts and subsequent mental retardation and other detrimental phenotypes. However, a diet low in phenylalanine does not lead to the buildup and the phenotypes are not seen. Thus, this disorder is conditional—under one set of conditions (high phenylalanine diet), one phenotype is seen (mental retardation); and under another set of conditions (low phenylalanine diet), a different phenotype is seen (wild type). **Temperature sensitive** alleles are a type of **conditional** allele. With temperature sensitive alleles, the phenotype varies based upon the temperature at which the mutants are grown or incubated.

PHENOTYPES

Wild type flies

Wild type flies have bright red eyes with approximately 800 facets per eye. The wild type eye is a spherical shape with a smooth surface and smooth edges. The wild type eye color allele is dominant (dominant wild type *white+* gene) over all other eye color alleles we will be observing in this lab.

At both temperatures we will be testing, wild type flies fly and hop around the vials.

Mutants

Eyeless is a recessive allele in which the eye size and number of facets is reduced, as compared to wild type. The eye may be only slightly reduced in size or may be less than one half of its normal size. This is an example of variable expressivity, because all of the true breeding stock have the same genotype but the phenotype varies. These flies have red eyes, but the eyes vary in size. Eyeless is an autosomal gene.

Lobe is a dominant autosomal allele that exhibits variable expressivity, in which the eye size and number of facets is reduced; the eye may also have a nick or nicks in the edge. *Lobe* also demonstrates incomplete penetrance; in the true breeding strain, the eye may also be wild type in shape and size. So, the eye may be wild type in size, only slightly reduced in size, or may be much smaller than wild type. These flies have red eyes, but the eyes vary in size.

The *white* eye allele is X-linked and recessive to all eye color alleles we will be looking at in this lab. We will only be looking at the white eye allele in respect to the eye colors, not the eye shape. This mutation is the total loss of function allele of the *white* gene (see Fig. 13.7, p. 345).

The eosin allele causes the eye to appear a pinkish-yellow, darker in color than wild type. Eosin is dominant to white and recessive to wild type, so it is an example of an allele of a gene with multiple alleles (the *white* gene). Eosin is an X-linked eye color mutation in which the gene dosage effect is best seen (p. 82–84). In true breeding females, the eyes are darker in color than the true breeding males' eyes. The eosin-white heterozygous female has the same eye color as the male with eosin eyes.

The apricot allele causes the eyes to appear a peachy color, lighter than wild type. Apricot is dominant to white and recessive to wild type, so it also is a multiple allele of the *white* gene. Apricot is an X-linked eye color mutation in which both dosage compensation and gene dosage effect are seen. As a result of dosage

compensation, hemizygous males (one copy on one X chromosome) and homozygous females (two copies on two X chromosomes) have the same phenotype. As a result of gene dosage effect, the apricot-white heterozygous female (one copy of the apricot allele) has a phenotype that is half as intense as the homozygous apricot female or the hemizygous male.

Bar is a dominant X-linked gain-of-function mutation. A gain-of-function allele is a variant of a gene in which the mutation has bestowed on the protein a new or enhanced function. The eyes in these mutants have fewer facets, corresponding to how many copies of the bar allele each fly has (p. 198–201). Thus, the more copies of the bar allele, the fewer facets the fly's eye has. This allele also demonstrates gene dosage effect. Female homozygous bar eye flies have fewer facets than hemizygous males.

shibire is an autosomal conditional (temperature sensitive) allele of the dynamin gene. Dynamin function is required for synaptic vesicle fusion. At the nonrestrictive temperature (low temperature, full protein function), the flies are wild type in terms of behavior. Incubation at the restrictive temperature (high temperature, loss of protein function), the flies are no longer able to release neurotransmitters, become paralyzed, and can have seizures. The flies will return to the wild type phenotype if transferred back to the nonrestrictive temperature.

Methods

1. Each lab group will obtain one vial of flies from the side counter. Double check with your instructor and then use Flynap to anesthetize the flies. CLEARLY LABEL the flies and place them under a dissection microscope for observation. Go around to each lab group station (each will have a different mutant or cross) and observe, as indicated in this lab handout.
2. For the eye mutants, observe and record each phenotype as indicated in this manual. Make sure you differentiate between the sex of flies in eosin, apricot, and bar eye flies. White, apricot, and eosin affect the eye color; and eyeless, *Lobe*, and bar affect the eye shape. Eyeless and *Lobe* are autosomal. White, apricot, bar, and eosin are X-linked.

VARIABLE EXPRESSIVITY AND INCOMPLETE PENETRANCE

Eyeless

- A. For true breeding eyeless, observe the variable expressivity of the trait. Look at the size and shape of the eyes. Count and record how many flies have almost a wild type eye, how many have a very small eye, and how many are in between. Observe at least 25 flies. Look at both eyes, not just one, as the phenotype will vary between individuals but also can vary between eyes on the same individual!

Lobe

- B. For true breeding *Lobe*, observe the variable expressivity and penetrance of the trait. Look at the size and shape of the eyes. Count and record how many flies have a wild type eye, how many have an almost wild type eye (e.g., may only have a small nick in the edge), how many have a very small eye, and how many are in between. Observe at least 25 flies. Look at both eyes, not just one, as the phenotype will vary between individuals but also can vary between eyes on the same individual!

MULTIPLE ALLELES

- C. Observe and record the eye color of the true breeding strains of wild type (red), eosin, apricot, and white, looking at both sexes. These are all alleles of the X-linked *white* gene.

GENE DOSAGE EFFECT

Eosin

- D. For the true breeding eosin mutant strain, observe the gene dosage effect in the flies. Note that the females and males differ in their eye color phenotypes. Record how much darker the females' eyes are than the males'. You are looking at eye color not shape in eosin flies.
- E. Observe the gene dosage effect in flies from the mixed stocks. These are F₁ and F₂ generation flies from the following cross: eosin eyes crossed to white eye (crossed to white so that we can see eosin, as eosin is dominant to white, but recessive to wild type). This cross was not performed with virgin females, and males and females of each phenotype were in the same vial, so the offspring are not all of the same genotype. Carefully sex, observe, count, and record the differing phenotypes of at least 25 different flies, noting when the females and males differ in their phenotypes. Compare these to the true breeding stocks, if necessary. In the mixed eosin stocks, you will be able to find a heterozygous female. What does the heterozygous female look like? How do you know this female is heterozygous? Show this heterozygous female to your instructor.

Apricot

- F. For the true breeding apricot mutant strain, observe dosage compensation. Note that the eyes of the females and males are basically the same color, even though females have two copies of the X-linked allele and the males have only one copy. You are looking at eye color not shape in apricot flies.
- G. Each lab group will observe the gene dosage effect in flies from the mixed stocks. These are F₁ and F₂ generation flies from the following cross: apricot eyes crossed to white eye (crossed to white so that we can see apricot, as apricot is dominant to white, but recessive to wild type). This cross was not performed with virgin females, and males and females of each phenotype were in the same vial, so the offspring are not all of the same genotype. Carefully sex, observe, count, and record the differing phenotypes of at least 25 different flies, noting when the females and males differ in their phenotypes. Compare these to the true breeding stocks, if necessary. In the mixed apricot stocks, you will be able to find a heterozygous female. What does the heterozygous female look like? How do you know this female is heterozygous? Show this heterozygous female to your instructor.

Bar

- H. Observe the gene dosage effect in the true breeding bar eye flies. For the true breeding bar eye flies, count and record (draw) the difference in facet number and size of the eyes between females and males, observing at least 25 different flies. Looking at the size and shape of the eyes, not the color. Record (draw) how much smaller the female eye is. If you observe a male fly with eyes the same size as females', a wild type fly, or a female with eyes smaller than other females' eyes, show these to your instructor.

CONDITIONAL (TEMPERATURE SENSITIVE) ALLELE

- I. Now observe *shibire* mutant flies and wild type flies. Do not anesthetize the flies!

1. Record the activity pattern of both flies stocks at room temperature.
2. Now warm up the wild type flies and the *shibire* mutants. Carefully time and record how long it takes for the *shibire* mutants to fall to the bottom of the vial and become paralyzed. Compare the *shibire* mutant activity to wild type.
3. Then return the *shibire* and wild type flies to room temperature. Carefully time and record how long it takes for the *shibire* mutants to resume wild type activity.

QUESTIONS

Answer these questions in your notebook.

1. Why did the *eyeless* and *Lobe* true breeding stocks show such variability of phenotype?
2. At the molecular level, what do you think is causing the phenomenon of variable expressivity? Answer in terms of protein function at a cellular or organ level.
3. At the molecular level, what do you think is causing the phenomenon of incomplete penetrance? Answer in terms of protein function at a cellular or organ level.
4. Design a series of crosses in which you can tell a fly with a *Lobe* allele but wild type phenotype from a true wild type fly.
5. What does hemizygous mean?
6. For the multiple alleles, white is a total loss of function allele and wild type (red) codes for a fully functional protein (Fig. 13.7, p. 345). What do you think is happening at the molecular, cellular, and organ (eye) levels in the apricot and eosin mutants? Describe what you think is occurring as it relates to protein level and function.
7. Eosin, apricot, white, and wild type are all alleles of the same gene. You know that wild type is dominant over all other alleles and white is recessive to all other alleles. Design a cross or series of crosses that would allow you to determine whether eosin is dominant over apricot (or vice versa).
8. In the true breeding stocks, why did the female eosin mutants have darker eyes than the eosin males? Answer in terms of protein levels as they relate to activity and function.
9. Why do the true breeding flies with apricot eyes have the same color eye regardless of sex, when apricot is X-linked? Hint: think about dosage compensation in flies (Table 7.1, p. 168). Answer in terms of protein levels as they relate to activity and function.
10. In the mixed eosin stocks, you will be able to find a heterozygous female. How can you tell the female is heterozygous? Why does she look that way? What are her protein levels? How do they compare to the homozygous female and the hemizygous male? How do her protein levels relate to activity, function, and phenotype?
11. In the mixed apricot stocks, you will be able to find a heterozygous female. How can you tell the female is heterozygous? Why does she look that way? What are her protein levels? How do they compare to the homozygous female and the hemizygous male? How do her protein levels relate to activity, function, and phenotype?
12. Why were eosin and apricot crossed to white rather than wild type?
13. Why don't all X-linked genes demonstrate the gene dosage effect? Answer in terms of protein levels and protein activity and function in *Drosophila*.
14. Bar is a gain-of-function mutation. How do you think the dominant gain-of-function bar protein is working in the cell?
15. Why, in the true breeding bar eye stocks, do females have fewer facets than males? How do you think the protein is working? What are the protein levels in the hemizygous male and the homozygous female?
16. Describe how the bar eye phenotype is governed by the gene dosage effect.
17. It is possible that you could find a wild type fly, a male fly with the female eye phenotype, or an ultrabar female. Why is this the case? In each of these cases what would the protein levels be? Hint: see p. 198–201.
18. Which phenotype(s) was/were the hardest to score? Why?

19. In this experiment, we looked at some mixed stocks and some true breeding stocks. Why do geneticists use true breeding stocks rather than mixed for their experiments?
20. What does restrictive temperature mean? What does nonrestrictive temperature mean?
21. What function does the protein dynamin specifically perform in the cell?
22. What is happening to the dynamin protein in the *shibire* mutants at the molecular level at the restrictive and nonrestrictive temperatures? Think in terms of protein structure, function, and activity.
23. Find and describe one other human conditional allele (not PKU).

RESULTS AND DISCUSSION

Students used mutants of *Drosophila* to examine and contemplate extensions of Mendelian inheritance. They were asked a series of postexercise questions about each mutant, focusing on the level, function, and/or structure of each protein in each mutant to direct their thinking towards the relationships between the protein coded for by each mutant allele and the phenotypes seen.

Quiz Questions and Answers

1. Define conditional allele.
2. Why does the heterozygote female in the eosin look different than the homozygous female?
3. Define the gene dosage effect.
4. Relate gene dosage in eosin eyes to protein levels.
If homozygous eosin-eyed females have 100% protein, then
Heterozygous eosin-eyed females have _____% protein
Homozygous white-eyed females have _____% protein
Eosin-eyed males have _____% protein
White-eyed males have _____% protein
5. In the conditional allele we are studying (*shibire*), the protein function
 - A. is always the same.
 - B. differs depending on the time of day.
 - C. is related to the diet of the flies.
 - D. changes depending on the temperature of the fly.
6. Why does the eosin allele need to be crossed into a white-eyed fly background to be seen?
7. If an eosin-eyed female is crossed to a red-eyed male (wild type), what will the female offspring look like?
 - A. white eyes
 - B. red eyes (wild type)
 - C. eosin
 - D. heterozygote eosin
8. The eyeless trait demonstrates variable expressivity. What does variable expressivity mean?
9. The lobe trait demonstrates incomplete penetrance. What does incomplete penetrance mean?
10. Why do the true breeding flies with apricot eyes have the same color eye regardless of sex, when apricot is X-linked? Answer in terms of protein levels as they relate to activity and function.

11. What is a dominant gain of function mutation?
12. Describe how the bar-eye phenotype is governed by the gene dosage effect.
13. Why don't all X-linked genes demonstrate the gene dosage effect? Answer in terms of protein levels and protein activity and function.
14. Eosin, apricot, white, and wild type are all alleles of the same gene. You know wild type is dominant over all of the other alleles and white is recessive to all other alleles. Design a cross or series of crosses to determine if apricot is dominant to eosin or vice versa.

Answers:

1. A conditional allele demonstrates one phenotype under one set of conditions and a differing phenotype under a different set of conditions.
2. Eosin demonstrates a dosage effect. The two copies of the eosin allele in the homozygous female make more pigment-producing enzyme than the single copy in the heterozygote, thus causing the homozygous female's eyes to appear darker.
3. The gene dosage effect is defined as a phenotype that differs depending on the number of copies of the allele present. Gene dosage is similar to incomplete dominance but is used to describe an X-linked gene. The more copies of the allele, the more intense the phenotype.
4. If homozygous eosin-eyed females have 100% protein, then
 Heterozygous eosin-eyed females have 50 % protein
 Homozygous white-eyed females have 0 % protein
 Eosin-eyed males have 50 % protein
 White-eyed males have 0 % protein
5. D. In the conditional allele we are studying (shibire), the protein function changes depending on the temperature of the fly.
6. The red-eye allele is dominant to all other eye color alleles at this locus.
7. B. red eyes (wild type)
8. Variable expressivity means that the phenotype varies, but the genotype is the same.
9. Incomplete penetrance means that an individual who carries the dominant allele may not always demonstrate the dominant phenotype.
10. In *Drosophila*, dosage compensation is seen between males and females for X-linked genes. Most X-linked genes in *Drosophila* males have turbo-charged expression so that the protein levels of the hemizygous males are the same as the XX female.
11. A dominant gain of function mutation results in a dominant allele in which the protein made demonstrates a new or enhanced function.
12. The Bar allele generates a protein that, during development, shapes the fly's eyes. The more protein that is present, the smaller the fly's eyes will be; the higher the gene dosage, the smaller the fly's eyes.

13. In *Drosophila*, most X-linked genes in the males have expression that is upregulated in relation to the females two Xs so that the male's expression is the same as the females. In mammals like humans and cats, one X chromosome is inactivated.

14. Cross an apricot fly to an eosin fly and look at the females in the resulting offspring. The phenotype that is seen in the females is dominant.

Assessment. Assessment was determined via pre- and postlab questionnaires on students' skill and knowledge (Table 1) and by a postexercise questionnaire on the students' perception of the activity (Table 2) (3, 6). Pre- and postlab answers were analyzed using a one-tailed homoscedastic student's *t* test (Table 1). Students were asked a series of survey questions pre- and postlab. Questions addressing students' self-assessment of knowledge and skills indicated that the students thought that they understood the concepts covered by the lab better after the lab exercise and felt that their skills of sexing, manipulating, and scoring phenotypes in *Drosophila* improved (Table 1). In all cases, except for the question concerning simple Mendelian inheritance, there was a significant difference between the pre- and postlab self-assessment of knowledge and skills, indicating that the students had in fact felt as though they had learned from the exercise. This lab was a worthwhile exercise to increase students' self-assessment of their knowledge and their actual understanding of the topics.

This lab was also successful in building students' fly manipulation skills. This exercise falls in our lab curriculum after an initial *Drosophila* inheritance lab in which the students set up crosses to generate the F₁ generation. This lab further enhanced the students' *Drosophila* manipulation skills. According to the students' survey answers, they did feel more comfortable manipulating and sexing flies after performing the exercise (prelab 3.47 ± 1.01 compared to postlab 4.15 ± 1.05 , *P* value of 8.25×10^{-5}). This lab also helped the students feel more comfortable in scoring *Drosophila* phenotypes (prelab 3.33 ± 0.93 compared to postlab 3.91 ± 1.06 , *P* value of 4.02×10^{-4}). The responses to the pre- and postlab survey indicate that the learning objectives of increased ability in fly manipulation are being met.

Additional postlab survey assessment indicated that the students felt the lab was a worthwhile exercise and should be kept in the curriculum. The responses to the postexercise survey indicated that students felt they had learned something, the lab exercise fit in well with the curriculum, the exercise was an active process, and the exercise made them ask questions and think; all responses to these survey questions were at least a 4 (agree slightly) on a 5-point Likert-type scale (Table 2). As a part of the postexercise survey, students were able to write additional comments about the lab, which are detailed in Table 3. These comments fall into two main categories, those that indicate the learning goals were achieved and those that indicate that the lab did not achieve the learning goals. The negative comments included that the lab or a particular phenotype was confusing. This lab can be confusing because the phenotypes of *Lobe* and *eyeless* are variable and can be subtle. One of the learning objectives is to acquaint the students with careful observation of the phenotypes. The other main complaint was that the counting was confusing or that they did not understand why they needed to count the flies. I specifically included the instructions about counting in the lab manual to ensure that the students looked at more than one fly per phenotype, as many of the phenotypes vary from fly to fly. Most student comments indicated that the lab was successful in its learning objective of obtaining skills of sexing and manipulating flies and scoring nonbinary phenotypes.

Additional student assessment was employed to determine if in fact the students were learning from the exercise. Pre- and posttesting was employed to determine if the exercise taught the students about extensions of Mendelian inheritance (Table 4). Students were asked a variety of questions about the topics covered in the lab exercise in a quiz given directly before the exercise and 1 week after the exercise (quiz questions are included in Supplemental Material). In all cases there were more correct answers to the questions after the exercise than before the exercise. Statistically significant increases, as analyzed by McNemar's test of paired enumeration data, were seen in students' understanding of gene dosage effect, multiple alleles, variable expressivity, dosage compensation, and dominant gain-of-function mutations. For questions 1, 2, and 5 there was not a statistically significant increase from the pre- to the posttest, perhaps because in each case over 86% of the students had the correct answer the first time. For question 4, students were to relate protein levels to gene dosage, and although there was an increase

in the percentage of correct answers, it was not statistically significant. For question 9, the increase from pre- to postexercise was almost statistically significant ($P = 0.0704$), perhaps because the question asked about incomplete penetrance and in the answers to the question several students confused this topic with incomplete dominance. Anecdotal experience indicates that students in our genetics course lack precision in their language, confusing topics like sex limited with sex influenced inheritance and transcription with translation. Question 7 asked about the phenotypic result of a cross between an *eosin* fly and a wild type fly. All of the students (six total), who answered incorrectly on this question in the posttest, answered that the resulting offspring would be heterozygous *eosin*, indicating that they did not remember wild type was dominant over *eosin*. More prelab lecture and postlab discussion should resolve the remaining issues with this lab. Pre- and postexercise testing indicated that for eight of fourteen quiz questions there was a significant increase in students' understanding, and in all cases of individual quiz questions, the percentage of correct answers on the quiz increased from pre- to postexercise (Table 4).

CONCLUSION

This lab exercise is a novel discovery-based method to demonstrate one concept in genetics: extensions of Mendelian inheritance. The objective was to allow the students to examine phenotypes in *Drosophila* that exhibited an extension of Mendelian inheritance and could be difficult to discern. This lab was developed to force the students to examine traits in terms of protein function and to give them more experience in manipulating, sexing, and scoring phenotypes in flies. Many of the phenotypes were variable and subtle; thus, this lab also forces the students to deliberately and slowly analyze the phenotypes, as opposed to moving quickly through the exercise, not truly paying attention to the concepts or recording the data to the fullest extent possible. The purpose of this exercise was to force students to spend some time analyzing flies so they could become comfortable with fly phenotype analysis and manipulation. Students should take responsibility for their education and knowledge acquisition; through discovery-based hands-on activities, the instructor can direct them to see the inherent value in their own self-directed learning.

This lab exercise will be easily adaptable to many genetics teaching labs, as many instructors of genetics are already familiar with *Drosophila* husbandry and manipulation. The stocks are easily accessible and grow robustly in the lab setting with a minimum of care. The topics covered in this lab are covered in most traditional genetics lectures but not usually in labs. This lab exercise will be an active hands-on learning activity for the genetics laboratory. This exercise will give students practice and build confidence in their abilities to manipulate and sex flies. Examining *Drosophila* mutants with these phenotypes and answering questions about the phenotypes in terms of protein levels and function should lead to enhanced student understanding of these extensions of Mendelism. Working with phenotypes that are not binary in nature will help strengthen students laboratory analysis skills, build self-confidence, and should move students forward in their cognitive development, such that they are confident that they can ask and answer questions that may not have "black and white" answers.

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