Bean Beetles, *Callosobruchus maculatus*, a Model System for Inquiry-Based Undergraduate Laboratories

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Abstract: Bean beetles, *Callosobruchus maculatus* (Coleoptera: Bruchidae), are tropical and subtropical agricultural pest insects. This species is among the most tractable and robust laboratory animal systems but it is not widely used outside of research laboratories. *C. maculatus* is extremely easy to manipulate, maintain and has a very rapid life cycle. Extensive past and present research on *C. maculatus* (more than 150 journal articles in the past 10 years) provides opportunities for connections between undergraduate laboratory studies and research in ecology, evolutionary biology and animal behavior. We present general information on bean beetles that is necessary for using this species in laboratory courses. This information can be found at www.beanbeetles.org. In addition, we introduce an experiment that evaluates oviposition substrate choice. The experiment can be readily implemented as an inquiry-based study in introductory or advanced undergraduate laboratory courses. In this experiment, we will test the hypothesis that female bean beetles discriminate between seeds of the species from which they emerged (natal seeds) and seeds of another species that also are a suitable oviposition substrate.

Introduction

In biology, studying organisms in their natural habitats and working with live animals are typically the most compelling and essential learning experiences for undergraduate students. Yet, they represent the most difficult means to address questions in the fields of ecology, behavior and evolutionary biology. However, the bruchid bean beetle, *Callosobruchus maculatus* is among most tractable and robust animal systems we know. Yet, it is not widely used outside of research laboratories. *C. maculatus* is extremely easy to manipulate and maintain and has a very rapid life cycle. Furthermore, there are none of the humane animal care issues that must be addressed prior to working with insects that are a significant barrier to working with vertebrate animals in undergraduate teaching laboratories. Extensive past and present research on *C. maculatus* (more than 150 journal articles in the past 10 years) provides opportunities for connections between undergraduate laboratory studies and research in ecology, evolutionary biology and animal behavior.

Bean beetles, *Callosobruchus maculatus* (Coleoptera: Bruchidae), are tropical and subtropical agricultural pest insects. Females lay their eggs on the surface of beans (seeds in Family Fabaceae). Eggs are deposited singly and 8-10 days after oviposition, a beetle larva (maggot) burrows directly from the egg into the bean. At 25°-30°C, pupation and emergence of an adult beetle occurs 25-35 days after an egg was deposited. Adults are mature 24-36 hours after emergence and they do not need to feed. Under these circumstances, adults may live for an average of 12-14 days during which time mating and oviposition occurs. Adult sexes can be distinguished by means of readily observed morphological differences that are easily seen with the naked eye. Females have dark stripes on each side of the posterior dorsal abdomen that are not found in males. Adults have an average mass of 4-6mg and an average body length of 4-6mm. The entire life cycle can be readily and successfully completed without the provision of water or any food source other than the dried beans upon which the eggs are laid. Females have a lifetime egg production ranging from 30 –100 in laboratory cultures. Adults will readily mate under laboratory conditions and males inseminate females with spermatophores that represents a substantial proportion of total body mass, as much as 20% of his body mass. The laboratory environment is very similar to the environment that these beetles would experience in nature. This unusual alignment between the natural and laboratory environments is a consequence of *C. maculatus* naturally infesting stored bean supplies. Virgin adults are easily isolated because females will disperse their eggs singly when provided with a large number of beans on which to oviposit. Isolating single-egg beans ensures that the emerging adult is a virgin. The adults are easy to handle with forceps or fly brush, and although they have fully functional wings, they prefer to crawl. Consequently, adults are very easy to pick from their culture container without anaesthesia or special tools. Cultures can be grown in a very wide range of containers: lidded plastic Petri dishes, screen covered jars, snap lid vials, and cotton plugged shell vials. Live bean beetle cultures are almost odorless and can be maintained at room temperatures without an incubator.

Our website (www.beanbeetles.org) provides information on the following:

- Laboratory methods
- Research
- Bibliography
- Laboratory activities
- Inquiry-based learning.

The laboratory methods section of the website includes information on:

- Natural history
- Life cycle
- Culture techniques
- Generation time at different temperatures
- Sex identification
- Handling techniques
- Mating beetles
- Isolating virgins

The research section includes links to the websites of researchers currently using bean beetles in their research laboratories. The bibliography section contains downloadable documents with a bibliography of ecology, evolution, and animal behavior research on the genus *Callosobruchus*. The bibliography is updated several times per year. The laboratory activities section will include inquiry-based experiments using bean beetles as a model system. New experiments will be added to the site as they are developed. The inquiry-based learning section has a brief discussion of inquiry-based learning and links to additional resources on this pedagogy.

Student Outline

Natal Bean Discrimination by Bean Beetles

Objective
Design and perform a set of experiments to evaluate whether female bean beetles (*Callosobruchus maculatus*) discriminate between two suitable species of beans.

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 21-30 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. 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. 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, *Phaseolus aureus*, for at least 10 generations. Supplies of organic mung beans and adzuki beans, *Phaseolus angularis*, another bean species that is a suitable substrate for normal bean beetle development, also will be available.

Experimental Design
Since the oviposition choices of females influence the survival and future success of their offspring, females may be very sensitive to the species and condition 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 two suitable species of beans. 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 mung or adzuki beans.
- 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

Janzen, D.H. 1977. How southern cowpea weevil larvae (Bruchidae *Callosobruchus maculatus*) die on non-host seeds. Ecology 58:921-927.

Mitchell, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* F. Ecology 56:696-702.

Wasserman, S.S. and D.J. Futuyma. 1981. Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabrivius (Coleoptera: Bruchidae). Evolution 35:605-617.

Instructor's Notes

Consult "A Handbook on Bean Beetles, *Callosobruchus maculatus*" for detailed information on growing cultures and handling techniques (available for downloading at: http://www.beanbeetles.org/handbook.html). In addition, tips on identifying the sexes including pictures of a male and female that could be used in class are available at: http://www.beanbeetles.org/handbook.html#IS.

Experimental Design

The two questions that students generally address in their experiments are:

- Do females prefer to visit the bean species from which they emerged (natal bean species) when given a choice between the natal bean and another suitable bean species?
- Do females preferentially lay eggs on their natal bean species when given a choice between the natal bean and another suitable bean species?

Note that in any experiment in which location preference is evaluated, some animals may prefer to move in one direction regardless of the treatment conditions. Students should consider the following questions in their experimental designs:

- How can you control for potential location bias?
- How will you measure whether a female prefers to visit one bean species over another?

Oviposition will readily occur during a 24-hour period when adult females are provided with single layer of beans in a small covered dish. Although most adult females in an active culture will have been inseminated, there are always some female that may have only recently emerged (and be infertile) and others that are near the end of their adult life (and laid most of their eggs). Students should consider the following questions in their experimental designs:

- How can you account for variation among females in the number of eggs they lay?
- If females lay eggs preferentially on their natal bean species, how will you detect that preference?

The following data entry sheet is provided as a potential guide for data collection. However, it is most appropriate to guide your students to design their own experiments, a guided inquiry process, rather than giving them explicit directions on how to conduct their experiments.

Data Collection

Location data may be in the form of the number of times each female was in a given location (mung, adzuki, or neutral zone) in a three-section arena. These data could be collected by starting an experiment and checking the location of a female at fixed time intervals, for example, every 2 minutes during a 30-minute trial. Alternatively, continuous observations could be made during a fixed period of time and the total time a female spent in each location would be calculated.

The actual number of eggs laid on each of two bean species during a 24-hour period could be evaluated in an oviposition preference experiment in which a female is presented with an equal number of mung and adzuki beans. These egg laying data do not need to be collected immediately after 24-hours but the females should be removed from the experimental arenas, so students can evaluate the initial bean species choices. The eggs are glued to the beans and will remain intact on the beans. Therefore, students may count the eggs one (or even two) weeks after the start of the oviposition experiment. A 48-hour period for egg laying may be used if too few eggs are laid in a 24-hour interval.

Data Analysis

In the location preference experiment, if the data consist of number of times a female was present in each section of the arena, then the appropriate test is a chi-squared analysis. In this analysis, students would be comparing the observed location counts to the expected location counts if the females behaved randomly. If the location preference experiment were conducted with continuous time in each section data, then a two-sample t-test could be performed to evaluate whether there were differences between the two bean species in female preference. Because females can spend time in a neutral zone, the time spent in one section with beans is independent of the time spent in the other section with beans. Therefore, a two-sample t-test is more appropriate than a paired t-test.

The data from the oviposition preference experiment should be the number of eggs laid on each of the two bean species. The appropriate statistical analysis for the egg count data is a binomial test to determine whether one bean species received more eggs than the other for a given female. The difference in the average number of eggs on each bean species across replicates also could be compared with a paired t-test.

Previous Results

Based on previous trials, the data from the location choice experiment indicate no significant preference for either the natal or non-natal bean species. Some females show a preference, but taken as a whole, no consistent preference was observed. However, when an individual female is provided with equal numbers of beans from the natal and non-natal species (mung and adzuki), and allowed to lay eggs for 24-hours, a very clear preference was observed in the bean species on which eggs are laid (Figure 1).

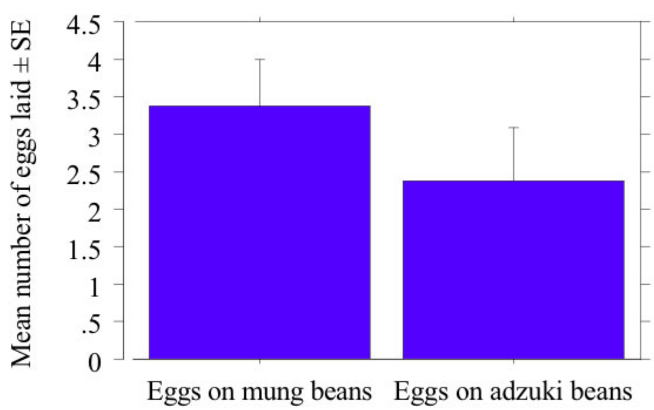


Figure 1. Mean number of eggs laid by a single bean beetle on mung and adzuki beans during a 24-hour period. The means are significantly different (paired t-test, t=2.72 df=37 p=0.0099). Each dish contained 10 beans of each bean species and one female beetle. These data were provided by Calvin Greene II, a Morehouse College student.

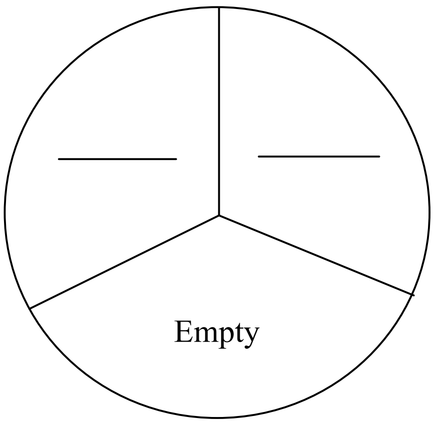
Bean Beetle Location Data Sheet

Date: ____________

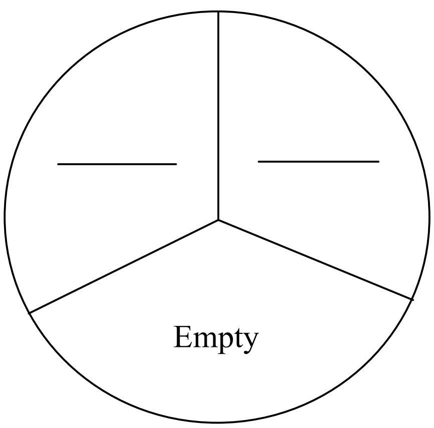
Beetle #: __________

Natal Bean (bean type from which female emerged) _________________

Trial 1 Orientation



Trial 2 Orientation



Location At:			
Trial 1		Trial 2	
2 minutes		2 minutes	
4 minutes		4 minutes	
6 minutes		6 minutes	
8 minutes		8 minutes	
10 minutes		10 minutes	
12 minutes		12 minutes	
14 minutes		14 minutes	
16 minutes		16 minutes	
18 minutes		18 minutes	
20 minutes		20 minutes	
22 minutes		22 minutes	
24 minutes		24 minutes	
26 minutes		26 minutes	
28 minutes		28 minutes	
30 minutes		30 minutes	

Bean Beetle Data Analysis Sheet

Bean Beetle Location Analysis

	Mung	Adzuki	Empty	
Random Expected Locations:	10	10	10	
Actual Observed Locations:	___	___	___	
Observed – Expected				
(Observed – Expected)^2				Sum: _______

Bean Beetle Oviposition Analysis

Egg Counts After 24 hours: _______ on Mung Beans ________ on Adzuki Beans

Conduct a binomial test on these data to evaluate deviation from a random distribution.

Equipment and supplies

For a class of 30 students working in pairs:

- 30 magnifiers 2.5x4" diameter self-standing with folding base (Fisher #14-648-19 or VWR #62379-535, approximately $50.00 per unit) or dissection microscopes
- 15 bean beetle cultures with newly emerged adults
- 15 plastic petri dishes to hold beetle cultures and for picking adults females from cultures
- mung beans (one 16 ounce container) dried beans, organically grown
- adzuki beans (one 16 ounce container) dried beans, organically grown
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps (Catalog No. 4748 or 4750)
- 30 petri dishes (plastic) for holding isolated beetles (35mm) (Falcon 351008) and for conducting oviposition choice experiment
- 30 petri dishes (three-section) for bean location preference experiment (make caulk line sections in standard plastic 100mm dishes using clear aquarium caulk)
- 15 countdown timers or stopwatches

Acknowledgements

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Literature Cited

An extensive bibliography of *Callosobruchus* research is available at our website: www.beanbeetles.org.

About the Authors

Christopher Beck earned his B.S. in biology from the College of William and Mary and his Ph.D. in ecology from the Institute of Ecology at the University of Georgia. He is a senior lecturer at Emory University in Atlanta, where he teaches evolutionary biology, ecology, and ecology lab. He is a member-at-large on the board of ABLE and an associate experiments editor for Teaching Issues and Experiments in Ecology (tiee.ecoed.net).

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