Ecology Lab 3-31-2010
Biology 229 Prof. Kerkhoff

Agents of selection: Predation by birds on Gall-making Flies

Natural selection can act only when **variation** is present in a population. Selection is a consequence of the **differential survival and reproduction** of individuals with particular **heritable phenotypic characteristics**, relative to other phenotypes in the population**.** Differential survival and reproduction often reflects the ability of individuals to deal with ecological challenges such as predation, competition, or abiotic stress. These factors are known as **agents of selection.** Because phenotypic characteristics are (at least to some degree) heritable, the more successful variants increase their representation in the population over time, if the pressures applied by the agents of selection do not change. That's natural selection.

Individuals in a population face an array of ecological challenges. Evaluating the evolutionary consequences of these pressures is the study of **evolutionary ecology.** In general, evolutionary ecologists are interested in how particular **selection events** affect the distribution of phenotypic (and increasingly, genotypic) variability within a population – that is, the **response to selection.** It is important to remember that natural selection is a culling process, so selection events are events that kill individuals or otherwise limit their reproductive success.

We can examine phenotypic variation using standard tools for examining statistical variables, e.g., graphical summaries like histograms or boxplots, and numerical summaries like the mean, median, standard deviation, and inter-quartile range. The **response to selection** is gauged by comparing the distribution of phenotypes (or genotypes) before and after the **selection event** occurs. If the distribution of phenotypes in the population is relatively *Normal* (i.e., it follows the familiar "bell-curve"), we can measure the strength of selection using the means and standard deviation of the pre- and post-selection phenotypes (more below). In particular, three patterns of selection are often described: Directional, Stabilizing, and Disruptive selection.

Are birds agents of selection on gall-making flies?

Natural History
You are all familiar with this study system from your work in the introductory biology lab. **Galls** are growth deformities induced in plants by various herbivorous insects. These interactions are often highly specific; a single species of insect may induce galls in only one specific tissue of a single plant species. For example, the "ball galls" we will be studying here in the stems of goldenrod

(*Solidago altissima*) are induced by the fruit fly *Eurosta solidaginis,* while the "elliptical galls" found on the same stems are produced by a moth (*Gnorimoschema gallaesolidaginus).* Yet another insect, a midge (*Rhopalomyia solidaginis*), produces the "rosette galls" sometimes seen on the apical tips of the goldenrods.

Galls are used by the insects as protected sites in which to grow and feed on plant tissue. **Characteristics of the gall depend on the chemical interactions between the insect and the plant.** The insect secretes chemicals that act as growth hormones. Thus, some features of the gall can evolve **in response to selection on the gall-former.** Previous work on the *Solidago-Eurosta* system has shown that gall diameter is a highly heritable character of the gall-making fly, as well as the plant. Here we are going to use gall diameter as a phenotypic characteristic of the fly, in order to examine whether predatory birds exert selection on *Eurosta* favoring the production of smaller and less detectable galls.

Goldenrods (*Solidago spp.*): Goldenrods are perennial herbs common in abandoned agricultural fields in the eastern and Midwestern US. The stems seen aboveground are often connected underground by modified stems (called rhizomes), forming extensive clones. Goldenrods are in the aster family (*Asteraceae*), and they produce an elongate, one-sided cluster of small, yellow flowers in the late summer and early fall. The flowers are visited by many insects, including bees, wasps, and beetles. Goldenrods depend on these animals for pollination and since the pollen is not carried by wind, it does not generally aggravate human allergies. The goldenrod genus has many different species, and identification is often confounded by the similar morphologies and a taxonomically inconvenient tendency to hybridize. At the BFEC, we will be studying goldenrods in the *Solidago canadensis* group, which some botanists divide into the late goldenrod (*S. gigantea*) and the tall goldenrod (*S. altissima*). The two forms interact with *Eurosta* in a similar way.

Flies (*Eurosta solidaginis)*: This fruit fly (Family Tephritidae) frequently parasitizes goldenrods in the *S. canadensis* group, forming a single "ball gall" on each parasitized stem. The choice of host is very specific, as sometimes dozens of different goldenrods can co-occur. In the spring – early May around here – newly emerged adult females lay their eggs. For each egg, the female finds a new terminal bud on a goldenrod shoot. After emerging from the egg, the fly larva tunnels into the stem just below the apical meristem (the growing tip), where is secretes compounds similar to normal plant growth hormones, The plant responds with abnormally high rates of cell division in the area of pith occupied by the larva, resulting in the spherical gall. The gall continues to grow (along with the plant) until about mid-July. *Eurosta* larvae eat some of the tissue lining the gall, growing to full size by early fall. Larvae form an escape tunnel in the pith of the gall before the plant stem becomes dry and hardened late in the fall. The

larvae overwinter in the gall and pupate in March or April. After metamorphosis is completed in May, the adult emerges to find a mate to complete the life cycle.

Eurosta mortality within the gall can result from a number of **selective agents.**

1. **Parasitoid wasps:** You studied these killers in Biol 109. Parasitoids are insects that lay their eggs in or on a host, and the resulting larvae consume the host, always resulting in host death – which distinguishes them from parasites which can sometimes coexist with the host. In particular, we studied two principal parasitoids: *Eurytoma obtusiventris* and *Eurytoma gigantea.* The female wasp uses her ovipositor to insert an egg into the central chamber of the gall, where the resulting wasp larva attacks and generally consumes the fly larva, then eats goldenrod tissue for the rest of the season. Recall that flies in smaller galls may be more vulnerable, since oviposition may be limited by the length of the ovipositor, particularly for *E. gigantea,* which attacks the galls later in the season. Thus, *E. gigantea* can produce **directional selection for larger galls.**
2. **Omnivores:** The goldenrod stems are also fed upon by many other herbivores. One common species, the beetle *Mordellestena unicolor,* lays its eggs on the surface of the gall in early summer, When many larvae burrow into the gall tissues they often encounter and consume the fly larva as well.
3. **Plant defense:** The goldenrods themselves may cause the death of *Eurosta* larvae, even in the absence of parasitoids. The mechanism for these cases of larval death is unknown, so we are not even sure that it involves the plants. However, even when the fly dies, the gall may continue to grow.
4. **Abiotic stress:** Since the flies overwinter within the gall, they are somewhat protected from the elements. Still, very cold conditions can present a serious physiological challenge, and flies may succumb. It is known that flies vary in their cold tolerance, and it is possible that large galls afford more protection, but the latter has never been studied.
5. **Bird predation:** Finally, the *Eurosta* larvae are also vulnerable to birds like the Downy Woodpecker (*Picoides pubescens*) and Chickdees (*Parus carolinensis).* Bird predation generally occurs during winter. The birds peck open the galls to extract the larvae, so this fate is easy to identify. **The birds are generally visual foragers, so it is reasonable to hypothesize that they select for *larger* gall borne *higher* on the stem.**

More detail about this fascinating system is available on the website of Dr. Warren Abrahamson, who has led much of the research on this plant-parasite-parasitoid-predator system: http://www.facstaff.bucknell.edu/abrahmsn/solidago/main.html.

As you know, Biology 109 students have been examining galls since 2002 to evaluate the evolutionary effects of the parasitoid wasps on gall size. The intensity of selection has varied, but often there has been strong selection by *Eurytoma gigantea* for larger gall size. In this lab, we will be examining the effects of the bird predators as selective agents on gall size.

Methods

By locating galls in the field, measuring the distribution of gall diameters and heights (**fly phenotypic variation**), and examining how the selective agents (bird predators) change the distribution, we can estimate the strength and form of selection on gall size for these populations of *Eurosta solidaginis.* By using data from the Biol 109 class, you will also be able to compare the relative strengths of birds and parasitoid wasps as selective agents.

We can also ask whether the two different species of birds might avoid competition by utilizing somewhat different sets of galls. For example, they might differ in the preferred **height of the gall or gall size**.

Sampling

The BFEC has several goldenrod-dominated sites. This year, we will be examining the goldenrod population in the BFEC prairie restoration area, just south and west of the farmhouse.

Our first task is to formulate a sampling strategy. When collecting a sample of individuals from a wild population, it is important to consider carefully how to avoid bias. Ideally, we would like to choose individuals from the population of galls at random, so that the condition of any gall is completely independent from all previously collected individuals. This is obviously not always practical, but we should be careful to avoid systematic bias – like selecting only the largest, most obvious galls. **What other potential biases must we avoid? Why is it hard to define random, independent observations in this system?**

- ***Describe the fundamentals of our sampling strategy in enough detail that you (or others) could replicate it?***

Next, consider the following details when measuring the galls *in situ*. When you encounter a gall,:

1. Measure the diameter at the widest point, with a precision of 0.1 millimeter. Be sure that the caliper is properly zeroed before making your measurement to avoid instrumental bias.
2. Measure the distance along the stem from the ground to the base of the gall to estimate the height of the gall.
3. Classify the state of the gall as one of the following:
    a. Undamaged (U), for galls with no holes or only the tiny exit hole for the fly
    b. Woodpecker (W), for a gall with a discrete hole
    c. Chickadee (C), for a gall with a "messy" hole

Measure **ALL** of the ball galls you encounter, but avoid elliptical and other galls. Carefully enter your data on the collection sheet, and collect as many data points as you can in the allotted time.

In from the field: Analysis

With data in hand, we can now analyze the strength of the birds as selective agents.

Phenotypic Variability and Assessing Normality

First (as always) we need to examine our data. Graphically summarize the distribution of phenotypic variability for both gall diameter and gall height.

- ***Describe the distributions for gall diameter and gall height for all of the galls taken together. Do the distributions appear to be approximately normal? Explain.***

- ***Now examine the distributions for only the galls that were not predated by the birds. Do they differ from the overall distributions? In what way?***

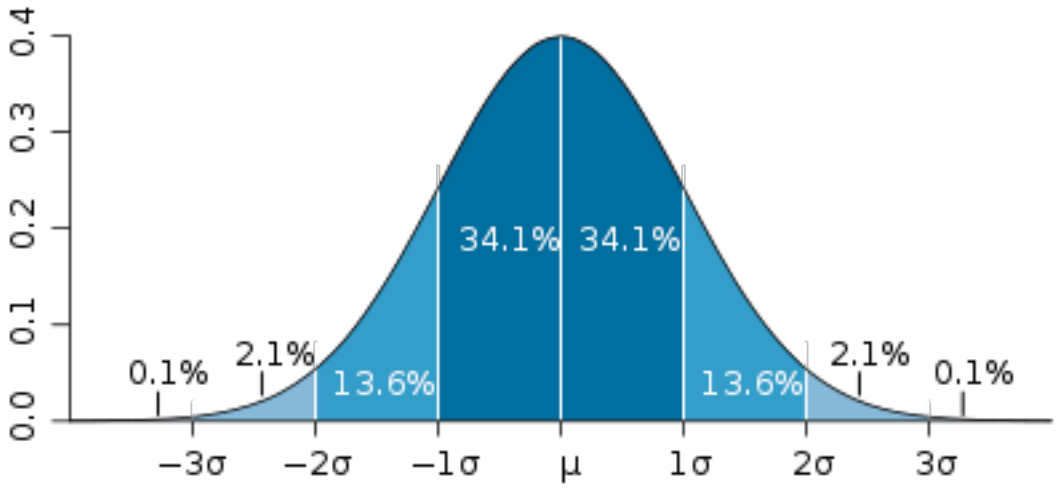
Selection Intensity Calculations

As described in Weiss and Abrahamson (1985), we can estimate the selection intensity as

$$\frac{\overline{X}_U - \overline{X}_{ALL}}{SD_{ALL}}$$

where the numerator is the difference between the mean phenotype of the "selected" (i.e., unpredated X_U) galls and the mean phenotype of all the galls (both predated and unpredated, X_{ALL}), while the denominator is the standard deviation of the phenotypic character across the entire population.

Thus, the numerator measures the difference in mean phenotype before and after selection. We divide through by the standard deviation in order to "normalize" the scale of this difference so we can consider it in "standard deviation units." That is, a selection intensity of, say, 1.45 means that the selection event has shifted the observed phenotype by 1.45 standard deviations in the positive direction (e.g., larger galls). Thinking in standard deviation units is helpful if we remember the empirical rule of thumb that for approximately normal distributions, ~68% of observations lie within one standard deviation, ~95% of observations lie within 2 standard deviations, and ~99.7% of observations lie within three standard deviations of the mean (see below).



So since we are measuring selection in standard deviations, even selection intensities of magnitude 1 are actually fairly strong, meaning that (in the case of positive selection) only individuals of about the 85th percentile of the phenotypic character (or higher) were selected. Likewise, selection coefficients around 2 or even 3 are remarkably strong, with selection only letting a very small fraction of individuals through the "filter." **Of course, the appropriateness of this interpretation depends on the assumption that the distribution of phenotypic variability is approximately normal.**

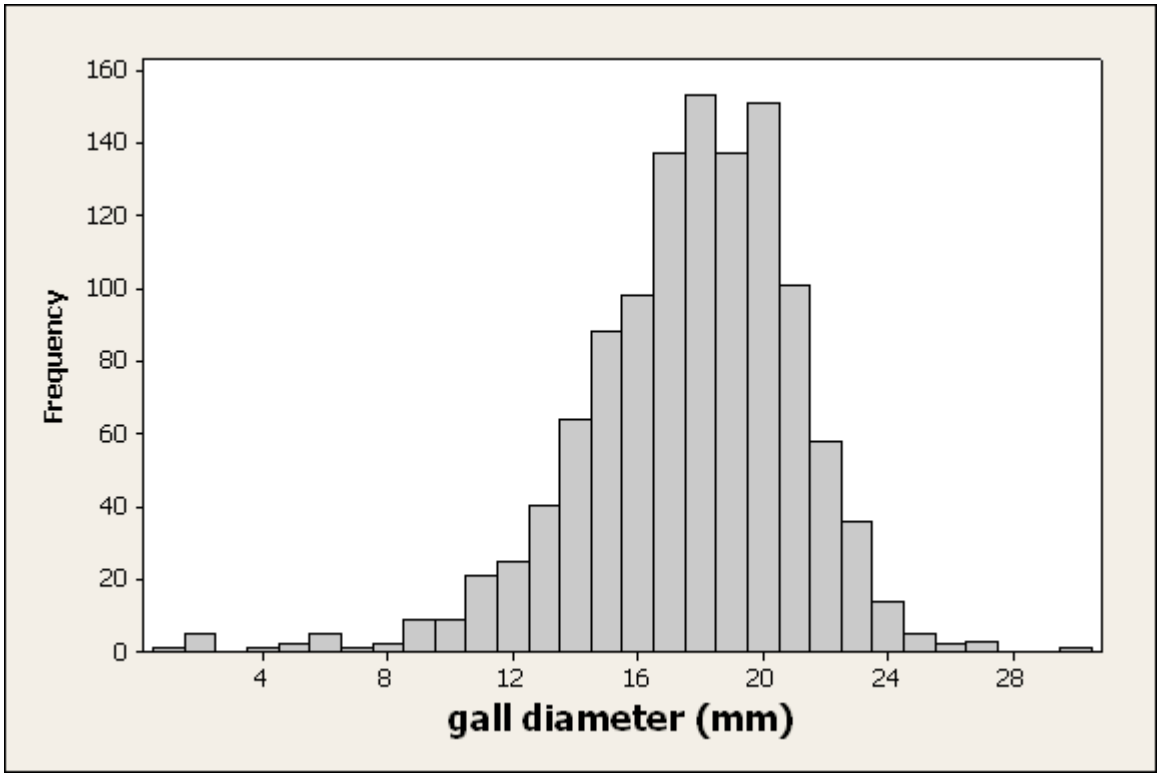
- ***Calculate the selection intensity of bird predation on gall diameter, for both predators together. Do the birds appear to be potent agents of selection? Explain your answer.***
- ***Based on your analysis of the distribution of phenotypic variability above, is this model of selection intensity appropriate for these data? Why or why not?***
- ***Compare the gall diameters and heights for the woodpecker and chickadee prey. Is there evidence that the two predators are foraging on different subsets of galls? Do you think one or the other is a stronger agent of selection?***

Evolution in the context of multiple selective agents

You know from your experience in Biol 109 and from reading Weiss and Abrahamson (1985), that the strength of selection by the parasitoids varies from year to year. A summary of data from the fall of 2008 is below.

Mean diameter of all galls: 17.62 mm
SD of gall diameters: 3.48 mm

Mean diameter of galls with surviving flies: 19.90 mm
SD of gall diameters with surviving flies: 2.76 mm



- ***Are the parasitoids or the birds stronger agents of selection?***
- ***How does selection by birds and by parasitoids interact? What is the resulting overall pattern of selection on* Eurosta *galls?***

For Next Week:
Compose a brief report summarizing our sampling methods and results, then discussing your conclusions. This is not a full-scale paper, but an organized concise write up of our research. Be sure to address each of the points in the boxed questions above, but write one continuous narrative. Support your conclusions with data in the form of graphical and numerical summaries and statistical inferences, as appropriate.