Teaching Principles of Experimental Design While Testing Optimal Foraging Theory

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Abstract

We describe a simple field study that we have found useful in introducing students to experimental design. Students manipulate the nutritive gain available from flowers to test the hypothesis that the foraging behavior of nectarivorous insects maximizes energy gain rate. They add sucrose solution to some flowers and water to others; additional flowers are left unmanipulated. Visit durations of foraging butterflies are then measured to test the prediction that individuals will forage longer at patches that offer higher energy gains. The project encourages students to consider how a study's design influences the results obtained, and helps to develop scientific reasoning skills.

Key Words: *Adaptation; evolution; experimental design; optimal foraging; coevolution; animal behavior.*

The concept of adaptation via natural selection can be demonstrated beautifully to students by investigations of animal morphology or physiology, but studies of the behavior of organisms can be equally valuable. Animal behavior is especially amenable to experimental testing of hypotheses about adaptation and, thus, provides an opportunity for students to learn basic principles of experimental design.

Here, we describe a simple study that we have found useful in introducing students to fundamental properties of well-designed experiments in the context of testing hypotheses about adaptation. We use the experiment as the first of a series of projects conducted in an undergraduate animal behavior laboratory class. The course is for upper-division students, and in addition to collecting data, our students also find and read relevant primary literature, enter and analyze the data, and prepare a lab report in journal-style format. These components of student participation could easily be adjusted so that the study could be conducted in introductory biology labs or advanced placement high school biology courses. The project addresses the content category "Science as Inquiry" of the *National Science Education Standards*, as well as the "Life Sciences" (Behavior of Organisms, Biological Evolution) and "History and Nature of Science" (Nature of Scientific Knowledge) categories (National Research Council, 1996).

> *This experiment tests one of the most basic hypotheses from optimal foraging theory: animal foraging behavior maximizes net energy gain per unit time.*

○ Background

Virtually any behavior can be used for testing hypotheses about adaptation, but foraging is especially convenient because animals do it frequently and because the costs and benefits of various options that a foraging animal might have can be quantified. The cost/benefit approach to the study of foraging behavior, known as "optimal foraging theory," was devised in the late 1960s through mid-1970s, and it has served to explain multiple aspects of the food-acquisition behavior of animals (for a thorough review, see Stephens & Krebs, 1986; for a discussion of the role of optimality models in studies of adaptation, see Parker & Maynard Smith, 1990). One general use of the theory is the generation of predictions about which types of food animals should include in their diets versus which should be ignored. One might ask, "If I offer an animal a choice of prey items, will it prefer those that are of highest nutritive value?" or "If I offer prey items that are time-consuming to handle (e.g., nuts in shells) and also prey items that can be readily consumed (nuts without shells), will the animals prefer the prey with lower time costs?"

An alternative application of the theory is to predict how long an individual should remain in a patch of food before leaving it to search elsewhere. If, for example, food patches are widely spaced (and so require a long time to get to), then one would generally predict that the amount of time spent foraging in each patch will be longer than if travel time between patches is very short. Or if environments vary from rich to poor, such that some habitats offer patches yielding high prey density and others have patches containing few prey, one would predict that animals in the rich environments should stay longer in each patch they visit. Ideas for laboratory exercises featuring the marginal value theorem (Wellborn, 2001), and predator decisions about which prey or patches to choose (Rop, 2001; Yahnke, 2006) have previously been offered in *The American Biology Teacher.* The experiment we describe here, like the projects described in Yahnke (2006), is a field study that we conduct on campus.

The American Biology Teacher, Vol. 73, No. 4, pages 238–241. ISSN 0002-7685, electronic ISSN 1938–4211. ©2011 by National Association of Biology Teachers. All rights reserved. Request permission to photocopy or reproduce article content at the University of California Press's Rights and Permissions Web site at *www.ucpressjournals.com/reprintinfo.asp.* DOI: 10.1525/abt.2011.73.4.10

This experiment tests one of the most basic hypotheses from optimal foraging theory: animal foraging behavior maximizes net energy gain per unit time. Students manipulate the nutritive value of food patches, and measure the time that individuals spend feeding at the patches. The prediction they test is that individuals will forage longer at patches that offer higher energy gains. "Patches" in the experiment consist of individual flowers, the foragers are nectar-feeding insects, and the energy gain available per patch is manipulated by adding sucrose solution to some flowers, water to others, and leaving some flowers unmanipulated. We have tried working with various insect/plant combinations since the project was first implemented 8 years ago; in recent years we have typically used *Lantana camara* flowers and skipper butterflies (Hesperiidae) for the project. We like this particular combination because butterflies in general have a low "creep-factor" for students, skippers are relatively abundant, and *L. camara* are commonly planted on our campus and are blooming well by late August, when we conduct the experiment. In addition, excellent background information is available on both butterfly foraging (e.g., Hainsworth et al., 1991; Goulson et al., 1997) and the volume and composition of *L. camara* nectar (Weiss, 1997).

○ The Experiment

As preparation, we have the students read the chapter on optimal foraging in their lecture textbook (Alcock, 2009). We also give them two handouts, one of which briefly summarizes optimal foraging theory and indicates the purpose and nature of the experiment. We explain that multiple factors could influence net energy gain per unit time, including the time or energy necessary to (1) locate, (2) collect or capture, and (3) consume the food, as well as (4) the quality or quantity (nutritive value) of the food. We indicate that they will be manipulating the last of these factors – nutritive value – and observing the effect of that manipulation on time spent feeding. The second handout provides instructions for data collection, along with a data sheet. Table 1 summarizes the experimental design.

○ Materials & Methods

We locate suitable plantings of (blooming) *Lantana* several days before the experiment, and verify that they are being visited by skipper butterflies during afternoon hours, when our class meets. The students work either in pairs or in groups of three for the project, with one person recording data and one or two others timing visits and/or watching for approaching butterflies. Each group is assigned to an approximately 2–3 m^2 cluster of plants. Various cultivars of *L. camara* differ in flower color, and if possible, it's probably a good idea to hold this constant. (On our campus, most plants have uniformly yellow flowers, so we usually use those.) Other flowering plants could be substituted for *Lantana*; the species simply needs to be attractive to skipper butterflies and have flowers that are sufficiently small and upright to hold a drop of sucrose or water.

Each group of students will need a stopwatch or other timing device (e.g., a cell phone with built-in stopwatch function), a permanent marker, a jar containing ~25 mL water, a jar containing ~25 mL sucrose solution, and two disposable 1-cc syringes (no needles). They also will need a pencil or pen for recording data and a data sheet consisting of three columns, one to record which treatment the visited flower had received, one for recording visit duration, and the last for noting general observations (e.g., interactions between the focal butterfly and other foragers or visible depletion of nectar); each row represents a visit.

Before the lab begins, we mix the sucrose solution, using one part table sugar to four parts water. This yields a concentration of 17.9% (w/w), which is within the range of the sucrose-equivalent of nectar from undepleted *L. camara* (Weiss, 1997). We distribute the sucrose solution and the water among the jars that the students will receive, and we label each jar lid with either a "1" or a "2" to identify its contents. We inform the students that they will be "blind" to experimental treatment during data collection.

Before leaving the classroom, we review the study's purpose and describe the physical appearance of both *L. camara* inflorescences and skipper butterflies, including a description of the proboscis. (Photographs of both *L. camara* and various skipper species are readily available on the Internet.) We explain to the students that they will compare the duration of butterfly visits to unmanipulated control, water-treated, and sucrose-treated flowers. We then go through the instruction handout. We have found it especially important to emphasize that everyone should use the same operational criterion when measuring visit duration, which should be timed as the number of seconds a butterfly's proboscis is in contact with the flower.

Table 1. Features of the project's experimental design and their consequences for the independent variable (IV: nutritive value) or dependent variable (DV: butterfly visit duration).

Feature	Effect
Sucrose treatment	Manipulates IV by increasing volume of available food
Water treatment	Manipulates IV by reducing concentration of naturally occurring nectar; controls for addition of colorless, odorless liquid to flower
Unmanipulated flowers	Holds IV at level equal to that of naturally occurring nectar
Having students blind to treatment	Controls for observer bias effects on DV
Restriction of treatment to central, fully open flowers	Reduces variation in DV due to flower location within inflorescence
Random distribution of treatments across plants	Reduces variation in DV due to location of inflorescence (e.g., proximity to observer)
Random order of sampling visits to unmanipulated flowers in relation to treated flowers	Reduces variation in DV due to changes across time
Operational definition of visit duration	Reduces variation in DV due to differences among observers; allows replication of study

Once each group of students has been assigned their *L. camara* plants, we usually find that the most common butterflies around are fiery skippers (*Hylephila phileus*), so we focus on them. The students verify that they can detect proboscis contact with a flower without getting so close to the butterfly that they disturb it, and they begin distributing the treatments. We suggest they start by adding one drop (~0.025 mL, which is vastly larger than the volume of nectar available from an unmanipulated flower) of one solution to each of 10 flowers, then dispense one drop of the other solution to each of 10 flowers, using a separate syringe for each solution. (They learn quickly that a drop added to a flower that is parallel to the ground simply rolls off!) The marker is used to label each experimentally treated inflorescence (or the stem, or a nearby leaf) as having received either a "1" (for solution 1) or "2" (for solution 2) treatment. Only one flower per inflorescence is treated. Because the nectar reward available from *L. camara* flowers is typically highest for newly opened, central flowers within an inflorescence (Weiss, 1997), we instruct the students to treat only flowers that are fully open and in the center of an inflorescence. We also ask that they distribute the two treatments relatively randomly across the cluster of *L. camara* plants.

After the treatments have been distributed (~15 minutes), the students begin collecting data on visit durations. Each time a butterfly visits a treated flower, they time the visit and record the treatment (1 or 2) and the visit duration. Visits to unmanipulated flowers will be far more common than visits to treated flowers, and not all of them need to be measured. For data on behavior occurring at unmanipulated control flowers, we ask the students to restrict their samples to visits at central, fully open flowers and to intersperse their samples of visit durations at unmanipulated flowers randomly in time among the sampling of behavior at treated flowers. However, some students inevitably become impatient with the infrequency of visits to treated flowers, as evidenced by data sheets showing a lengthy string of visit durations at unmanipulated controls followed by data for the rarer visits to treated flowers. We advise students to replenish any treated flowers that become depleted (using the same solution) and to treat additional flowers if visits are infrequent. We aim for samples of 25–50 visits per treatment; if 8–10 groups of students are collecting data, this takes about 1.5–2 hours, depending on butterfly density.

○ Results

Our students use SPSS software to obtain descriptive statistics on visit durations at unmanipulated, water-treated, and sucrose-treated flowers (Figure 1) and then to run a one-way analysis of variance (ANOVA) with pairwise comparisons. Our classes routinely find that visits at sucrose-treated flowers are substantially longer than visits at unmanipulated or water-treated flowers, and the one-way ANOVA results (comparing differences among the three treatments) also are routinely significant. However, the results from pairwise comparisons have varied across years (e.g., visits to water-treated flowers were significantly shorter than visits to unmanipulated flowers in one year).

The level of data analysis can be tailored according to student background. For introductory courses, instructors may wish to have their students simply use Excel to calculate the mean and standard deviation or 95% confidence interval of visit durations at the three types of flowers, and then to prepare a figure depicting the means for each treatment. Alternatively, for more advanced students, the project offers an opportunity to discuss the assumptions of ANOVA, whether the data should be transformed and, if so, which transformation to use, the role of planned versus post hoc comparisons, effect size, and power analyses. Few of our students have had a course in statistics, and nearly all are unfamiliar with SPSS when they conduct this project; consequently, we focus primarily on very basic statistical concepts, such as P values and variance.

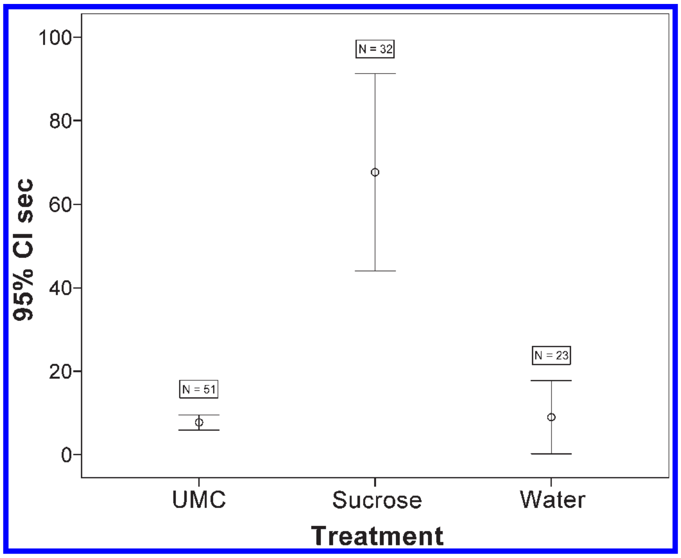

Figure 1. Visit durations (mean, 95% confidence intervals) at unmanipulated control flowers (UMC), sucrose-treated flowers, and water-treated flowers; sample results from fall 2009.

○ Discussion Questions & Ideas for Additional Activities

1. Were visit durations longer, on average, at flowers that offered sucrose than at flowers that offered water? Were they longer at sucrose- or water-treated flowers than at unmanipulated flowers?
2. What are water-treated flowers "controlling for"? What does the addition of water do to the concentration of nectar within the flower? Would you expect visits to be longer at water-treated flowers than at unmanipulated flowers?
3. If butterflies stayed longer at sucrose-treated flowers than at water-treated flowers, what would that imply with respect to our hypothesis? If they stayed equally long at sucrose- and water-treated flowers, what would be the implication?
4. Why is it good practice to have observers blind to treatment?
5. What is the advantage of operationally defining visit duration? What do you think the data would look like if different observers used different criteria for measuring visit durations?
6. Why do you think that visits at sucrose-treated flowers varied so much in length? What factors might affect how much food an individual consumes?
7. Why would it not be a good idea to collect all data from, say, unmanipulated flowers early in a day, and then collect all data from sucrose-treated flowers in the afternoon?
8. How else could you test this same hypothesis using skipper butterflies as your subjects? What would you manipulate, and what would be your prediction? (Two ideas from the 2009 class were to offer alternative sucrose concentrations or to compare visit durations at central and peripheral flowers. After reading Weiss [1997], students opted to do the second of these and developed operational criteria for "central" and "peripheral" flowers. They found that, as they had predicted, visits lasted longer at central flowers, but the difference was not significant; their follow-up discussion focused

on sample size and depletion of nectar within central flowers as possible limitations of the study.)

9. This experiment could easily be extended into investigations of plant–pollinator coevolution, thus addressing Life Sciences content standard "Interdependence of Organisms" (National Research Council, 1996). Some *L. camara* feature flowers of variable color; this variation represents an actual change in floral color across days and correlates with the nectar reward level of the flowers. Weiss (1991) provides a very interesting and readable account of floral color change and its effects on pollinator foraging behavior.
10. Opler et al. (2009) provide online species accounts and photographs of many North American butterflies, and students can search by state to identify the Hesperiidae species that are known to occur in their county and learn more about their life history.
11. If students in your class have had previous exposure to optimal foraging theory, they may assume that, because the question concerns how long to remain in a patch of food, this project tests the marginal value theorem. We usually point out that the marginal value theorem applies only if the return on time investment in the patch diminishes gradually across time (see Stephens & Krebs, 1986) and that the amount of nectar ingested through a proboscis across time probably does not meet this assumption. Similarly, students familiar with the basic prey-choice model may assume they are testing butterfly "preferences" for sucrose-treated flowers and that the number of visits each type of flower received is an indication of which was preferred (even though many more unmanipulated flowers are available than treated flowers). We typically ask them to consider whether their frequency of driving a particular vehicle, consuming particular soft drinks purchased from campus vending machines, etc., actually reflects their preference.
12. We postpone the students' involvement in research design until after they have collected data for this experiment, analyzed and interpreted the results, and completed background reading on foraging behavior of nectarivorous insects. Other instructors may choose to have their students participate in the design of this study, in which case we suggest that the class first spend some time observing butterflies foraging and derive hypotheses for why variation exists in which flowers the butterflies visit and the amount of time they spend feeding on them.

○ Summary

Judging from a pretest we gave our students on the first day of class this past fall, only about half of them are able to distinguish between hypotheses and predictions when they enter our course (see McPherson, 2001). During our follow-up discussion of this project, we have been especially pleased with the ideas they generate for testing the same hypothesis in a different way (question 8, above) and their ability to derive predictions from the hypothesis; for the past 3 years, we have had them discuss the merits of each candidate idea, and then select one to implement the following week. It seems unlikely, also, that many students previously have thought much about experimental design, but participation in the study encourages them to consider how aspects of a study's design can affect the results they obtain. Indeed, in their lab reports on the project, we often have students point out that some of the variation in their visit duration data probably stemmed from our lack of control over the feeding history of individuals, windy conditions, interference by foraging bees, the presence of a courting male, and so on. One might debate whether such comments merit inclusion in their papers when the results support the hypothesis; nevertheless, they reveal an appreciation for the general value of standardizing factors that introduce variation in the dependent variable. The comments also demonstrate that simply by spending a relatively short time actually watching animals, students observe first-hand some of the impediments faced by foraging organisms (see Krupa, 2000) and, consequently, develop an understanding of potential constraints on behavioral adaptations.

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