

Inducing Evolution in Bean Beetles

Instructor's Notes

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

The student handout is written as a guided inquiry that allows students to design their own experiments, rather than instructors giving students explicit directions on how to conduct their experiments. No matter the exact experiment that students design, the experiments will require 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. Newly emerged cultures work better for this experiment than older cultures.

Instructors should caution students to prevent the accidental release of bean beetles from the laboratory environment. *Callosobruchus maculatus* is a potential agricultural pest insect that is not distributed throughout the United States and Canada. It is essential that you keep your cultures secured in a laboratory environment to ensure that they are not released to the natural environment. Disposal of cultures (and beans (seeds) exposed to live beetles of any life cycle stage) requires freezing (0°C) for a minimum of 72 hours prior to disposal as food waste. If you have any questions about the handling or disposal of bean beetles, please contact Larry Blumer at lblumer@morehouse.edu or 404 658-1142 (voice or FAX). Information also is available at: www.beanbeetles.org in the Handbook section.

Experimental Design

This study is different from most experiments in that we are asking students to design an experiment to induce a predicted outcome and assess whether that outcome occurred. Students will test the hypothesis that they can induce evolution in bean beetles. The most obvious traits that are highly variable and readily measured are body mass and development time (time from egg-to-adult emergence). Two types of experiments are possible:

- A directional selection experiment in which one extreme phenotype is selected to start a new population or
- A genetic drift experiment in which a population bottleneck is created.

Directional Selection This experiment could be performed by students selecting one extreme phenotype to start new populations. The extreme phenotype could be at either end of the trait distribution, so either up-selection or down-selection is possible. For example, the variation in body mass of pre-experiment control populations could be evaluated so that individual males and females with body mass in the top 5% of the distribution (up-selection) [or the bottom 5% of the distribution (down-selection)] could

be selected to be founders of a new population. Similarly, the distribution of development time could be evaluated in pre-experiment control populations so extreme selection criteria could be proposed. We suggest that new populations should be started with 15 to 25 males and females (using the same numbers for both selection treatments and control populations). New control populations should be run simultaneously with selection treatment populations to ensure that any changes seen in the selection treatments are a consequence of selection and not an environmental effect. Since development can be completed in as few as 3 or 4 weeks at 30°C, several generations of a directional selection experiment could be performed in a semester course. However, data collection requires that students attend to cultures every day once adult beetles begin emerging. Data on development time requires a record of the date each adult emerged, so each day all newly emerged adults would need to be collected and removed from each culture. Similarly, data on body mass requires that beetles are weighed within 24 hours of emergence because adults do not feed and body mass decreases with age and as a consequence of mating (for males) and egg laying (for females). Data collection should occur for 7-10 days once adults begin emerging. Having each pair of students run one control and one selection treatment replicate culture will minimize the amount of data collection each student must perform while ensuring adequate replication in a class of 20-30 students. Conducting a selection experiment to induce evolution requires the assumption that the observed variation in the trait being selected is caused by genetic differences between individuals (variation must be heritable). For example, we assume that parents with greater than average body mass will produce offspring with greater than average body mass. Egg-to-adult development has very small or zero heritability so even very extreme selection is unlikely to yield evolutionary change.

Genetic Drift This experiment is simpler than a selection experiment since the characteristics of the treatment populations need not be quantified at the start of the experiment. Students could start control and genetic bottleneck treatment cultures by manipulating the number of adult beetles that start a new culture. Our students have done this with control cultures of 15 or 25 randomly chosen males and females and bottleneck cultures of 3 or 5 randomly chosen males and females. As in the selection experiment, the control and bottleneck treatments must be run simultaneously to ensure that any changes observed in the bottleneck treatments are due to drift and not environmental effects. Once adult beetles begin to emerge after 3-4 weeks data must be collected each day for 7-10 days from every culture. Data on development time requires a record of the date each adult emerged, so each day all newly emerged adults would need to be collected and removed from each culture. Similarly, data on body mass requires that beetles are weighed within 24 hours of emergence because adults do not feed and body mass decreases with age and as a consequence of mating (for males) and egg laying (for females). Having each pair of students run one control and one selection treatment replicate culture will minimize the amount of data collection each student must perform while ensuring adequate replication in a class of 20-30 students. In smaller classes, we have had each student run one control and one bottleneck treatment replicate. Heritability may be a factor in the occurrence of genetic drift since sampling error causing an increase in homozygous genotypes must actually be reflected in a change in phenotype frequency. When heritability is at or near zero, either a population is already

homozygous for the gene(s) that underlie a given trait or genotypic differences play a minor role in the observed variation in a trait.

Data analysis—The data from either experiment will be the sex of the adult beetle, its development time or its body mass. The mean development time or body mass should be calculated for each culture along with the minimum, maximum and variance. These statistics should be calculated separately for males and females. In selection experiments, differences between control populations (run simultaneously with the treatment populations) and the treatment populations may be evaluated with two sample t-tests. In genetic drift experiments, the same statistics will be calculated, but statistical tests are not clearly applicable. The expected outcomes of genetic drift are random changes from the control populations, so the average values for all control populations should be compared to each individual bottleneck population. The bottleneck treatment populations could be categorized as having drifted in a positive direction or a negative direction and separate t-tests (or non-parametric equivalent Mann-Whitney U) could be performed to compare those groups with the control populations. Alternatively, the characteristics of adults starting each population (founders) could be compared to the characteristics of the individuals emerging (descendants) from the same populations. Genetic drift may occur in all populations but we expect greater drift (more phenotypic change) in bottleneck populations than in control populations. Comparisons between founder and descendant data could be evaluated using a pairwise test (paired t-test). Additional analyses may be performed by addressing two predictions about population changes expected as a consequence of genetic drift. We expect the variation among bottleneck populations to be greater than that among control population since genetic drift should cause more change in the bottleneck populations than in the controls. This prediction could be tested with Levine's test for equality of variances. We also predict a pattern in the variation within each independent population. Bottleneck populations are predicted to exhibit less variation (regardless of the direction of drift) than control populations. This prediction could be tested by means of a t-test on the collection of variance values calculated for each population to compare the control and bottleneck populations.