INQUIRY & INVESTIGATION

Acorn Caching in Tree Squirrels: Teaching Hypothesis Testing in the Park

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Abstract

We developed an exercise for a university-level ecology class that teaches hypothesis testing by examining acorn preferences and caching behavior of tree squirrels (Sciurus *spp.). This exercise is easily modified to teach concepts of behavioral ecology for earlier grades, particularly high school, and provides students with a theoretical basis for examining commonly observed squirrel behavior. Students gain experience in testing hypotheses and revising predictions. They evaluate how well predictions of competing hypotheses are supported by statistically analyzing and interpreting class data using t-tests and chi-square tests.*

Key Words: *Behavioral ecology; ecology lab exercise; plant–animal interactions.*

Developing effective exercises for ecology courses is challenging, given time and fiscal constraints and scarcity of nearby habitats. Ideally, exercises would develop critical-thinking skills by introducing students to a scientific topic through hypothesis testing, data collection, data analysis, and interpretation. If exercises were linked to existing research, students could explore the topic in more depth using library databases. Here, we describe a laboratory exercise on acorn caching by squirrels that was originally developed for a junior-level general ecology class at the University of Illinois Springfield. Learning objectives are related to hypothesis testing, with students developing predictions based on existing hypotheses regarding squirrel behavior. Our exercise makes use of research that documented differences in tree squirrel (genus *Sciurus*) foraging in response to acorn characteristics (Steele et al., 2001; Steele & Smallwood, 2002; Steele, 2008).

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Not all acorns are alike, and tree squirrels respond to the differences in their feeding and food-storing behavior. Acorns of different oak species (genus *Quercus*) have different concentrations of fat and tannin and differences in germination schedules (Table 1; Smallwood et al., 2001). Broadly, acorns from the white oak (WO) subsection (including white oak, *Quercus alba*) have low fat, low tannins, and germinate when acorns ripen in the fall. By contrast, acorns from the red oak (RO) subsection (including northern red oak, *Quercus rubra*) have high fat, high tannins, and germinate in the spring following fall maturation. Tree squirrels preferentially cache (bury) acorns of the red oak group and eat acorns from the white oak group (Steele et al., 2001). One hypothesis explaining this behavior is the perishability hypothesis, which predicts that squirrels will eat perishable food items and cache hardier food items (Hadj-Chikh et al., 1996). WO acorns are a short-lived food resource because they germinate in the fall and can become seedlings before squirrels can recover the buried acorn. An alternative hypothesis explaining squirrel behavior is the handling-time hypothesis (Jacobs, 1992), which predicts that squirrels will cache acorns with long handling times and eat acorns with short handling times. It is proposed that squirrels are constrained by the time they have to cache acorns in the autumn and should, therefore, bury as many as possible and spend less time eating.

In this exercise, students test these hypotheses in a city park. They present acorns of two species, one from each group, to either fox squirrels (*Sciurus niger*) or eastern gray squirrels (*S. carolinensis*), then record squirrel behavior. Damaged and undamaged acorns are also presented to squirrels, allowing students to test multiple predictions of the perishability hypothesis. Specifically, they can test (1) that damaged acorns will be preferentially eaten and undamaged ones cached and (2) that WO will be eaten more than RO. In a 40-student class, enough data are typically generated in two 3-hour lab periods to have a subsequent lab on data analysis and interpretation. Some students also create an oral presentation in which they discuss results in the context of the primary literature. One question that inevitably arises is how such behavior could have evolved. This exercise therefore

The American Biology Teacher, Vol. 74, No. 7, pages 509–512. ISSN 0002-7685, electronic ISSN 1938-4211.
DOI: 10.1525/abt.2012.74.7.14

Table 1. Comparison of acorn properties in white oak versus red oak acorns.

Property	White Oak	Red Oak
Germination[a]	Fall of ripening	Spring following winter dormancy
Tannins[a]	Low	High
Lipids[a]	Low	High
Caching rates	Low	High
Eating rates	High	Low

[a]Modified from Smallwood et al. (2001) and Steele et al. (2001). See table 1 in Smallwood et al. (2001) for primary references and ranges of tannin and lipid concentrations. Also, see Vander Wall (2001) for detailed attributes of acorns of individual oak species.

provides a field and computer experience that could be followed by the role-playing exercise developed by Riechert et al. (2011) on evolution of squirrel caching behavior.

○ Classroom Preparation

Before the lab, students receive a lecture on animal behavior that discusses how ecologists develop hypotheses to predict behavior using evolutionary theory (Krebs & Davies, 1991). Animals should behave so as to maximize their fitness (i.e., maximize their survival as well as offspring production and survival). Optimal foraging theory is also reviewed. In its simplest form, this theory predicts that animals should forage to maximize the energy (calories) they ingest while minimizing time, risk, and calories expended (Krebs & Davies, 1991).

Once students understand the context in which animal behavior is studied, we cover the unique environment that tree squirrels (genus *Sciurus*) have evolved in and the constraints this places on foraging behavior. Squirrels not only have a variety of food items to choose from when making foraging decisions; they also can either store a food item for winter or immediately consume it. Optimal foraging theory predicts that squirrels should respond to food items differently if they differ in their characteristics. This is where broad differences found among acorn types can help students in understanding hypotheses and their associated predictions.

The perishability and handling-time hypotheses are then discussed with the students, and as a class we develop predictions based on them. Because acorns in the RO group (subsection *Lobatae*) have higher fat than those in the WO group (subsection *Quercus*; Nixon, 1993), they provide more calories for squirrels and are a higher-valued food item. One prediction that students test is whether squirrels invest more time in burying RO acorns and whether RO acorns are cached at greater distances from their source. Because ROs also have a delayed spring germination schedule, an RO acorn buried in autumn will not become a seedling until the following spring. A squirrel can therefore use this food item throughout the winter. By contrast, WOs germinate in the autumn the acorns mature. If buried, they can germinate and develop a long tap root, changing the seed into a less desirable food item (seedling). The perishability hypothesis therefore predicts that squirrels will cache RO acorns, which will last through winter, and eat WO acorns, which will not. The handling-time hypothesis predicts that squirrels will cache whichever acorn has a longer handling time. Students therefore measure acorn handling times in the field to determine the caching predictions under this second hypothesis.

Students can also test a second prediction under the perishability hypothesis by collecting data on acorn damage. Some acorns have shell damage or evidence of infestation by acorn weevils (small round exit hole; *Curculio* spp.) Some weevil larvae will typically emerge from collected acorns prior to the lab, and these larvae are shown to students when discussing factors that alter food perishability. It has been shown that squirrels prefer weevil-infested acorns (along with the weevil) over sound (non-infested) acorns even when there is no obvious external evidence of infestation (Steele et al., 1996).

Students should be able to reevaluate predictions on the basis of new evidence. Scientists do this as information shifts in their fields of study. This experience is simulated by withholding information about a crucial squirrel behavior until after students have collected their data. Students are prompted during data collection to watch squirrels closely because they may observe a behavior that modifies the predictions for one of the hypotheses. The behavior we are secretive about is embryo excision. Squirrels use their incisors to excise embryos from the apical tip of WO acorns prior to caching (Fox, 1982; Pigott et al., 1991). This kills the acorn and therefore shifts a perishable food source (sound WO acorn) into a nonperishable food source (dead acorn).

The following initial predictions are discussed with students prior to lab:

(1) *Perishability Hypothesis Predictions:* Squirrels will cache nonperishable food items (RO or undamaged) and eat perishable (WO or damaged).

(2) *Optimal Foraging Prediction:* Squirrels will take longer to cache RO acorns and will cache them at greater distances because of their higher lipid content.

(3) *Handling-Time Hypothesis Prediction:* Squirrels will cache species with longer handling times and eat species with shorter handling times.

○ Learning Objectives

This exercise combines a field lab (data collection) with a computer lab (data analysis) and a final oral presentation. Students involved in all three sections should be able to

(1) describe differences between acorn types within *Quercus*,

(2) describe squirrel foraging behavior in the context of evolutionary theory,

(3) explain the perishability and handling-time hypotheses,

(4) develop predictions under these hypotheses for a specific species and its environment,

(5) reevaluate predictions when given new information,

(6) test predictions using data obtained in the field using standard statistical tests,

(7) evaluate whether results of statistical tests support or refute hypotheses,

(8) develop logical explanations to explain results, and

(9) present results and discuss them in the context of the primary literature.

○ Field & Computer Labs

Field Lab. For the field exercise, students are divided into groups of three to four. The following materials are provided to each group:

(1) Two labeled paper bags with 20 acorns each, one containing WO acorns and the other RO acorns. (Fresh WO and RO acorns should be collected each year. We try to use *Q. alba* and *Q. rubra* if possible. These can be stored in a refrigerator prior to use.)

(2) Binoculars

(3) Stopwatch (can substitute a watch second hand)

(4) Meter tape (optional)

(5) Data sheet with spaces to record the following: squirrel species, acorn type (WO/RO), whether acorn was damaged, type of damage, whether acorn was eaten or cached, time spent eating (handling time), time spent caching, caching distance, and additional observations.

The lab is conducted in the fall in an urban park where squirrels are abundant (Washington Park, Springfield, IL). Identifying characteristics of fox squirrels (orange fur on belly and underside of tail) and gray squirrels (white fur on belly and tail) are reviewed. Over 7 years of implementing this lab and another 25 conducting similar research with undergraduates, there has never been a negative interaction between students and squirrels. Acorns are tossed to the squirrels from a considerable distance (typically >3 m), minimizing risk. For safety reasons, instructors should never let students hand-feed squirrels.

Students are then given the following instructions:

(1) Approach a squirrel *slowly*. Get close enough to toss an acorn gently in front of the squirrel. You can get the squirrel's attention by making a clicking sound and showing the acorn. Toss the acorn in front of the squirrel. Start with either acorn type, but be sure to change acorn type on each subsequent toss. Make sure that you have recorded acorn data *before* throwing the acorn!

(2) Record the following: whether the acorn is eaten or cached, time spent eating the acorn, time spent caching the acorn, distance to cache from the site where the acorn was presented, and any interesting behavior.

(3) Continue to present acorns, alternating acorn type and recording until you have filled your data sheet. Make sure to record which data were collected for the same squirrel. Multiple data points from the same squirrel are okay.

Over two 3-hour lab sessions, 40 students typically generate ~80 data points. The data are compiled in Excel for analysis in the following lab.

Computer Lab (Data Analysis). Using Excel and a handout, students perform statistical tests and answer a number of questions regarding interpretation of the data (Table 2). The tests used are the chi-square test of independence (caching frequencies) and Student's t-test (handling times and dispersal distances). We discuss these tests with the students and, in particular, emphasize how to interpret P values. Students use an alpha value of 0.05 when deciding whether differences are found in their data.

Students first analyze frequency data for the two oak species, constructing 2 × 2 tables of the number of eaten and cached acorns for each species. In addition, they construct 2 × 2 tables of damaged and undamaged acorns, scoring whether the acorns were eaten or cached. These frequency data are then analyzed in Excel using the chi-square test of independence. Although results have varied between years, we typically find no difference in rates of eating and caching between the oak groups, RO vs. WO ($P > 0.05$). However, damaged acorns tend to be eaten rather than cached more frequently than undamaged acorns ($P < 0.05$). We also typically find that squirrels take longer to cache RO acorns than WO and that RO acorns are moved farther than WO when cached ($P < 0.05$). Students write interpretations of the results and reflect on whether the results are consistent with the initial predictions (Table 2). They are also asked to explain why the results might differ from initial predictions. We expect them to reflect on how embryo excision alters predictions for the perishability hypothesis. Students need to determine that the lack of difference in caching rates between RO and WO may still be consistent with the perishability hypothesis if squirrels can make a perishable food source (germinating WO acorns) nonperishable via embryo excision.

Alternative Simplified Approach. This lab can be modified if supplies and equipment listed above are not available. The simplest version would require only the two acorn types. Students could observe squirrel behavior with the naked eye and record time with watches or cell phones. Distances could be estimated using pacing, or the portion of the lab that requires measurement of distances could be dropped (questions 12–14 from Table 2).

○ Assessment & Response

This lab achieves the desired learning objectives listed above. Scoring of lab reports for fall 2011 showed that 100% of students met learning objectives 1, 7, and 8; 87% met learning objective 3; and 93% met learning objectives 4 and 6. Student response to this lab has been overwhelmingly positive. In anonymous evaluations for fall 2010, 96% of the students rated this lab as "definitely keep." This exercise fosters critical-thinking skills in students by simulating the scientific process, including reworking predictions in light of new data. Follow-up activities can include oral presentations of class results presented in the context of a primary literature review and/or role-playing exercises in which students learn how natural selection could produce some of the complex squirrel behavior observed (Riechert et al., 2011). Copies of handouts and data files that we use for the lab can be obtained by contacting the lead author.

○ Acknowledgments

We thank University of Illinois Springfield (UIS), Wilkes University, and the Howard Hughes Medical Institute for financial support. Special thanks to BIO 371 students at UIS.

Table 2. Questions from data analysis lab.

Data	Test Performed	Question
2 × 2 frequencies: Number cached vs. number eaten by acorn type	Chi-square test of independence	(1) Do squirrels cache red oak acorns more than white oak? Write your conclusion, justifying it with the P value. (2) Does your result support our prediction under the perishability hypothesis that RO would be cached more than WO? Why or why not? (3) What squirrel behavior alters our initial prediction? Explain why this behavior alters the prediction.
2 × 2 frequencies: Number cached vs. number eaten by damaged/undamaged status	Chi-square test of independence	(4) Do damaged acorns differ from undamaged in the frequency with which they are eaten vs. cached? Use P value to decide. (5) Is this result consistent with the perishability hypothesis? Explain why or why not.
Handling time for eaten acorns by acorn type	Student's t-test	(6) What are the average handling times (minutes) for white oak vs. red oak? (7) Is there a significant difference in handling time between acorn types? Use P value to decide. (8) Does the handling-time hypothesis offer an alternative explanation that explains the red and white oak caching rates we observed? Why or why not?
Caching time data for cached acorns by acorn type	Student's t-test	(9) What are the average caching times (in minutes) for white oak vs. red oak? (10) Is there a significant difference in caching times between white and red oak acorns? Use P value to decide. (11) What might explain any difference you find in caching time between acorn types?
Caching distance for cached acorns by acorn type	Student's t-test	(12) What are the average caching distances (in meters) for white oak vs. red oak? (13) Is there a significant difference in caching distance between white and red oak acorns? Use P value to decide. (14) Are caching time and distance results consistent with our original predictions? What might explain any inconsistent results?

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