



Outside Resources

Laboratory Exercise:

Revised College Genetics Laboratory Exercise for Witnessing Phenotypic and Molecular Evolution in the Fruit Fly

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Overview:

Our team recently published a laboratory exercise in which college students observe both phenotypic and molecular evolution through natural selection in a population of *Drosophila simulans* over the course of a few months. This exercise takes advantage of eye color variation and molecular variation in a live model organism to demonstrate the transmission genetic concepts of X-linked inheritance and linkage as well as the evolutionary genetic concepts of selective sweeps and hitchhiking. To make this activity more accessible to a broader audience of novice fly handlers, we have constructed strains of *Drosophila melanogaster* that eliminate the difficult, time-consuming steps of collecting virgin females to be used in crosses, and designed new markers to be used with this species. We describe the changes to the activity, how the strains were constructed, and results from a test-run of the revised activity. An overview of the activity, supplementary detailed teacher instructions, and supplementary student handouts are presented here.

Core Concepts Addressed:

Nature of genetic material: What are the mechanisms by which an organism's genome is passed on to the next generation?

Transmission/ patterns of inheritance: How does the phenomenon of linkage affect the assortment of alleles during meiosis? How can one deduce information about genes, alleles, and gene functions from analysis of genetic crosses and patterns of inheritance?

Evolution: What are the processes that can affect the frequency of genotypes and phenotypes in a population over time?

Core Competencies Addressed:

Observational strategies/ Hypothesis testing/ Experimental design

Evaluation of experimental evidence

Audience:

Intermediate undergraduate, biology/genetics majors

Activity Type:

Open-ended laboratory

Activity Length:

4-6 activity days spread across 2-3 months

Original Activity and Justification for Revision:

Our research group recently published a hands-on laboratory exercise in which students observe evolution by natural selection in a live population of *Drosophila simulans* (Heil et al. 2012). In this exercise, students place a single red-eyed male fly into a population of white-eyed flies and observe the spread of the advantageous red-eyed phenotype over three to four generations, with individual generations taking approximately eleven days. Students observe that male flies with white eyes are less successful at mating than their red-eyed (wild-type) counterpart. Thus, the higher fitness associated with flies having red eyes is favored, and this trait increases in frequency in subsequent generations through the process of natural selection.

Not only are students able to witness phenotypic evolution in living organisms over a few months with this activity, students also study molecular evolution, partaking in a meaningful and valuable hands-on experience with molecular techniques that include DNA extraction, PCR, and gel electrophoresis. Here, the students examine two molecular markers, one near the eye color locus (*white*) and one on the opposite end of the same chromosome, and see that, while allelic variation is maintained at the locus far away from the eye color locus, a single allele at the marker near the eye color gene spreads with the red-eyed flies. Overall, this lab demonstrates the genetic principles of X-linked inheritance, linkage, selective sweeps, and hitchhiking.

Since the efficacy of this lab depends on the increase of the red eye allele over time, the white-eyed female flies in the cross set up by the students on Day 1 must be virgins. If non-virgin white-eyed females are used in this activity, the activity may not progress as planned (e.g., perhaps more white-eyed offspring will be observed than expected). Likewise, the proper demonstration of selective sweeps and hitchhiking requires that the initial populations of white-eyed flies the students cross on Day 1 have ample allelic variability at the two molecular markers. If non-virgin white-eyed females are used in the first cross set up by teachers during the in-advance preparation, the experiment could fail, create and propagate misconceptions, and/ or cause an undetectable (or no) spread of the red eye allele over the course of the experiment. Given that most faculty and staff in charge of coordinating and running undergraduate teaching labs have limited experience handling flies, the likelihood of non-virgin females being used in the lab is non-trivial.

As a way of side-stepping this problem and the time-consuming, error-prone nature of virgin collecting, we have constructed white-eyed heat shock male-lethal strains of *Drosophila melanogaster* that will ensure the collection of virgin females. We further develop new molecular markers by leveraging available transposable element (TE) insertion strains, and crossing the heat-shock-sensitive Y-chromosome into those strains. We amend the protocol published previously (Heil et al. 2012) for use with these new strains. **This new approach virtually eliminates the possibility for use of non-virgin females, and simplifies the exercise for faculty and staff setting up the activity.**

Heat shock treatment begins when larvae start encasing themselves in pupal cases and ends when adult flies begin to emerge (which will all be virgin females!). The treatment consists of placing vials of pupae in an incubator (or oven) set at 37°C (98.6°F) for one hour each day or every two days, leaving them at room temperature otherwise. Once adults begin to emerge, females can be collected until there are no more adults left in the vial and heat shock treatment continued until all females needed for the activity are collected. This treatment will not harm the larvae, and must be done in the pupal stage of development to work.

Heat shocking vials of these flies produces all females, while leaving the flies at room temperature produces both males and females. Educators will still need to separate the males and females by sex in vials left at room temperature to collect and isolate males.

In summary, the activity presented here utilizes the protocol and concepts addressed in Heil *et al.* (2012) with the added improvements of using heat shock *D. melanogaster* strains as opposed to *D. simulans* strains. For students, the overall activity will appear identical to the one first described in Heil *et al.* (2012), but the present version dramatically reduces setup time and effort and mitigates the risk of non-virginity of the flies in the crosses. We have included revised versions of the Teacher Instructions and Student Handouts to accommodate changes made to the lab exercise associated with the new heat shock strains.

Links to ORIGINAL *D. simulans* Activity and Supplemental Materials:

Heil *et al.* 2012:

<http://dx.doi.org/10.1007/s12052-012-0447-5>

Teacher/Student Instructions and Powerpoints:

<https://sites.google.com/site/noorlabduke/fly-evolution-advanced>

Protocol Used for Creation of the Heat Shock *D. melanogaster* Lines:

We created a white-eyed, heat shock Y-susceptible strain by crossing a *D. mel w(1)* strain obtained from Carolina Biological Supply to a heat shock susceptible strain (stock #25679) obtained from the Bloomington Drosophila Stock Center at Indiana University. F1 males from this new white-eyed hybrid strain were backcrossed to *D. mel w(1)* virgin females for 10 generations, then tested for male heat shock susceptibility and maintained in lab. Males from the heat shock hybrid strain were then crossed to *D. mel (w1118)* females containing an Mi{ET1} transposable element both near the eye color gene on the X chromosome (stock #25634) AND *D. mel (w1118)* females containing a Mi{ET1} transposable element on the opposite end of the X chromosome (stock #25312). Both of these strains were obtained from Bloomington Drosophila Stock Center at Indiana University. F1 males were then backcrossed to pure females (25634 and 25312) to ensure that the females remained homozygous for the inserted genetic element. The new transposable element heat-shock susceptible strains were then tested to confirm the successful transmission of Y-heat shock susceptibility and female non-susceptibility and maintained in lab. The Mi{ET1} white-eyed strains were chosen to aid in the development of markers close to and far from the eye color locus on the X-chromosome for the molecular component of the lab. We refer to these new strains as heat shock white-eyed strain 60739 (has TE insert near the eye color gene) and heat shock white-eyed strain 60740 (has TE insert far from eye color gene) in the protocol.

NOTE: The *D. melanogaster* strains used in this laboratory are available to be ordered at Bloomington Drosophila Species Stock Center under stock numbers:

60739: heat-shock-sensitive-Y, white-eyed flies with TE insert near eye-color gene

60740: heat-shock-sensitive-Y, white-eyed flies with TE insert far from eye-color gene

60741: wild-type (red-eyed) flies from Zimbabwe (strain S29), neither TE insertion

Documentation of Spread of Red Eye Phenotype with Revised Protocol:

We executed a test-run of our revised procedure to ensure that the red eye phenotype still increases in abundance. We started with 20% of the males having red eyes (4 white-eyed males, 1 red-eyed male). If the red-eyed fly had equal fitness to the white-eyed flies, then 20% of the female progeny in generation 1 should have red eyes. Among generation 1 vials we examined, every vial (10/10) had a higher fraction of red-eyed females than 20%, averaging closer to 60% (range 29%-91%). As expected for an X-linked trait, none of the generation 1 males had red eyes. The fraction of flies (male + female) with red eyes in generations 3 and 4 averaged 41% (range 8%-62%) and 44% (range 8%-85%) respectively.

Learning Objectives:

By the end of the laboratory exercise, students will be able to:

- Explain the process of evolution by natural selection in terms of fitness-related traits and allele frequencies
- Describe the concept of X-linked inheritance
- Perform DNA extractions, PCR, and gel electrophoresis
- Analyze molecular data as a means of understanding an evolutionary process
- Explain the concepts of selective sweeps, hitchhiking, and linkage

Overview of Student Activities:**Day One: Set Up Fly Crosses and Observe Matings** Time: 30+ min.

In an introductory lecture, students are given a brief overview of the concepts of inheritance and evolution by natural selection. Students are then given two vials of flies: one with four white-eyed females, and one with four white-eyed males and one red-eyed male. Once the students have examined their flies and feel comfortable telling apart the differences between males and females and between eye colors in the vial of males, students are asked to predict what will happen to the percentage of red-eyed flies in future generations if the having red eyes confers a fitness advantage or confers a lower fitness (is disadvantageous). Students then combine males and females into a single vial and observe the behavior of the flies. The students should see courtship behavior and mating within 5-10 minutes, though sometimes this may take longer. The first mating that students almost always observe is between the red-eyed male and a white-eyed female. Thus, students infer that the better vision and health associated with having red eyes helps the red-eyed male find and pursue his mates more easily, giving him an advantage at passing on his alleles to the next generation relative to the white-eyed males.

Day Two: Examine Generation 1 Offspring and Observe X-linked Inheritance Time: 30+ min.

Approximately two weeks later, students are given back the offspring (referred to as generation 1) from the crosses they set up and observed on Day One. Students put the flies to sleep using either CO₂ or FlyNap, and sort them by sex and eye color. They will notice that there are no red-eyed males. If having red eyes is advantageous, especially for males in terms of finding mates, why do none of the males have red eyes? The teacher then explains to the students that eye color in *Drosophila* is X-linked, and that the red eye allele is dominant over the white-eye allele. Thus, when a red-eyed male mates with a white-

eyed female, all of their daughters will have red eyes, but all of their sons will have white eyes since the only X chromosome they inherit will come from their white-eyed mother.

Day Three: See Evolution by Natural Selection and Begin Molecular Component

Part 1: Evolution by Natural Selection Time: 20-30 min.

Approximately five weeks after Day Two, students are given back the fourth (or third) generation offspring from their Day One crosses. Once again, the students sort the flies by sex and eye color, and calculate the frequency of red-eyed flies in their populations. Students should see that close to half of the flies have red eyes. Thus, if the frequency of red-eyed flies in their population is greater than the starting frequency of red-eyed flies in the original population on Day One (1/9 or 11%), then the students have witnessed evolution by natural selection. Students are able to confirm that evolution by natural selection occurred in the following ways:

1. The definition of evolution is change in a population over time. The change in frequency of the white-eyed and red-eyed individuals over multiple generations demonstrates this.
2. The population was variable, containing both red and white-eyed individuals. This allows the opportunity for natural selection to favor one trait over another.
3. The variation was heritable since eye color is inherited in an X-linked manner.
4. The spread of red eyes occurred in a predictable manner, with the proportion of red-eyed flies increasing in frequency in subsequent generations.

Part 2: DNA Extraction Time: 45-60 min.

Here, students are introduced to the concept of examining molecular evolution by looking at variable "markers" within an organism's genome. The students randomly select seven male flies (preferably 4 of one eye color and 3 of the other, with 1 negative control) and place them each in a separate well of 0.2 ml microcentrifuge strip tubes. They then add a mixture of buffer and proteinase K to each well, and crush the body of the flies to release DNA into the solution using the end of a sterile pipette tip. Once all seven of the flies have been thoroughly squished, the strip tubes are placed in a thermal cycler for 32 minutes to prepare the DNA samples for PCR.

Day Four: Polymerase Chain Reaction (PCR) Time: 30 min. prep, 75 min. thermal cycler

Students set up two PCRs using the seven DNA samples they prepared to amplify two markers along the X chromosome: one near the eye color locus (referred to as the "NEAR" marker) and one on the opposite end of the chromosome (referred to as the "FAR" marker). Thus, students end up with a total of 16 amplified DNA samples (including two negative control samples) that they visualize on a gel in the following class period.

Depending on the amount of time in each lab period, this step can be combined with Day Three activities. If needed, students can set up their PCRs and put them in thermal cyclers, while the lab instructor or prep staff comes back approximately an hour and fifteen minutes later to put the finished PCR products in the refrigerator until needed in Day Five.

Day Five: Gel Electrophoresis Time: 75 min.

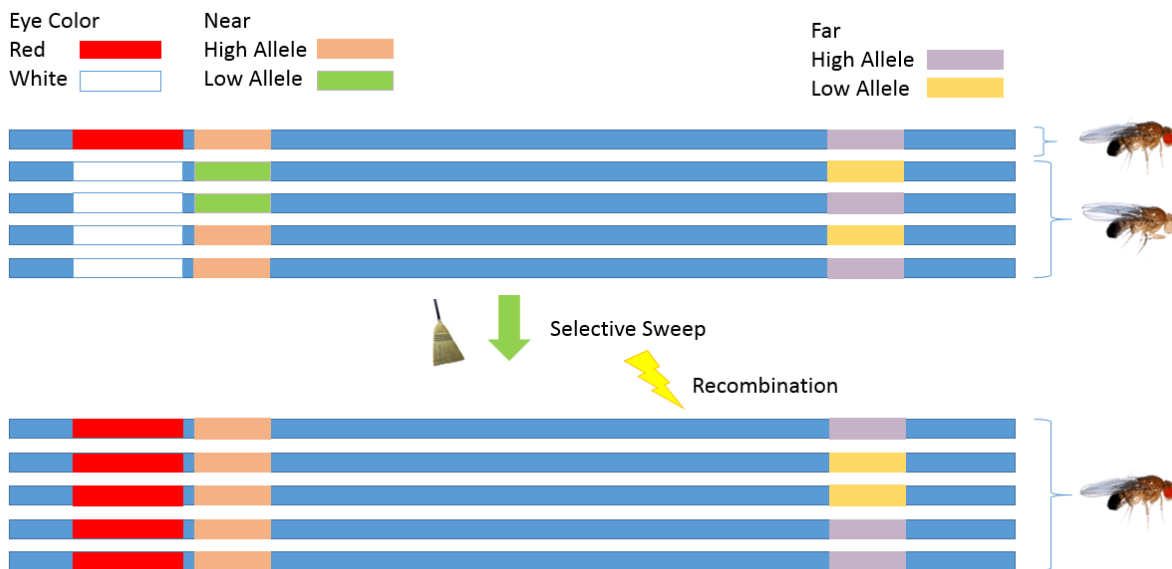
Students first practice their gel loading skills by pipetting loading dye into petri dishes containing agarose. Once students feel comfortable loading a gel, their PCR products from Day Four are returned to them. They then load their 16 samples into an agarose gel and let it run. The gel is then imaged and used by students for analysis.

Note: Gel electrophoresis and data analysis can be combined in the same day depending on the amount of time allotted for each lab period.

Day Six: Data Analysis: Observation of Selective Sweep/Genetic Hitchhiking Time: 30 min.

Students examine their gel images and record the alleles that both the red and white-eyed flies have at both the NEAR and FAR markers. Students should see that all of the red-eyed males have the larger size allele at the NEAR marker, while the white-eyed flies have both the larger size and smaller size alleles. At the FAR marker, both the red and white-eyed males will have two alleles. The loss of variation in alleles at the NEAR marker in red-eyed males demonstrates a selective sweep and the hitchhiking of a neutral variant along with a selected variant at the eye color locus. In this case, the larger size allele is genetically linked to the red eye allele at the eye color locus, and thus increases to high frequencies with the spread of the red eye allele. Alleles at the FAR locus are not linked to the eye color locus, and are therefore separated by recombination and minimally affected by the selective sweep occurring at the eye color locus.

Concept Summary:



Revised Figure from Heil *et al.* (2012)

The initial population consists of white-eyed flies that carry two alleles at the NEAR marker (pink or green), two alleles at the FAR marker (purple or yellow), and the white allele at the eye color gene. A

red-eyed fly is introduced that carries the pink allele at the NEAR marker, purple at the FAR marker, and red at the eye color gene. The red eye allele is advantageous and sweeps to higher frequencies in the population. The pink allele at the NEAR marker is located close to the advantageous eye color and hitchhikes with the red eye color. The FAR marker is located on the opposite end of the chromosome. Recombination breaks down linkage between eye color and the FAR marker, so both purple and yellow alleles persist in the population.

Sample Timeline for Crosses Based on Our Trial Run:

Teacher Cross 1 (near insert females with far insert males): Set up December 23

Teacher Cross 2 (F1 brother-sister cross): Set up January 3

F2 offspring start emerging: January 15

Student Cross Day 1 (4 white-eyed females, 4 white-eyed males, 1 red-eyed male): set up January 20

Generation 1 emerges: January 30

Generation 2 emerges: February 10

Generation 3 emerges: February 22

Generation 4 emerges: March 4

References:

Heil, C. S. S., M. J. Hunter, J. K. F. Noor, K. Miglia, B. Manzano-Winkler, S. R. McDermott, and M. A. F. Noor. 2012. Witnessing phenotypic and molecular evolution in the fruit fly. *Evolution: Education and Outreach*, 5: 629-634.