HOW TO DO IT

Witnessing Evolution First Hand: A K–12 Laboratory Exercise in Genetics & Evolution Using *Drosophila*

RECOMMENDED FOR *AP Biology*

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Abstract

We present a laboratory exercise that leverages student interest in genetics to observe and understand evolution by natural selection. Students begin with white-eyed fruit fly populations, to which they introduce a single advantageous variant (one male with red eyes). The superior health and vision associated with having the red-eye-color allele confers a fitness advantage, and the students can watch the spread of the allele within the population. The increasing numbers of red-eyed flies they observe over generations demonstrate evolution by natural selection. The students concurrently learn genetic principles, including basic inheritance and X-linkage.

Key Words: Drosophila*; evolution; genetics; natural selection.*

Evolution is defined simply as “change through time.” However, despite the simplicity of this concept and the obviousness of such change occurring, many in the broader public view most or all evolution as “controversial,” particularly when applied to humans. Many students graduate from high school or college without having a clear idea that evolution by natural selection is frequent and ubiquitous (Smith, 2010). Educators are sometimes hesitant to delve too deeply into evolution for fear of backlash from students, parents, or even school administrators. However, understanding evolution is fundamental to understanding all of biology: “Without that light [of evolution] it becomes a pile of sundry facts some of them interesting or curious but making no meaningful picture as a whole” (Dobzhansky, 1973).

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In 1859, Charles Darwin’s *On the Origin of Species* was published, laying out the theory of evolution by natural selection. This theory rests on three simple ideas: (1) individuals in a population are variable, (2) this variation is inherited from parent to offspring, and (3) inherited variations that help individuals to survive and reproduce better than others will become more abundant in the population. The outcome of this process is that the composition of the population changes from one generation to the next in a predictable manner.

Examples of evolution by natural selection abound, and some of these examples affect our everyday lives. For example, a new influenza vaccine is produced each year to combat new variants of the associated viruses that arose and spread because they were resistant to the old vaccine. Recent studies have also identified mutations in human lactase genes that occurred within the past 10,000 years and allow us to consume dairy as adults (Schaffner & Sabeti, 2008); before these mutations, all adult humans were lactose intolerant.

However, examples of viral or human evolution are difficult for the general public to “observe” directly. Indeed, one of the issues hindering evolution education is the misconception by students and educators that it is slow and, thus, not amenable to laboratory exercises, except perhaps via computer simulations or digital organisms (e.g., http://avida-ed.msu.edu/). Therefore, experiential learning may be stronger if students also have the opportunity to observe evolution in real organisms, making evolutionary change seem less like a story and more like nature.

Here, we present a multiday laboratory exercise using the fruit fly, *Drosophila melanogaster*, as a model organism to illustrate basic concepts of genetics and demonstrate that evolution by natural selection can happen before students’ very eyes. We have collaborated with Carolina Biological Supply to produce kits that allow easy access to all the materials needed for these exercises, although the materials may also be purchased from other science-education supply companies and applied using the approaches described here. Sample associated PowerPoint lectures and detailed instructions are available for download at http://www.biology.duke.edu/noorlab/flyevolution.html, and educators are free to modify them for their individual use.

○ Materials

Carolina Biological Supply Natural Selection with Drosophila Kit (includes below items plus Teacher Manual and Student Guide): 171995.

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These items are included in the kit or can be purchased individually:

- Carolina Biological Supply: Drosophila (fruit fly), white: 172220
- Carolina Biological Supply: Drosophila (fruit fly), wild-type: 172100
- Carolina Biological Supply: Drosophila Kit (FlyNap brand anesthetic, 72 vials, 72 plugs, media, labels, transfer cards): 173052
- Sorting brushes

Additional materials needed (not included in kit):

- Sharpie markers

Optional materials (not included in kit):

- Magnifying glasses
- Carolina Biological Supply: Carolina Easy Fly brand Sex-Linked Inheritance Drosophila Culture, white: 172633
- PowerPoint lectures (samples available online) and projector
- Carolina Biological Supply: Drosophila Stand: 173030

Preparation

The details of preparation are presented in the kit and online materials, but basically, the teacher should anesthetize and separate the flies that will be used in day 1 at least one day ahead of time to minimize any effects of anesthesia on behavior and to improve the alacrity of the laboratory's execution.

Day 1

Students are divided into groups of two and are given two vials of flies, one containing five white-eyed females and one containing five white-eyed males and one red-eyed male. The teacher should ask students to carefully observe their flies and take note of any physical differences between the flies (e.g., one male has red eyes). Students carefully tap all flies from one vial into the other, then immediately lay the vial on its side on a white piece of paper to observe. The teacher should ask students to watch for any mating activity, and if mating is observed, what color eyes the male has. In our experience, most classes observe 3–5 matings, nearly always involving a red-eyed male. Note: the preferential mating of the red-eyed male is unexpected by chance because 83% of the males have white eyes. At this point, it is fun to note that *Drosophila* have a courtship song and dance, similar to courtship behavior in more familiar animals like peacocks, big-horned sheep, and deer. If students look closely, some will see the male extending his wing and vibrating it. Many videos and recordings (e.g., http://www.youtube.com/watch?v=KzWIuhXMUko, http://www.biology.duke.edu/noorlab/song.html) of *Drosophila* courtship behavior are available online.

Before concluding the exercise, the teacher should explain the differences in white and red eye color. Although the "population" the students have consists mainly of white-eyed flies, the red-eyed fly is actually the wild-type fly. The white eye color is caused by a mutation in a gene in the pigment pathway, discovered in 1910 by the father of modern genetics, Thomas Hunt Morgan (Morgan, 1910). The "white" mutation is located on the X chromosome and results in lack of pigment, poor eyesight, and lower fitness, so no one ever sees a white-eyed fly in the wild (Reed & Reed, 1950; Jones & Probert, 1980). The teacher should ask the class two questions. First, if all the males had the same number of offspring, what fraction of the daughters would have red eyes? (Correct answer: 1/6, reflecting the fraction of red-eyed fathers.) Second, based on the matings the students observed, which eye-color flies would have more offspring? (Correct answer: the red-eyed male.)

Day 2 (~2 weeks later)

The first-generation offspring of the flies the students set up in the previous lab are returned to them. Using FlyNap or another anesthetic, students should anesthetize their flies (this takes a couple of minutes). Meanwhile, each group should take out a white piece of paper, divide it into quadrants, and label each: red male, red female, white male, white female. When all of the flies are asleep, students should tip their flies out on the paper, sort them into the four categories, and count the totals in each.

On the board or projector, tally up the totals in each category. Because the eye-color gene is located on the X-chromosome, there will be no red-eyed males (see Figure 1). Red-eyed males always give a Y-chromosome to their sons instead of the X-chromosome with the red-eye allele. This is a powerful demonstration of basic heredity and sex linkage.

Additionally, the students may note another significant finding. Although each group started with a 1/11 frequency of red-eyed flies (and 1/6 of red-eyed males), in just one generation, close to half of the females have red eyes. Is this what the students predicted last class? The teacher can ask the students why they think this is, and if

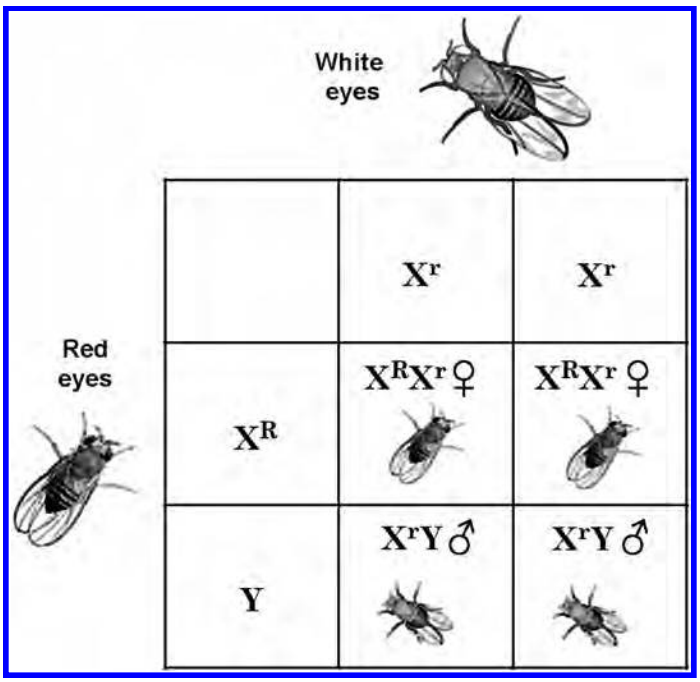

Figure 1. The eye color gene is located on the X chromosome (one of the sex-determining chromosomes of *Drosophila*). White eye color (X^r) is recessive. When a red-eyed male (X^RY) mates with white-eyed females (X^rX^r), their daughters will have red eyes (X^RX^r), but their sons will have white eyes (X^rY). Therefore, there can be no red-eyed males in the first generation, but they will appear in the second and later generations.

necessary remind them what they observed in the first lab (the red-eyed fly mated first!). This change in the composition of the population is evolution, and the high consistency with which red-eyes become more abundant illustrates natural selection as the driver of this evolution.

Occasionally, an experimental group vial will have all white-eyed flies. Rather than treating it as an "error," the teacher should use this example as instructive – ask the students why this may be an outlier (for example, maybe the red-eyed male got stuck in the food or was unhealthy). Evolution will not proceed identically in every replicate because random events can happen; this is known as genetic drift.

Note: If desired, a third lab day can be added after 3 or 4 more generations (approximately 2–3 months later). This requires minimal alterations to the procedure outlined above and serves as an even stronger demonstration of the increase in frequency of red eye color by natural selection. For a more advanced version of this exercise involving molecular techniques, see Heil et al. (2012).

○ How Do We Know It's Evolution by Natural Selection?

Now to go back to Darwin's postulates and apply them to this lab. (1) Individuals in a population are variable; in this case, the variation was white or red eye color. (2) This variation is inherited; we saw this using the Punnett square to demonstrate X-linkage. Finally (3), individuals with variations that help them to survive and reproduce better than others will be selected: we observed the red-eyed parent mating more on day 1 and learned that the red-eyed flies are more "fit." The outcome is that the composition of the population changes from one generation to the next – in this case, the increase in the frequency of red eye color, also known as evolution by natural selection. This evidence is robust: it is replicated across almost all student vials and across all classes (in our fall 2011 demonstration, we observed an increase in the abundance of the red-eye allele in 26 of 32 vials), demonstrating that such evolution is nonrandom (binomial test $p = 0.0005$) and almost certainly a product of natural selection.

○ Assessments & Standards

Two levels of evaluation are possible with this exercise. First, the students may be administered a pretest and a posttest to assess learning of major concepts covered, preconceptions with respect to evolution in particular, and their level of interest in science. In our pretests, we found that most middle school students had never heard of natural selection and had no opinion on evolution, whereas 40% of the high school students argued before the demonstration that evidence for evolution was lacking. Given that these students attended the same school system (the middle school fed into the high school), one can hypothesize that skepticism toward evolution increases between grades 6 and 12. This observation argues for greater academic intervention in that period. Our posttests at the high school level indicate that this exercise increased interest and understanding in the topics addressed (of 69 students surveyed, 62, or 90%, responded that this exercise increased their interest).

Second, students can present their findings as a formal laboratory report, including hypotheses tested and outcomes observed both for their group and for the class as a whole. Recall that some of the experimental groups will fail to observe the spread of the advantageous variant, and this failure provides a valuable lesson to the group and to the class as a whole: if one "rewinds the tape of evolution" and plays it again, as the late Stephen J. Gould (1989) described it, one does not necessarily get the same result.

The described experiments complement the current *National Science Education Standards* (http://www.nap.edu/catalog/4962.html). Although connections can be drawn with each standard, the experiments that we present are mainly in line with Standard A – Facilitating inquiry-based science; Standard B – Teachers should guide and facilitate learning; Standard D – Teachers of science should design and manage learning environments that provide students with the time, space and resources needed for learning science; and Standard E – Science teachers should create communities of science learners. By conducting any of the laboratory exercises, the teacher is adapting his or her curriculum in a way that creates authentic experiences for their students and ultimately enhancing the science program at their school – a goal of every dedicated science educator.

○ Conclusions

Evolution is a major foundation for all of biological thought, and evidence for evolution by natural selection is abundant. These laboratory exercises build on a classic genetics example dating back more than 100 years (Morgan, 1910; Green, 2010) and on student interest and engagement in genetics to demonstrate basic principles of both genetics and evolution using a living model system. In the end, students will have a greater understanding of inheritance (including X-chromosome linkage) and will have literally observed evolution by natural selection within their class.

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