Cost of Reproduction in Bean Beetles

Instructor Notes

This laboratory activity was designed as part of an introductory level integrative physiology class for biology majors. It corresponds with an introduction to general reproductive physiology in their lecture class. By the time this lab is introduced, students have already had practice designing their own experiments within a guided inquiry type environment and have been introduced to basic statistical analysis techniques.

Introduction of Lab

Students are given the bean beetle handbook (www.beanbeetles.org/handbook) prior to the first lab to familiarize themselves with their new study organism. At the beginning of lab, we review the bean beetle lifecycle as a class before breaking into groups to answer the questions posed in the student handout. We then reconvene as a class to discuss the questions and review the materials available to them before individual groups are asked to design their own experiments. This lab can be successfully performed in small groups (3-4) or can be set up as a class depending on the type of question asked, the number of beetles available and the number of replicates you wish to conduct.

Sample Experimental Question

The initial question asked in this lab was simply what are the costs of reproduction in bean beetles? Students identified multiple components of reproduction that could be easily studied including 1) the cost of producing gametes 2) the cost of mating and 3) the cost of egg laying.

> **Alternative/follow-up questions asked by students:**
> Does the number of eggs laid by the female affect longevity?
> Does the natal bean influence the amount of energy stores available to (and therefore longevity of) the beetle?
> Does the species of bean available to lay eggs on affect beetle longevity?
> Does the time at which a mate is introduced affect longevity?
> Does the number of mates/number of mating events affect longevity?

Sample Experimental Protocol

As the beetles do not feed as adults, the cost for each of these acts was measured by simply calculating longevity for each beetle. Students recorded the adult emergence and death days for each individual in order to determine lifespan. After identifying the reproductive components that they wanted to study, students were asked to consider how they would isolate each component (gamete production, mating and egg laying). The following treatment groups were then selected:

1) Unmated, where newly emerged virgins were placed in individual petri dishes for the duration of the experiment in order to determine the cost of simply being female versus male (gamete production).

2) Mated, where a male and female pair were placed together in a petri dish.
3) Mated with beans, where a mated pair was placed in a petri dish with a layer of beans on which to lay eggs.

Students were provided with bean beetle cultures that were within a few days of having adults emerge and asked to isolate beans that had an identifiable pre-emergence window present on the bean. Note: you may have the students place beans into individual wells in 6 or 12 well plates to isolate emerging virgins however it is not advisable to use these plates for the entire experiment as we have found that given time beetles will manage to climb out of the wells into the spaces between the lid and the curvature of the well. For this reason we used petri dishes to house individuals after emergence. Although sex ratios tend to be 1:1 in beetle cultures, isolation of extra beans was encouraged in order to insure an adequate number of beetles would emerge.

For the first few days, students came in daily to separate beetles into treatment groups as they emerged. After that daily checks were made to determine the death date for each individual. The experiment continued until all beetles had died.

Data Collection

We originally piloted this lab as a class (15 students) in order to have a large number of replicates (50 for each treatment group) and still insure that the outside time commitment was reasonable for the students (as this lab requires students to check on the beetles every day including weekends, student groups signed up on a calendar to check on the beetles so that the individual time commitment outside of lab was not as great). Students were given a 2 hour time frame in which they needed to collect the data each day. As error rates tend to increase as the number of individuals involved increase, I have found that if you are doing this as a class experiment, the easiest way to collect the data is to have the students record the emergence and death days directly onto the individual petri dishes as they check them rather than relying on the use of a separate data sheet. Petri dishes whose beetle(s) are deceased can then be placed on a designated benchtop for the data entry into a central spreadsheet. If working in smaller groups, the number of replicates can be cut back in order to make daily data collection reasonable for your students. I find it helps to reiterate what "dead" means to the class prior to starting the data collection otherwise less active beetles tend to be classified as such before they have actually died.

Data Analysis

Two types of data could potentially be collected. One type would be the lifespan of the beetles, which could be analyzed using an ANOVA. A second possible type could be the number of eggs laid. This could be compared to the lifespan of the female using a correlation analysis.

Equipment and Supplies

For a class of 30 students working in groups of 3:

- 10 bean beetle (*Callosobruchus* maculatus) colonies (on mung beans) with beetles ready to emerge within a few days (additional colonies on black eyed peas can be provided for students wishing to examine effects of natal bean type)
- 16 ounces of mung beans, *Vigna radiata*, dried beans
- 16 ounces of one or more of the following bean species, for students wishing to examine the effect of bean type: black-eyed peas (*Vigna unguiculata*), garbanzo (*Cicer arietinum*), kidney, pinto, black beans (*Phaseolus vulgaris*), soy beans (*Glycine max*), lima beans (*Phaseolus lunatus*), and green pea (*Pisum sativum*)
- 30 soft forceps, BioquipTM featherweight forceps (Catalog No. 4748 or 4750) or 30 small paint brushes for moving beetles from one dish to another
- 120 plastic 150mm Petri dishes for each replicate of the oviposition substrate choice experiment (only 4 dishes per replicate for each group as the female and male mated beetles can be housed together)
- 30 Sharpie marking pens to record dates on petri dishes

This experiment was written by Emily Boone, 2014 (www.beanbeetles.org).