Chapter 15

Variability and Selection in Natural Populations of Wood Lice

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Introduction

Introductory students of evolutionary biology often focus on the consequences of natural selection without having a solid grasp of the underlying importance of character variability. By examining variation in a natural population of real organisms and subjecting them to artificial predation, students are able to explore the relationship between variation and selection.

Our students perform this exercise in the introductory laboratory course they take during their second year. Variations on the experiment are also taught at local high schools (AP Biology) and community colleges. The availability of wood lice and the simplicity of the materials make this exercise especially attractive.

Notes for the Instructor

We have chosen to do this exercise with wood lice because they are excellent experimental subjects and they are readily available in our area. Students collect their experimental animals, which provides an introduction to the organism as well as to its habitat and behavior. In many regions, this would not be possible year-round and animals could be maintained in the lab or ordered from a biological supplier. Alternatively, laboratory reared animals such as mealworms or guppies might be substituted.

We do the exercise that follows in the manner described in the Student Outline in order to finish the entire experiment in one 3-hour laboratory period. However, pedagogically, the exercise makes more sense if students first measure several characters and then characterize the extent to which they vary. Based on their assumptions and knowledge of the biology of these organisms, the next step would be for students to construct one or more hypotheses about how predation might be a selective force on the traits examined. Once predictions have been made, the artificial predation experiment would be performed. Following predation, survivors and victims would be examined to see if character variation in the two groups differed. When we conducted the parts of the exercise in this more logical sequence, it took at least four hours to complete. Hence, we chose to reverse the order of activities, beginning with performing artificial predation and then characterizing variation.

All students are asked to measure body length, number of dorsal plates, and sprint speed. These characters were chosen to represent a range of character types (e.g. physiological, morphological, and behavioral) as well as to demonstrate both variable and invariant characters. Other traits our students have chosen to measure include body width, mass, color, number of legs, etc. Variability in some of the traits examined has a heritable component, while in others it may be strictly environmental and/or developmental. This is not dealt with explicitly in the exercise but may come up in discussion and is an important point.

Student Outline

(Modified from Berkelhamer and Watkins, 1997)

Summary

Many traits vary considerably in natural populations; other traits do not vary at all. Variation in a trait is necessary for that trait to evolve through natural selection, and the amount of variation can influence the rate at which the trait evolves. In this lab, we will measure the amount of variation in a natural population of terrestrial wood lice (Class Crustacea, Order Isopoda) and then determine which traits are subject to selection by predators by performing a simulated predation experiment.

Goals

By the end of this exercise, you should be able to:

- Draw a frequency distribution of sample data
- State and evaluate hypotheses about which traits confer a survival advantage in a simulated predation experiment
- Collect and record a large number of data in an organized fashion

Means and Variation in a Population

A population of organisms almost never consists of individuals that are all exactly alike. For example, youíve probably noticed quite a bit of variation in the size, shape, color and firmness of apples in a grocery store bin. Nevertheless, if you were to describe apples to a friend who had never seen one before, you might describe one that had a size, shape, color and firmness that were not particularly extreme but which you felt best represented apples in general. Such an apple would be an average, or mean, with respect to the particular traits you used to describe it. In biology, the mean value is useful for a number of reasons. First, it is sometimes the best representation of the population as a whole. Since classical times, naturalists have described organisms in terms of their mean characters in order to categorize them. The statement ìLeopards have spots but tigers have stripesî is based on the average color patterns of these two cats and is used to classify them as separate species. Second, the mean can be used for comparing populations or samples. For instance, a plant ecologist might compare the mean heights of a shrub species living in damp and in dry habitats in order to understand the speciesí growth response to moisture.

But despite our tendency to think about average organisms, for some traits there remain considerable variation among individuals in a population and this variation is biologically very important. For example, the coats of some tigers are spottier than others and the coats of some leopards are more striped than others. Darwin was the first to understand the importance of such variation in organisms. He suggested that differences we observe *between* species (that is, their average properties) might arise from variation in those properties *within* species. In his words (Darwin, 1859:45),

> "No one supposes that all individuals of the same species are cast in the very same mould. These individual differences are important for us, as they afford materials for natural selection to accumulate, in the same manner as man can accumulate in any given direction individual differences in his domesticated productions."

(Here ìdomesticated productionsî refers to the breeding of livestock for specific qualities such as egg production or docility. Such animal husbandry is an ancient practice upon which Darwin drew much of his argument for similar processes in nature).

The mean and variation of a population are easily seen by graphing the number of individuals that have a trait of a given value. Suppose you measured the sizes of 100 apples and sorted the fruit into seven different size classes. You would probably find some large apples, a some small ones, and more of intermediate size. A graph of the numbers of apples in each size class (Figure 15.1) is called a frequency distribution. In this case the distribution is symmetrical (bell-shaped) and so the mean value would lie close to the middle of the graph. The amount of variation about the mean is indicated by the breadth of the distribution.

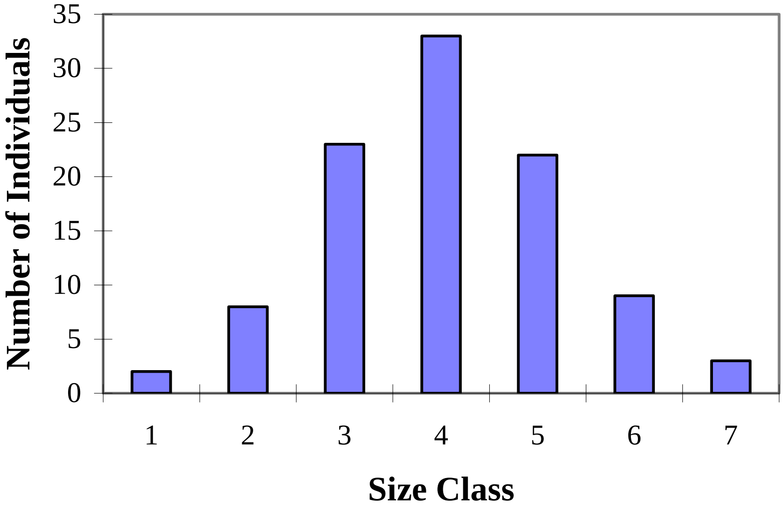

Figure 15.1. Size distribution for a sample of 100 apples.

Natural Selection

Ever since Darwin, it has been known that variation in a trait is absolutely necessary for that trait to evolve through natural selection. Recall that natural selection is simply defined as variation in survival and reproduction that is associated with variation in a particular trait. In a study conducted by a UCI faculty member (Campbell et al., 1991), *Ipomopsis aggregata* flower width varies considerably among individuals. The hummingbirds that visit these flowers remove more pollen per visit from wide flowers than from narrow flowers. Thus, natural selection acting through hummingbirds on male reproductive success favors wide flowers. One might expect the continued evolution of ever-wider flowers. There are, however, many different forms of selection in the wild. One can imagine that flower diameter might also influence female reproductive success, or the ability of a plant to survive an attack by herbivores or parasites, or its ability to synthesize sugars and grow, and so on. Furthermore, flower shape might even impose selection on hummingbird bill shape or foraging behavior. A full analysis of natural selection can be extremely complex!

Darwin proposed that over time the net effect of the various forms of selection acting on a population of organisms can result in the evolution of new species. For example, the famous Galapagos finches vary considerably in bill shape and size. This variation among species seems to have evolved in response to the distribution and abundance of different kinds of seeds among the islands of the Galapagos archipelago (Grant, 1986). Deep billed individuals within a population survive better when they have access to large seeds, while small-billed individuals survive better when they have access to small seeds. Over time, this form of selection has resulted in different species with very different bill shapes. Presumably, the distribution and abundance of different finches has also generated complicated patterns of selection on seed size and shape, but this possibility has received less attention.

In this experiment, we will investigate: 1) variation in several traits of wood lice, a common terrestrial crustacean, 2) whether and how some traits confer a survival advantage in the presence of simulated foragers and 3) whether such survival advantages depend on the kind of forager.

Pre-Lab Questions

1. Are the isopods you collected a random sample of those that live in the wild, or may you have unconsciously selected for certain traits when you caught them? If so, what traits do you think you may have selected for?
2. How much variation (none, some, a lot) do you think each of the 5 traits will exhibit? Why might some traits be more variable than others?
3. What might be the relationship between the amount of variation in a trait and the intensity of selection on that trait in the past? What might be the relationship between the amount of variation and the opportunity for selection in the present?

Materials

Per Group:

- 50 wood lice
- 50 capped vials
- 2 rulers marked in centimeters
- 2 dissecting microscopes with lights
- 2 stopwatches
- 2 racetracks with paintbrushes
- 1 predation arena with refuges
- 2 release rings
- 2 plastic cups with lids

Per Lab Section:

- 2-4 electronic balances (mg sensitive)
- 8 plastic spoons
- 8 plastic forks
- 4 plastic combs

Observations and Experiments

A. Before Coming to Lab

Prepare before coming to lab by reading the sections of your text book that discuss evolution and doing the following tasks:

1. One person from each group of six students should capture 50 wood lice and place them with a moist paper towel into the container provided by your TA. (We use small plastic urine collection

containers with lids that we perforate with a dissecting needle. Any lidded container with small air holes would work.) These animals are quite common under dense ground cover (e.g. ivy) on the UCI campus, and are most easily found in the morning or evening. Collect all 50 isopods from one location and make sure they are all the same species (Use either pill bugs or sow bugs but not a mixture of the two. In our area, pill bugs roll up into a ball when disturbed while sow bugs remain flat.). You may collect them one or two days before your class meets; if you do, keep the container in the refrigerator.

2. Design a well-organized table for recording the relevant traits (including repeated measurements and calculations) for 50 individuals. Be sure to indicate the appropriate units of measurement, with the correct number of significant figures.

3. For one of the traits you will measure in the lab, state a hypothesis about whether or not the trait will confer a survival advantage in a simulated predation experiment. Briefly describe how you will decide whether the trait confers such an advantage.

B. Simulated predation experiment

For the first part of todayís exercise, you will be the predator on your population of prey isopods.

1. Assign one person in your group to be the artificial predator and decide which of the following tools (i.e. morphological adaptations) this predator will have: spoons, forks, or combs. Other members of the group should be responsible for counting animals as they are caught and making sure none escape the arena.

2. Assemble the predation arena as demonstrated by your instructor. Divide your 50 isopods evenly into the two release rings at the center of the arena. Allow the animals to recover for about one minute, then when the ëpredatorí is ready, release the 50 isopods simultaneously Using only the chosen tools, the predator should pick up isopods *without injuring them* and place captured prey in a marked plastic cup. Collect the isopods until 25 have been captured; you may need to repeat the predation trial if many isopods escape to the refuges. Be sure to keep the victims and survivors in separate cups.

C. Measuring traits

It is most efficient to gather today's data by having each person measure one variable and pass each animal to the next person after measuring it. Since it is difficult to mark wood lice in a manner that does not interfere with their behavior, the first person in line should transfer each animal from its holding container (labeled ìvictimsî or ìsurvivorsî) to a clearly numbered vial after recording data on that animal. *It is very important that the other students NOT know whether a given animal was a victim or survivor. Such information may bias their data unintentionally.* The following measurements should be made in whatever order you think will be most efficient.

1. To measure the length of an animal, place it on cotton in a Petri dish. (Be sure that the cotton is slightly damp - *not soaked!*). When the dish is covered, the cotton will hold the animal in place for observation under the dissecting microscope. Measure the total length in millimeters using the ruler.

2. Count the number of dorsal plates under the microscope. If you are unfamiliar with isopod anatomy, consult an invertebrate zoology book (e.g. Barnes, 1980).

3. Two people are needed to measure sprint speed. One person should release an animal behind the starting line of the race track and prod it gently with the paint brush. As the animal crosses the starting line, the second person should start the timer. Record the time and the distance to the finish line (either 20 or 40 cm, depending on the individual animal). Repeat this a second time for each animal and compute the mean burst speed in cm/second. You are strongly advised to practice this procedure on non-experimental animals before actually running your tests.

4. Choose two additional characters that can be measured easily and quickly on all 50 animals.

D. Data analysis

Be sure you have data for all fifty individuals. If there was insufficient time for your group to gather all the data, obtain results from another group to pool with your own.

1. For each of the five characteristics you measured, calculate the mean and range. Enter these values at the bottom of each column of your data table.

2. Make five frequency distributions, one for each characteristic measured. Be sure to label the distribution with a title and axis labels. For the x-axis, you will need to establish an appropriate number of bins, each of the same range, in which to place the values for each character. For example, for speed you might have five bins, each with a range of 5 cm/second (0-5, 6-10, 11-15 cm/second, etc.). You may have to decide through trial and error on the number and width of the bins.

3. Evaluate your hypothesis about the survival advantage conferred by one trait of your choice by comparing the distributions of those traits in the survivors vs. the victims. Compare the means of the two distributions by performing a t-test (Sokal and Rohlf, 1995). Report the mean and variance of the survivors and victims separately, the value of t, the degrees of freedom, and the associated probability. You may compare the survivors and victims with respect to the other traits. You should calculate your t-test by hand; further t-tests may be done with a computerized data analysis program.

Post-Lab Questions

1. What is the relative strength of selection acting on the various traits in this simulation? What might be the consequences of these different selection intensities?

2. If there are observed differences between predator types in the patterns of selection on the traits, how might these differences be explained?

3. For those traits that were subject to selection in the simulation, why does variation in those traits persist in the wild?

Possible Answers to Post-Lab Questions (for Instructors)

1. Our students have quantified strength of selection by calculating a selection differential for each trait they have studied. Speed tends to have a relatively high selection differential (selection intensity) while number of doral plates and antennal length have low selection differentials. Characters such as color, mass, and length vary with predator type. High selection intensities should result in directional selections through time. Low selection intensities should result in little change in the character as a result of selection.

2. Different predator types are responding to different prey characters. Therefore, it is likely that selection intensity for a character will depend on the type of predator. For example, a visual predator may select strongly against conspicuously colored individuals. A predator that relies on a particular size and shaped feeding apparatus may select against a certain size class of prey more strongly than against other sizes.

3. Our laboratory simulation limited selection to one factor, predation. In the wild, there are many selection factors which may result in the persistence of considerable trait variation. Fast animals may escape predation by may require more energy than their slower counterparts and be selected against in times of nutrient shortage. Additionally. some of the characters that we studied may vary developmentally (e.g. body length). For example, strong selection against small animals may not result in the elimination of small individuals. All sow bugs must be small before they become large.

Acknowledgments

This exercise was first designed, written and taught to students at U.C. Irvine by Jeffrey Kaufmann, a former graduate student in the Department of Ecology and Evolutionary Biology. Instructions that Jeff prepared have been revised and rewritten several times by Rudi Berkelhamer and, most recently, by Timothy Watkins, a recent graduate student at U.C. Irvine. Over the years, many improvements in exercise procedures and instructions have been made as a result of helpful suggestions by both graduate teaching assistants and undergraduate students.

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Appendix A

Materials

Specifics about materials used in the Wood lice lab at U.C. Irvine follow. Measurements for non-scientific materials not ordinarily available in metric units in the United States are given in inches.

Wood lice: If wood lice are not available locally, two varieties are collected and sold by Carolina Biological Supply Company: pill bugs (*Armadillidium*) and sow bugs (*Porcellio*). We use both pill bugs and sow bugs from our area and they provide an interesting comparison with sow bugs being considerably faster. Since they are caught locally, we release the isopods when we are through with the exercise. Be sure that each student group has 50 individuals of a single species.

Capped vials: We use 20 ml glass scintillation vials (e.g. Fisher #03-337-4) for marking and holding individual wood lice. Vials with white screw caps are easily pre-marked (1-50) and can be used repeatedly. Scintillation vials are especially handy because they are usually packaged in cardboard trays that can be used in class to organize the vials.

Racetracks: The racetracks we use were constructed in our shop. We use a strip of wood 2 x 20 x 3/4 inches as the track base. The sides of the track are 1/4 inch thick black Plexiglas 2 3/4 inches high. (We have also used brown corrugated cardboard for the track sides.) We enclose (Plexiglas, cardboard, etc.) the ìfinishî end of the track so that it provides a shelter.

Predation arenas with refuges: Predation arenas are constructed by making a square boundary for a region of the lab bench. We have taken new corrugated cardboard boxes (12 x 12 inches) and sliced them into 2 inch deep strips. Care must be taken to make the cuts clean and straight so that the arena boundaries sit flush on the lab bench. Our arenas are 12 x 12 inches and we have constructed refuges to cover half the arena area. For each arena, we make four triangular refuges that fit flush into the four corners. Refuges are cut from mat board (6 inch right angle isosceles triangles) and are raised from the substrate by gluing a bottle cap to the underside.

Release rings: Animals are placed into the center of the arena in two release rings. Once the animals have had a short period of time (usually about one minute) to recover from the disturbance of being transferred, they are all released simultaneously by lifting the rings. Rings need be only about 2 cm high and about 5 cm in diameter. It is important that the wood lice not climb up and over the rings. We find that Plexiglas works well for the rings, but thick cardboard tubing would also work. If the ring diameter were somewhat greater, one ring per arena might suffice.

Predation tools: In the past, we have only used plastic utensils to simulate predator bird bills. This year we are experimenting with a variety of other ìbillî types. We plan to try aspirators and sticky dowels in addition to the spoons, forks and combs listed.