

# Natal Bean Discrimination by Bean Beetles

## Instructor's Notes

Consult “A Handbook on Bean Beetles, *Callosobruchus maculatus*” for detailed information on growing cultures and handling techniques (available for downloading at: <http://www.beanbeetles.org/handbook.html>). In addition, tips on identifying the sexes including pictures of a male and female that could be used in class are available at: <http://www.beanbeetles.org/handbook.html#IS>.

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

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?

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 over 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?

A sample data entry sheet is available in the [Downloads](#) section as a potential guide for data collection. However, it is most appropriate to guide your students to design their own experiments, a guided inquiry process, rather than giving them explicit directions on how to conduct their experiments.

## **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.

## **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 per unit) or dissection microscopes
- 15 bean beetle cultures with newly emerged adults

- 15 plastic petri dishes to hold beetle cultures and for picking adults females from cultures
- mung beans, *Vigna radiata*, (16 ounces) dried beans, organically grown
- adzuki beans, *Vigna angularis*, (16 ounces) dried beans, organically grown
- 30 small paint brushes
- 30 soft forceps, Bioquip™ featherweight forceps ([Catalog No. 4748 or 4750](#))
- 30 petri dishes (plastic) for holding isolated beetles (35mm) ([Falcon 351008](#)) and for conducting oviposition choice experiment
- 30 petri dishes (three-section) for bean location preference experiment (make caulk line sections in standard plastic 100mm dishes using clear aquarium caulk)
- 15 countdown timers or stopwatches
- marking pens