Oviposition Substrate Choice by Bean Beetles, *Callosobruchus maculatus*

Lawrence S. Blumer[1] and Christopher W. Beck[2]

[1]Department of Biology
Morehouse College
830 Westview Dr.
Atlanta, GA 30314
lblumer@morehouse.edu

[2]Department of Biology
Emory University
1510 Clifton Rd.
Atlanta, GA 30322
christopher.beck@emory.edu

Abstract: Bean beetles, *Callosobruchus maculatus*, are tropical agricultural pest insects. Females lay eggs on the surface of beans (Family Fabaceae). The most important choice a female makes for her offspring is the prey bean, as it will influence their growth, survival, and future reproduction. Students design and conduct experiments to evaluate the substrate choices of female bean beetles, and address three questions: Do bean beetles discriminate between bean species? Does the bean species from which a female emerged influence her subsequent choice of oviposition substrate? What are the consequences of a female's choice of substrate on offspring survival and characteristics?

Introduction

This laboratory study is a continuation of our project to develop bean beetles, *Callosobruchus maculatus*, as a model system for undergraduate laboratories in ecology, evolution, and animal behavior. Detailed resources on this insect model system may be found our website (www.beanbeetles.org) including a downloadable handbook on the biology, culturing, and handling of bean beetles.

In this study we challenge students to design experiments to determine whether female bean beetles distinguish among bean species in their oviposition choice (Experiment 1) and to determine whether female bean beetles distinguish between their natal bean species and another suitable host species in their oviposition choice (Experiment 2). The student handouts are intentionally missing detailed protocols because we have developed this study as a guided inquiry exercise. This method of teaching and learning requires that instructors guide the development experimental protocols by students and then permit students to conduct their experiments. It is necessary and appropriate to expect some variation between classes and instructors in the specific experimental design used to address a given question.

Finally, students may examine the consequences of female oviposition choice (Experiment 3) by following the development of beetles to the time of adult emergence. Sample data are presented on all three types of experiments with recommendations on methods for data analysis.

This study would appropriate for introductory courses conducting a single experiment and could be adapted for more advanced courses by permitting students to conduct a sequence of experiments. All the experiments could be replicated sufficiently, even by individual laboratory groups within a class, to permit robust statistical analyses.

Student Outline

Experiment 1
Oviposition Substrate Choice by Female Bean Beetles

Objectives

- Evaluate characteristics of the prey (beans) of bean beetles, *Callosobruchus maculatus*, that could influence prey choice.
- Design and perform an experiment to determine whether female bean beetles distinguish among prey species.
- Design and conduct an experiment to evaluate the consequences of bean species choice by female bean beetles.

Introduction

Bean 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. Larval growth and pupation occur inside the bean and are consequently difficult to observe. 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 7-10 days 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. The choice of prey bean is the most critical choice a female makes for her offspring, as it will influence their growth, survival, and future reproduction (Mitchell, 1975; Wasserman and Futuyma, 1981).

Methods and Materials

Prior to class, design an experiment in which you could address the following question: Do female bean beetles choose prey beans randomly? Alternatively, do female bean beetles prefer some bean species and avoid others?

Material

In class, you will be provided with live bean beetle cultures and supplies of dried beans (seeds) from a variety of species. The seeds available for this experiment will include: mung beans (*Phaseolus aureus*), black-eyed peas (cowpea) (*Vigna unguiculata*), garbanzo (*Cicer arietinum*), kidney, pinto, navy, and black beans (*Phaseolus vulgaris*), soy beans (*Glycine max*), adzuki beans (*Phaseolus angularis*), urad beans (black gram) (*Vigna mungo*), fava beans (horsebean) (*Vicia faba*), lima beans (sieva bean) (*Phaseolus lunatus*), and green pea (*Pisum sativum*). 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 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 determine whether female bean beetles discriminate among 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:

- List characteristics of bean species that might be important to a female bean beetle, and how you would measure these characteristics.
- Describe an experimental design for evaluating whether female bean beetles discriminate among different bean species.
- Describe an ADDITIONAL experiment to evaluate the consequences of females laying eggs on different bean species.
- 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 of Lab and Field.* Sinauer Associates, 194 pages.

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.

This experiment was written by L. Blumer and C. Beck (www.beanbeetles.org) and is based on an experimental protocol originally published by Luther Brown and Jerry F. Downhower (Brown and Downhower, 1988).

Experiment 2
Natal Choice by Female Bean Beetles

Objectives

- Design and perform a set of experiments to evaluate whether female bean beetles (*Callosobruchus maculatus*) discriminate between their natal host and another suitable species of bean.
- Design and conduct an experiment to evaluate the consequences of bean species choice by female bean beetles.

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. Larval growth and pupation occur inside the bean and are consequently difficult to observe. 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. 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*) or adzuki beans (*Phaseolus angularis*). Supplies of organic mung beans and adzuki beans 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 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 their natal host and another suitable species of bean 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 mung and adzuki beans.
- Describe an ADDITIONAL experiment to evaluate the consequences of females laying eggs on 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

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

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.

This experiment was written by L. Blumer and C. Beck (www.beanbeetles.org).

Materials

Equipment and supplies

For a class of 30 students working in pairs:

Experiment 1

- 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
- 15 plastic 150mm Petri dishes for picking adults females from cultures
- 15 plastic 150mm Petri dishes for carrying out oviposition substrate choice experiment
- 30 plastic 35mm Petri dishes for holding isolated beetles
- 16 ounces of each the following bean species, dried and organically grown, if possible: mung beans, adzuki beans, black-eyed peas, garbanzo, kidney, pinto, navy, black beans, soy beans, urad beans, fava beans, lima beans, and green pea
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps (Catalog No. 4748 or 4750)
- 30 vernier calipers for measuring bean characteristics
- 0.1mg analytical balance for weighing beans

Experiment 2

- 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
- 15 plastic 150mm Petri dishes for picking adults females from cultures
- 30 plastic 35mm Petri dishes for holding isolated beetles
- 30 plastic 100mm Petri dishes divided into three sections using clear aquarium caulk for location preference experiment
- 30 plastic 35mm Petri dishes for oviposition preference experiment
- 15 countdown timers or stopwatches

Experiment 3

- 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
- 35mm Petri dishes for holding individual beans OR flat-bottom tissue culture plates (6 or 12 well)
- 0.1mg analytical balance for weighing beetles
- 30 vernier calipers for measuring beetle characteristics

Notes for the Instructor

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

We include details of three experiments that can be used independently or in combination for a multi-week exercise. All the experiments are written as guided inquiries that allow students to design their own experiments, rather than instructors giving students explicit directions on how to conduct their experiments.

All of the experiments 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. The number of eggs that one female will lay depends on her age and reproductive history prior to use in an experiment. Using randomly selected females from an active stock culture will almost certainly be fertilized but the number of eggs laid by a given female may range from zero to 150 or more. In the Previous Results reported below, we observed 27±6 eggs laid per female (mean ± SE) among those females that laid any eggs (N=31 of 40 females) with a range from 2 to 150 eggs per female.

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

Experiment 1: Oviposition substrate choice by female bean beetles

A similar exercise that is not inquiry-based is described in Brown and Downhower (1988). Questions that students generally address in their experiments include:

- Do females prefer to lay eggs on a particular species of bean?
- Do females actively avoid laying eggs on a particular species of bean?
- Is oviposition substrate choice by females determined by the size of a species of bean?

In their experimental designs, students should consider the following questions:

- How would you control for female preference for the bean species from which she emerged?
- How would you control for the possibility that females will lay their eggs on the first species of bean they encounter?

Oviposition will readily occur during a 48-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 females 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 a particular bean species, how will you detect that preference?

Data collection—The actual number of eggs laid on each of ten bean species during a 48-hour period could be evaluated in an oviposition preference experiment in which a female is presented with an equal number of each bean species. In this experiment, we do not use the natal bean species to control for a bias toward that species. Generally, about 10 beans of each species in a 150mm Petri dish are appropriate. If the beans are randomly arranged throughout the dish, females will be equally likely to encounter each bean species. Egg laying data do not need to be collected immediately after 48-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 ensures that sufficient numbers of eggs are laid.

Data analysis—The data from the experiment should be the number of eggs laid on each bean species. The appropriate statistical analysis for the egg count data is a chi-squared test to determine whether the distribution of eggs on the bean species differed from random. The null hypothesis is that females will lay an equal number of eggs on each bean species. The chi-squared test can be carried out for individual replicates or for all replicates pooled. The difference in the average number of eggs on each bean species across replicates also could be compared with a one-way ANOVA with bean species as the factor and number of eggs as the dependent variable.

Experiment 2: Natal choice by female bean beetles

A simpler version of this experiment was presented as a mini-workshop at ABLE in 2006 (Beck and Blumer, 2006).

The 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 bean species on which they will lay eggs?
- Do females preferentially lay eggs on their natal bean species when given a choice between the natal bean and another bean species on which they will lay eggs?
- Does the identity of the other bean species influence the strength of female preference for her natal bean species?
- Does the identity of the natal bean species influence the strength of female preference for her natal bean species?

This experiment can be used as a follow-up to Experiment 1, in which students determined on which species of beans, other than their natal host, females will lay eggs. The results from Experiment 1 can be used to determine the alternate bean species for this experiment. Different groups of students in the class may want to carry out the same experiment with different alternate bean species and then compare their results. Different groups of students also could use beetles that are from different natal 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 rather than another?

Oviposition will readily occur during a 48-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 females 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?

Data Collection—Location data may be in the form of the number of times each female was in a given location (natal host, alternative species, 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 48-hour period could be evaluated in an oviposition preference experiment in which a female is presented with an equal number of the natal species and alternative species. These egg laying data do not need to be collected immediately after 48-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 ensures that sufficient numbers of eggs are laid.

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.

Experiment 3—Consequences of oviposition choice

A similar experiment is suggested in Brown and Downhower (1988), but is limited to emergence success. This experiment is an extension of Experiment 1 or Experiment 2. We have incorporated this experiment as a part of the student outlines for both Experiments 1 and 2. The exact questions students address will depend on which experiment is being continued. However, the dependent variables that are measured are generally the same.

Examples of questions that students may ask include:

- What is the effect of host species on offspring characteristics?

- Do offspring characteristics differ between beetles that emerge from natal and non-natal hosts?

The most challenging part of the experimental design for students is determining what offspring characteristics to measure. Below is a list of characteristics that can be measured in a reasonable time span.

- Time to emergence
- Size at emergence (either mass or body length)
- Emergence success

The quality of the data for emergence success will depend on the ability of students to identify eggs on beans. Students may suggest other offspring characteristics, such as lifespan, reproductive success, hatching rate, and sex ratio. Characters such as lifespan could be measured, but would add another two or more weeks to the experiment. Other dependent variables are appropriate, but difficult to measure (i.e., reproductive success and hatching rate). Finally, for other offspring traits like sex ratio, the predictions are not clear.

In their experimental designs, students should address the following questions:

- What factors other than host type might affect offspring traits?
- How would you control for these factors?

Data collection—One of the biggest confounding factors is the number of eggs laid on beans. If more than one egg is laid on a bean, then the larvae will compete for resources. As a result, beans with single eggs should be used in all experiments. In advanced classes, students may want to record the identity of the female that laid the egg to be able to consider differences among females in their analysis. However, data on female identity is not essential. As a continuation of Experiment 1 or Experiment 2, students can isolate beans of each species with single eggs into the wells of tissue culture plates or small Petri dishes. As the beetles emerge, students can record the offspring characteristics that they chose to measure.

Data analysis—Most of the offspring traits that students measure will be continuous. As a result, ANOVA can be used to determine the effect of host species or natal versus non-natal species on offspring characteristics. For emergence success, the data would be the number of emerged and non-emerged beetles from each host species. Differences in emergence success could be determined using a chi-squared test. If only two host species are used (e.g., in Experiment 2 with natal and non-natal bean species), a Fisher's exact test could be used.

Previous Results

The results reported below were collected William D Shipman III, a Morehouse College undergraduate.

Oviposition Substrate Choice

A fertilized female bean beetle was permitted to lay eggs on ten bean species, not including the natal bean species. The natal bean was mung (*Phaseolus aureus*) in this experiment. A total of 17 independent trials were conducted, each with one female. In each trial, 10 beans of each of the following were presented to a female: black-eyed peas (BEP, cowpea) (*Vigna unguiculata*), garbanzo (*Cicer arietinum*), kidney, pinto, navy, and black beans (*Phaseolus vulgaris*), soy beans (*Glycine max*), adzuki beans (*Phaseolus angularis*), lima beans (sieva bean) (*Phaseolus lunatus*), and green pea (*Pisum sativum*). Females were permitted to lay eggs for 96 hours before they were removed and the eggs were counted. The difference between bean species in the mean number of eggs laid per female was highly significant (ANOVA, $F_{9,160}$=15.1, p<0.0001, Figure 1). An alternative statistical analysis could be based on the frequencies of eggs (the total eggs counts) on each bean species. That analysis also indicated a highly significant difference between bean species in the number of eggs laid (X^2=717.9, df=9, p<0.0001).

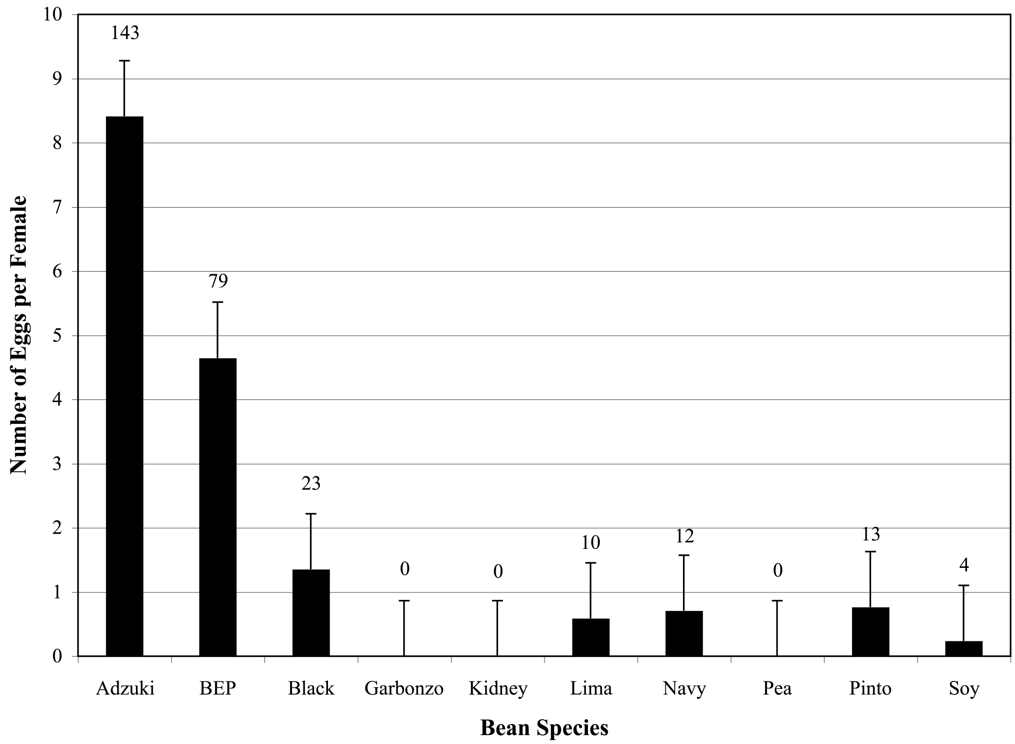

Figure 1. Number of eggs laid per female. The mean ± SE of eggs laid per female on each of 10 bean species is shown for the 17 independent trials conducted with females from mung beans. Each of the 17 replicates contained 10 beans of each of the 10 species, so these means are based on 170 beans of each species. The value shown at the top of each histogram bar is the total number of eggs laid on each bean species.

Consequences of Oviposition Substrate Choice

The elapsed time from egg laying to the emergence of an adult (development time) and the mass of the newly emerged adult beetle was evaluated for each bean species on which eggs were laid. Beans with eggs were followed for a total of 40 days after egg laying to permit adequate time for adults to emerge. Adults only successfully emerged from adzuki beans and black-eyed peas (BEP) and the development time from black-eyed peas was significantly faster than from adzuki beans (ANOVA $F_{1,23}$=24.4, p<0.0001, Figure 2). There were no significant differences between the sexes in development time emerging from a given bean species (ANOVA $F_{1,23}$=0.5, p=0.49, Figure 2). The mass of newly emerged males and females was significantly greater for adults emerging from black-eyed peas compared to adzuki beans (ANOVA $F_{1,15}$=4.7, p<0.05, Figure 3). Females had significantly greater mass at emergence than males (ANOVA $F_{1,15}$=6.1, p<0.03, Figure 3).

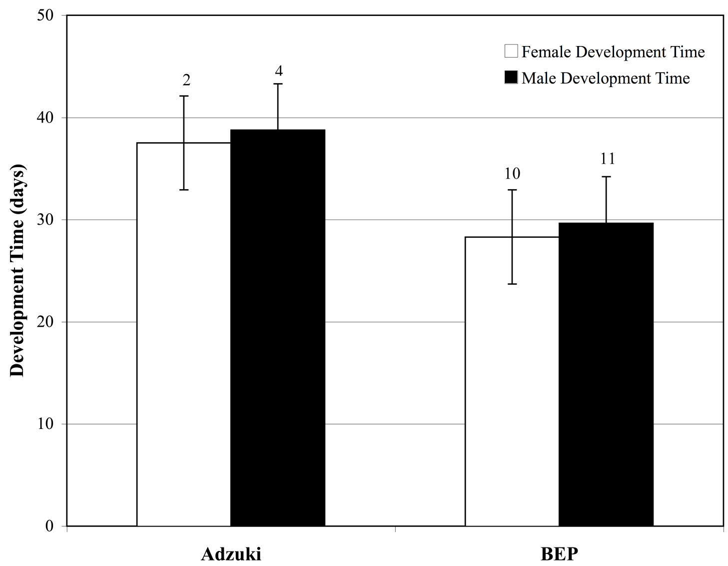

Figure 2. Development time of bean beetles emerging from adzuki beans and black-eyed peas (BEP). The mean ± SE development time (egg laying to emergence of adult) is shown for males and females emerging from the two bean species from which adults successfully emerged. Sample sizes are shown at the top of each histogram bar.

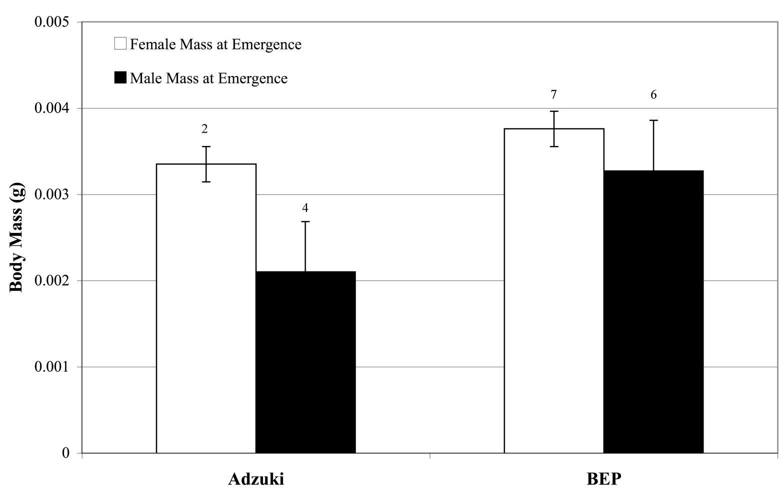

Figure 3. Body mass of bean beetles emerging from adzuki beans and black-eyed peas (BEP). The mean ± SE mass at emergence is shown for males and females emerging from the two bean species from which adults successfully emerged. Sample sizes are shown at the top of each histogram bar.

Natal Choice

A fertilized female was permitted to choose between laying eggs on mung beans or adzuki beans in this experiment. A total of 14 independent trials were conducted in which 10 beans of each species were presented to a different female that had emerged from mung beans. As in the Oviposition Substrate Choice experiment, females were allowed 96 hours to lay eggs before being removed. The mean number of eggs laid per female on adzuki beans was significantly greater than on mung beans (paired t-test, $t=2.602$, $df=13$, $p=0.02$, Figure 4). An alternative statistical analysis could be based on the frequencies of eggs (the total eggs counts) on each bean species. That analysis also indicated a highly significant difference between bean species in the number of eggs laid (binomial test, $p<0.001$).

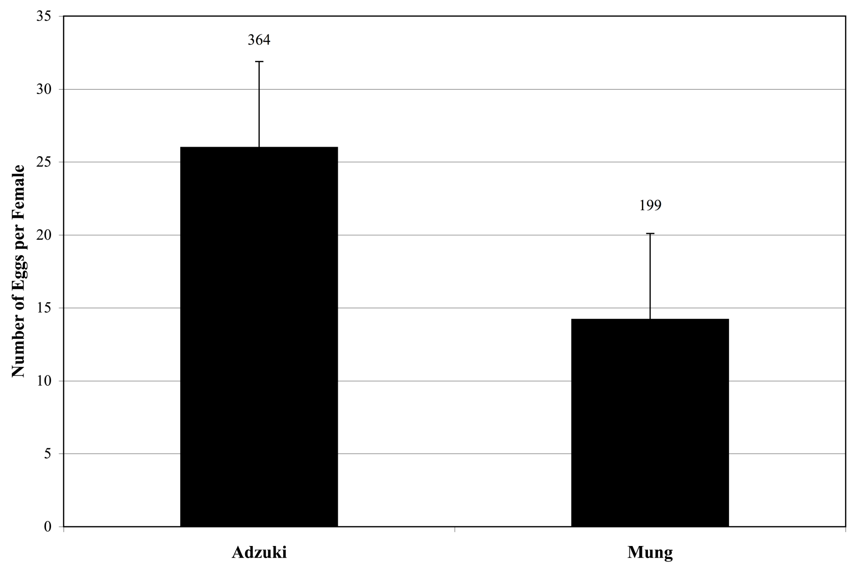

Figure 4. Number of eggs laid per female. The mean ± SE of eggs per female laid on either adzuki or mung beans is shown for the 14 independent trials conducted with females from mung beans. Each of the 14 replicates contained 10 beans each of these two species, so these means are based on 140 beans of each species. The value shown at the top of each histogram bar is the total number of eggs laid on each bean species.

Consequences of Natal Choice

The elapsed time from egg laying to the emergence of an adult (development time) and the mass of the newly emerged adult beetle also was evaluated for the two bean species in this experiment. Beans with eggs were followed for a total of 40 days after egg laying to permit adequate time for adults to emerge. The development time from mung beans was significantly faster than from adzuki beans (ANOVA $F_{1,73}=116.9$, $p<0.0001$, Figure 5). There were no significant differences between the sexes emerging from a given bean species. The mass of newly emerged males and females was significantly greater for those adults emerging from mung beans compared to adzuki beans (ANOVA $F_{1,73}=9.9$, $p=0.002$, Figure 6). Females had significantly greater mass than did males (ANOVA $F_{1,73}=22.3$, $p<0.0001$) and there was a significant interaction between bean and sex on emergence mass (ANOVA $F_{1,73}=5.7$, $p=0.02$, Figure 6). Although the number of eggs on, and therefore larvae in, a given bean can influence development time and adult mass at emergence, there were no consistent significant relationships between either development time or mass and the number of eggs laid on a given bean for either males or females. This suggests that these results are not confounded by competition between larvae in beans that received more than one egg.

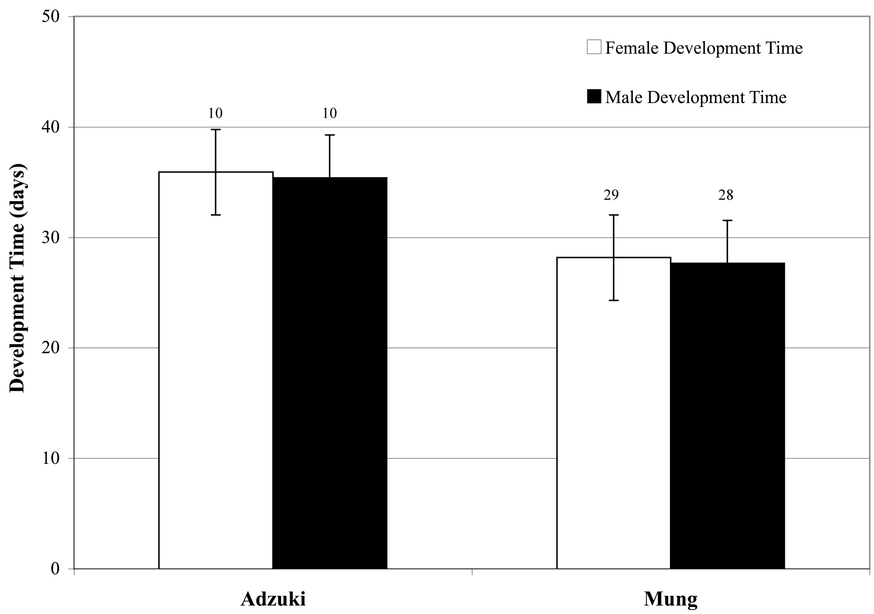

Figure 5. Development time of bean beetles emerging from adzuki and mung beans. The mean ± SE development time (egg laying to emergence of adult) is shown for males and females emerging from these two bean species. Sample sizes are shown at the top of each histogram bar.

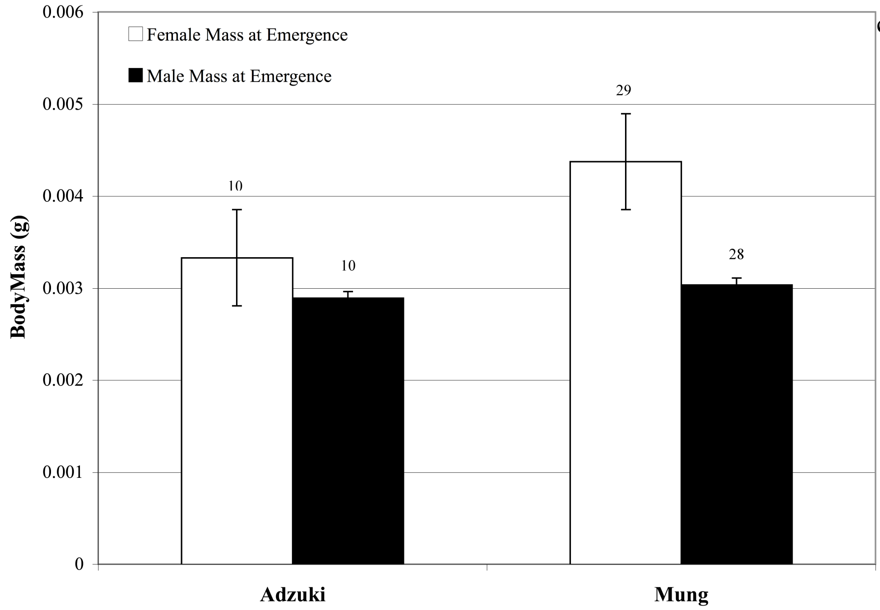

Figure 6. Body mass of bean beetles emerging from adzuki and mung beans. The mean ± SE mass at emergence is shown for males and females emerging from these two bean species. Sample sizes are shown at the top of each histogram bar.

Acknowledgments

This project is supported by the National Science Foundation, DUE-0535903, "Developing Bean Beetles as a Model System for Undergraduate Laboratories." We thank William D. Shipman III, a Morehouse College student, for conducting the experiments and providing the data reported in Previous Results. Disclaimer: Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessary reflect the views of the National Science Foundation, Emory University, or Morehouse College.

Literature Cited

Beck, C.W. and Blumer, L.S. 2006. Bean Beetles, *Callosobruchus maculatus*, a Model System for Inquiry-Based Undergraduate Laboratories. Pages 274-283, *in* Tested Studies for Laboratory Teaching, Volume 28 (M.A. O'Donnell, Editor). Proceedings of the 28th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 403 pages.

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

About the Authors

Larry Blumer earned his Ph.D. from the University of Michigan in 1982 and he is Professor of Biology and Director of Environmental Studies at Morehouse College. He teaches Ecology, Environmental Biology, and Introductory Biology. His research interests are in the development of effective pedagogy in the sciences, and the evolutionary biology and social behavior of insects and fishes.

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, chair of the education section of the Ecological Society of America, and an associate experiments editor for Teaching Issues and Experiments in Ecology (tiee.ecoed.net).