Introduction

The determination of how natural selection acts in contemporary populations constitutes an important link between the studies of ecology and evolution. While we have referred to natural selection as a “force” that causes populations to evolve, it is perhaps more properly considered as an outcome of an interaction between phenotypic variation in a population and the current environment that population experiences (where environment is broadly construed to include abiotic and biotic factors). This interaction leads to differences in survivorship and/or reproduction between phenotypic variants, one of the criteria for natural selection to operate. Understanding how biotic interactions and/or the physical environment create selection may provide a clue as to how the current characteristics of a population have been molded through evolution. Through such studies, we come to appreciate the link between ecological interactions and their evolutionary effects.

If we constrain our study of selection to differences in viability (as we will in this lab), we are looking for significant associations of phenotypic variants with the probability of surviving. The effects of different probabilities of survival can have three different types of effects on phenotypic variation:

1) Directional selection - The population of survivors can have a higher or lower mean value for the characteristic than before selection acted.
2) Stabilizing selection - The population of survivors can have a reduced variance compared to the original population, if individuals with extreme phenotypes have higher rates of mortality than individuals with intermediate phenotypes.
3) Disruptive selection - The population of survivors can have a higher variance compared to the original population, if individuals with intermediate phenotypes have higher rates of mortality than individuals with extreme phenotypes.

Natural History of the Solidago - Eurosta System

Galls are deformities induced in certain plants by various insects. These interactions are frequently species-specific, with a particular species of insect inducing galls in a specific tissue of one species of plant. Galls are used by the insects that induce them as sites for larval development and as food. Characteristics of the gall are often under the influence of both the insect that provides the stimulus for gall formation and the plant producing the gall. Therefore, some features of gall morphology may evolve in response to selection on the gall-forming insect. Previous work on the goldenrod insect-plant system has shown that gall diameter is a heritable characteristic of the insect, as well as the plant. In this laboratory an analysis of gall diameter will be used to determine whether there is selection on the gall-inducing insect for gall size.
Solidago gigantea, or late goldenrod, is a common perennial of the eastern and midwestern United States that is frequently parasitized by the gall fly, Eurosta solidaginis. In the spring, adult female gall flies lay a single egg in each of many terminal buds of developing goldenrod shoots. The fly larva tunnels into the stem just below the apical meristem, where it secretes compounds believed to be similar to normal plant growth substances. As a result the plant undergoes abnormally high rates of cell division in the area occupied by the larva, resulting in the formation of a spherical or ball gall (Figure 1). Gall fly larva feed off the plant tissue, growing to full size by early fall, overwintering in the gall, and pupating in the spring. After metamorphosis is completed in May, the adult emerges from the gall to seek a mate. [Note that a related species of goldenrod, Solidago altissima, is also attacked by a separate, reproductively isolated host race of this fly species. The natural history of this interaction is almost identical to that between the fly and S. gigantea, and has received a greater amount of study.]

Sources of Eurosta Mortality

Mortality of fly larvae within galls may result from a variety of different causes, including interactions with predators or other herbivores of the goldenrod. The following lists the major, diagnosable causes of mortality in this system:

1) Parasitoid wasps - Parasitoids are insects that lay their eggs on or in a host, but whose effect is to kill the host (unlike a true parasite). The wasp Eurytoma gigantea is such a species - a female wasp inserts its eggs into the central chamber of goldenrod galls. The resulting wasp larva eats the fly larva and then switches to a vegetarian diet, eating gall tissue the rest of the growing season. Flies in smaller galls may be more susceptible to attack by this parasitoid wasp because wasps can attack only those fly larvae that are within reach of the wasp’s ovipositor. If this is the case, then attack by wasps may be a factor causing directional selection on the size of galls induced by the flies.

2) Bird predators - During the fall and winter, downy woodpeckers (Picoides pubescens) and black-capped chickadees (Parus atricapillus) also prey on the gall fly larvae. These birds peck through the tissue of the gall and extract the soft-bodied fly larva. These birds are visual predators and thus larvae living in galls more easily seen by birds may suffer higher rates of mortality. If gall size is a determinant of which fly larvae are attacked by birds, then predation by birds will cause directional selection on the size of galls induced by flies.

3) Other herbivores - The stems and galls of the goldenrods are attacked by a large number of herbivorous insects. One common herbivore found in the galls is Mordelistena unicolor, a beetle species that lays its eggs on the surface early in the summer. When many larvae burrow into the gall tissues they often cause the death of the fly larva. If gall size is a determinant of which galls are attacked by this herbivore, beetle attack will cause selection on the size of the galls induced by the flies.
4) Plant interactions - Plants may have mechanisms to resist herbivory, in some cases causing the death of the herbivore. This may be an explanation of the phenomenon of Early Larval Death for Eurosta flies - the gall continues to form although the fly had died early during gall formation. This is a common cause for mortality for flies on S. gigantea, and often leads to smaller than average gall sizes due to the early death of the gall inducer.

By collecting galls, measuring the phenotypic distribution of gall sizes and determining the mortality of flies from these sources, you will be able to estimate the strength and form of selection on this phenotype for these populations.

Methods

**Sampling galls** - When collecting a sample of individuals from a population, it is important to consider carefully how the methods used to choose measured individuals may bias the results. Sampling is a complicated area of ecology, with different techniques used in different situations. Ideally, we want to choose individuals from a population randomly, although sometimes this is not always practical. The technique described below does not produce a truly random sample of the population of galls, but it should guard against systematic biases in the sample (can you think of biases that might be introduced by other methods of sampling?):

1) Lay out a 30 m measuring tape along one edge of the population and determine the end-points of belt transects along this tape using a random number table.
2) Run a belt transect into each population perpendicular to the end-point line using the 30 m measuring tapes.
3) Collect all galls within 0.5 m of the measuring tape. Make sure you do not miss small galls!
4) Place the galls in plastic bags and put in labels with date and location of gall collection.

**Data collection to be done in lab on Nov. 2:**
1) Measure the diameter at the widest point of each gall by using the calipers. Read the diameter in mm for each gall.
2) Determine the fate of the fly that induced the gall. If the gall has been attacked by birds, the gall will have very obvious peck marks. If the fly survived and bored out of the gall, there will be a small bore hole from which the fly emerged. However many galls will have no obvious external sign of the fate of that fly. Then you must carefully cut open the gall to check the fate of the fly.
   a) If a cream-colored, fat larva or a tan-colored fly pupal case is present in the gall, that fly has clearly survived all the mortality agents discussed above. All galls that contain anything other than a fly larva or pupa were induced by a fly that did not survive.
   b) After examining each gall, categorize the fate of the fly that induced the gall. Examples of galls from the different mortality classes will be available for
comparison. For each gall you will record the gall diameter and the fate of the fly on the data sheet.

**Data analysis:**

We will combine all the data collected by the class so that we have a fairly large sample size.

1) Calculate the mean, variance, and standard error of the gall width for all the galls (this will be the pre-selection set of galls). Then calculate the mean, variance, and standard error for the gall width for the survivors. Then calculate the mean, variance, and standard error for the gall width for each group of gall flies killed by different mortality agents. You may calculate the mean and variance by hand or by using a calculator or computer program.

2) Plot the means and use the standard errors to construct error bars for each mean. This will allow you to compare the mean widths of the galls in the pre-selection, surviving, and different mortality agent groups.

3) It is common to calculate the strength of directional selection by using a statistic called the **intensity of directional selection**, which is defined as:

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i = \frac{\text{mean after selection} - \text{mean before selection}}{\text{variance before selection}}\]

You should do this for the survivors and for each group of gall flies killed by a different mortality agent. Note that the intensity of directional selection gives a unitless measure of the strength of the change in the mean phenotype, scaled by the amount of variation available for selection. Calculate the intensity of directional selection for gall size due to each mortality agent in this population.

4) We can determine whether stabilizing or disruptive selection is occurring by using a statistical test called the **F-test** which determines whether the variances of two samples are significantly different. The F-test works based on the following idea – suppose you have two populations that actually have the same variance, but you can’t measure all individuals within both populations. Therefore you collect samples to estimate the population variance. If you take samples of just a few individuals it is quite likely that by chance your estimates of the variances will be different. The more individuals you measure for each sample, the more likely that your estimated variances will be equal. The F-test uses this principle by asking you to express the difference between the variances as a ratio (here the larger variance divided by the smaller) called the F-ratio. The null hypothesis is that the variances are equal, i.e., that the ratio is equal to 1. The F-test asks whether the deviation of the calculated ratio from 1 could happen easily by chance (given the number of individuals you sample). If the probably of getting this ratio by chance is less than 5%, then we accept the alternative hypothesis that the variances are significantly different.

To test whether there is significant stabilizing or disruptive selection, calculate the ratio of pre- and post-selection variances (larger variance divided by smaller variance)
and compare that ratio to the appropriate critical value in an F table (the “green book” A Handbook of Biological Investigation by Ambrose et al. has an F table as will any statistics text). Remember that d.f. = n-1. (d.f. means degrees of freedom) If your d.f. value doesn’t appear in the table then use the largest value in the table that is less than your actual d.f. If the ratio is higher than the critical value, you can reject the null hypothesis of equal variances with 95% confidence.

What do your analyses suggest about the evolution of gall size? Do we see evidence of directional or stabilizing selection? If different mortality agents cause directional selection, do they cause selection in the same direction? What are some possible constraints on adaptation by the gallmaker to such selection?

This lab report will be due in lab on Tuesday Nov. 3.

Acknowledgments

Special thanks to Dr. Jackie Brown of Grinnell College who first introduced me to this system and gave me the idea to do this lab.

References


Necessary Formulae:

mean = \frac{\sum x}{n}  
variance = \frac{\sum x^2 - (\sum x)^2}{n}  
standard error = \frac{variance}{\sqrt{n - 1}}

where x is each individual measurement and n is the sample size
Figure 1. Golden rod gall types.

*The goldenrod ball gall:* This is a globular stem swelling, 1-2 in. (3 cm) diameter, common to various *Solidago* species. Good-sized patches of goldenrods are sometimes hosts, and two galls are occasionally found on a single stem. Goldenrod ball galls often form at approximately the same height on all the stems in a patch. They are incited by the gall fly *Eurosta solidaginis* Fitch, a fairly large fly with brown marked wings.

*The elliptical goldenrod gall:* This elongate spindle-shaped stem gall is caused by the whitish caterpillar of the gall moth *Gnorimoschema gallaesolidaginis* Riley, and is found on *S. canadensis*. The caterpillar keeps the interior walls smooth and neat, and its castings can be found packed in the bottom of the cavity.

*The goldenrod bunch gall:* A number of gall midges affect the goldenrods. The common gall midge *Rhopalomyia solidaginis* Loew, attacks *S. canadensis* leaf buds, producing globular masses of deformed leaflets at the stem's apex to shelter its maggots.

*The black blister gall:* The gall midge *Asteromyia carbonifera* Felt (*A. euthamiae* Gagné) affects the leaves of *Euthamia graminifolia* (formerly *S. graminifolia*). Resulting black blister galls give the leaves the appearance of having been spattered with black oil.
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Possible Fly Fates: 1) survived; 2) eaten by bird; 3) early larva death; 4) killed by parasitoid; 5) killed by beetle