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Substrate Size Selection by Bean Beetles

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Live animal systems that are easily manipulated and permit rapid data collection would be ideal for teaching non-science majors and introductory-level majors the methods of science. In this study, we use the well-developed bean beetle, *Callosobruchus maculatus*, model system to guide students in the development and implementation of an experiment that is an authentic scientific study from which they can easily collect meaningful data. Bean beetles are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae) and the entire pre-adult parts of the life cycle occur inside the host bean. In this study, students design and conduct experiments to evaluate whether female bean beetles discriminate between beans of different sizes within one bean species. We also will review the on-line resources available for laboratory studies with the bean beetle model system and present an assessment strategy for student understanding of the scientific process.

Keywords: bean beetles, substrate size discrimination, reproductive behavior, *Callosobruchus maculatus,* insect behavior

Introduction

Teaching undergraduates how science is done, the scientific method, is typically among the first subjects addressed in any science course, yet it is rare that scientific research skills are developed early in introductory courses with animal model systems and authentic experimental studies. The bean beetle model system is ideal for addressing this need and providing introductory biology students (or non-science majors, or secondary school biology students) with an easily manipulated and highly flexible experimental system. We developed this experimental study to serve as a vehicle for students to examine the nature of science and to foster the development of scientific research skills. This study could be coupled with an assessment instrument, such as an experimental design case study (an example is included in the **Appendix**), that could provide students with feedback on their experimental design skills and assist faculty in better fostering the development of those skills that we deem to be essential for all students, majors and non-majors. We present this study in the context of understanding how science is different from other ways of knowing, and describing what living things do. Rather than lecturing or giving students a list, we ask our students the questions: How is science different from other ways of knowing?, What do living things do?, What is the most important activity of any living thing?, and solicit the lists from them. Instructors also could use the model of the process of science outlined on the University of California, Berkeley, Understanding Science website:

http://undsci.berkeley.edu/article/0_0_0/howscience-works_02

We then follow with an introduction to the life history of the bean beetle, *Callosobruchus maculatus* to ask the question: Do female bean beetles choose their egg laying substrate on the basis of bean size? The Student Handout is written for an experimental design that is developed by students in a guided-inquiry process that permits students to design an experimental protocol that they may conduct immediately. The introduction, experimental design, and initiation of an experiment can readily be accomplished in a 2-3 hour class meeting. Data collection may be conducted as soon as 24 hours to as late as 3 weeks after an experiment is initiated. The data collection takes another 2 hours of class time. In Supplemental Materials, we have included the PowerPoint slide set we used in our workshop presentation and "A Handbook on Bean Beetles, *Callosobruchus maculatus*" (Beck and Blumer, 2010) that was distributed to workshop participants and also is available at **www.beanbeetles.org**.

Student Outline

Objective

Design and perform a set of experiments to evaluate whether female bean beetles (*Callosobruchus maculatus*) discriminate between different size beans of the same species.

Introduction

Bean beetles (cowpea seed beetles), *Callosobruchus maculatus,* are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae). Eggs are deposited (=oviposition) singly and several days after oviposition, a beetle larva (maggot) burrows into the bean. At 30°C, pupation and emergence of an adult beetle occurs 25-30 days after an egg was deposited. Adults are mature 24-36 hours after emergence and they do not need to feed. Adults may live for 1-2 weeks during which time mating and oviposition occurs. A female may lay as many as 100 eggs during her lifetime, with the majority of egg laying concentrated early in adult life. Since larvae cannot move from the bean on which an egg was deposited, the oviposition choice of a female determines the future food resources available to their offspring (Brown and Downhower, 1988). As a result, it is the most critical choice a female makes for her offspring, because it will influence their growth, survival, and future reproduction (Mitchell, 1975; Wasserman and Futuyma, 1981). Although females can be induced to lay eggs (oviposit) on a wide range of bean species, very few bean species result in normal development and the successful emergence of adults. Some bean species are very clearly toxic to *Callosobruchus maculatus* larvae (Janzen, 1977).

Materials

In class, you will be provided with live cultures of bean beetles containing adults that have been raised on mung beans, *Vigna radiata*. Supplies of organic mung of various sizes also will be available. Female beetles are easily identified in the live cultures because they have two dark stripes on the posterior of the abdomen, whereas the posterior abdomen of males is uniformly light in color.

Experimental Design

Since the oviposition choices of females influence the survival and future success of their offspring, females may be very sensitive to the size of the beans on which they are depositing eggs. Prior to the laboratory class, each group should design a set of experiments to address whether female bean beetles discriminate between different bean sizes within one bean species and the consequences of those choices. Each group will present their designs to the class and common experimental approaches will be discussed.

After you have read the background information and before the laboratory class meeting:

- Describe at least TWO experimental designs for evaluating whether female bean beetles discriminate between different bean sizes with one bean species.
- Describe an ADDITIONAL experiment to evaluate the consequences of females laying eggs on beans of different sizes.
- Predict the outcomes for each experiment.
- Identify and list the variables you would manipulate in each experiment.
- Identify and list the variables you would keep constant in each experiment.
- List the data you would collect to determine if your predictions were true.
- Describe the statistical analyses that you would carry out to test your predictions.

Come to class prepared to present your experimental designs.

Literature Cited

Brown, L. and J.F. Downhower. 1988. *Analyses in Behavioral Ecology: A Manual for Lab and Field*. Sinauer Associates Publishers, 194 pages.

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Appendix

Science Experimental Skills Quiz: Pre-test Assessment Date ________________

Name (print) ______________________ Course ___________ Section ________

This quiz is being given to you to in an attempt to assess your understanding of the scientific process. This quiz will count as part of your course grade but it also is part of a research project. Results of this study only will be reported in aggregate and your name will not be reported.

If you do NOT want your quiz data included in this research, please check here [ ].

The Problem:

Bean beetles, *Callosobruchus maculatus*, get their lifetime nutrition from the bean seeds (family Fabaceae) on which their mother lays her eggs. The specific bean on which each egg is laid determines the future success of the larva that develops from that egg. Choosing where to lay an egg is the most important decision a female bean beetle will make for her offspring, yet there are many different species of bean plants in the natural tropical environment of this species. Bean beetle larval development is not the same on each bean species and some beans are completely toxic to *Callosobruchus*. A visit to a local farmers market would provide you with as many as 12 species of beans such as adzuki beans, black beans, black-eye peas, soy beans, green peas, lima beans, kidney beans, mung beans, navy beans, garbonzo beans, fava beans, and pinto beans.

What **question** can you pose given the data described in The Problem?

What **variables** would you evaluate to address your question?

State the **Null Hypothesis:**

Given the data described above, **what do you predict** if the null hypothesis is true?

State an **Alternative Hypothesis:**

Given the data described above, **what do you predict** if the alternative hypothesis is true?

Materials

Equipment and supplies

For a class of 30 students working in pairs:

- 30 magnifiers 2.5x, 4" diameter self-standing with folding base (**Fisher #14-648-19** or **VWR #62379-535**, approximately $50.00 US per unit) or dissection microscopes
- 15 bean beetle cultures with newly emerged adults (Carolina Biological **#144180**)
- 60 plastic 60 mm Petri dishes for new cultures
- Plastic 35 mm Petri dishes for isolating adults (minimum 60, but reused)
- 32 ounces each of dried mung beans and split mung beans
- 30 small paint brushes
- 30 soft forceps, BioquipTM featherweight forceps (**Catalog No. 4748 or 4750**)
- 0.1 mg analytical balance for weighing beans (more than one balance would permit more efficient data collection by students)
- 30 vernier calipers for measuring bean size
- permanent markers for labeling Petri plates

Notes for the Instructor

Consult "A Handbook on Bean Beetles, *Callosobruchus maculatus"* (Beck and Blumer, 2010) for detailed information on growing cultures, handling techniques, and methods of safe disposal (available for downloading at: **http://www.beanbeetles.org/handbook** and in the supplemental materials for this major workshop). In addition, tips on identifying the sexes including pictures of a male and female are available at: **http://www.beanbeetles.org/handbook/#IS**.

The student handout is written as a guided inquiry that allows students to design their own experiments, rather than instructors giving students explicit directions on how to conduct their experiments. No matter the exact experiment that students design, the experiments will require having dense cultures of bean beetles from which females can be isolated. If new cultures are initiated approximately 2 months before the lab period, there will be sufficient time for two generations of beetles, which will result in dense cultures. When possible, we supply one culture to each group of students. However, cultures should have sufficient beetles for multiple groups. Newly emerged cultures work better for this experiment than older cultures.

Instructors should caution students to prevent the accidental release of bean beetles from the laboratory environment. *Callosobruchus maculatus* is a potential agricultural pest insect that is not distributed throughout the United States and Canada. It is essential that you keep your cultures secured in a laboratory environment to ensure that they are not released to the natural environment. Disposal of cultures (and beans (seeds) exposed to live beetles of any life cycle stage) requires freezing (0°C) for a minimum of 72 hours prior to disposal as food waste. If you have any questions about the handling or disposal of bean beetles, please contact Larry Blumer at **lblumer@morehouse.edu** or 404-658-1142 (voice or FAX). Information also is available at: **www.beanbeetles.org** in the Handbook section.

Experimental Design

Our students have successfully conducted this experiment using both very short-exposure and long-exposure protocols. Students typically design experiments in which female beetles choose between two categories of beans and are permitted to lay eggs on those beans. The species of bean is the same but the quantity of the bean resource is different. For example, we have had students conduct experiments in which each replicate contained 15 whole mung beans and 15 split mung beans in a 60 mm dish (or 10 of each category in a 35 mm dish). A female and a male bean beetle were introduced to each dish and the female was permitted to lay eggs for a minimum of 24 hrs or as long as 7 days. Alternatively, students could sort whole mung beans (visually judging size) to create sets of large beans and small beans. A similar experiment with 15 large and 15 small beans per replicate could be conducted, and run for 24 hrs to 7 days. If similar numbers of beans of each size category were presented to a female, the experiment could be permitted to run until each female laid all her eggs and died (7-10 days from adult emergence). The difference in the quantity of the bean resource may be documented at the start of an experiment by weighing the beans in each size category or measuring beans with vernier calipers. In all experiments, the data collected are the number of eggs laid on beans in each category of size.

This experiment may be readily modified for more advanced students by using it as an alternative means of studying intra-specific competition. Beck and Blumer (Beck and Blumer, 2009 and Blumer et al., 2010) previously presented a competition protocol in which female beetles chose between beans with or without an egg from another female, so females were choosing based on the number of competitors already present. In the current study, female beetles would choose between beans based on differences in the resources actually available, not the number of potential competitors. The experiment could be elaborated by using other bean species (such as black-eye peas or adzuki beans) and by evaluating differences in successful development and adult body size for eggs laid on different size beans.

Data Analysis

The total number of eggs laid on each of the two size categories may be analyzed using a binomial test. This is more appropriate than a chi-squared test since there are only two categories in a given experiment. Alternatively, the mean number of eggs laid on each bean size category per replicate may be evaluated using a paired t-test or a non-parametric pairwise test (Wilcoxon sign-rank test). These statistical tests

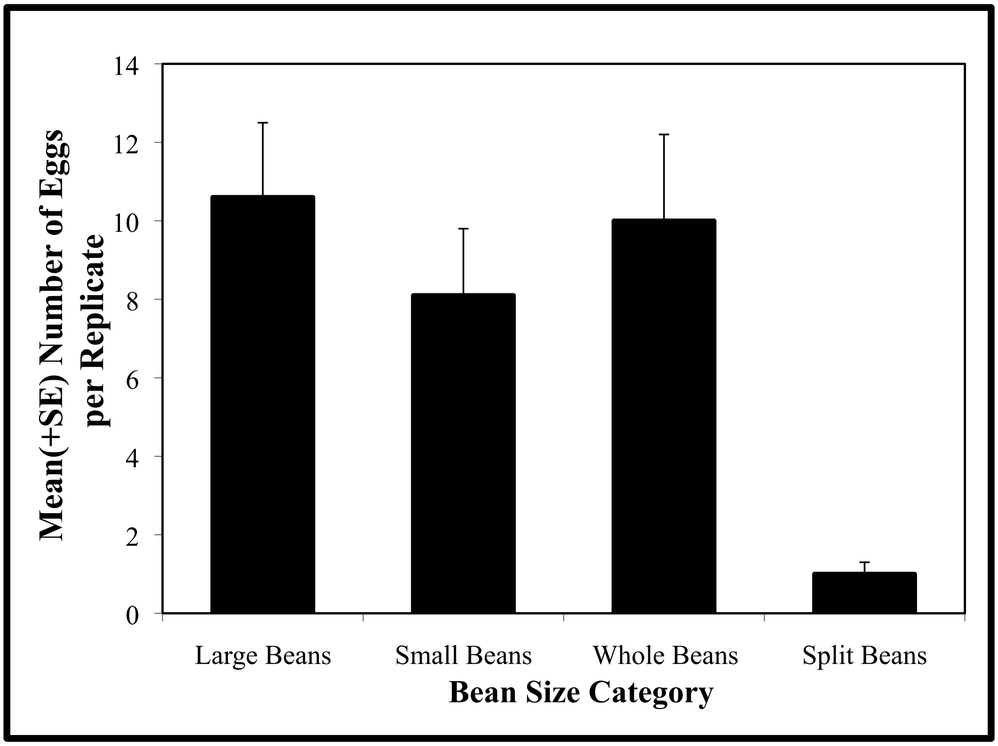

Figure 1. Female discrimination of bean size in short-exposure experiments. The mean±SE of the number of eggs laid by female bean beetles per replicate are shown for 16 replicates containing 15 large and 15 small mung beans, and 26 replicates of 15 whole and 15 split mung beans. The differences in eggs laid on large versus small and whole versus split were significant (paired t-tests, $p<0.05$ for both comparisons).

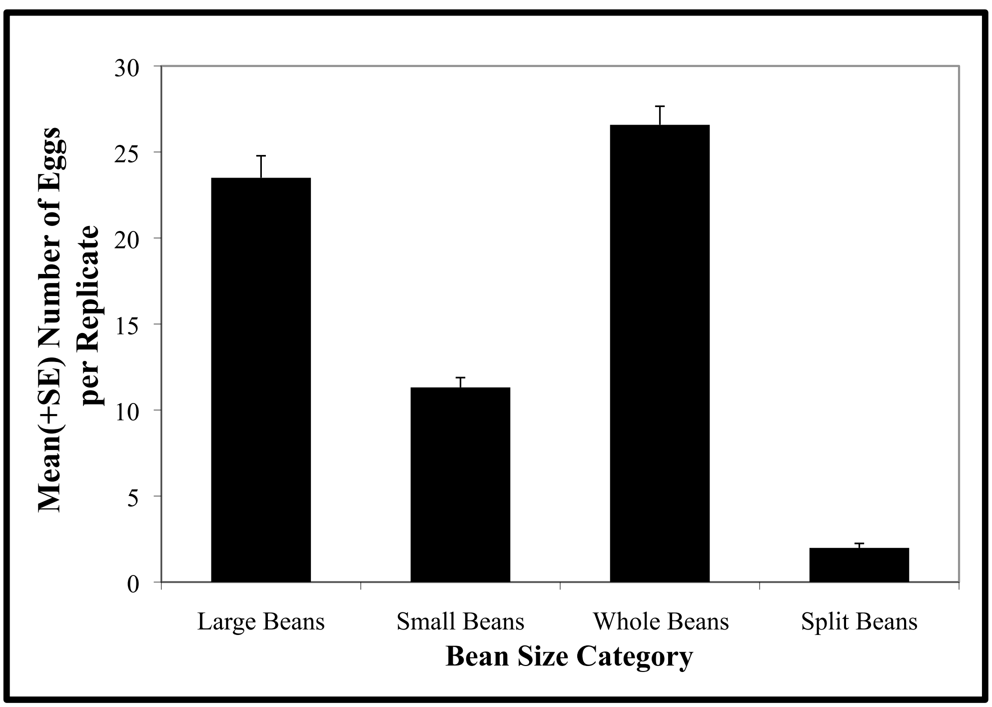

Figure 2. Female discrimination of bean size in long-exposure experiments. The mean±SE of the number of eggs laid by female bean beetles per replicate are shown for 76 replicates containing 10 large and 10 small mung beans, and 76 replicates of 10 whole and 10 split mung beans. The differences in eggs laid on large versus small and whole versus split were highly significant (paired t-tests, $p<0.000001$ for both comparisons).

are easily performed using the on-line VassarStats website **http://faculty.vassar.edu/lowry/VassarStats.html.**

Previous Results

The following data were collected by students in the non-majors Science and Society course at Morehouse College in the Fall 2009 in a short-exposure experimental protocol. A total of 26 replicates of 15 whole and 15 split mung beans were conducted. Each replicate contained one female and one male bean beetle and egg laying was permitted for 48 hours. The average per bean mass of 15 whole beans was 0.060 g and that of the split beans was 0.028 g. There were significantly more eggs laid on whole beans (260) compared to split beans (26) (binomial test, $p<0.000001$) with an average per replicate of 10 eggs on whole beans and only 1 egg on split beans (paired t-test, $t=4.22$ $df=25$ $p=0.0003$) (Figure 1). A total of 16 replicates of 15 large and 15 small (all whole beans) also were conducted. The average per bean mass of 15 large beans was 0.071 g and that of the small beans was 0.054 g. Again, there were significantly more eggs laid on large beans (169) compared to small beans (130) (binomial test, $p=0.027$) with an average per replicate of 10.6 eggs on large beans and only 8.1 eggs on small beans (paired t-test, $t=3.48$ $df=15$ $p=0.003$) (Figure 1). Note that split beans differ from whole beans in both size and quality, which likely accounts for the much greater discrimination by female bean beetles.

A long-exposure protocol experiment was conducted by students at the University of Northern British Columbia in Spring 2010. A total of 76 replicates were prepared that consisted of 10 whole and 10 split mung beans on which a single inseminated female was permitted to lay eggs until she died. The average per bean mass of the whole beans was 0.059 g and that of split beans was 0.036 g in this experiment. As in the short-exposure experiment, there was a significant bias toward whole beans. The bias was highly significant in this long-exposure experiment with more eggs laid on whole beans (2020) than on split beans (151) (binomial test, $p<0.000001$). The average number of eggs per replicate laid on whole beans was 27 while on split beans it was 2 (paired t-test, $t=23.8$ $df=75$ $p<0.0001$) (Figure 2). A total of 76 replicates of 10 large and 10 small (all whole beans) also were conducted. The average per bean mass of 10 large beans was 0.089 g and that of the small beans was 0.037 g. There was a highly significant bias by females toward larger beans as there were significantly more eggs laid on large beans (1786) compared to small beans (860) (binomial test, $p<0.000001$) with an average per replicate of 23.5 eggs on large beans and only 11.3 eggs on small beans (paired t-test, $t=12.67$ $df=75$ $p<0.0001$) (Figure 2).

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