

Intraspecific Competition in Bean Beetles

Instructor Notes

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. 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 pair of students. However, cultures should have sufficient beetles for multiple student 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.

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

References on Competition in *Callosobruchus*

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