**INQUIRY & INVESTIGATION**

# Using Brassica Butterflies as a Model Organism for AP Biology Lab 11


KATHY VAN HOECK



## Abstract

*Advanced Placement Biology Lab 11 has used pillbugs as the model organism and choice chambers so that they can travel between two environments. I used cabbage white butterflies as the model organism. Each student was given their own larva and observed metamorphosis, choice behaviors of the larva, and mating behaviors of adult butterflies.*

**Key Words:** *Biological inquiry; animal behavior; AP Biology; Wisconsin Fast Plants; cabbage white butterfly.*

Recent studies of American science education have highlighted a need for more inquiry-based lessons. Advanced Placement laboratory exercises have been criticized as being "cookbook" rather than inquiry-based. This criticism is particularly evident in the program of 12 labs that must be done by each student in AP Biology. Questions on the all-important AP exam usually assume that the students are familiar with these labs and sometimes contain specific questions about them. However, teachers are encouraged to adapt the labs as long as the overarching principles are the same. Many teachers of AP Biology are trying to incorporate inquiry into these adaptations.

The conventional AP Lab 11 has used small crustaceans known as pillbugs as the model organism, and choice chambers are built using two Petri dishes that have adjoining passageways so that the pillbugs can travel back and forth between two environments (e.g., light/dark or wet/dry). Other model organisms that have been used to demonstrate choice include fruit flies (geotaxis) and crayfish (sand vs. rocks for hiding) (Brown, 1998).

> *It is amazing how even seniors in high school… are still excited at the prospect of raising and caring for their own larva.*

The Wisconsin Fast Plants group has used *Pieris rapae*, the cabbage white butterfly, as a model organism to demonstrate the interdependence of these butterflies and rapid-cycling *Brassica rapa*, or the Fast Plant, developed by Paul Williams at the University of Wisconsin-Madison (Williams & Hill, 1986). "Brassica butterflies" are so called because they lay their eggs only on plants in the brassica family and the larvae eat only brassica plant material.

Fast Plants have been used in classrooms across the country for many years now and can be incorporated into lessons for students as young as preschool, for developing observation skills and learning about plant life cycles. They are suitable for lessons in K–12 and are even used in college laboratories. Fast Plants are very easy to grow and require no special equipment; plant light boxes can be constructed from computer-paper boxes with compact fluorescent bulbs as a light source. The Fast Plant Web site (Lauffer & Williams, 2007) has complete planting directions.

This year, I used cabbage white butterflies as the model organism for Lab 11 in my AP Biology class. First of all, it is amazing how even seniors in high school are fascinated with living things and are still excited at the prospect of raising and caring for their own larva. Each student was given their own larva and required to observe it on a regular basis and keep a journal. They were able to see the complete metamorphosis of an insect, hone their observation skills, and learn to measure and log the changes in morphology.

## ○ Experimental Methods

To begin the experiment, a few pots of Fast Plant seeds were planted (purchased from Carolina Biological Supply Company). Radish seeds can be substituted for Fast Plant seeds but should be planted a few days earlier because they don't grow as quickly. Brassica-butterfly egg strips must be purchased from Carolina also, and arrangements should be made for them to arrive at least 5 days after seeds have been planted. They come on a strip of waxed paper in a microcentrifuge tube (Figure 1). The strip must be placed on brassica cotyledons as soon as possible, as some eggs may have hatched during shipping (Figure 2). These larvae go through five instars and are almost microscopic in their L1 stage. They need soft plant material to eat. Small holes in the leaves and/or presence of frass (larval feces) are evidence of larval hatching (Figure 3). Allow the students to view the egg strip under a dissecting scope or a magnifying glass and have them make their first observations in their journals. Last year, I used radish plants in AP Lab 9 (Transpiration), and by the time we started Lab 11 the plants were quite mature. Radish plants are studded with trichromes (i.e., they are hairy). This is a defense against herbivory, and the L1 larvae were repelled by them. This slowed down our experiment a bit, because we ended up needing to replant seeds. Once the larvae reached the late L2–L3 stage, each student adopted a larva to put in their "brassica barn,"

*The American Biology Teacher*, Vol. 72, No. 6, pages 361–364. ISSN 0002-7685, electronic ISSN 1938–4211. 
DOI: 10.1525/abt.2010.72.6.9

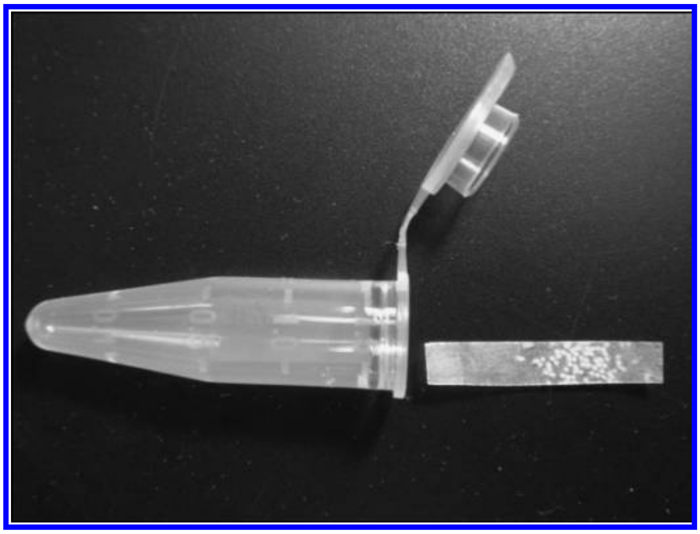

**Figure 1.** Egg strip as it arrives from Carolina Biological Supply Company.

**Figure 2.** Egg strip laid on brassica cotyledons.

**Figure 3.** Evidence of larva eating brassicas. Note frass on leaves.

**Figure 4.** "Brassica barn" with Brussels sprout.

which consisted of an individual yogurt container with a clear lid into which air holes had been punched. A few layers of paper towel cut into circles were placed in the bottom of the container to catch the frass that accumulated. This paper needs to be changed regularly.

A piece of broccoli, cauliflower, or Brussels sprout is suspended from the top of the container and held in place by a tack punched through the lid from the top. This way, the frass falls to the bottom of the container (Figure 4).

## ○ Choice Experiment

To cover the choice aspect of the lab, the Fast Plants group has an activity called "The Salad Bar" (http://www.fastplants.org/pdf/activities/Butterfly_Activity.pdf [p. 23]). A piece of moist filter paper is fitted inside the bottom of a Petri dish. Various food choices are placed on the paper in a clockwise fashion. There should be a variety of brassicas: broccoli, Brussels sprouts, bok choi, cabbage (green and/or red), cauliflower, kale, radish, and mustard are some common choices. There should also be non-brassicas: carrot, parsley, spinach, celery, beet, etc. A larva is placed in the middle of the dish and observed for its food preferences (Figure 5). The students can observe the larvae for a class period, or the larvae can be left in the dishes overnight and observed again the next day. The students should discuss with their lab partners the differences and similarities in behavior between their individual larvae. Some are disoriented at first and just stay in the middle of the dish. Others immediately start trying to get out of the dish and circle round and round the perimeter. Some begin sampling the different foods, and others go straight to their first food choice and stay there. Not only will the larvae eat only the brassicas, they sometimes prefer one brassica over another. The evidence of feeding is frass where the food was the day before. One larva may eat mostly kale and a little cabbage, for example; another may eat only cabbage and not sample any other food. Or there may be little bites out of many different brassicas. If there are any bites out of non-brassicas, they will be minimal but should be noted (Figure 6). This can demonstrate to the students that there is individual choice within the species and challenge them to state the selective advantage of this. On its surface, this is a very simple lab and can be done with preschoolers. However, AP students can be challenged to relate the behavior of these larvae to niche, competitive exclusion, generalist vs. specialist feeding, coevolution, plant defense against herbivory, adaptation to plant defense, embryology, genetics, and more. What is it specifically that the larvae are attracted to?

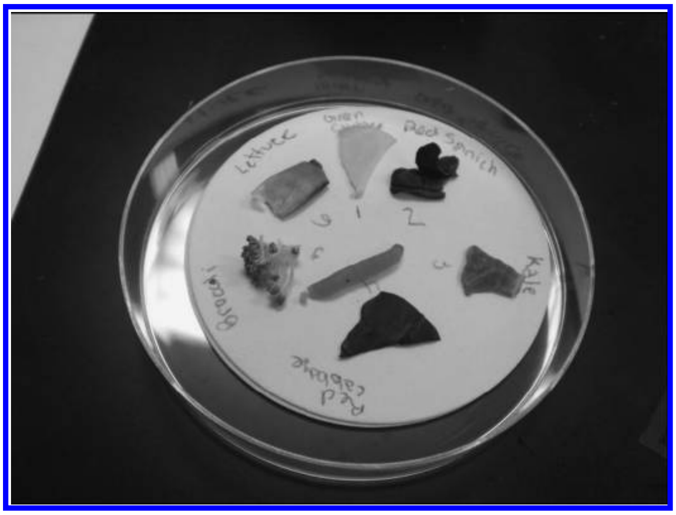


**Figure 5.** "Salad bar" set up with larva.

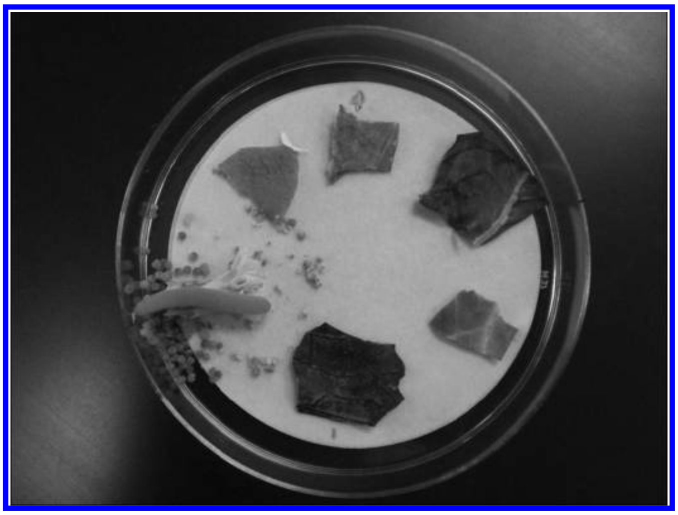

**Figure 6.** Results of Salad Bar experiment, day 2. Larva has preferentially eaten the broccoli.

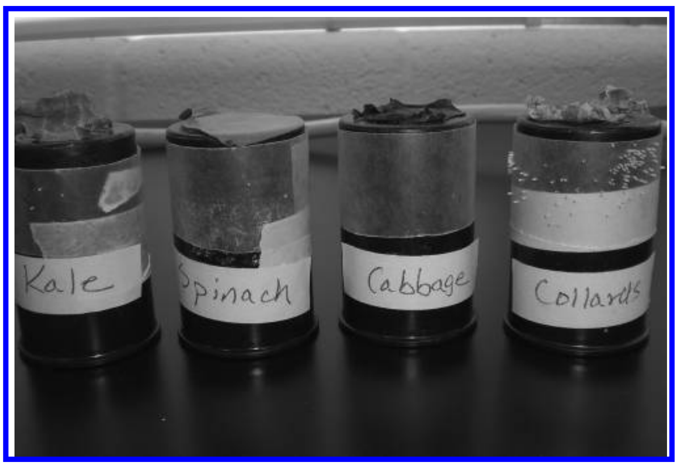


**Figure 7.** Results of egg-laying experiment showing preference for collard greens.

The Salad Bar experiment should take place while the larvae are in their L4 or very early L5 stage, when they are voracious eaters and leave large frass pellets. However, one year, I made the mistake of waiting just a day too long and the larvae pupated overnight. Once they pupate, the food source and all frass should be removed.

## ○ Mating Behavior

Lab 11 also includes a section on mating behavior, using *Drosophila* as the model organism. This, too, can be demonstrated with the adult butterflies. Before the pupae began to hatch, I moved the barns to butterfly boxes and removed the lids from the barns. Ideally, there will be one box per group so that mating behaviors can be observed by a minimum of students. (See the Fast Plants Web site for instructions for making butterfly boxes.) As soon as the adults emerge from the pupae, they need to find a vertical surface to unfold their wings and hang in order to allow them to pump up their wings. They will also be thirsty. Artificial nectar can be purchased or made. We have had luck with Melon Fierce Gatorade poured onto pieces of cellulose sponges placed in a Petri dish. Once their wings are pumped up and their exoskeletons harden, mating takes place immediately. If there are males and females in the box together, the males will immediately attempt to mate. In a butterfly box, mated females exhibit a very distinctive male-avoidance posture that students will probably misinterpret the first time they see it. If the female is not receptive, it positions its abdomen at a 90° angle to its body. Insects mate "tail to tail," and this prevents the male from capturing the female. It is sometimes difficult to tell the males from the females morphologically, but this behavior makes it easy; the males will be fluttering around the females, which are perched on the wall of the box. Males generally also have one black spot on each wing, whereas females have two. Once a male captures a female for mating, the process can take an hour or more.

## ○ Egg-Laying Behavior

As soon as mating is finished, the female will be looking for brassica plant material to lay its eggs on. Another experiment showing plant preference is to offer different types of plants for egg laying, or oviposition. My students glued brassica leaves and non-brassica leaves to film canisters. A strip of waxed paper was glued around the top of the canister, also called an "ovipositron" (see http://www.fastplants.org/pdf/activities/Butterfly_Activity.pdf). The females curl their abdomens to deposit their eggs on the underside of a leaf. They end up depositing the eggs on the wax-paper strip instead. Placing two or more ovipositrons in a butterfly box with an egg-laying female makes it easy to observe which plant material it prefers to lay its eggs on (Figure 7). This is where inquiry can be introduced. What is it that attracts the female to a certain plant? I have asked students to hypothesize what it is about the Fast Plants that are attractive to the butterflies. We brainstorm and come up with alternative hypotheses. For example: is it a scent, is it the color of the flowers (brassicas generally have yellow flowers), is it the size of the flowers, is it the leaves? (They generally lay their eggs on leaves). Are the leaves smooth, hairy, dark green, light green? Each group designs an experiment, performs it, and reports the findings to the class. Studies have shown that the chemical glucosinolate attracts the females. This plant-defense chemical discourages herbivory of the brassicas and gives them their peppery taste. The cabbage white butterfly has adapted to this chemical and is a specialist feeder. This creates its particular niche in the ecosystem.

Because butterflies are ectotherms, the length of their life stages can be slowed or sped up somewhat by controlling the temperature of their environment. Students will probably want to release their butterflies when the lab is over. They should not be allowed to do this, because the larvae are agricultural and garden pests. The butterflies don't live long after laying their eggs. A second life cycle can be observed using the eggs that are laid. The adults and/or eggs can also be euthanized by placing them in the freezer.

## ○ Assessment

As I mentioned above, these experiments have been done by students from preschool to college. For AP Biology students, an assignment to describe the different behaviors of the organism at various points in its life cycle is appropriate. They can determine why larvae eat specific food types, why females lay eggs on these food types, what it is that the butterflies are attracted to, how they detect the stimulus, what plants use these chemicals for, the concept of herbivory and chemical inhibition of herbivory by plants, the niche of cabbage white butterflies (why they lay their eggs on certain plants, why they are not repelled or killed by the plant), and the coevolution of these butterflies and brassicas. This lab is deceptively simple yet can demonstrate complex behavioral and ecological questions.

I did some preliminary Internet research to see how difficult it would be for my students to find the pertinent information and decided to give them some URLs, although I didn't tell them why I chose the sites I did. I have listed these sites below.


## ○ Acknowledgments

I thank Paul and Coe Williams, and Dan Lauffer of the Wisconsin Fast Plants group, for always being just an email or a phone call away.


## ○ Additional Resources

Bryn Mawr College: Caterpillar and Butterfly Behavior Activity
http://www.brynmawr.edu/biology/franklin/brassica_page/cat_butterfly_webpage.html

Nearctica: Cabbage White (*Pieris rapae*)
http://www.nearctica.com/butter/plate3/Prapae.htm

University of California, Irvine: Cabbage White
http://mamba.bio.uci.edu/~pjbryant/biodiv/lepidopt/pieridae/cabbagem.htm

Dr. Karowe's Research
http://homepages.wmich.edu/~karowe/research.html
(See what you can glean about the larvae from lurking on this page.)

Art Shapiro's Butterfly Site: *Pieris rapae*
http://butterfly.ucdavis.edu/butterfly/Pieris/rapae

Department of Biochemistry: Glucosinolates in *Arabidopsis thaliana*
http://www.ice.mpg.de/ger/research/glucosinolates/glucosinolates.htm

Imperial College, London: Resisting Insect Attack
http://www.imperial.ac.uk/P2767.htm

Insect Physiology Online: Plant-Insect Interactions – A Synthesis
http://www.faculty.ucr.edu/~insects/physiolecol/plenuryeb.html

Highlights aus Pflanzenforschung
http://www.mpg.de/forschungsergebnisse/wissVeroeffentlichungen/forschungsberichte/PF/aktuell.html#200803-24
(Scroll down to "The genetic basis of a plant-insect coevolutionary key innovation.")

Michigan State University: RadishDB: Background
http://radish.plantbiology.msu.edu/index.php/RadishDB:Background#Herbivory_has_a_major_impact_on_plant_fitness.2C_and_is_a_major_challenge_for_agriculture
(Read "Herbivory has a major impact on plant fitness….")

## References


Brown, J. (1998). Lab 5 – Animal Behavior. Grinnell College, Grinnell, Iowa. Available online at http://web.grinnell.edu/individuals/brownj/edu/136_lab5.html.

Lauffer, D. & Williams, P. (2007). Wisconsin Fast Plants. Available online at http://www.fastplants.org.

Williams, P.H. & Hill, C.B. (1986). Rapid-cycling populations of *Brassica*. *Science*, *232*, 1385–1389.



KATHY VAN HOECK is a biology teacher at York Community High School, 355 W. St. Charles Road, Elmhurst, IL 60126; e-mail: kvanhoeck@elmhurst205.org.