Investigation 1

ARTIFICIAL SELECTION

Can extreme selection change expression of a quantitative trait in a population in one generation?

BACKGROUND

There are only a few possible laboratories available and appropriate for the high school classroom environment that can explore real-time natural selection with multicellular organisms. For reasons of time and resources, trying to measure natural selection is problematic. Many lab investigations that help students derive an understanding of natural selection are either computer simulations or structured simulations. However, a promising alternative is to have the students study and carry out an artificial selection investigation using Wisconsin Fast Plants (*Brassica*). Just as Darwin relied on examples of artificial selection in cattle, domestic pigeons, and other farm animals to make his case in *On the Origin of Species*, students can gain important insights into natural selection by studying artificial selection. In addition, this particular investigation on artificial selection provides an easy transition into student-generated explorations that look for possible advantages or disadvantages that selected traits might confer on individuals in different environmental conditions.

For the first part of the investigation, students will perform one round of artificial selection on a population of Wisconsin Fast Plants. First, they will identify and quantify several traits that vary in the population and that they can quantify easily. They then will perform artificial selection by cross-pollinating only selected plants. Students will collect the seeds, plant them, and then sample the second-generation population and see if it is different from the previous one. Their results will generate questions, and they will have a chance to test their own ideas about how selection works.

PREPARATION

Materials and Equipment

Per Class:

- Lighting: light box systems (as per the Wisconsin Fast Plants website, ***http://www.fastplants.org***)

Per Team/Student:

- Growing system: reused plastic soda or water bottles
- Wicking: #18 nylon mason twine
- Fertilizer: Miracle-Gro Nursery Select All Purpose Water-Soluble Plant Food, or Peters Professional with micronutrients
- Soil: Jiffy-Mix (soil mix, not potting soil)
- Vermiculite
- Fast Plant seed (C1-122 works well and provides some additional options explained in The Investigations; it can be purchased through the catalog of the Rapid Cycling Brassica Collection [RCBC], ***http://www.fastplants.org/pdf/rcbc.pdf***. Other seed stocks, such as standard Fast Plant seeds that can be purchased from Carolina Biological or Nasco, work as well.)
- Bee sticks for pollination
- Digital cameras to record the investigation
- Plastic magnifiers
- Laboratory notebook

Timing and Length of Lab

The first part of this investigation, Procedure, minimally involves growing one generation of Wisconsin Fast Plants from seed to seed, followed by an additional 10-day growing period for the second generation of plants. The total time is approximately seven weeks. Almost all days will be short, with students taking care of plants and making notes. Occasionally, more time (5-10 minutes) will be needed — for planting, quantifying variation and selection, pollinating plants, and scoring the second generation.

The time needed to fully investigate questions generated by students in the second part of the investigation will need to be determined by you and your students. As in the first part, much of the work in the student-led part can be carried out in a part-time manner at the beginning and/or end of class. Another option would be after school.

Safety and Housekeeping

When growing plants under lights, be careful to avoid any situation where water or fertilizer could come in contact with the electrical wires.

ALIGNMENT TO THE AP BIOLOGY CURRICULUM

This investigation can be conducted during the study of concepts pertaining to natural selection and evolution (big idea 1). As always, it is important to make connections between big ideas and enduring understandings, regardless of where in the curriculum the lab is taught. The concepts align with the enduring understandings and learning objectives from the AP Biology Curriculum Framework, as indicated below.

Enduring Understanding

- EVO-1: Evolution is characterized by a change in the genetic makeup of a population over time and is supported by multiple lines of evidence.

Learning Objectives

- EVO-1.F: Explain how humans can affect diversity within a population.
- EVO-1.G: Explain the relationship between changes in the environment and evolutionary changes in the population.

Science Skill

- 4.B: Describe data from a table or graph.

ARE STUDENTS READY TO COMPLETE A SUCCESSFUL INQUIRY-BASED, STUDENT-DIRECTED INVESTIGATION?

It is great to implement this investigation at the beginning of the year. Students need minimal content background to begin this investigation and complete the first part of the lab. In general, students find this lab to be very accessible and enjoyable. For the most part, skills are developed as the lab progresses. However, essential to the success of this investigation is the student's ability to make and record good observations. This is best done in a laboratory notebook.

Skills Development

The students can use this particular experience to build good laboratory notebook skills. A lab notebook should demonstrate originality and reflection while serving as a record of the investigator's work. Planting, quantifying variation, recording images/drawings of that variation, maintaining plants, and recording results all make for prime lab notebook subject matter. By tending their own population of plants each day and recording daily observations, students develop their own particular style and rhythm of writing in the lab notebook. These activities require only about 10 minutes of class time and are essential to the student-led part of the investigation. While working through the Procedure, students naturally generate questions regarding the traits they are working with and the variations they observe. Often these questions are not recorded and are soon forgotten. Encourage the students to record the questions that come to them as they work intimately with these plants and to reflect on those questions in writing.

The instructor needs to decide when to start this investigation. The students may benefit from having an understanding of natural selection prior to beginning this lab, but this lab might best be used to introduce the concept of natural selection. Think about how you wish to approach this as an instructor.

Potential Challenges

As with all long-term lab investigations, management of time and the calendar can be challenging. To coordinate with school calendars, start the investigation on a Monday or Tuesday. Make sure that the water reservoirs are full before every weekend. Keeping track of multiple sections and their various plants can present a challenge as well. You might want to consider smaller growth chambers for each class in order to keep the different populations separate.

In general, most classrooms have minimal plant pests, but if your classrooms have a large population of plants year-round, you may experience pest outbreaks in your Fast Plants®. Soapy water sponged on the plants controls some pests, such as white flies. Insecticidal soap comes in ready-to-use spray or in concentrate, and it is safe to use indoors. Another safe way to control insect pests is summer horticultural oil. There are two kinds of summer oil, one extracted from neem seeds and one from citrus peels. Mix them according to the package label directions. Another option is dusting plants with diatomaceous earth, which is simply mined, powdered glass skeletons of marine diatoms, you can control soft-bodied pests like aphids. The powder is not harmful to humans or pets.

With this size of plant population students can sometimes get in one another's way as they move plants in and out of growing areas. It is generally during these times that plants are damaged. Take care to minimize the movement of the plants or develop a system whereby the plants can be protected.

Trying to standardize trichome (plant hair) counting or measurement of other variable traits is another challenge. Present students with questions that will help them develop both a procedure for counting hairs and a method to ensure the fidelity of the counts.

THE INVESTIGATIONS

Getting Started: Prelab Assessment

Investigating biology requires a variety of skills. The skills reinforced and introduced vary across the laboratories in this manual. The skills emphasized in a laboratory dictate whether a prelab assessment is appropriate.

This particular investigation provides a lab environment, guidance, and a problem designed to help students understand how populations of organisms respond to selection. To gain the maximum benefit from this exercise, students should get started and not do too much background preparation so that they can build understanding from their own work.

Designing and Conducting Independent Investigations

To set the stage for student-centered investigations, consider presenting a number of probing questions to the class that center on artificial selection in agricultural crops or inadvertent natural selection, such as antibiotic resistance and pesticide resistance. Through questioning, focus on the common features of these events: extreme selection, rapid changes in populations, and preexisting variation in the population. Use questions to help students recognize appropriate quantitative traits in plants that are growing in the classroom. Likely you'll need to ask questions to help students develop an understanding of quantitative traits that are polygenic. They usually have little problem coming up with a design for a selection experiment once they have an appropriate trait selected. In Fast Plants, appropriate traits include number of trichomes, amount of purple anthocyanin, and plant height.

Logistically, the first part of the lab requires quite a bit of coordination and sharing of duties among all students in the class. Artificial selection experiments require a relatively large population of plants with ample phenotypic variation. The numbers involved are not very workable for the individual student or even for a small group of students. For this reason, it is recommended that the first step of this lab be conducted at the class level. The minimal population size for part one is about 120–180 plants per class. Require each student in your class to care for enough plants to achieve this population size. This size of population will generally express adequate phenotypic variation for a trait, such as trichomes. Consider directing your students toward this trait because trichomes are quantifiable. There is no need to count every hair — just a sample. One possible sampling procedure would be to count the hairs along the edge of the right side of the first true leaf. (See the following document for more information about Fast Plants: ***http://www.fastplants.org/pdf/activities/WFP_growth-development-06web.pdf***.)

For the trichome trait, if the top 10% of hairy plants are selected, that will generate a selected parent stock of about 12–15 plants — an adequate number to produce the seed for the next generation.

Your students will need a magnifier to study trichomes. Don't be surprised if many plants have few or no hairs. The hairs are often more visible if backlit and held against a dark background. Help your students develop a system to keep track of their counts. Somehow they will need to mark each individual plant. One possible method is to record the number of trichomes on a small plastic stake for each plant. Students record the number of hairs on a stake and place it near the appropriate plant. (Stakes can be created by cutting a plastic milk jug into 1 cm x 10 cm strips.)

As an instructor, you might consider utilizing Fast Plant seed stock C1-122 for this investigation. This stock offers a unique advantage in addition to expressing some variation in hairiness. That is, it is heterozygous for two Mendelian traits, green/light green leaves and with anthocyanin (purple stems) and without anthocyanin. In other words, these are F1 plants from a dihybrid cross. By using this stock and carefully managing the pollination and the offspring, your class can begin two separate investigations with one seed generation. Your class can investigate artificial selection with the quantitative trait of hairiness or stem color, and with the same plant population you can raise an F2 generation of a dihybrid cross for a classical Mendelian investigation on genetics. The advantage is that the 90% of the population not selected for hairs can continue to be grown by the individual students to produce an F2 generation. The seed from this cross can be used in a genetic cross demonstration/experiment, as described in the Fast Plant publication "Who's the Father?" (***http://www.fastplants.org/pdf/WTF_di.pdf***).

It is recommended that you build your own light racks and growing systems following the instructions available from the Wisconsin Fast Plant website. However, complete systems are available from supply companies. Light systems constructed by you are generally more cost effective than commercial products and can be custom designed for your room. Be sure to check with your school administration first.

Allow students to grapple with the data analysis and ways they will report their data. Refer students to Chapter 3 in their lab manual. In case they struggle, you might suggest that they graph the frequency distribution of the trait (the number of plants within a specific interval) by constructing histograms like Figures 1 and 2 in their report.

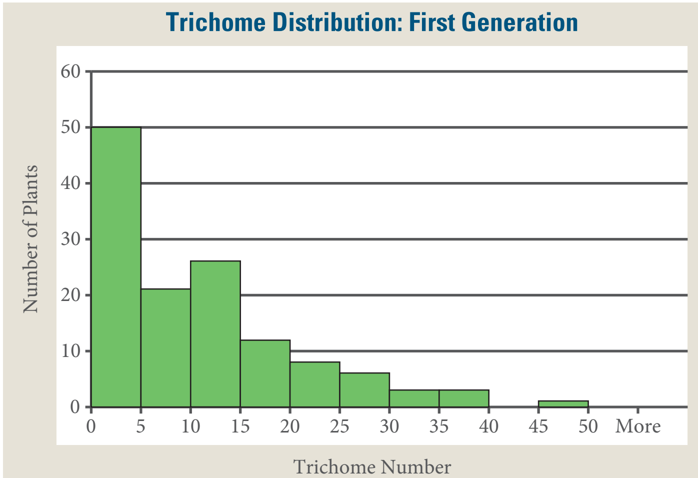

Figure 1. Trichome Distribution: First Generation

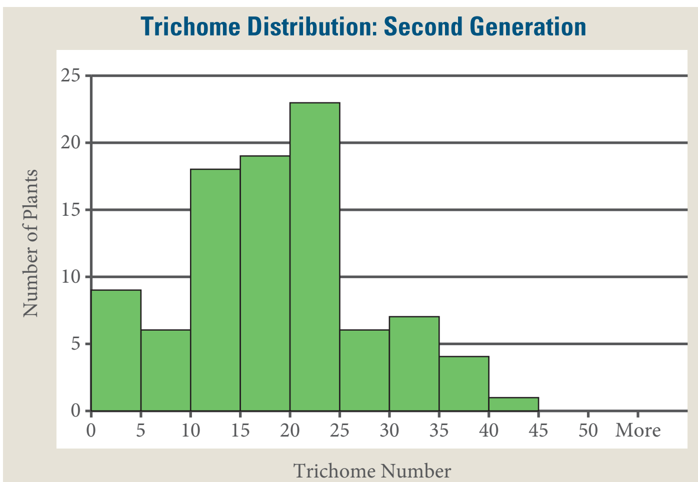

Figure 2. Trichome Distribution: Second Generation

Summative Assessment

For the first part of the investigation, you might want to have students or student groups develop individual online or digital presentations of the compiled work on artificial selection. While the class shares results and data collection methods, the data analysis and presentation of results are still the responsibility of the individuals or groups. This work would be enhanced if illustrated with digital images taken by students over the course of the selection experiment. The true summative assessment for this work will be revealed in the quality of the questions and work that the students propose for the final part of the investigation.

Consider having the students construct and present miniposters that represent their research as a summative assessment. First, have them present and defend posters to each other and provide peer review. Encourage the students to utilize the same rubric that you choose to evaluate their research project. Give them an opportunity to modify their posters before you evaluate the work with the same rubrics.

Miniposters have an advantage over traditional posters by not requiring quite so much time. If the students are working in research teams, you might consider emulating a professional society's poster session. When students put in this amount of work, it is appropriate to display their work publicly. Displaying posters in the science hall is an excellent way to provide a sense of authenticity to the research.

Where Can Students Go from Here?

An essential component of this investigation is to take it beyond the simple selection experiment. With the skills and knowledge gained in the selection experiment, students should be able to design new experiments to investigate the adaptive characteristics of the trait they studied — particularly if they selected for a quantitative trait like trichomes. For instance, they could select for the amount of purple color in the plants. This would involve students designing a system that would "quantify" color and look into the possible function(s) of purple pigment. The Supplemental Resources section includes the descriptions of a number of very accessible investigations related to the work that students conduct in the first part of the lab. Encourage students to explore concepts such as phenotypic plasticity or herbivore responses to trichomes. Cabbage white butterfly (*Pieris rapa*) larvae make a good herbivore for such a study.

A commonly asked question is *Why do these plants produce these small hairs?* It must take energy to produce the hairs. Is there an environment in the natural world where the hairs might serve as an advantage for those plants that express them? This is the start of a hypothesis that students can investigate.

Students may have other questions to investigate as well. They should start with a question of their own regarding hairs or some other variable quantitative trait, such as plant height, stem color, or flower number. For instance, in a closely related plant, one investigation demonstrated that herbivore damage early in the plant's development led to increased trichome numbers in later leaves. Could herbivore damage influence the hairy trait expression?

Several hypotheses have been proposed as a possible explanation for the role that trichomes play. One hypothesis is that trichomes provide a degree of protection from herbivores — either by discouraging herbivores, such as insect larvae, or by discouraging egg laying. A common herbivore that feeds on Fast Plants is the cabbage white butterfly. Students could choose a question related to the trichomes and their importance to a plant, such as one that explores the relationship between herbivory and hair production, or they could choose a different trait and design and carry out an investigation to answer a question related to it.

SUPPLEMENTAL RESOURCES

The following resources are presented to provide students and teachers with examples of research that focus specifically on the concepts and organisms in this laboratory. The hope is that students and teachers will find inspiration for their own work in these references.

Agrawal, A. A., S. Y. Strauss, and M. J. Stout. 1999. Costs of Induced Responses and Tolerance to Herbivory in Male and Female Fitness Components of Wild Radish. *Evolution* 53, no. 4 (August): 1093–1104.

Agren, Jon, and Douglas W. Schemske. 1993. The Cost of Defense Against Herbivores: An Experimental Study of Trichome Production in *Brassica rapa. The American Naturalist* 141, no. 2 (February): 338–350.

———. 1994. Evolution of Trichome Number in a Naturalized Population of *Brassica rapa*. *The American Naturalist* 143, no. 1 (January): 1–13.

Bidart-Bouzat, M., Gabriela, Richard Mithen, and May R. Berenbaum. 2005. Elevated CO_2 Influences Herbivory-Induced Defense Responses of *Arabidopsis thaliana*. Oecologia 145, no. 3 (September): 415–424.

Callahan, Hilary S. 2005. Using Artificial Selection to Understand Plastic Plant Phenotypes. *Integrative and Comparative Biology* 45, no. 3 (June): 475–485.

Handley, R., B. Ekbom, and J. Agren. 2005. Variation in Trichome Density and Resistance Against a Specialist Insect Herbivore in Natural Populations of *Arabidopsis thaliana*. Ecological Entomology 30, no. 3 (June): 284–292.

Schmaedick, M. A., and A. M. Shelton. 2000. Arthropod Predators in Cabbage (*Cruciferae*) and Their Potential as Naturally Occurring Biological Control Agents for *Pieris rapae (Lepidoptera: Pieridae). Canadian Entomologist* 132, no. 5 (October): 655–675.

Siemens, David H., and Thomas Mitchell-Olds. 1998. Evolution of Pest-Induced Defenses in Brassica Plants: Tests of Theory. *Ecology* 79, no. 2 (March): 632–646.

Sleeman, Jonathan D., and Susan A. Dudley. 2001. Phenotypic Plasticity in Carbon Acquisition of Rapid Cycling *Brassica rapa* L. in Response to Light Quality and Water Availability. *International Journal of Plant Sciences* 162, no. 2 (March): 297–307.

Strauss, Sharon Y., David H. Siemens, Meika B. Decher, and Thomas Mitchell-Olds. 1999. Ecological Costs of Plant Resistance to Herbivores in the Currency of Pollination. *Evolution* 53, no. 4 (August): 1105–1113.

Traw, M. B., and T. E. Dawson. 2002. Differential Induction of Trichomes by Three Herbivores of Black Mustard. *Oecologia* 131, no. 4 (May): 526–532. DOI: 10.1007/s00442-002-0924-6.

Williams, Paul H., and Curtis B. Hill. 1986. Rapid-Cycling Populations of *Brassica*. Science 232, no. 4756, New Series (June 13): 1385–1389.