Intraspecific Competition in Bean Beetles

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Biography

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 currently 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).

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.

Introduction

This laboratory study is one of a growing number of inquiry-based laboratory investigations using bean beetles (*Callosobruchus maculatus*) as a model system for undergraduate laboratories. 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 (1) whether females attempt to minimize competition among their offspring and (2) what the effects of competition are offspring life history traits. The student handout does not give students a detailed protocol for the experiment 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.

Sample data are presented on the experiments with recommendations on methods for data analysis. Given the complexity in interpreting the data, this study would appropriate for upper-level courses in ecology. The experiment that examines whether females minimize competition among their offspring would be appropriate for introductory courses, as the analysis and interpretation of the data is more straight-forward than the experiment that examines the effects of competition.

Student Outline

Intraspecific Competition in Bean Beetles

Objectives

1. Evaluate characteristics of female bean beetles, *Callosobruchus maculatus*, egg laying (oviposition) patterns.

2. Design and perform an experiment to evaluate whether competition is occurring between females as they lay eggs on beans, and whether competition is occurring between larvae in individual 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 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).

Experimental Design

Since the oviposition choices of females influence the survival and future success of their offspring, females may be very sensitive to the presence of potential competitors in (and on) the beans on which they are depositing eggs. Address the following questions. Come to class ready to discuss your answers.

- In bean beetle populations, who are potential competitors of a female's offspring? Do female bean beetles attempt to minimize competition among their offspring? Design an experiment or set of experiments to test the hypothesis that females will attempt to minimize competition.
- The hypothesis that females will attempt to minimize competition is based on what underlying assumption? Design an experiment or set of experiments to test this assumption.

For each of the experiments you designed above, you should:

- predict the possible outcomes for the experiment that would support your hypothesis

- identify and list the variables you would manipulate in your experiment
- identify and list the variables you would keep constant in your experiment
- list the data you would collect to determine if your predictions were true

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.

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
- 15 plastic 150mm Petri dishes for picking adults females from cultures
- Plastic 35mm Petri dishes for female oviposition (minimum 30)
- 16 ounces of dried mung beans, organically grown, if possible
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps (Catalog No. 4748 or 4750)
- 0.1mg analytical balance for weighing beetles
- flat-bottom tissue culture plates (6 or 12 well) for holding individual beans
- Very fine tip permanent markers

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.

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. In addition, because students in our courses are required to write scientific papers based on their results, we intentionally do not review the literature on competition in bean beetles in the student handout. For instructor's who wish to provide a more detailed introduction in the student handout, many studies have examined competition in bean beetles (Colegrave, 1993; Colegrave, 1995; Cope and Fox, 2003; Credland et al., 1986; Fox and Savalli, 1998; Giga and Smith, 1981; Giga and Smith, 1991; Guedes et al., 2003; Horng, 1997; Kawecki, 1995; Lale and Vidal, 2001; Messina, 1991a; Messina, 1991b; Messina and Renwick, 1985; Messina and Tinney, 1991; Ofuya and Agele, 1989; Ofuya, 1989; Ofuya and Reichmuth, 1994; Sakai et al., 1986; Toquenaga, 1993; Toquenaga and Fujii, 1991; Vamosi, 2005).

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

Generally, students suggest that females will lay eggs in order to avoid competition between their offspring and the offspring of another female. However, they frequently do not think about the possibility of competition among siblings. Two assumptions underlie the hypothesis that females will attempt to minimize competition. First, the hypothesis assumes that females are able to distinguish between beans with eggs and beans without eggs. Second, the hypothesis assumes that the fitness of offspring from beans with more than one egg is less than the fitness of offspring from beans with a single egg.

The hypothesis that females attempt to minimize competition and the first assumption can be tested by giving females a choice between beans without eggs and beans with eggs. The eggs on the beans could be from the same female or from a different female depending on the exact hypothesis that is being tested. Each of the beans should be marked with the number of original eggs, and a single female added to a 35mm Petri dish with a minimum of five beans of each type. After a 24-48-hour period, students can evaluate the oviposition pattern of the females. Egg laying data do not need to be collected immediately after this period but the females should be removed from the experimental arenas, so students can evaluate the initial choices of females. 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 24-48-hour period for egg laying ensures that sufficient numbers of eggs are laid. An oviposition period longer than 48-hours might lead to egg dumping by females that would obscure female choice. In some cases, students suggest that they need treatments with only beans with no eggs and treatments with only beans with eggs to serve as "controls." However, since we are interested in whether females exhibit a preference, and these treatments do not give females a preference, these treatments do not address the question at hand. The instructor could have the students think about what these treatments would tell us.

To test the second assumption, students will need to isolate beans with different numbers of eggs and examine offspring traits such as emergence success, time to emergence, and size at emergence. This experiment could be considered a continuation of the first experiment. Both time to emergence and size at emergence require checking isolated beans on a daily basis beginning about 4 weeks after egg laying, which may be feasible only in smaller, more advanced classes. However, emergence success can be determined on a single day after a reasonable amount of time following oviposition (approximately 40 days). Emergence can be determined by counting adult beetles or emergence holes in beans.

The first experiment requires having beans with varying numbers of eggs on them. We have used two different approaches. One approach is to have students set up 35mm Petri dishes with mung beans, introduce one female to each dish, and permit her to lay eggs for one week. About five dishes per student should be sufficient. The following week in lab, students can sort the beans based on the number of eggs and set up the experiment. The other approach is for the instructor to set up four of these dishes per student 5-7 days before the lab period. The longer oviposition period ensures that there will be beans with multiple eggs. If by some chance these initial cultures do not result in sufficient beans with eggs to run the experiment, students could run an alternative experiment. Students could determine whether females distinguish between beans without eggs that have been exposed to another female and beans that have never been exposed to a female. This experiment would allow students to explore potential mechanisms for females to avoid competition (e.g., pheromone trails).

Data analysis

The data from the experiment should be the number of eggs laid on beans with and without eggs. The appropriate statistical analysis for the egg count data is a binomial test to determine whether the distribution of eggs on the two types of beans differed from random. The null hypothesis is that females will lay an equal number of eggs on each bean type. The binomial test can be carried out for individual replicates or for all replicates pooled. The difference in the average number of eggs on each bean category (with or without previously laid eggs) across replicates also could be compared with a paired t-test, using the total number of eggs laid on each bean type in each replicate as the dependent variable. A paired t-test is most appropriate as females only have a choice between two different types of beans (those with eggs and those without). The above analyses assume that students classify beans solely based on the presence or absence of eggs. However, students also might use a finer grade classification for beans with eggs by dividing them based on the number of eggs. In this case, chi-squared tests and ANOVA are the appropriate statistical analyses. Yet, the number of beans with more than one previously-laid egg may be too small to permit such an analysis.

For the second experiment, students will compare emergence success, time to emergence, or mass at emergence for beetles emerging from beans with one egg (i.e., no competition) or from beans with more than one egg (i.e., intraspecific competition). For emergence success, the frequency of eggs leading to emerged versus unemerged adults can be compared between the two bean types using a chi-squared test or Fisher's exact test. Time to emergence and mass at emergence can be compared between beans with one egg and beans with more than one egg using a t-test. Again, the above analyses assume that students classify beans solely based on the presence or absence of eggs. However, students also might use a finer grade classification for beans with eggs by dividing them based on the number of eggs. In this case, chi-squared tests and ANOVA are the appropriate statistical analyses. Yet, the number of beans with more than one previously-laid egg may be too small to permit such an analysis.

Previous Results

The following data were collected by students in the Ecology Laboratory course at Morehouse College during spring semester 2007. A total of 17 independent trials were conducted in which five mung beans without eggs and five mung beans with a single egg laid by a previous female were placed together in a 35mm Petri dish. One female (a different female than the one who had previously laid single eggs on beans) was placed in each Petri dish and permitted to lay eggs for 24 hours. A total of 100 new eggs were laid in the 24 hour period and there were significantly more eggs laid (71) on beans without previously laid eggs (binomial test, $p<0.001$) than on beans that had one egg from a previous female (29). An alternative statistical analysis comparing the mean number of eggs laid on beans without previously laid eggs to those beans that had an egg already present also showed a highly significant preference for beans without eggs (Figure 1, paired t-test, $t=3.7$, $df=16$, $p=0.002$) suggesting that female bean beetles are very sensitive to the potential for competition among their offspring in a given bean.

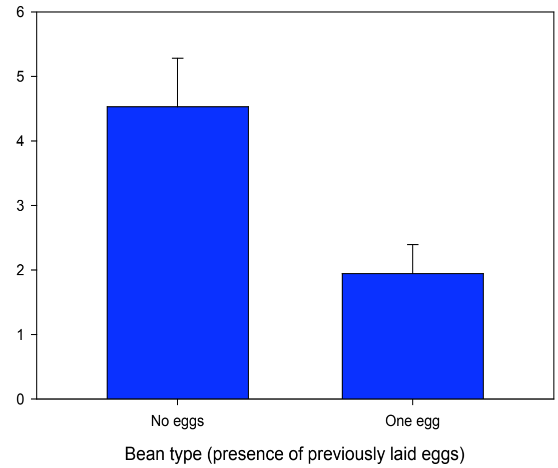

Figure 1. Number of eggs laid by female bean beetles on beans with or without previously laid eggs. The mean ± SE of the number of new eggs laid by a second female are shown (n=17). The total number of new eggs laid on mung beans that had no previous eggs and mung beans that had one egg from a previous female are given at the top of each bar.

Mung beans (N=137) with one, two, or three eggs were isolated individually in the wells of 12-well tissue culture plates and allowed to develop for 5-weeks. Beans were evaluated for the number of adult beetles that emerged from each bean. Only 38 of the 137 beans with eggs successfully yielded adult beetles, and only one adult emerged from a bean regardless of the number of eggs that had been laid on that bean. Consequently, among those beans from which an adult emerged, eggs on two egg beans had a 50% success rate and eggs on three egg beans had a 33% success rate. This suggests that competition does affect emergence success.

Across all replicates, the per egg success rates (i.e., percentage of all eggs laid that yielded adults) varied among beans with different numbers of eggs (Figure 2; 18%, 21% and 24% for beans with one, two and three eggs, respectively), but these differences were not significant (X^2=0.44, df=2, p=0.80). These results may have been a consequence of the relatively poor success rate of single eggs (18%) and the small sample size for two-egg (41 cases) and three-egg (7 cases) beans.

Morehouse students repeated this experiment in the fall semester 2007 on a total of 502 mung beans of which there were 355 one-egg beans, 121 two-egg beans, and 26 three-egg beans. Again, the per egg success rate varied among beans with different numbers of eggs (Figure 3; 32%, 35% and 19% for one, two and three egg beans, respectively), and the differences were significant (X^2=6.69, df=2, p=0.03). Furthermore, more than one adult emerged from only four of 147 multiple egg beans. The female preference experiment also was repeated and females again showed a significant preference for beans without eggs (binomial test, p<0.001). Thus, all the results collected in the fall 2007 indicate competition between larvae.

The inconsistent evidence on competition may be due to factors that were not controlled in this experiment, for example, bean volume and bean mass. In addition, the avoidance by female bean beetles of beans that had previously received eggs might be a response to factors other than competition between larvae in the bean. In nature, larvae are subject to parasitoid attack and beans containing more than one larva may attract parasitoids more readily than a single larva in a bean (for example, Mbata et al., 2005). Ambiguous results such as these could be a very good starting point for discussion about redesigning the experiment to better address the question of competition.

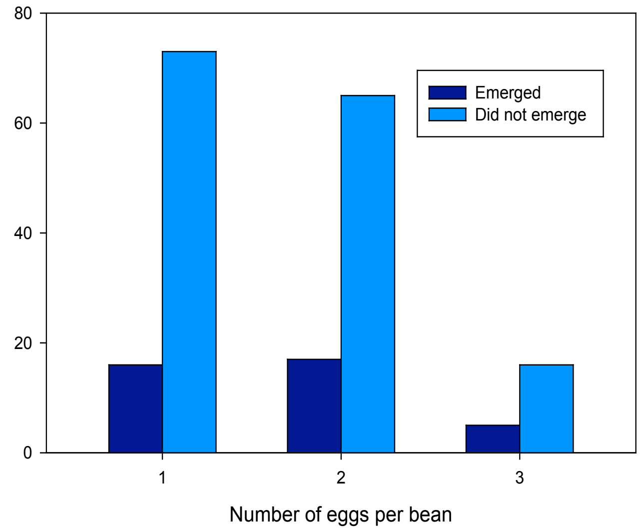

Figure 2. The effect of the number of eggs per bean on the number of emerged and non-emerged adults (spring 2007 data).

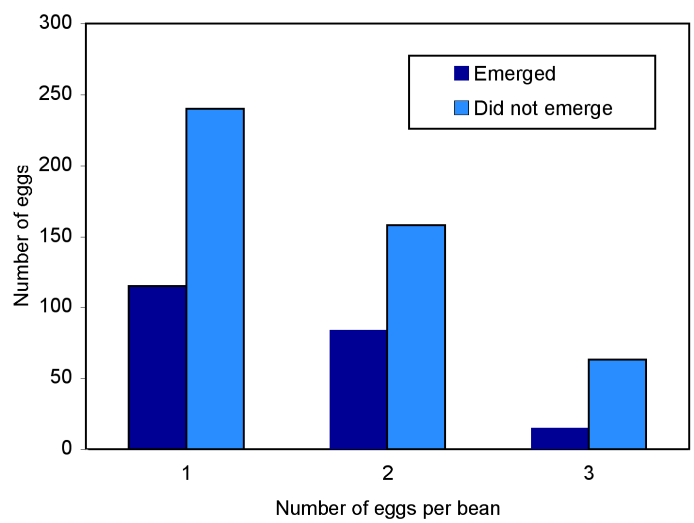

Figure 3. The effect of the number of eggs per bean on the number of emerged and non-emerged adults (fall 2007 data).

In fall 2007, students in the Ecology course at Emory University examined the effect of previous exposure of beans on female oviposition preference. Females were given access to 3 beans exposed to another female and to 3 unexposed beans, and allowed to oviposit for 7 days. Based on 14 replicates, the frequency of eggs laid did not differ between the two types of beans (binomial test, $p = 0.37$; Figure

3). Furthermore, the mean number of eggs laid on the two bean types was not significantly different (paired t-test, t=0.49, df=13, p=0.63).

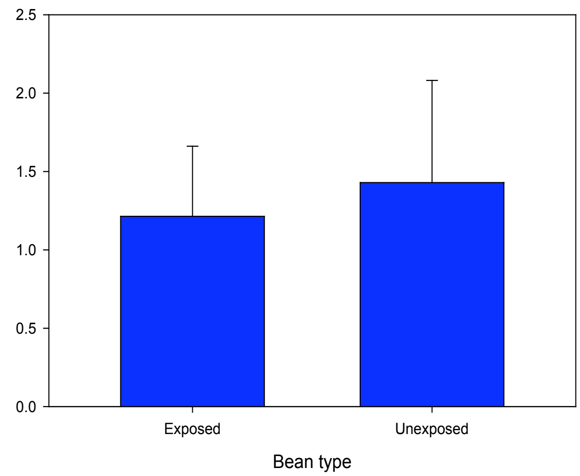

Figure 3. Number of eggs laid by female bean beetles on with or without previous exposure to another female. The mean ± SE of the number of new eggs laid by a second female are shown (n=14). The total number of new eggs laid on mung beans that had no previous exposure to a female and mung beans that had been exposed to a previous female are given at the top of each bar.

Acknowledgments

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