Evolution Lab with *Drosophila*

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Abstract: In order to demonstrate certain aspects of evolution, a hands-on laboratory exercise was designed. Two distinct populations of *Drosophila*, wildtype and *ebony*, were used in the exercise. *Ebony* flies were chosen for three reasons: 1) they can be distinguished by the naked eye from the wildtype morph; 2) the allele causing *ebony* phenotype is recessive; and 3) they have a decreased fitness in comparison to wildtype flies. Each one of these characteristics helped simplify the mechanics of the experiment. Given an introduction on raising *Drosophila*, Mendelian genetics, and population genetics, students were well prepared to conduct the exercise, predict the outcomes, and analyze the results. Students displayed their understanding of the semester long exercise by writing a journal article based on the results and participating during an open discussion period about Mendelian genetics and population dynamics.

Keywords: evolution, *Drosophila*, laboratory exercise

INTRODUCTION

Students tend to learn about evolution at the macro level through textbooks and at the micro level through games. Few laboratory exercises are created to teach evolution principles by using living organisms. This situation may lead students to think of evolution as an abstract and untestable concept. An upper-level evolution biology course was designed to include laboratory exercises with living organisms. This paper describes one of those exercises that demonstrate significant population genetic change over a thirteen-week period by using the common fruit fly, *Drosophila melanogaster*. In fact, the genetic change was observable even after the first five weeks of the experiment. The flexibility of the laboratory exercise is not only one of time, but also of depth of the lesson. Depending on the level of student understanding and objectives of the course, the instructor may choose from the following list of topics and expand on them: 1) Drosophila life cycle and Mendelian genetics; 2) maintenance of Drosophila populations with proper feeding conditions; 3) density dependence of populations; 4) geometric growth of populations; 5) carrying capacity; 6) population genetics; 7) microevolution; 8) natural selection and fitness; and 9) evolution.

With today's technologies and access to the World Wide Web, computers have added another dimension to biological education and have become a mainstay in educational initiatives. For example, one can download a population genetics program that allows manipulation of imaginary populations to show microevolution in graphic form. The laboratory exercise presented here provides the benefits of a hands-on experience with living organisms allowing students to see microevolution in real time.

A previous paper titled, "Using Species of *Drosophila* to Teach Evolution," (Rosenthal, 1979) briefly discusses using breeding competition experiments in which two species of *Drosophila* were placed into the same container. The students then assess the "reproductive success…by counting the number of each species in the container" (p.554). Rosenthal's experiment is an eloquent demonstration of competition, but not of evolution.

MATERIALS

The following are materials necessary for either fifteen students working alone or thirty students working in pairs:

200 *Drosophila melanogaster* - wildtype *
400 *Drosophila melanogaster* - ebony *
15 plastic rectangular containers with lids
anti-mite paper *
450, 15ml conical tubes
sterile cotton
dry Drosophila food *
active dry yeast
2 to 6 stereoscopes
15 paintbrushes or fly brushes *
2 carbon dioxide tanks or Fly-Nap*

30, 250ml beakers
a source of distilled water
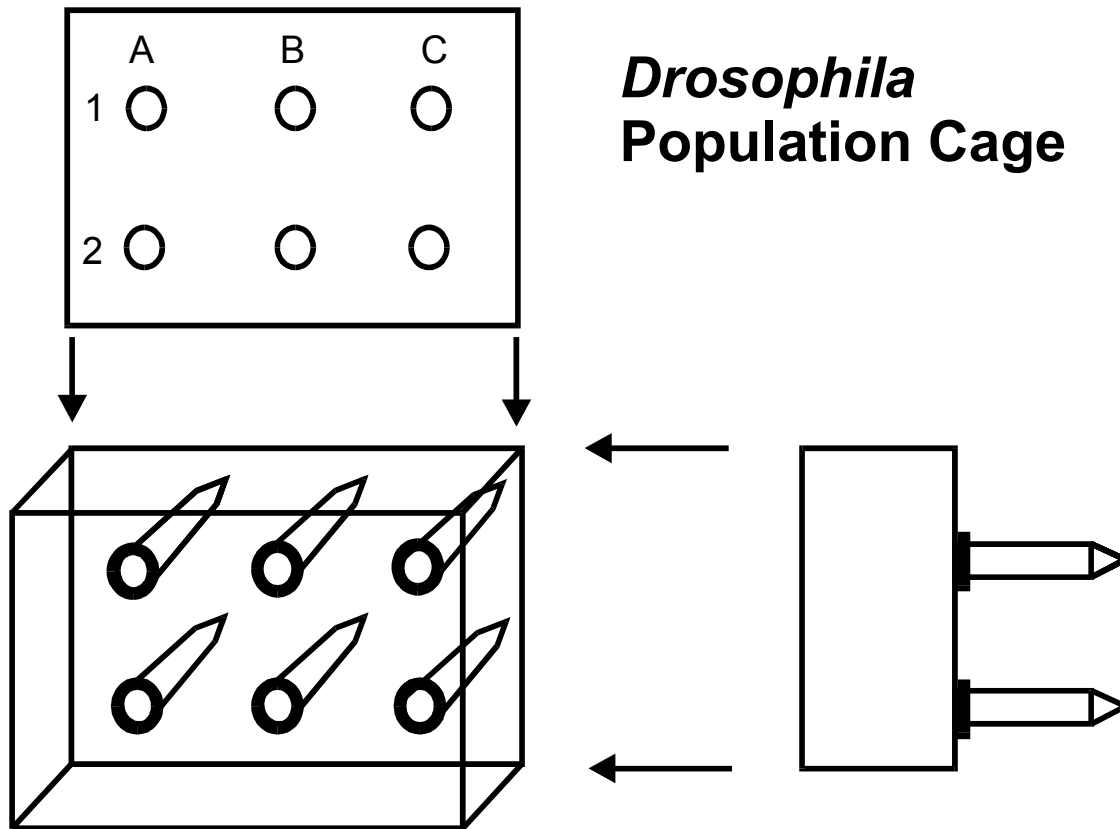*from Carolina Biological Supply*

EXPERIMENTAL DESIGN AND METHODS

This experiment demonstrates evolution principles with a simple design - one favored allele (wildtype over *ebony*). *Ebony* flies were chosen for three reasons:1) they can be distinguished by the naked eye from the wildtype morph; 2) the allele causing the *ebony* phenotype is recessive; and 3) they have a decreased fitness in comparison to wildtype flies (Lindsley and Zimm, 1992). Each of these factors helps simplify the mechanics of the experiment.

Food storage containers were adapted for use as population cages. Each container was fitted with six 15ml conical tubes. Six holes, no larger than the caps of the 15ml conical tubes, were created in the bottom of the container. Holes were made in the tube caps such that the threads were still intact. Then the tube caps were adhered to the container over the holes with a silicon-based adhesive, and left to dry overnight. Four empty conical tubes were screwed into four caps (B1, B2, C1, and C2) of the storage container (Figure 1). Two conical tubes were each filled to the 1.5ml

mark with dry fly food, distilled water, and five to ten pellets of active dry yeast. They were screwed into caps A1 and A2. Then in order to begin with a high *ebony* allele frequency in the population containers, fifteen wildtype and thirty *ebony* fruit flies from homozygous stocks were added to each food storage container. All transfers of fruit flies were done using CO_2 fly pads under stereoscopes with paintbrushes to gently separate the flies. On every third day subsequent to starting the population cage a fresh food tube was exchanged for an empty tube or sample tube. The order of replacement was as follows: B1, B2, C1, C2, A1, A2, B1, and so on. This allowed fifteen days for the original food tubes, placed in A1 and A2, to give rise to a second generation of flies.

During these fifteen days students began an additional experiment which would help them explain their results from the population cages. They estimated the relative fitness of the *ebony* flies by isolating virgin flies of *ebony* and wildtype and doing all four crosses (Figure 2). The total number of offspring from *ebony* females was divided by the total number of offspring from wildtype females to calculate relative fitness

A   B   C

1 O   O   O

2 O   O   O

Drosophila
Population Cage

Figure 1. *Coordinate system for placement of food tubes and schematic for cage construction.*

	Females	
Males	*ebony*	wildtype
ebony	47 (n=3)	55 (n=4)
wildtype	27 (n=4)	41 (n=4)
Total Offspring	74	96

Relative Fitness 74/96 = 0.77

Figure 2. *The relative fitness of ebony was calculated by the students in a separate experiment by crossing virgin fruit flies and counting offspring of ebony and wildtype females (n=number of matings, each cell contains the average number of offspring per mating).*

On the fifteenth day the first sample tube collected was plugged with cotton and dated. All adult flies trapped in the tube during collection were discarded. Flies that emerged from the sample tube's food over the next few weeks were identified by sex and morph type (either wildtype or *ebony*). Each student kept a record of his/her individual population cage's data. At the end of the semester all data were compiled and tabulated. Students individually wrote the combined population cage results as a journal article according to a sample layout provided to them.

RESULTS

The students followed the instructions for the exercise. Variation existed between individual cages; however, the general tendency for the homozygous ebony population to decrease in frequency occurred (Figure 3). The students' calculation of ebony's relative fitness to wildtype was 0.77 (Figure 2). The reference text states that ebony's relative fitness is, "about 80%" (Lindsley and Zimm, 1992). Students submitted journal articles that expressed their understanding of the nine topics mentioned in the introduction of this article. One student applied the lesson in population genetics and fitness in a graphic display of the decline of the ebony allele in the population (Figure 4).
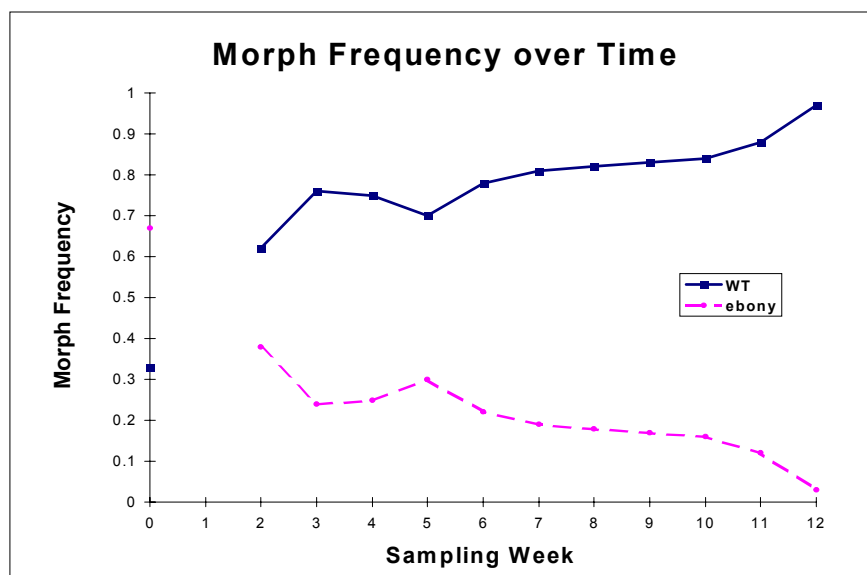

Figure 3. *Data compiled and averaged from fourteen individual population cages. The ebony morph frequency decreases from 67% to less than 5% over a thirteen-week course.*
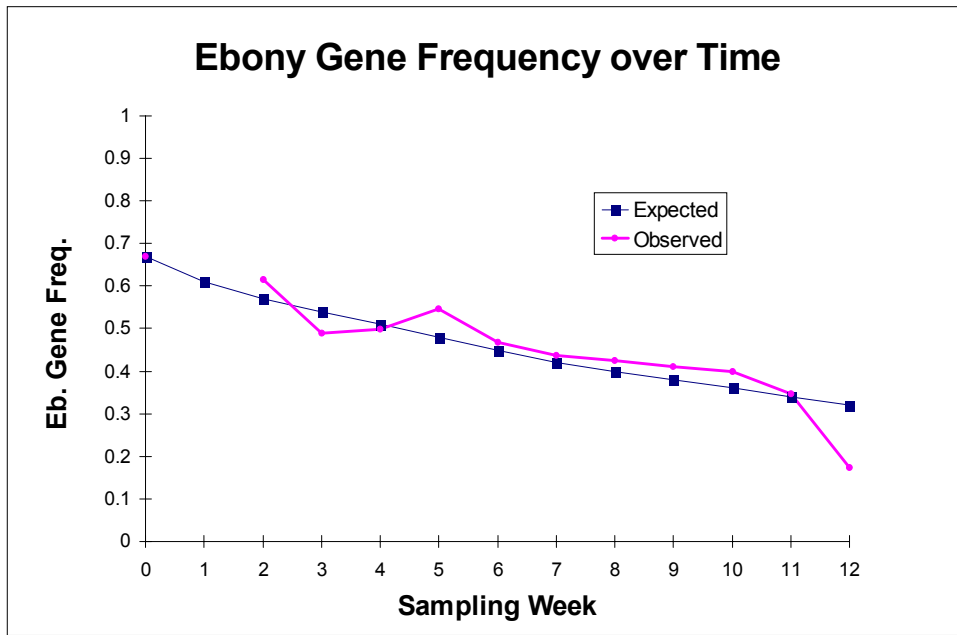
Figure 4. *Assuming ebony gene frequency could be extrapolated from the number of ebony morphs identified, a student created this figure for his journal article.*

DISCUSSION

This exercise can be adapted for different laboratory courses. It can be part of an evolution course or incorporated into a genetics, population biology, or molecular biology laboratory. Some variations of the exercise are also possible. The relationship between homozygous wildtype, homozygous ebony, and the heterozygous cross can be developed into a deeper discussion on dominance and competition amongst the three types. Rendel (1951) provided evidence that the *ebony* males are more successful when mating in the dark. Possibly one variation of the laboratory exercise is to have half of the population cages in a dark room. Kyriacou et al. (1978) showed that the heterozygote cross of wildtype and *ebony* may even have a selective advantage over both homozygotes. This leads to the interesting question, "How many of the wildtype morphs identified in the laboratory exercise are actually heterozygotes?" A molecular identification of the *ebony* locus, perhaps using specific oligos for PCR and a subsequent DNA agarose gel, would provide the students with the opportunity to learn another common laboratory technique. Backcrossing the sample flies with the parent population would be more time consuming, but it would also provide an answer to the same question. Even without the quantification of the heterozygote population in the samples, the information from the aforementioned journal articles could be used to deepen the student discussions.

NOTES

1. Fly-Nap from Carolina Biological Supply may also be used according to the instructions of the manufacturer without stereoscopes.
2. Virgin flies may be provided to the students at the beginning of the fifteen days if isolating them is not one of the course objectives. The experiment can also be dropped since the relative fitness of ebony homozygotes to wildtype is known (Lindsley and Zimm, 1992).
3. Sample tubes may be collected a day early or late if the third day falls on a weekend; however, tubes should be collected as close to the third day as possible.

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