Consequences of Oviposition Substrate Choice by Bean Beetles

Instructor's Notes

Consult the Laboratory Methods section of www.beanbeetles.org for detailed information on growing cultures and handling techniques, as well as tips on identifying the sexes.

The experiment requires 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.

Experimental Design

A similar exercise that is not inquiry-based is described in Brown and Downhower (1988), and the consequences of oviposition choice are limited to emergence success.

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?

For examining the consequences of oviposition choice, students generally propose to address the question of whether the host species affects offspring characteristics. 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

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.

For the consequences of oviposition choice, one of the biggest confounding factors is the number of eggs laid on individual beans. If more than one egg is laid on a bean, then the larvae may compete for resources. Therefore, beans with single eggs should be used. 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. 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. Accurate data on time to emergence and mass at emergence require that students check for emergence on a daily basis. As a result, measuring these life history traits may be feasible only in smaller, more advanced classes. Emergence success could be determined on a single day after sufficient time for emergence (approximately 40 days). Therefore, emergence success is more tractable for larger classes.

Data Analysis

The data from the ovipositition choice 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 may be conducted 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.

Most of the offspring traits that students measure will be continuous. Thus, ANOVA may be used to determine the effect of host 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.

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
- 30 plastic 150mm Petri dishes for each replicate of the oviposition substrate choice experiment

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