TIEE

Teaching Issues and Experiments in Ecology - Volume 11, August 2016

EXPERIMENTS

Egg size plasticity in bean beetles (*Callosobruchus maculatus*): Does host bean species matter?

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ABSTRACT
In this exercise, students design a series of experiments (1) to test whether female bean beetles adjust the size of their eggs on different host bean species and (2) to determine whether differences in egg size based on host bean species are optimal. Most often, students design experiments in which different females are allowed to lay eggs on different bean species that vary in nutritional quality. After approximately 6 weeks, students measure egg size, female body size, and emergence success of offspring. Experimental design and initial setup require one laboratory period. Data collection requires 1-2 laboratory periods depending on the number of eggs measured. In class, students discuss approaches to data analysis and analyze their data. Outside of class, they write a scientific paper based on their results in the format of *Ecology*.

KEYWORD DESCRIPTORS

- **Ecological Topic Keywords:** Egg size, phenotypic plasticity, evolution, adaptations, life history
- **Science Methodological Skills Keywords:** Data analysis, evaluating alternative hypotheses, experimental design, hypothesis generation and testing, quantitative data analysis, scientific writing, statistics, graphing
- **Pedagogical Methods Keywords:** Authentic assessment, Cooperative learning, Guided inquiry, Student Teaching and Learning

CLASS TIME

One 2-3 hour class period for experimental design and initial setup and 1-2 3-hour class periods 6 weeks later for data collection and data analysis.

OUTSIDE OF CLASS TIME

Students will spend several hours conducting library research and writing papers based on their results.

STUDENT PRODUCTS

Each student prepares a written scientific paper in the style of *Ecology* based on the pooled data from the entire class. If different laboratory groups develop and implement different experimental designs, groups could present posters or oral presentations to the rest of the class.

SETTING

The experiment is carried out entirely in the laboratory.

COURSE CONTEXT

The experiment as described is used in a stand-alone upper-level undergraduate ecology laboratory course with a maximum of 12 students.

INSTITUTION

This experiment has been conducted at a mid-sized private research university. Bean beetles have been used in teaching labs across a wide range of institution types.

TRANSFERABILITY

This experiment is transferable to other levels, given that it is a guided inquiry exercise. At the lower level, students will need additional support and direction as they design the experiment and analyze data. A simpler version of the experiment may be appropriate for introductory level undergraduate students. For example, students could examine egg size plasticity among different bean species or between large and small beans of the same species. Bean beetles have been used for other experiments in high school biology classes and are reliable experimental organisms. Other phytophagous insects that are easily reared in the laboratory on a variety of host plants and for which eggs can be easily identified and measured could be used in this experiment. For example, tobacco hornworms, *Manduca sexta*, and the Brassica butterfly, *Pieris rapae,* are available through biological supply companies.

ACKNOWLEDGEMENTS
This experiment is one of a growing number of laboratory exercises using the bean beetle (*Callosobruchus maculatus*) as a model species. See www.beanbeetles.org for additional experiments. We thank the ecology students at Emory University who pilot-tested the experiment and provided feedback. This work would not have been possible without prior support of the National Science Foundation to Morehouse College and Emory University (DUE-0535903, DUE-0815135, and DUE-0814373). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

SYNOPSIS OF THE EXPERIMENT

Principal Ecological Question Addressed

Do female bean beetles exhibit adaptive phenotypic plasticity in egg size based on host species?

What Happens

First, students work individually before class and then in small groups in class to design experiments to test (1) whether female bean beetles produce eggs of different sizes based on host bean species, and (2) if variation in egg size based on host bean species is optimal. Second, groups present their proposed experimental designs to the class and are guided by the instructor to a consensus experiment. Third, students work together to set up the appropriate replicates of each treatment. Approximately 6 weeks later, students collect data on egg size, female body size, and emergence success of resulting adults. The data are analyzed to determine if female bean beetles exhibit adaptive phenotypic plasticity in egg size based on host bean species.

Experiment Objectives

1. Design an experiment to evaluate whether bean beetles (*Callosobruchus maculatus*) produce eggs of different sizes based on host bean species.
2. Design an experiment to determine if variation in egg size based on host bean species is optimal.
3. Conduct consensus experiments to evaluate whether there is adaptive plasticity in egg size in bean beetles.

4. Analyze and interpret the resulting data to determine whether variation in egg size based on host bean species occurs and whether the variation is adaptive.

Equipment/ Logistics Required

The experiment requires having dense cultures of bean beetles from which females can be isolated. Consult the Handbook on Bean Beetles for detailed instructions on culturing and maintaining beetles. Cultures that are initiated approximately 2 months before the laboratory period (sufficient time for two generations of beetles) will result in dense cultures. Beetles should be from cultures reared on one particular host. Each culture should have sufficient beetles for use by multiple student groups. As bean beetles are a tropical species, they develop most rapidly in warmer temperatures. The time estimates for the experiments are based on rearing beetles in incubators at 30˚C. Beetles can be reared at room temperature. However, this will extend larval development by 1-2 weeks. Beetle cultures are available from Carolina Biological and Ward's.

Below is a list of materials for a class of 24 students.

- 24 dissection microscopes with ocular cameras. We have used Motic cameras, such as Moticam 1 (approximately $200/each). Alternatively, ocular micrometers or microscope adapters for cell phone cameras could be used with stage micrometers as scales. If fewer microscopes are available, students can work in groups of two or three.
- Image analysis software, such as ImageJ
- Bean beetle cultures with newly emerged adults
- 12 Plastic 150mm Petri dishes for picking adults females from cultures
- Plastic 35mm Petri dishes for oviposition by isolated beetles (for example, 72 dishes would be needed to evaluate oviposition on three different bean species with 24 replicates each)
- Many 35mm Petri dishes for holding individual beans or flat-bottom tissue culture plates (6 or 12 well)
- Soft forceps (such as Bioquip™ featherweight forceps #4748 or 4750) or *Drosophila* brushes for handling beetles

- Dried beans (preferably organic) of the species used for culturing the beetles (see Handbook on Bean Beetles). Beetles will emerge successfully from mung beans (*Vigna radiata*), adzuki beans (*Vigna angularis*), blackeye peas (*Vigna unguiculata*), pigeon peas (*Cajanus cajan*), and hyacinth beans (*Lablab purpureus*). Adzuki and hyacinth beans are of lower nutritional quality than the other bean species (https://ndb.nal.usda.gov/ndb/search).
- Index cards and white glue for gluing beans and beetles for measurement

Summary of What is Due

During the first laboratory period, students will produce an experimental design to examine egg size plasticity in bean beetles. After collecting and analyzing the data, each student will write a scientific paper based on the pooled results of the class. Data analysis is conducted in class with facilitation by the instructor.

DETAILED DESCRIPTION OF THE EXPERIMENT

Introduction

All organisms must acquire resources (i.e., food) and allocate those resources to the competing demands of growth, reproduction, storage, and maintenance, while at the same time avoiding death. For energy allocated to reproduction, females have a further decision on how to partition that energy between the number of offspring and the size of offspring. With a given amount of energy, females can either produce a few large offspring or a large number of small offspring. In a seminal paper, Smith and Fretwell (1974) proposed that natural selection will act such that females produce offspring of an optimal size that is determined by the relationship between offspring size and offspring fitness.

Bean beetles (cowpea seed beetles), *Callosobruchus maculatus*, are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans of several species in the family Fabaceae. Specifically, they can successfully emerge from mung beans (*Vigna radiata*), adzuki beans (*Vigna angularis*), black-eye peas (Vigna *unguiculata*), pigeon peas (*Cajanus cajan*), and hyacinth beans (*Lablab purpureus*). Eggs are laid on the surface of bean seeds (=oviposition) singly. Several days after oviposition, a beetle larva (maggot) burrows into the bean and cannot move from the bean on which an egg was deposited. As a result, the quality of the food resources available in a bean will influence the developing individual’s growth, survival, and future reproduction (Mitchell 1975, Wasserman and Futuyma 1981). At 30°C, pupation and emergence of an adult beetle occurs 21-28 days after an egg is deposited, completing one generation of the life cycle. Adults are mature 24 - 36 hours after emergence and they do not need to feed. Adults may live for 1-2 weeks during which time mating and oviposition occurs. Because host beans differ in nutrient quality (USDA Agricultural Research Service) and secondary compounds (Bisby et al. 1994), we would expect the relationship between offspring size and offspring fitness to vary depending on host type. Therefore, optimal offspring size should also differ among host types.

In bean beetles, egg size is known to be affected by both genetic (Fox 1993b) and non-genetic factors (Fox 1993a, Fox and Dingle 1994, Kawecki 1995). In particular, larger eggs are produced by younger females (Fox 1993a), larger females (Kawecki 1995), females who have multiply mated to the same or different males (Fox 1993a), and females reared at high adult densities (Kawecki 1995). These results suggest that females exhibit plasticity in egg size and have the potential to respond to differences in host type.

Materials and Methods

Overview of Data Collection and Analysis Methods: In class, you will be provided with live cultures of bean beetles containing adults that have been raised on mung beans. Supplies of three bean species (mung, adzuki, black-eye pea) also will be available. Female beetles are easily identified in the live cultures because they have two dark stripes on the posterior of the abdomen, whereas the posterior abdomen of males is uniformly light in color (Figure 1).

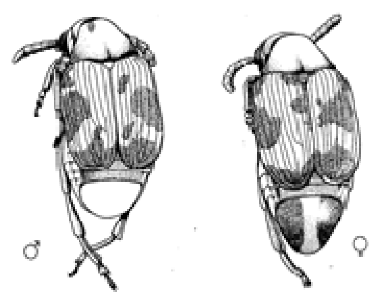

Figure 1. Dorsal view of male and female *Callosobruchus maculatus*. The sex specific coloration of the posterior abdominal plate (pygidium) is shown (Figure from Brown and Downhower 1988).

Prior to the laboratory class, you should design an experiment or set of experiments to determine whether female bean beetles produce eggs of different sizes based on host type and if this variation represents variation in optimal egg size. You will discuss your experimental design with others in a small group, and each group will present a consensus design to the class. Based on the experimental designs presented by the groups, we will discuss common experimental approaches for the entire class.

First, carefully read the background information above and research differences in nutrient quality among bean types on the USDA National Nutrient Database for Standard Reference (http://www.nal.usda.gov/fnic/foodcomp/search/). Then, before the laboratory class meeting:

- Design an experiment to evaluate whether bean beetles (*Callosobruchus maculatus*) produce eggs of different sizes based on host bean species.
- Design an experiment to determine if variation in egg size based on host bean species is adaptive (optimal).
- For each experiment, list the dependent variables you would measure to determine if your predictions were true.
- For each experiment, identify and list the variables you would manipulate in each experiment.
- For each experiment, identify and list the variables you would keep constant in each experiment.
- For each experiment, describe what comparisons between treatments you would need to make to test your predictions.

In class, you will share your experimental design with members of your lab group. Then, as a group you will develop a consensus experimental design that will be shared with the entire class. As a class, we will come to an overall consensus experimental design. Then, we will conduct the experiment. Based on analysis of the data that the class collects, you will write a scientific paper in the format of *Ecology*. In your paper, you should consider the questions below.

Questions for Further Thought and Discussion:

1. Based on your results, do female bean beetles lay different size eggs on different host bean species?

2. What confounding factors may influence egg size? How did you control for confounding factors that may influence egg size in your experiments? If female age influences egg size, how would that change your interpretation of your results?

3. Did the average egg size you observed on beans of one host species correspond to the optimal size for offspring production?

4. Does egg size in bean beetles influence development time? Is there a relationship between egg size and development time in other egg laying animals?

5. Bean beetles are at one end of the continuum for adult lifespan, with an adult lifespan of only 1-2 weeks and no overlap between generations. If bean beetles lived longer and guarded the beans on which eggs were laid until their offspring emerged, would that change your expectations about egg size variation?

6. Host quality may be uniformly good or poor. Alternatively, environments might be heterogeneous and females may encounter both good and poor quality hosts. Based on literature research, how would egg size vary depending on whether environments are heterogeneous or homogenous? Would your answer change if females were able to exhibit phenotypic plasticity (change their egg size) at small spatial scales?

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Tools for Assessment of Student Learning Outcomes:

You will be assessed based on a scientific paper that you write in the format of *Ecology*. Prior to writing the paper, you will be involved in developing the rubric for the paper by "dissecting" a published paper from *Ecology* to see how it is written (see *Anatomy of a Scientific Paper* handout).

NOTES TO FACULTY

Challenges to Anticipate and Solve

Challenge #1: Making appropriate predictions: Students often make conflicting predictions as to how egg size should vary with host quality. Some students argue that if host quality is low, then females should not invest much energy in eggs and therefore eggs will be smaller on lower quality hosts. In contrast, other students reason that females will need to give offspring a head start if the offspring are on a low quality host. As a result, eggs will be larger on lower quality hosts. Both of these arguments are reasonable and it provides instructors with the opportunity to have students practice their reasoning skills. The prediction that females would not invest much energy in eggs laid on poor quality hosts assumes that females would have an alternative host available and that females can distinguish between low and high quality hosts. If females are restricted to a low quality environment, they are predicted to produce larger eggs (e.g., McGinley et al. 1987, Fox and Czesak 2000).

Challenge #2: Determining appropriate dependent variables: Especially when designing the experiment to test whether variation in egg size among hosts is optimal, students often have difficulty determining what dependent variables to measure. Time to emergence, body size at emergence, and emergence success can be measured in a reasonable time span. Students may suggest other offspring characteristics, such as egg hatching rate, sex ratio, lifespan, or adult reproductive success. Life history traits 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., adult reproductive success and egg hatching rate). Finally, for other offspring traits like sex ratio, the predictions of how egg size would influence sex ratio on different quality hosts are not clear.

Challenge #3: Identifying female beetles: Although bean beetles are sexually dimorphic (see Figure 1), some students have difficulty distinguishing males from females. We begin by showing students a picture of male and female beetles and describing the key difference (the coloration of the posterior abdominal plate or pygidium). In addition, we have students isolate females from stock cultures to a petri dish and check that they are females before the beetles are added to the smaller petri dishes with beans.

Challenge #4: Identifying eggs on beans: Students may have difficulty distinguishing between eggs and frass on the bean surface. Eggs are larger, smooth, and have a distinct dome shape when viewed with magnification. Typically, if students are shown examples of eggs on beans, they are better able to find them on their own. An instructor could place small petri dishes with beans with eggs attached at each lab station. We often rotate through the room and show individual students beans with eggs.

Challenge #5: Linking data from female beetles to her offspring: Since female body size may influence egg size, linking data from female beetles to her offspring is critical. If students are allowed to develop their own labeling system for females and offspring, they often come up with unnecessarily complicated and non-intuitive systems that make linking the data difficult. We have had better success by explicitly discussing how females and their offspring should be labeled and how to record the appropriate data. Females can be lettered and offspring numbered. For example, “A-11” would be offspring 11 from female A.

Challenge #6: Data collation: This experiment can generate a large amount of data. As a result, collating the data from students in a uniform fashion to reduce the amount of data clean-up is essential. For small classes, we have used Google sheets and created the format of the spreadsheet first. For large classes, we have used Google forms where the data entered into the form populates a Google sheet. In either case, we have students indicate who collected the data in case we need to recheck inconsistent data.

Challenge #7: Statistical comparisons: Students may have difficulty determining the appropriate statistical comparisons and then interpreting the results depending on their statistical training prior to the course. After allowing the students to discuss the comparisons in groups, the instructor should review the possible comparisons and their interpretation. We have this discussion after the final data are collected. It could take place after the consensus experimental design is determined during the first lab period. However, given the time span between the initial lab period and the data analysis, students may have difficulty recalling the discussion if it takes place during the initial lab period. Given the complexity of some of the statistical comparisons, after we discuss approaches to data analysis, students work in small groups in class to conduct the analysis with the instructor available to address questions that arise.

Comments on Introducing the Experiment to Your Students:

Prior to this exercise, we present the concepts of phenotypic plasticity, adaptive plasticity, and life history traits to students in the lecture portion of the course. Since this exercise is designed as a guided inquiry, students working on their own begin by developing potential experimental designs as a pre-lab assignment that could be submitted for a grade. At the beginning of the first lab session, we often review a little of the natural history of bean beetles and the materials that will be available to the students and answer any questions that students might have. Then, students discuss their designs with members of their lab group. The instructor can facilitate this process by moving among groups and asking them questions about their designs. Finally, each group contributes their group design to a whole-class discussion of approaches to answering the experimental questions. We guide students to a consensus experiment that is conducted by the entire class. However, individual groups could carry out their own experimental designs. In this case, each student will need to increase the number of replicates that they set up in order to have sufficient replication for statistical analysis. Typically, a minimum of 20 replicates per treatment group is required. In our experience, most groups propose very similar experiments and therefore the process of coming to a consensus experiment for the class is more a confirmation of the ideas of each group rather than rejecting the ideas of most groups in preference to that of a single group.

To determine if females exhibit phenotypic plasticity in egg size based on host type, female beetles are placed in a 35mm petri dish with a single layer of a single type of bean. After oviposition, egg width and egg length can be measured. Students might suggest placing females in a petri dish with several types of beans. However, since females exhibit a preference for bean types, they may not lay eggs on low quality hosts. An alternative approach would be to move females sequentially from one bean host to another. To prevent confounding host type with female age, the order in which females are placed with a particular type of bean would need to be randomized. Although an elegant design, we have found it to be unnecessarily complicated.

To determine if variation in egg size based on host quality is adaptive, effects of egg size on some measure of offspring fitness needs to be determined. We have students isolate beans with single eggs (to avoid the confound of competition within the bean) into wells of 12-well tissue culture plates. Then, we measure adult emergence success and size at emergence. For size at emergence, we use linear measures of body size (elytra length), because beetles begin to lose weight as soon as they emerge from the bean as an adult. If students are able to check on beetle cultures on a daily basis, they also could measure time to emergence.

Comments on the Data Collection and Analysis Methods Used in the Experiment:

Bean Beetle Cultures

Detailed information on growing cultures and handling techniques, as well as tips on identifying the sexes are available in the Laboratory Methods section of the Bean Beetle website (www.beanbeetles.org).

The experiment requires having dense cultures of bean beetles from which females can be isolated. Beetle cultures are available from Carolina Biological (www.carolina.com) and Ward's Science (www.wardsci.com). 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 working in pairs. However, each culture should have sufficient beetles for use by multiple student groups. Once cultures are established, they do not need to be monitored or recultured until 2 months later.

Experimental Design

To determine if egg size varies based on host bean type, we have each student set up a minimum of 5 replicates of each bean type being used. The number of replicates per student will depend on class size, but a minimum of 20 replicates per treatment should be used. To set up a replicate, a single female from a stock culture is added to a 35mm Petri dish with a monolayer of beans. Oviposition will readily occur during a 48-hour period. Although most adult females in an active culture will have been inseminated, some females may have only recently emerged (and be infertile) and others are near the end of their adult life (and laid most of their eggs). Replication in the class will allow for failures in egg laying.

To measure egg size, beans are glued with white glue to index cards with the egg facing upward. Multiple beans can be glued to the same index card. One approach is to glue all of the beans with eggs laid by a particular female on one card. Egg width and egg length then are measured for each egg individually under a dissecting microscope using a microscope camera and image analysis software or an ocular micrometer with a stage micrometer for scale. Egg area can be estimated using the formula for an ellipse (egg length x egg width x π x ½). The same approach is used for measuring female body size. For adult body size, students measure the length of the elytra (wing covering). Since the eggs are affixed to the beans and the shell remains after hatching, egg size measurements can be made at any time after egg laying. If students are examining the consequences of egg size variation on offspring fitness, egg size can be measured at the very end of the experiment. However, female body size can only be measured at the end of the oviposition part of the experiment.

Statistical Analysis

Female body size might affect egg size. Consequently, we use female body size as a covariate in an ANCOVA with host bean type as a fixed effect and egg size (egg width, egg length, or egg area) as the dependent variable to determine if egg size varies based on host bean type, assuming that female body size and measures of egg size are normally distributed. Since the ANCOVA assumes that the slope of the relationship between female body size and egg size is the same for all host bean types, we begin by including the interaction term (female body size x host bean type) in the model. If the interaction is not significant, it can be removed from the model and the model rerun. If the interaction is significant, we inspect individual scatterplots of female body size versus egg size for each host type. If a simpler approach to statistical analysis is preferred, students could conduct two separate ANOVAs. First, they could determine that female body size does <u>not</u> differ based on host bean type and therefore is unlikely to be a confound. Then, they could evaluate whether mean egg size differs among host bean types.

To determine if variation in egg size based on host bean type is adaptive, effects of egg size on some measure of offspring fitness needs to be evaluated. Adult emergence success and size at emergence (elytra length) are the most tractable traits to measure. However, if students are able to check on beetle cultures on a daily basis, they also could measure time to emergence. After egg laying is complete, we have students isolate beans with single eggs (to avoid the confound of competition within the bean) into wells of 12-well tissue culture plates. To avoid confusion, only beans with eggs from the same female should be put in a particular tissue culture plate. After approximately 4 weeks, depending on incubator temperature and host bean type, adult beetles should emerge. Any eggs that have not resulted in an emerged beetle should be scored as non-emerged. The body size of emerged adults can be measured in the same way as described above. So that we do not need to wait for the emerged adults to die, we euthanize the emerged adults by placing the tissue culture plates in a -80C freezer for 10 minutes. In addition to measuring adult body size, the sex of the adults needs to be determined and recorded, as bean beetles are sexually size dimorphic.

To determine the effect of egg size on emergence success, we use binary logistic regression with egg size as the independent variable and emergence success (yes or no) as the dependent variable. For simplicity, we carry out the analysis separately for each host bean species and then determine if the relationships differ qualitatively. However, a more complex statistical model could be used that includes the host bean type and the interaction between egg size and host bean type as additional independent variables. To determine the effect of egg size on the resulting adult body size at emergence, we use simple linear regression with egg size as the independent variable and adult body size at emergence as the dependent variable. Separate analyses are done for each host bean type and each sex. Statistical models with host bean type and the interaction between host bean type and egg size also could be used.

Comments on Questions for Further Thought:

1. Based on your results, do female bean beetles lay different size eggs on different host bean species?

We predicted that females would lay smaller eggs on higher quality hosts (mung beans and black-eyed peas) and larger eggs on lower quality hosts (adzuki beans).

In the first semester in which we carried out the experiment, female body size had a significant effect on egg size (estimated as egg area), but the relationship varied based on host bean type (ANCOVA, host bean type x female body size interaction, $F_{2,526}$=4.38, P=0.013) (Figure 2). For smaller females, eggs laid on black-eyed peas (BEP) were significantly smaller than those laid on mung and adzuki beans. However, for larger females, egg size did not differ between bean types.

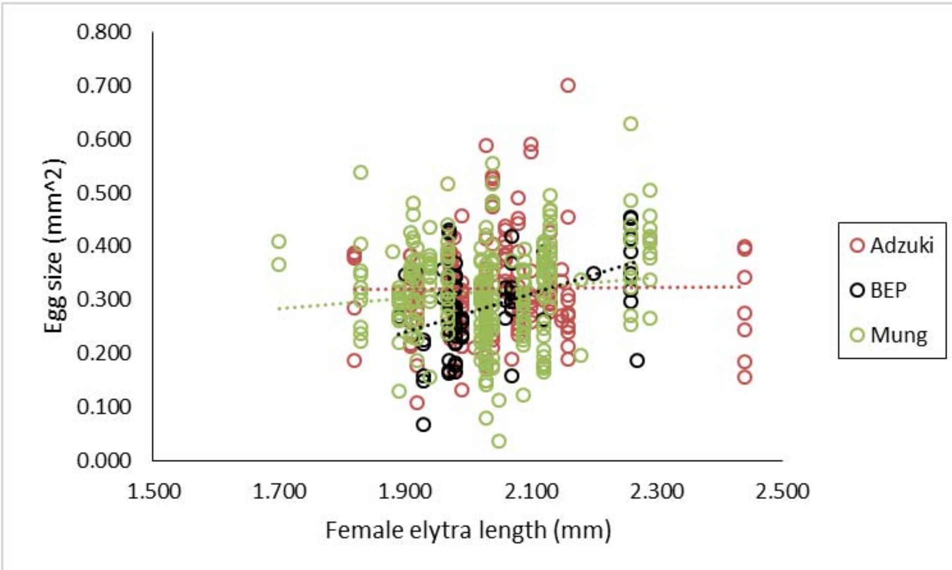

Figure 2: Effect of female body size on egg size (estimated as egg area) for three different host bean species. The interaction between female body size and host bean type was significant in an ANCOVA.

When we repeated the experiment, neither the interaction between female body size and host bean type (ANCOVA, $F_{2,880}$=1.01, P=0.37) nor female body size (ANCOVA, $F_{1,882}$=0, P=1) had a significant effect on egg size. However, eggs laid on BEP were significantly smaller than those laid on adzuki beans and mung beans (ANCOVA, $F_{2,883}$=6.61, P=0.001) (Figure 3).

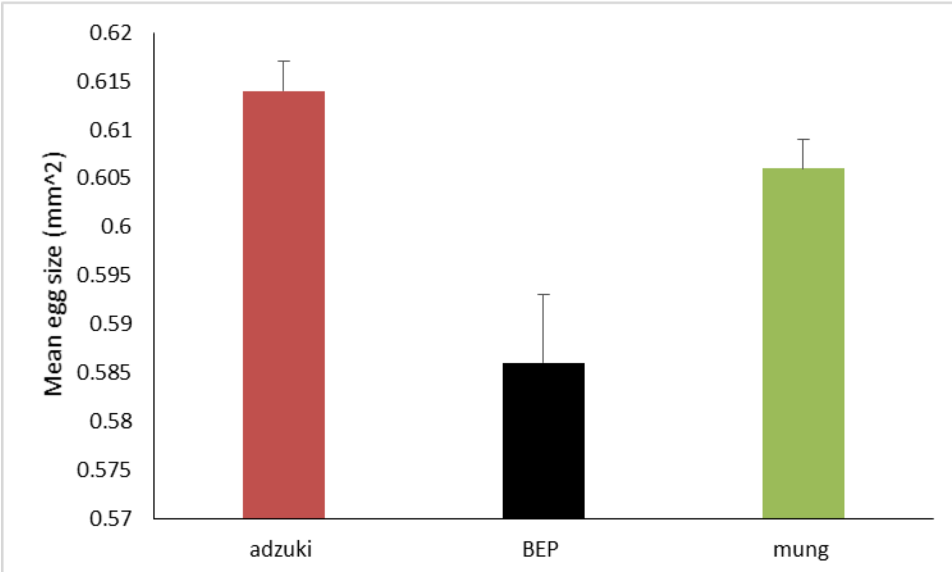

Figure 3: Phenotypic plasticity in egg size based on host bean type. Values are means ± SE.

Our data partially support the prediction that females will lay smaller eggs on higher nutritional quality host beans. BEP and mung beans have more protein and lipids than adzuki beans (USDA National Nutrient Database for Standard Reference). Females did lay smaller eggs on high quality BEP hosts. However, egg size did not differ between eggs laid on adzuki beans (a low nutritional quality host) and mung beans (a high nutritional quality host). Female bean beetles are sensitive to the size of beans on which they lay their eggs and prefer larger beans as a means of minimizing the potential negative effects of competition between larvae within a bean (Beck, Migabo and Blumer 2011). Therefore bean size may be another quality of host beans that influences egg size.

2. What confounding factors may influence egg size? How did you control for confounding factors that may influence egg size in your experiments? If female age influences egg size, how would that change your interpretation of your results?

Female body size, female age, and female mating history are known to affect egg size. Our students control for female body size by recording the size of each female so that information may be used as a covariate in an analysis of covariance that enables them to look at egg size differences between different bean species independent of female body size (see Figure 2). It is possible to control for female age by using only newly emerged and mated virgin females in the study. However, this is logistically challenging and would add a minimum of 4 weeks additional time to the study if students staged cultures to obtain virgin females (see www.beanbeetles.org/handbook for a protocol on staging virgins). In practice, having students obtain mated females from a mass culture provides a random collection of females of different ages. This adds some noise to the data, but does not create a systematic bias that would confound the analysis, as long as the sample size of females is large (>25).

3. Did the average egg size you observed on beans of one host species correspond to the optimal size for offspring production?

In our first experiment, egg size had a significant effect on emergence success in all three host bean species (binomial logistic regressions, adzuki: Wald chi-square=12.3, P<0.001; BEP: Wald chi-square=9.6, P=0.002; mung: Wald chi-square=6.0, P=0.015). The relationships between egg size and emergence success suggest that optimal egg size should be larger for eggs laid on mung and adzuki beans compared to BEP (Figure 4). However, egg size was unrelated to adult size at emergence for both males and females emerging from any host (simple linear regression, Figure 5).

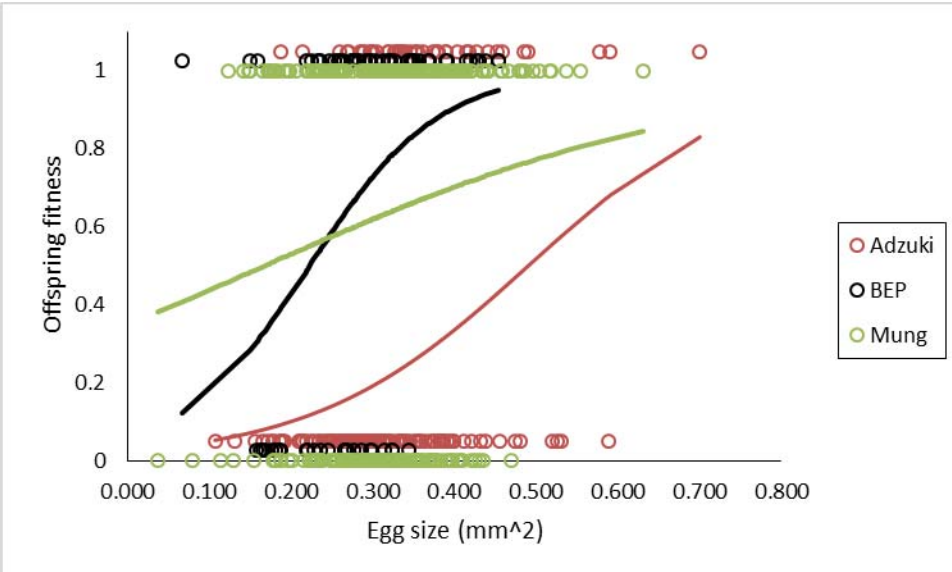

Figure 4: Effect of egg size on offspring fitness in different host bean species. Offspring fitness was estimated as emergence success (emerged=1 or did not emerge=0).

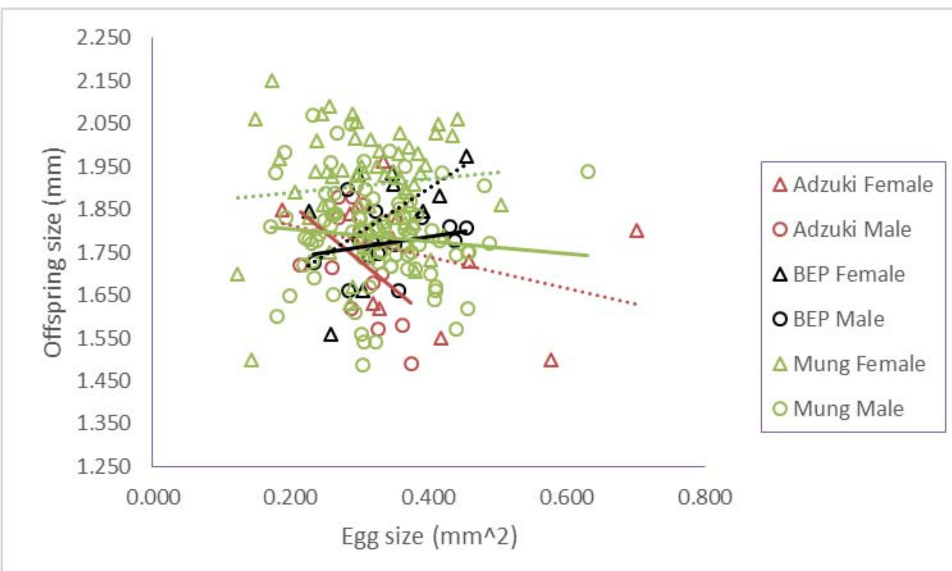

Figure 5: Effect of egg size on offspring size at emergence in different host bean species. Trend lines are solid for males and dotted for females.

When we replicated the experiment, egg size significantly affected emergence success only for eggs laid on mung beans (binomial logistic regressions, adzuki: Wald chi-square=0.51, P=0.48; BEP: Wald chi-square=1.3, P=0.26; mung: Wald chi-square=6.4, P=0.011) (Figure 6). Again, egg size did not have a significant effect on size at emergence for either sex on any host (simple linear regression, Figure 7). As a result of the absence of an effect of egg size on emergence success and size at emergence, a clear prediction about optimal egg size cannot be made. Alternative measures of offspring fitness might suggest differences in optimal egg size for eggs laid on different host bean types.

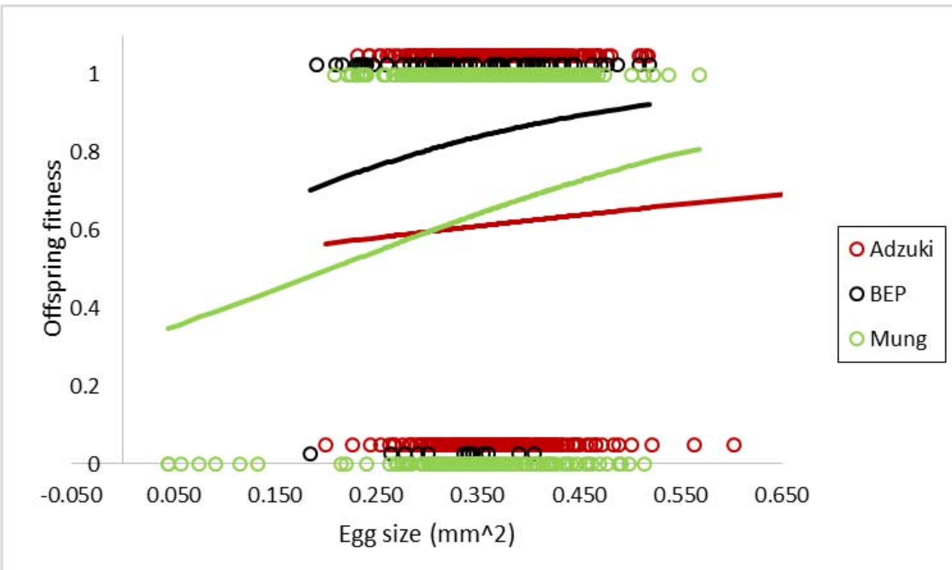

Figure 6: Effect of egg size on offspring fitness in different host bean species. Offspring fitness was estimated as emergence success (emerged=1 or did not emerge=0).

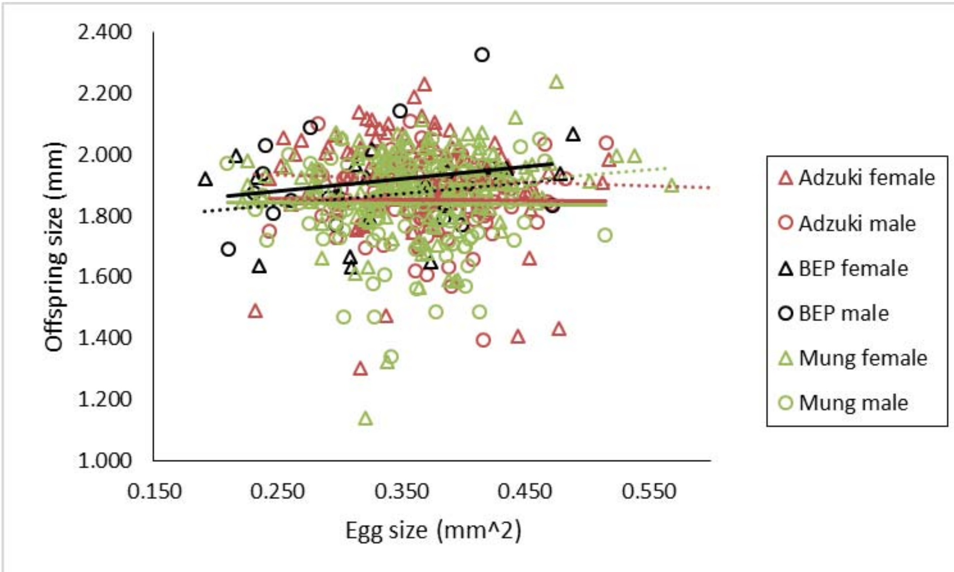

Figure 7: Effect of egg size on offspring size at emergence in different host bean species. Trend lines are solid for males and dotted for females.

4. Does egg size in bean beetles influence development time? Is there a relationship between eggs size and development time in other egg laying animals?

In bean beetles, development time (the time from an egg being laid to the time of adult emergence) is influenced by egg size for eggs laid on a given host species (Fox 1994). Fox (1994) found that larger eggs developed faster than smaller eggs and yielded larger adults in an experiment using BEP host beans. Similar relationships between egg size and development rate have been documented for other insect species (see references in Fox 1994) and among marine invertebrates but the relationship may not be a simple negative linear function (Levitan 2000). Furthermore, the relationship between egg size and development rate may be complicated by the presence of a feeding or non-feeding larval form in the life history of a given species (Strathmann 1985). Among some marine invertebrates, larger eggs take a longer time to hatch, but larger eggs yield larger larvae that feed for a decreased time and consequently metamorphose to adults in a shorter time period (Strathmann 1985). For example, larger eggs generally have shorter total development times to adult metamorphosis than smaller eggs among six marine gastropods (*Conus*), because small larvae must feed longer prior to metamorphosis (Strathmann 1985). Similarly, among fishes with pelagic eggs, larger egg size results in a longer time to hatching at a given temperature and a longer time for yolk sac absorption in non-feeding larvae, but will yield larger juveniles than smaller eggs (Pepin 1991).

5. Bean beetles are at one end of the continuum for adult lifespan, with an adult lifespan of only 1-2 weeks and no overlap between generations. If bean beetles lived longer and guarded the beans on which eggs were laid until their offspring emerged, would that change your expectations about egg size variation?

You might predict that egg size would increase if guarding resulted in decreased offspring mortality or decreased competition with other larvae (see Nussbaum and Schultz 1989). However, parental care would not change the differences in nutritional quality between bean species, so no change in the variation in egg size among bean type is predicted.

6. Host quality may be uniformly good or poor. Alternatively, environments might be heterogeneous and females may encounter both good and poor quality hosts. Based on literature research, how would egg size vary depending on whether environments are heterogeneous or homogenous? Would your answer change if females were able to exhibit phenotypic plasticity (change their egg size) at small spatial scales?

McGinley et al. (1987) showed that females should produce smaller eggs in uniformly good environments and produce larger eggs in uniformly poor environments. In heterogeneous environments, optimal egg size depends on the relative frequency of good versus poor environments and the relative difference in host quality (McGinley et al. 1987). If good environments are relatively common or the difference in host quality is high, females should produce small eggs (i.e., behave as if they are in uniformly good environments) even though the small eggs will not produce successful offspring on poor hosts. In contrast, if good environments were less frequent or the differences in host quality were small, females are predicted to produce eggs that are intermediate between those produced in good and poor environments (McGinley et al. 1987).

If females are able to exhibit phenotypic plasticity at small spatial scales (i.e., they can examine a bean, determine its quality, and adjust egg size appropriately), then we might expect females to lay eggs of different sizes on different bean species even in a heterogeneous environment.

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Comments on the Assessment of Student Learning Outcomes:

We ask students to develop their own rubric for science writing assignments. This is an effective way to have students learn about science writing and it can increase buy-in from the students on how they are assessed (see *Anatomy of a Scientific Paper* handout). Having students submit the rubric filled out in reference to their own writing assignment may insure greater consideration of the rubric by the students. However, we have not tried this technique in our own teaching.

If you would rather develop your own rubric rather than have students develop it, we highly recommend the rubric bank (www.rsc.org/suppdata/rp/c2/c2rp00023g/c2rp00023g_5.pdf) developed by Kishbaugh et al. (2012) that helps faculty design rubrics for both lower-order and higher-order skills using a variety of assignments.

Kishbaugh, TLS, S Cessna, SJ Horst, L Leaman, T Flanagan, D Graber Neufeld, and S Siderhurst. 2012. Measuring beyond content: a rubric bank for assessing skills in authentic research assignments in the sciences. Chemistry Education Research and Practice 13:268-276

Comments on Formative Evaluation of this Experiment:

Students in the ecology course at Emory University (Fall 2012 and Fall 2013) were asked to rank each experiment they completed on a ten point scale with respect to how useful each experiment was in reinforcing their knowledge and understanding of the subjects covered in the lecture portion of the course. A score of 10 meant that the study was the most useful. In addition, the students were asked which studies were the most and least enjoyable and which study best increased their understanding of the scientific method. The students completed four experiments (including the bean beetle experiment) plus an independent project during the semester.

In 2012, 14 students in the course completed the course evaluation. The average ranking of the current experiment was 8. Only one student considered it the most enjoyable experiment; however, three students thought that this experiment was the one that best increased their understanding of the scientific method, including one student who thought that it was the least enjoyable. The majority of students thought that the independent projects were most important to their understanding of the scientific method. Most negative comments about the experiment related to the frustration with the confusing nature of the data. Much of this seemed to be related to the complicated identification scheme that students developed themselves. As a result, in the following semester, students were more directly coached in the development of an identification system (see Challenge #5).

In 2013, 13 students in the course completed the course evaluation. The average ranking of the experiment was 7.8. Two students considered it the most enjoyable and three considered it the most useful, including one student who thought that it was the least enjoyable. Again, the majority of students thought that the independent projects were most important to their understanding of the scientific methods. Most negative comments related to the tedium of measuring large numbers of beetles and eggs. Students measured 886 eggs and 516 emerged adults. Instructors could limit the number of eggs and emerged adults measured if this is an issue.

Although student writing assignments and end-of-semester surveys can be used for formative evaluation that allows for adjustments from semester to semester, students could be evaluated before completion of the experiment. For example, students bring to class written proposals for experimental designs that could be evaluated. In addition, after the class has discussed experimental approaches, students could be asked to write a minute paper explaining how the consensus experimental design tests the hypotheses that females exhibit phenotypic plasticity in egg size based on host bean type and that this plasticity is adaptive. Both of these types of formative evaluation would allow an instructor to determine how well students understand adaptive plasticity and how to test it, which may influence how instructors discuss the results of the experiment with their class at a later date.

Comments on Translating the Activity to Other Institutional Scales or Locations:

1. translating this experiment to larger scales if you teach at a larger school and vice versa,

   Because bean beetles are easy to rear in large numbers and the materials for the experiment are inexpensive, this experiment could easily be scaled up to larger institutions.

2. using this lab in different regions of the country or world, in different seasons, or using different study species or systems,

   Because the experiment is a laboratory experiment, it could be used in most regions. The only restrictions would be those associated with the shipment of bean beetles. This experiment could be conducted with other phytophagous insects that are commercially available, easily reared in the laboratory and can be induced to use a variety of host plants, such as tobacco hornworms, *Manduca sexta*, and the Brassica butterfly, *Pieris rapae.* However, the eggs in these species are relatively fragile and are more likely to disintegrate when the larvae hatch, so eggs would need to be measured before hatching.

3. using this activity to teach ecology to students with physical or other disabilities, and

    This experiment may be difficult for students with physical disabilities, as it requires the manipulation of small insects. In addition, students with visual disabilities may have difficulties distinguishing between the sexes and identifying eggs on beans.

4. using this activity to teach ecology in pre-college settings (K-12).

    Although this experiment has not been tested in pre-college settings, bean beetles have been used for other experiments in high school biology courses. For example, see Natal Bean Discrimination by Bean Beetles.

STUDENT COLLECTED DATA FROM THIS EXPERIMENT

We have included student-collected data from this experiment from two different iterations of the experiment.

COPYRIGHT STATEMENT

Lastly, we request that you return your students' and your comments on this activity to the TIEE Managing Editor (tieesubmissions@esa.org) for posting at this site.

GENERIC DISCLAIMER

Adult supervision is recommended when performing this lab activity. We also recommend that common sense and proper safety precautions be followed by all participants. No responsibility is implied or taken by the contributing author, the editors of this Volume, nor anyone associated with maintaining the TIEE web site, nor by their academic employers, nor by the Ecological Society of America for anyone who sustains injuries as a result of using the materials or ideas, or performing the procedures put forth at the TIEE web site, or in any printed materials that derive therefrom.