Inducing Evolution in Bean Beetles

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Abstract: Demonstrating and measuring evolution in a laboratory environment with animal species is typically very challenging. However, insects are among the most amenable species for such studies and the bean beetle, *Callosobruchus maculatus*, is the easiest species in which such studies may be conducted. Bean beetles are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae) and the entire pre-adult parts of the life cycle occur inside the host bean. In this study, students design and conduct experiments to evaluate whether evolution by natural selection (or alternatively, genetic drift) may be induced in laboratory populations.

Introduction

This laboratory study is one of a growing number of inquiry-based laboratory investigations using bean beetles (*Callosobruchus maculatus*) as a model system for undergraduate laboratories. Detailed resources on this insect model system may be found our website (www.beanbeetles.org) including a downloadable handbook on the biology, culturing, and handling of bean beetles.

In this study, we challenge students to design and conduct experiments to evaluate whether evolution by genetic drift (or alternatively evolution by natural selection,) may be induced in laboratory populations. The student handout does not give students a detailed protocol for the experiment because we have developed this study as a guided inquiry exercise. This method of teaching and learning requires that instructors guide the development experimental protocols by students and then permit students to conduct their experiments. It is necessary and appropriate to expect some variation between classes and instructors in the specific experimental design used to address a given question.

Sample data are presented on a genetic drift experiment with recommendations on methods for data analysis. Given the complexity in interpreting data from a genetic drift experiment, this study would appropriate for upper-level courses in ecology. An experiment inducing evolution by natural selection could be successfully implemented in both introductory and advanced courses.

Student Outline

Inducing Evolution in Bean Beetles

Objectives

- Design and perform an experiment to determine whether evolution by genetic drift or directional selection can be induced in laboratory populations of bean beetles, *Callosobruchus maculatus*.

- Evaluate control and experimental populations to measure evolutionary change.

Introduction

Evolution is defined as genetically based phenotypic change that occurs over generational time spans. Although natural selection is typically the most potent cause for evolution and is the principal cause for evolutionary change, other processes, such as mutation, migration, and genetic drift also can cause evolution (Freeman and Herron 2007). Natural selection occurs when a trait, such as body size, varies in a population and individuals differ in their survival and reproductive success as a consequence of the particular character of a trait. For example, if adult body mass varied in a population and the risk of predation were greater among the smallest individuals in the population, then the larger individuals would have greater survival and consequently greater reproductive success than the smaller individuals. Directional selection, such as that described for body mass, may result in directional evolution if the variation in the trait (body mass variation in this example) were caused by genetic differences among individuals. In other words, if the variation in body mass were heritable, then directional selection on body mass would cause directional evolution in body mass. The other potential causes for evolution (mutation, migration, and genetic drift) are real, but all cause random phenotypic changes in a population. Mutation is the spontaneous change in the genotype of an individual that may cause a change in the phenotype of the offspring of that individual. Migration is the movement of individuals into a population (immigration) or movement out of a population (emigration). In either type of migration, the mean value for a trait and variation in that trait in a given population may change as a result of such movement. Genetic drift results from random genotypic change that can occur when a population has very few individuals among whom reproduction occurs. When a population contains few individuals, even random mating may result in the loss of alleles and an increased frequency of homozygous genotypes compared to populations with greater numbers (Futuyma 1986). Genetic drift is a form of reproductive sampling error. To the extent that random changes in genotype frequencies result in changes in phenotypes, phenotypic evolution may occur as a consequence of genetic drift.

In this study, you will design and conduct experiments to induce evolutionary change in an insect species, bean beetles (cowpea seed beetles), *Callosobruchus maculatus*. Bean beetles are agricultural pest insects of Africa and Asia. Females lay their eggs on the surface of beans (Family Fabaceae). Eggs are deposited (=oviposition)

singly and several days after oviposition, a beetle larva (maggot) burrows into the bean. At 30°C, pupation and emergence of an adult beetle occurs 25-30 days after an egg was deposited. Adults are mature 24 - 36 hours after emergence and they do not need to feed. Adults may live for 7-10 days during which time mating and oviposition occurs (Mitchell 1975). Adult body mass, linear body dimensions, and egg-to-adult development-time are variable traits in *C. maculatus*. Consequently, these easily measured traits are candidates for inducing evolutionary change in laboratory populations. Previous studies have found that variation in body mass is heritable in both sexes but failed to find heritable variation in egg-to-adult development-time (Fox et al. 2004).

Experimental Design

Your instructor will announce the focus of your study, either inducing evolution by natural selection or genetic drift. Address the following questions. Come to class ready to discuss your answers.

- What is the importance of trait variation if you were to induce natural selection?
- Design an experiment or set of experiments to test the hypothesis that you can induce evolution in a trait by natural selection or genetic drift.

- What assumption must you make about the cause for trait variation if selection were to result in evolution? Can natural selection result in evolution in a trait if variation were caused entirely by environmental variation?

For each of the experiments you designed above, you should:

- predict the possible outcomes for the experiment that would support your hypothesis
- identify and list the variables you would manipulate in your experiment
- identify and list the variables you would keep constant in your experiment
- list the data you would collect to determine if your predictions were true

Literature Cited

Fox, C.W., M.L. Bush, D.A. Roff, and W.G. Wallin. 2004. Evolutionary genetics of lifespan and mortality rates in two populations of seed beetles, *Callosobruchus maculatus*. Heredity 92:170-181.

Freeman, S. and J.C. Herron. 2007. Evolutionary Analysis. 4th edition. Benjamin Cummings. 800 pages.

Mitchell, R. 1975. The evolution of oviposition tactics in the bean weevil, *Callosobruchus maculatus* F. Ecology 56:696-702.

Futuyma, D.J. 1986. Evolutionary Biology. 2nd edition. Sinauer Associates. 600 pages.

Materials

Equipment and Supplies

For a class of 30 students working in pairs:

- 15 dissection microscopes with eyepiece video camera (Moticam 352, Carolina Biological 591282) to measure elyta length
- 15 computers with USB port to attach Moticam video camera and run image analysis software (either MacOS or Windows PC)
- 2 or more analytical balances (0.1mg) for weighing beetles
- 15 bean beetle cultures with newly emerged adults
- 150 x 25mm Petri dishes (Falcon 351013) to contain control and treatment cultures (30, 2 per pair)
- Plastic 35mm Petri dishes for isolating adults (minimum 30)
- 4-5 lbs. of dried mung beans (*Vigna radiata*), or black-eye peas (*Vigna unguiculata*), organically grown, if possible, and free of powdered debris
- 30 small *Drosophila* paint brushes (Carolina Biological 173094)
- 30 soft forceps, Bioquip™ featherweight forceps (Catalog No. 4748 or 4750)
- 30 needle-tip forceps to manipulate dead adults and glue to file cards
- marking pens, file cards, white glue (Elmers™ type glue)

Notes for the Instructor

Consult "A Handbook on Bean Beetles, *Callosobruchus maculatus*" for detailed information on growing cultures, handling techniques, and methods of safe disposal (available 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 pair of students. However, cultures should have sufficient beetles for multiple student 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. Information about handling and proper disposal of bean beetles 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, body length, 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.

Body mass should be evaluated immediately after each adult emerges from their natal bean since adults do not feed and therefore body mass decreases with age. The loss of male body mass that results from spermatophore production and insemination and the gain in female body mass that immediately follows insemination are additional reasons to evaluate body mass immediately following adult emergence. Body length may be the most tractable measurement to make on bean beetles because such measurements may be made on hard body parts, such as elytra (the wing covers), that do not change with adult age and could be made accurately on dead animals. Collecting linear measures may be facilitated by using an inexpensive microscope video camera on the eyepiece of a dissection microscope (such as the Moticam 352, Carolina Biological 591282).

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 or length 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. Linear measures may be collected on adults after they have died, for example, the founders of a given population could be collected and measured after the 7-10 days of a typical adult lifespan, and before the emergence of the next generation. Descendent adults could be measured after a standard development time, for example, 5-weeks, after which cultures could be frozen for 72 hours. Dead adults can be readily sorted by sex and glued to file cards with a non-toxic white glue. Having beetles glued to cards, dorsal surface up, will make it easier to manipulate them under a dissection microscope. Digital images at a given magnification must first be calibrated with a known length scale (included with the Moticam 352). Once calibrated, rapid, accurate measurement of elytra length or other body dimensions may be made. 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 and the same for body length. 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 on body mass and development time 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). If body length data are collected, founders could be removed from cultures after they have died but before the next generation emerged, two weeks after the start of an experiment. These dead founders could be glued to file cards and measured under a dissection microscope. Measurements

on the next generation of beetles need not be made as they emerge since length based on hard body parts, such as elytra, will not change with age and will remain stable even after the adult dies. We have typically let control and bottleneck cultures develop for 4-5 weeks at 30°C to ensure that most adults have emerged, then freeze all cultures for 72 hours at 0°C. The dead adults from these cultures can then be glued to cards and readily measured under a dissection microscope. 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.

Minimizing Data Bias—In both selection and genetic drift experiments, there is the potential for measurement bias given the expected results hypothesized by students. The potential for such bias may be greatest when the data collection is on body length since that requires judgments on the start and end of specific structures. Minimizing such bias may be accomplished by having students prepare each file card of glued animals with a second card attached that identifies the source of those animals. These double cards should be submitted to the instructor who would then write a random code number on both cards, separate the cards, and have another student make the measurements without that student knowing the identity of the animals being measured. Students could submit coded data in a spreadsheet that would be decoded once all the data were submitted. This blind data recording should be done for both the founders and the descendents from each culture.

Data analysis

The data from either experiment will be the sex of the adult beetle, its development time, body length (such as elytra length), or body mass. The mean development time, body length, 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 the characteristics of adults starting each population (founders) should be compared to the characteristics of the individuals emerging (descendents) 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 each founder and descendant population pair could be evaluated using a two sample t-test. We would expect a greater number of these pairs to be significantly different for the bottleneck populations than for the control populations. An alternative analysis could be conducted by calculating the extent of change from founder population to descendents by calculating the absolute value of

change that has occurred expressed as a percentage of the founder population mean. We expect the bottleneck populations to exhibit a greater change (in any direction) than control populations, so a simple two-sample test may be performed to make this comparison. 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 Levene'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.

Previous Results

A genetic drift experiment was conducted by Morehouse College student Ben Davids by creating genetic bottlenecks in cultures that were started with very few individuals (5 males and 5 females) and compared those to controls that were started with larger founding populations (25 males and 25 females). Males are typically much smaller in mass and body length than are females (Figure 1) so the sexes must be evaluated separately. For example, a control culture started with 25 males and 25 females randomly selected as founders yielded males that were 3.72±0.59 mg (mean±SD) and females that were 4.68±0.69 mg total body mass at emergence.

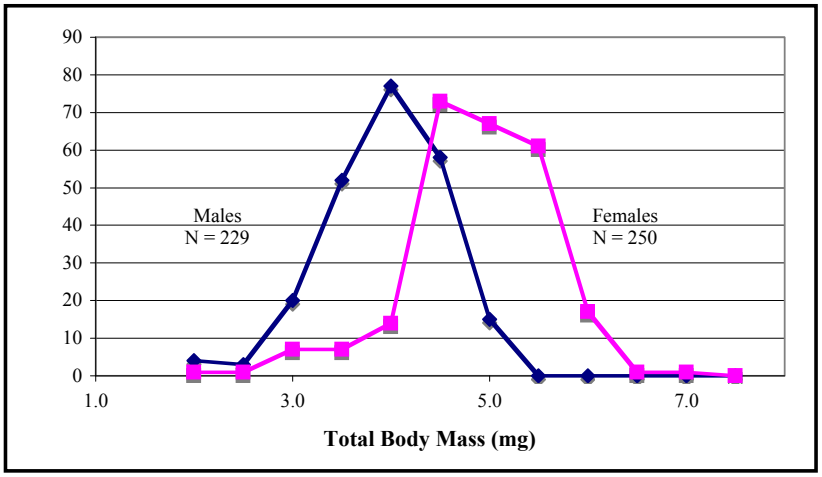

Figure 1. The frequency distribution of total body mass (mg) of males and female from a control population at adult emergence. The frequency distribution of 229 males and 250 females produced by one control culture founded by 25 males and 25 females (randomly selected) shows the sexual size dimorphism that characterized *Callosobruchus maculatus*. Females are typically larger and have a greater mass than do males.

The absolute value of the difference between founders and descendents was tabulated for the total body mass of males and females from ten control cultures and ten genetic bottleneck cultures. These differences, expressed as the percentage change from the mean founders body mass, were greater in the genetic bottleneck cultures than in the controls (Figure 2) but the difference only was significant among females. Among males, the change in control cultures was 6.4±2.7% (mean±SE) and in the genetic bottleneck cultures the mean change was 8.2±1.5% (Mann Whitney, U=72, p=0.052 one-tailed). Among females, the change in control cultures was 2.9±0.8% (mean±SE) and in the genetic bottleneck cultures the mean change was 5.1±0.6% among females (Mann Whitney, U=87, p=0.003 one-tailed).

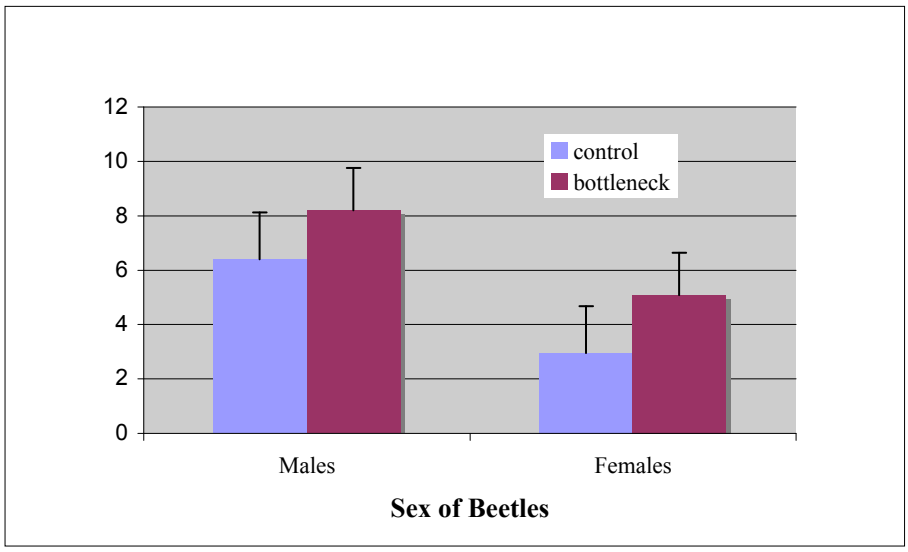

Figure 2. Body mass change due to genetic drift. The absolute value of mean change from founders to descendants in 10 control cultures and 10 genetic bottleneck cultures is shown as a percentage of the founders mean mass. Control cultures were started with a random collection 25 males and 25 females and the genetic bottleneck populations were started with a random collection of 5 males and 5 females.

A comparison of the within culture variances in total body mass revealed that for both males and females within culture variance was significantly smaller in the genetic bottleneck cultures (N=10) than in the control cultures (N=10) (Mann Whitney, U=20, p=0.013 one-tailed, for both sexes). The median variance for males was 3.68×10^{-7} in controls and 2.74×10^{-7} in bottleneck cultures. Among females, the median variance was 5.44×10^{-7} in controls and 3.66×10^{-7} in bottleneck cultures. This pattern conforms to the variance differences predicted for genetic bottleneck populations. The variation in body mass between the control and genetic bottleneck cultures (the offspring produced) was expected to be greater for the bottleneck populations than for the controls. However, the observed variances were not significantly different for either males or females (Levene's

F-tests, for males F=2.31 p=0.11, for females, F=3.05, p=0.056, df_1 and df_2 =9 in both comparisons). Among control males the variance in total body mass was $1.29x10^{-7}$ and among bottleneck males is was $5.57x10^{-8}$. Among control females the variance in total body mass was $2.14x10^{-8}$ and among bottleneck females it was $6.55x10^{-8}$. Overall, these results indicate evolutionary changes in total body mass in response to genetic bottlenecks.

Acknowledgments

This project is supported by the National Science Foundation, DUE-0535903, "Developing Bean Beetles as a Model System for Undergraduate Laboratories." We thank Morehouse College students William D. Shipman III and Benem-orom A. Davids for collecting extensive preliminary data and testing protocols, and the students in BIO 320L, Ecology Laboratory, at Morehouse College who conducted the first trial-run of the genetic bottleneck study using elytra length. Disclaimer: Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessary reflect the views of the National Science Foundation, Emory University, or Morehouse College.

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