

Consequences of Natal Bean Discrimination 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 group of students. However, cultures should have sufficient beetles for multiple groups.

Experimental Design

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 an experiment on oviposition substrate choice ([Oviposition substrate choice by bean beetles](#)), in which students determine on which species of beans, other than their natal host, females will lay eggs. The results from that experiment 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?

For examining the consequences of natal choice, students generally propose to address the question of whether the host species (natal or non-natal) 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

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.

For the consequences of oviposition choice, 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. 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. Consequently, measuring these life history traits may be feasible only in smaller, more advanced classes. Linear measures of body size (such as elytra length) or emergence success could be determined on a single day after sufficient time for emergence (approximately 40 days). Therefore, body size and emergence success variables are more tractable for data collection in larger classes.

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.

Most of the offspring traits that students measure will be continuous. As a result, t-tests can be used to determine the effect of natal versus non-natal host species on offspring characteristics. For emergence success, the data would be the number of emerged and non-emerged beetles from each host species type. Differences in emergence success could be determined using a chi-squared test or Fisher's exact test.

Materials and Supplies

- 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
- 35mm Petri dishes for holding individual beans OR flat-bottom tissue culture plates (6 or 12 well)
- 15 countdown timers or stopwatches
- 0.1mg analytical balance for weighing beetles
- 30 vernier calipers for measuring beetle size or inexpensive microscope eyepiece video cameras (Moticam 352, Carolina Biological 591282) that permit image capture and measurement on a computer via USB connection