Tested Studies for Laboratory Teaching
Proceedings of the Association for Biology Laboratory Education
Vol. 34, 409-417, 2013

Predator Sense and Prey Defense: A Lab Exercise in Evolutionary Hypothesis Formulation and Experimentation

Cynthia Bennington, Rachel Burnett, and John Jett

Stetson University, Department of Biology,Campus Box 8264, DeLand FL 32723 USA
(**cbenning@stetson.edu; rburnett@stetson.edu; jjett@stetson.edu**)

This open-ended laboratory exercise was designed to improve students' ability to formulate and test evolutionary hypotheses. Presented over three weeks, students first read a short scientific paper that described the results of a prior experiment conducted by the instructor(s) designed to investigate the evolution of distastefulness in prey species, then developed a testable prediction related to the evolution of distastefulness that could be answered using domestic crickets and field-caught wolf spiders. Laboratory exercises related to understanding the power of natural selection are often limited to physical or computer simulations. Our open-ended approach, which allows students to create phenotypic variation (i.e., manipulate cricket palatability through the application of distasteful substances) and to measure fitness (i.e., cricket survival in the presence of a generalist predator) provides students with a dynamic illustration of selection using real organisms.

Keywords: *Hogna* spp., natural selection, evolution, hypothesis formulation

Link to Original Poster

http://www.ableweb.org/volumes/vol-34/poster?art=45

Introduction

Evolution is a core concept in modern biology, but laboratory exercises illustrating evolutionary mechanisms rarely use macroscopic organisms in realistic settings. Perhaps most commonly, physical simulations with artificial "prey" phenotypes (e.g., different colors of bits of yarn; different sizes of beans) and student "predators" are used to demonstrate the evolution of yarn color or bean size over several rounds of "natural selection". While physical, as well as computer, simulations can be powerful tools for demonstrating change over time within a single class period, both types of exercise require students to make the connection between yarn or avatars and living organisms. The laboratory exercise we describe here uses macroscopic, dynamic organisms to illustrate the effect of organismal phenotype on individual survival, allowing students to test an evolutionary hypothesis related to the adaptive significance of a trait.

Most of the undergraduate students we teach have at least some misconceptions about evolution when they enter our course, and many have difficulty applying their knowledge of science to the topic of evolution. For this reason, we developed an exercise to improve student understanding of both the mechanism of natural selection and the formulation of scientific hypotheses related to evolution. In order to emphasize the process of science, we adopted a technique described by Witzig et al (2010), where we provide students with a mini-journal article (Appendix A) describing a simple experiment, conducted by us, that tested the hypothesis that crickets (*Acheta domesticus*) dipped in 5 mM quinine would have a bitter taste and would thus have higher survival than those dipped in water when they were each exposed to wolf spiders (*Hogna lenta*). The article introduces students to the basic experimental system and methodology that will provide the framework for their own experiment, supplies references to other articles that have addressed evolutionary questions, and presents students with a model for good scientific writing. In addition, the Discussion section of the article explicitly describes limitations and potential extensions of our results. After reading the paper, we allow each group of three to four students in the classroom to suggest a new hypothesis, related to the evolution of prey distastefulness, that they present orally to the class. All 16 – 20 students in the laboratory then discuss possible hypotheses and decide upon one that they will test the following week.

As described here, this experiment is best suited for second-year undergraduates who have had some experience with open-ended investigations. It could easily be modified to give students more or less agency in the experimental design, if appropriate. In addition, while we spread this lab exercise over two weeks, with some time for data analysis in a third week, it could be condensed, again depending on the degree to which students are responsible for generating their own hypothesis and experimental design. The instructor preparation for the lab depends in part on the experiment the students choose to conduct. We require students to collect the wolf spiders they will use, though these could be ordered from a biological supply company.

Student Outline

Learning Objectives

Successful students will be able to:
- Formulate testable scientific hypotheses
- Synthesize information from a journal article to develop unique research questions
- Work collaboratively to design and conduct an experiment
- Use and/or create phenotypic variability in live organisms to address an evolutionary hypothesis
- Use standard statistical techniques to analyze data
- Summarize data graphically

Week 1 Procedure

Evolutionary hypotheses formulation and experimental design

Before coming to lab this week, please carefully read the mini-journal article written by your instructors. Next week, you will be conducting an experiment using the experimental system described in the paper.

Introduction

Much of your biology education up to this point has been centered on understanding "how" biological systems work. "How" questions seek *proximate* causation, or those factors that are immediately responsible for some natural phenomenon. Evolutionary biologists typically ask "why" questions, which address *ultimate* causation, seeking to find evolutionary explanations that come from thousands of generations of natural selection (Mayr 1961).

Let's use a simple observation about a living organism to consider the difference between "how" and "why" questions.

Observation: Cheetahs can run faster over short distances than any other mammal.

How Question: How do cheetahs run so fast?
Possible answer: Compared to other cats, cheetahs have a relatively lightweight body, large nostrils (for easy breathing) and long legs. They also have a very long tail, which improves balance when running.

Why Question: Why do cheetahs run so fast?
Possible Answer: Cheetahs live in the open savanna where there are many large herbivores and few places to hide. Unlike some other big cats, cheetahs do not hunt in packs, so the ability to quickly stalk and chase prey has been favored by natural selection, and cheetahs have evolved into very fast runners.

With your lab partners, make an observation about some natural phenomenon (it could be anything). Then, create a "why" and a "how" question related to the phenomenon.

Observation:

Questions:

Both "how" or "why" questions, can be converted to scientific hypotheses that can be subjected to experimentation. Remember that scientific hypotheses must be **testable** (i.e., it must be possible to design a test of the hypothesis that will produce a measurable outcome) and **falsifiable** (i.e., if it is not possible to design a test that would potentially disprove a hypothesis, then the hypothesis is not a scientific one). In science, big ideas are typically tested by specific examples. For example, one big question might be, "Why are the males of many bird species often very showy?" A hypothesis could be created to address a specific example.

Is this hypothesis both testable and falsifiable?

Hypothesis: Peacocks have showy tail feathers to attract peahens.

A good scientific hypothesis can generally be used to generate predictions that, if true, support the hypothesis ("IF…THEN" statements).

IF peacocks have showy tail feathers to attract peahens, THEN peacocks with the largest, showiest tails should be more attractive to females than those with smaller, duller tails.

Which of the following are good scientific hypotheses (i.e., they are both testable and falsifiable)? Can you make bad ones better? For at least two of these hypotheses, make an IF…THEN prediction (you may need to first narrow the scope of the hypothesis).

- There are other planets in our universe that have life forms with DNA.
- Living near a nuclear facility increases the risk of developing cancer.
- Our universe is surrounded, and influenced by, a larger universe with which we will never have contact.
- The shape and smell of the flowers of the orchid *Ophrys bombyliflora* have evolved in response to selection from bumblebee pollinators.
- Rodents can be taught to use tools.
- The bacteria *Staphylococcus aureus* can evolve resistance to the antibiotic penicillin.
- Rats are stupid.

Now that you are familiar with hypothesis formulation, and you have some knowledge of the evolution of distastefulness in prey based on your reading of the mini-journal article you are ready to formulate your own scientific question. In groups of three or four, formulate one testable, falsifiable IF…THEN prediction that addresses some aspect of prey distastefulness and that can be tested using wolf spiders and crickets.

Once the entire class has agreed on an experimental question, we will arrive at an appropriate experimental design by considering the following questions:

1. How many replicates will we have?
2. How many treatments will we have? What is our control?
3. How will crickets be introduced to spiders? What will the experimental arena look like?
4. What will we measure? (We might measure the time it takes for a spider to touch a cricket, strike a cricket or consume a cricket. We will certainly want to measure cricket survival.)
5. How will we handle the situation of a spider that does not eat?
6. How will we control for variability in the size of crickets and spiders?
7. If we are artificially manipulating cricket phenotype, how will we do so?
8. How will we analyze the data?

Week 2 Procedure

Today you will conduct your experiment to quantify the strength of natural selection on distastefulness in crickets. Before beginning, create a spreadsheet that you will use to collect data. You may also want to assign certain tasks to individual students.

Notes for the Instructor

Week 1

If students are collecting their own wolf spiders, they will need to start the collection process two or three weeks prior to the lab. They should house them individually in containers with a damp cotton ball. Students tend to worry that the spiders will starve if they don't feed them, but housed at room temperature with access to water, spiders can live for weeks without food.

We have each group of students present a hypothesis to the entire class, with the whole class eventually agreeing on one experiment to perform. We do this mainly to ensure that the sample size for the experiment is large enough to be meaningful, but it is certainly possible to have each group conduct separate experiments.

Even though the experiment is not conducted in the first week of the lab, it is a good idea to have several wolf spiders, crickets, and testing arenas available so that the students will have a good understanding of the basic parameters to which they must adhere. Demonstrating the introduction of a cricket to a wolf spider during this lab period tends to get students interested in the exercise and shows them what to expect in their own experiment.

Week 2

The necessary materials and general guidelines for the experiment are given in the Methods section of our mini-journal article (Appendix A). This article could be modified or used as is to suit the instructor's needs. We encourage instructors to use the model of Witzig et al. (2010) to conduct and write up their own experiment, as we found it to be a very useful teaching tool.

It works well to allow students to identify with particular jobs that need to be done during the experiment. Some students will not want to handle spiders or crickets, but can be assigned jobs of timing spider-cricket interactions, or compiling data as it is being collected. It is important to ensure that all students understand the jobs being done by everyone else and that, when more than one student is doing the same job, that they do it in the same way. There is likely to be a little classroom chaos, but the chaos forces students to take responsibility for the quality of their data collection. Mistakes will be made, but these can be used as opportunities to teach the importance of details in the scientific process.

Once students have completed their experimentation and the data are entered on a computer in a spreadsheet, we project the data to the front of the classroom and go through each row to make certain that there are no mistakes. If time allows, the data could be analyzed then. We have found, however, that it works well to take 30 minutes or so in the following lab period to perform data analysis.

Week 3

Data analysis

We typically walk students through the data analysis and then allow them time to create graphs and/or tables that they will use to summarize the data in their lab report. In our course, we build up to a major lab report at the end of the semester. Because this lab occurs in the first half of the semester, we have students write an abbreviated lab report for this assignment. The report includes: Title, Hypothesis statement, Methods (detailed), Results (detailed, including both text and figures/tables), and Conclusion.

Acknowledgments

We are grateful to Dr. Paul Andreadis for suggesting the use of wolf spiders as predators in this experiment, to Dr. Terry Farrell for constructive comments on the implementation of the lab, and to Dr. Peter May for helpful comments on the mini-journal article and for photographs of spiders and crickets.

Literature Cited

Mayr, E. 1961. Cause and effect in biology: Kinds of causes, predictability, and teleology are viewed by a practicing biologist. *Science*, 134: 1501-1506.

Witzig, S. B., N. Zhao, S. K. Abell, J. C. Weaver, J. E. Adams, and F. J. Schmidt. 2010. Achievable Inquiry in the college laboratory: The mini-journal. *Journal of College Science Teaching*, 39:14-23.

Appendix

Bitter taste does not increase survival of crickets exposed to predatory wolf spiders (*Hogna lenta*)

by C. C. Bennington, R. Burnett, and J. Jett

Abstract

Prey species can defend themselves from predation by producing toxic or unpalatable substances. Predators that can recognize and avoid such chemically-defended prey will have higher fitness than those that cannot. We hypothesized that crickets with a bitter taste (due to the topical application of quinine) would have higher survival than non-treated crickets when they were individually exposed to a common predatory wolf spider (*Hogna lenta*). We found large amounts of variability in spider willingness to eat and no significant difference in survival between distasteful and palatable crickets. We conclude that the bitterness of the quinine was not sufficient to enhance cricket survival when individual spiders were not given a choice of prey or a range of quinine concentrations from which to choose.

Introduction

Insects have evolved a diverse array of chemical defenses to provide protection against predation (see review in Laurent et al. 2005). Fisher (1930) suggested that the evolution of such “nauseous flavours” could not be explained by natural selection on individuals because predators are only aware of the trait if they eat (and hence kill) the prey item. He therefore suggested that kin selection was responsible for the evolution of distastefulness, since one distasteful individual might be killed, but related individuals that live in close proximity, and that carry the distasteful trait, are spared because the predator has learned to avoid them. Kin selection need not be invoked, however, if a predator is capable of perceiving the threat before killing the prey, and hence individual fitness is increased by distastefulness. There is some evidence, for example, that predators instinctively avoid certain color patterns that are associated with prey toxicity (Smith 1975; Lindstrom et al. 1999) and there is significant experimental data supporting the idea that predators learn to avoid distasteful prey more quickly if they have conspicuous color patterns (e.g., Gittleman and Harvey 1980; Sillen-Tullberg 1985). Such experiments provide an adaptive explanation for the evolution of aposematism, where prey are both noxious and brightly colored (Guilford 1990).

While aposematic coloration is widespread among invertebrates (Bowers 1992), there are some examples of distasteful, but cryptically-colored prey species (Joron 2003). Given that not all predators use color vision to detect feeding cues, cryptically-colored prey species that use chemical, rather than visual, cues to advertise their toxicity may have increased fitness. For example, Terrick et al. (1995) found that garter snakes used both visual and chemosensory information to learn to avoid unpalatable fish. Like snakes, invertebrate predators also rely on chemical cues to detect prey (Bristowe 1958). Most spiders, which are ubiquitous predators of insects, have relatively poor vision despite having up to four pairs of eyes (Savory 1964). Spider leg hairs, or sensilla, however, can function as chemoreceptors, to detect chemical cues from prey (see review in Toft 1999).

Numerous spider species have been demonstrated to use chemical cues to find mates (Willey and Jackson 1993; Roberts and Uetz 2005), to recognize prey (Persons and Uetz 1996) and to avoid predation (Persons et al. 2001). Few studies, however, have tested whether spiders can use chemical cues to avoid distasteful prey. In one notable study of a web-building spider (*Araneus diadematus*), Holden (1977) found that spiders avoided bitter-tasting quinine-treated flies and, with repeated exposure to the quinine flies, took longer to respond to prey in their web.

We used the predatory Florida wolf spider (*Hogna lenta*) as an agent of natural selection to ask whether a distasteful (i.e., bitter), but cryptically-colored, prey item would have higher rates of survival than a more palatable prey when exposed to spiders.

Methods

We caught 20 wolf spiders (*Hogna lenta*) in a residential yard in DeLand, FL between Jan. 16 -19, 2012. Captured spiders were kept individually in plastic containers with a moist piece of cotton at room temperature (approximately 21^{o}C) in the lab. We used crickets (*Acheta domesticus*), purchased from a local bait shop as our prey species.

Experimental trials were conducted on Jan. 27, 2012. Of the 20 spiders captured, the 13 largest ones ($\bar{x}$ = 0.59 g + 0.25 SD) were used in the experiment. We individually placed spiders into clear, open-topped Pyrex® bowls (6.5cm tall and 19.5cm in diameter) on lab benches, and allowed them to become acclimated to the new environment for more than five minutes. Because spiders are sensitive to vibration (Savory 1964) we avoided all contact with the bowls and lab benches during the acclimation period and feeding trial.

We also selected 13 crickets ($\bar{x}$ = 0.47 g + 0.12 SD) for use in the experiment. We created six distasteful crickets by spraying each with a 5 mM solution of quinine hydrochloride. Another seven crickets were sprayed with tap water to serve as a control. Crickets were temporarily housed individually in plastic petri dishes (5.5 cm diameter), where they remained until they were offered to a spider.

At the end of the acclimation period, we dropped a single cricket directly over the center of a bowl containing a spider. We again avoided touching the bowl or lab bench while we observed the spider from a distance of 1 m while waiting for it to choose a cricket to eat. We recorded the time (in seconds) until the spider made initial contact between the spider's legs and the cricket as well as the time until first strike, when the spiders' fangs made contact with the cricket. We measured survival as the ability of a cricket to successfully avoid attack after 5 minutes in the arena.

We performed two separate t-tests to compare time to first contact and time to first strike between control and quinine groups. To ask whether survival was higher for crickets sprayed with quinine, we used a chi-square test with the null expectation of no survival difference between the two groups.

Results

There was no significant difference in time to first contact (Fig. 1) or time to first strike (Fig. 2) between crickets in the water and quinine treatment groups. Although four of the six crickets treated with quinine survived exposure to a spider and two of seven control crickets survived exposure to a spider, there was not a statistically significant effect of treatment on survival χ^2 = 1.931, p = 0.1647).

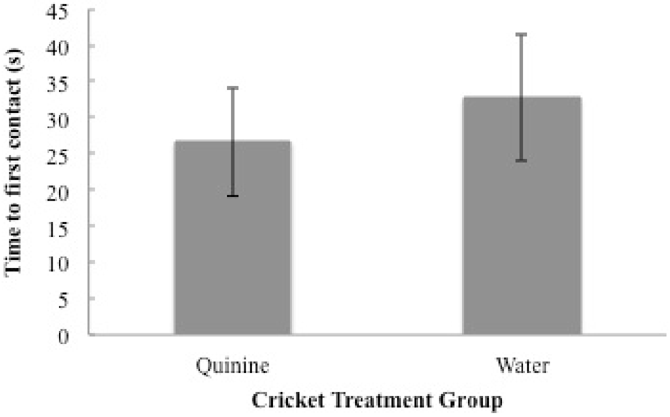

Figure 1. Time to first contact between wolf spiders and crickets treated with water and quinine. No statistically significant difference was observed between the two treatment groups (t = 0.52; p = 0.6151). Error bars represent ±1 standard error.

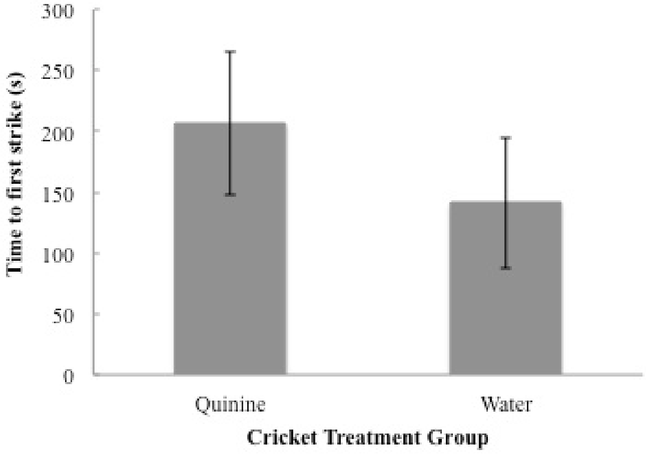

Figure 2. Time to first strike of wolf spiders on crickets treated with water and quinine (t = 0.82; p = 0.4303). Error bars represent ± 1 standard error.

Discussion

Our results do not support the hypothesis that cryptically-colored distasteful prey have higher survival than more palatable prey when exposed to wolf spiders. Spiders did not avoid contacting, attacking, or eating quinine-treated crickets. In general, there was large variability in feeding behavior among the 13 spiders, much of which had no apparent relationship to any attribute of the crickets.

One possible source of this variability is the prior experience of the spiders in the experiment. Our spiders were captured and then maintained indoors at room temperature for one week, but we have no way of knowing the prey types they had previously experienced or when they ate their last meal prior to capture. Since wolf spiders have been known to survive without food for more than six months (Anderson 1974), a one-week starvation period may have been insufficient to create hunger if a spider was satiated just prior to capture. Reducing this source of variability in a future experiment would improve our ability to detect differential survival of palatable and distasteful crickets.

An alternative explanation for the lack of cricket survival differences is that the bitter taste of quinine did not "taste" bad to spiders. This seems unlikely, however, given that bitter tastes are ubiquitous in nature. In addition, there are ample data to suggest that birds (Skelhorn and Rowe 2009), reptiles (Stanger-Hall et al. 2001) and mammals (Biondolillo et al. 2009) have taste aversions to bitter tastes such as quinine. The only other study we are aware of that has investigated the response of a spider to the bitter taste of quinine is by Holden (1977) who found that the web-building spider (*Araneus diadematus*) rejected flies that were ground up and mixed with quinine. Unfortunately, the author did not report the concentration of quinine used, so we cannot compare her experiment to ours. It is possible that, if wolf spiders frequently encounter bitter prey in their natural diet, they may have a threshold for bitter taste that exceeds the concentration used in this experiment (Glendenning 1994).

The evolution of predator-prey relationships in natural communities is affected by the ability of predators to discriminate among appropriate and inappropriate food sources. In natural arthropod communities, the relative fitness of both predator (e.g., spider) and prey (e.g., cricket) depends in part upon the tastefulness of the prey and the ability of the predator to detect the taste prior to killing the prey. Although our experiment did not allow spiders to choose between prey items, wolf spiders are generalist predators (Nentwig 1986), and, in natural settings, must make prey choices that can be affected by prey behavior (Persons and Uetz 1997) and nutritional quality (Toft 1999; Mayntz et al. 2005) as well as palatability. The choices made by predatory invertebrates affect not only the selective environment of the prey, but can also shape the structure of the macroinvertebrate community.

Literature Cited

Anderson, J. F. 1974. Responses to starvation in the spiders *Lycosa lenta* (Hentz) and *Filistata hibernalis* (Hentz). *Ecology,* 55: 576-585.

Biondolillo, J. W., L. A. Williams, M. S. King. 2009. Blocking glutamate receptors in the waist area of the parabrachial nucleus decreases taste reactivity behaviors in conscious rats. *Chemical Senses*, 34:221-230.

Bowers, M. D. 1992. The evolution of unpalatability and the cost of chemical defense in insects. Pages 216-244, in: *Insect chemical ecology: an evolutionary approach*, Roitber, B. D., Isman, M.B., (editors). New York: Chapman and Hall.

Fisher, R. A. 1930. The genetical theory of natural selection. Clarendon Press, Oxford, 318 pages.

Gittleman, J. L. , P. H. Harvey. 1980. Why are distasteful prey not cryptic? *Nature*, 286:149-150.

Glendenning, J. I. 1994. Is the bitter rejection response always adaptive? *Physiology and Behavior*, 56:1217-1227.

Guilford, T. 1990. The evolution of aposematism. Pages 23-61 in: *Insect defences: adaptive mechanisms and strategies of prey and predators* (Evans, D.L. and J. O. Schmidt, editors). Albany (NY): State of New York Press.

Holden, W. 1977. Behavioral evidence of chemoreception on the legs of the spider *Araneus diadematus* Cl. *Journal of Arachnology*, 3: 207-210.

Laurent, P.J., C. Braekman, and D. Daloze. 2005. Insect chemical defense. *Topics in Current Chemistry*, 240: 167–229.

Mayntz, D., D. Raubenheimer, M. Salomon, S. Toft, and S. J. Simpson. 2005. Nutrient-specific foraging in invertebrate predators. *Science*, 307:111-113.

Nentwig W. 1986. Non-webbuilding spiders: prey specialists or generalists? *Oecologia* 69: 571-576.

Person MH, Uetz GW. 1996. Wolf spiders vary patch residence time in the presence of chemical cues from prey (Araneae, Lycosidae). *The Journal of Arachnology,* 24: 76-79.

Persons MH, Uetz GW. 1997. The effect of prey movement on attack behavior and patch residence decision rules of wolf spiders (Araneae: Lycosidae). *Journal of Insect Behavior* 10: 737-752.

Persons MH, Walker SE, Rypstra AL, Marshall SD. 2001. Wolf spider predator avoidance tactics and survival in the presence of diet-associated predator cues (Araneae: Lycosidae). *Animal Behaviour,* 61: 43-51.

Roberts, J. A., G. W. Uetz. 2005. Information content of female chemical signals in the wolf spider, *Schizocosa ocreata*: male discrimination of reproductive state and receptivity. *Animal Behaviour*, 70: 217-223.

Savory, T. 1964. Arachnida. Academic Press, New York, 291 pages.

Sillen-Tullberg, B. 1985. Higher survival of an aposematic than a cryptic form of a distasteful bug. Oecologia, 67:411-415.

Skelhorn, J, Rowe C. 2009. Distastefulness as an antipredator defence strategy. *Animal Behaviour*, 78:761-766.

Smith, S. M. 1975. Innate recognition of coral snake pattern by a possible avian predator. *Science*, 187: 759-760.

Stanger-Hall, K. F., D. A. Zelmer, C. Bergren, S. A. Burns, A. H. Price. 2001. Taste discrimination in a lizard (*Anolis carolinensis,* Polychrotidae). *Copeia*, 490-498.

Toft, S. 1999. Prey choice and spider fitness. *Journal of Arachnology*, 27: 301-307.

Willey, M. B., R. R. Jackson. 1993. Olfactory cues from conspecifics inhibit the web-invasion behavior of *Portia*, web-invading araneophagic jumping spiders (Araneae: Salticidae). *Canadian Journal of Zoology*, 71: 1415-1420.

Mission, Review Process & Disclaimer

The Association for Biology Laboratory Education (ABLE) was founded in 1979 to promote information exchange among university and college educators actively concerned with teaching biology in a laboratory setting. The focus of ABLE is to improve the undergraduate biology laboratory experience by promoting the development and dissemination of interesting, innovative, and reliable laboratory exercises. For more information about ABLE, please visit **http://www.ableweb.org/.**

Papers published in *Tested Studies for Laboratory Teaching: Peer-Reviewed Proceedings of the Conference of the Association for Biology Laboratory Education* are evaluated and selected by a committee prior to presentation at the conference, peer-reviewed by participants at the conference, and edited by members of the ABLE Editorial Board.

Citing This Article

Bennington, C., R. Burnett and J. Jett. 2013. Predator Sense and Prey Defense: A Lab Exercise in Evolutionary Hypothesis Formulation and Experimentation. Pages 409-417 in *Tested Studies for Laboratory Teaching,* Volume 34 (K. McMahon, Editor). Proceedings of the 34th Conference of the Association for Biology Laboratory Education (ABLE), 499 pages. http://www. ableweb.org/volumes/vol-34/?art=45

Compilation © 2013 by the Association for Biology Laboratory Education, ISBN 1-890444-16-2. All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the copyright owner.

ABLE strongly encourages individuals to use the exercises in this proceedings volume in their teaching program. If this exercise is used solely at one's own institution with no intent for profit, it is excluded from the preceding copyright restriction, unless otherwise noted on the copyright notice of the individual chapter in this volume. Proper credit to this publication must be included in your laboratory outline for each use; a sample citation is given above.