

Lab 1 - Natural Selection in the Goldenrod Gall Fly System: Effects of Predators and Parasites

Week 1: What is natural selection and how can we measure it?

Objectives:

- Understand the concepts of natural selection, heritable variation, and selection pressures
- Become familiar with the experimental system in which we will measure natural selection (read this lab **and** the *Natural History* article “Just Lookin’ for a Home” - which is posted on Blackboard - **before you come to lab**).
- Develop testable hypotheses regarding natural selection in the goldenrod-gall fly system
- Determine the environmental and physical parameters necessary to effectively evaluate hypotheses
- Collect galls and information on environmental variables in the field
- Measure gall sizes and record their contents in the laboratory, and prepare to analyze data next week

Introduction:

Natural selection is a term that most of us have used at one time or another, but what does it mean, how does it happen, and how do we measure its effects? Natural selection can be defined as a difference between the survival or fecundity of individuals with certain phenotypes compared to individuals with different phenotypes. Evolution by natural selection requires that: (1) individuals within species are variable, (2) some of these variations have a genetic basis and are heritable (i.e., they can be passed from parents to offspring), (3) more offspring are produced than can survive, and (4) those individuals with favorable variations are those that survive and reproduce most successfully.

What causes the differential survival or reproduction of individuals? There are numerous potential selective pressures (factors that could cause differential survival or reproduction) in nature. For example, weather patterns could affect food quality or availability, or predation of young could result in fewer offspring. What is important is to isolate the various potential effects and identify those selective pressures that are most important to the population in question.

Perhaps the best way to understand natural selection and its components is to go into the field and look closely at organisms in their environment, just as Darwin did. In this lab, we will study natural selection in the goldenrod gall fly system, and investigate several potentially important selective pressures. This will be accomplished by collecting goldenrod galls in the field, bringing them into the lab and making several observations and measurements of them.

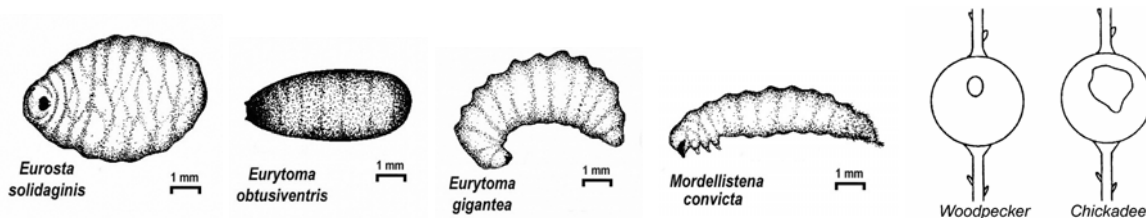
Experimental system:

During the summer in the northeastern United States, fallow fields are often covered with tall goldenrod *Solidago altissima*, meter-high wildflowers that have clusters of minute golden flowers. By the winter months, all that are left are dry vertical stems. If you look carefully at these dead stems, you will find that many have conspicuous bulges about half-way up the stems. These bulges are produced by the larvae of the golden-rod gall fly, *Eurosta solidaginis*. The larvae grew from eggs that were deposited by the mother fly in the terminal buds of the goldenrod the previous spring. A newly hatched larva then burrowed into the dividing meristematic plant tissue within the

bud. While burrowing, it secreted chemicals that act like plant growth hormones, causing growth of plant tissue around the larva. The round growth, called a gall, provides food for the larva and protection from the weather and other organisms that find gall fly maggots to be particularly tasty.

If you cut open galls at this time of year, you could find a variety of things. For example, you might find a...

- Gall fly larva (*Eurosta solidaginis*) – a large ovoid, cream colored maggot in the gall chamber
- Pupa containing the parasitic wasp, *Eurytoma obtusiventris* – This parasitoid hatched from an egg that an adult wasp inserted directly into the egg or developing embryo of the fly (before the fly larva hatched and burrowed into the stem). The parasitoid larva subsequently consumes the fly larva, causing the fly to pupate early. All that is left of the fly larva is the pupal case and bits of debris inside.
- Wasp larva (*Eurytoma gigantea*) – The larva of this parasitoid wasp is teardrop shaped and has dark mouthparts. It feeds on the gall fly larva by tearing it apart with its sharp, curved jaws. After this wasp larva eats the gall fly larva, it feeds on the nutritive tissue inside the gall chamber.
- Beetle larva (*Mordellistena convicta*) – This predator of the gall fly tunnels through the gall to feed on the fly larva and then leaves the gall chamber to tunnel into other parts of the gall.
- Empty gall with a large hole – The hole indicates that whatever was in the gall (the gall fly larva or any of its three predators) was taken from the outside by a larger predator – either a chickadee or a woodpecker – by chopping a hole into the gall chamber. Woodpeckers usually make smaller, clean holes, whereas chickadees make larger, messy holes.



These are the subjects of the lab for the next two weeks. We will study the interactions of these organisms (the host plant *Solidago altissima*, its gall fly parasite *Eurosta solidaginis*, and the gall fly's various parasitoids and predators) and consider how natural selection affects these interactions. For example, do birds prefer to attack larger galls, and do larger galls contain larger larvae? Do birds attack galls closer to the edge of the woods where they can retreat for protection from predators (small hawk)? Such associations would be consistent with the idea that natural selection is important in this system.

The goldenrod gall fly and its predators have been studied extensively, in particular, by Warren Abrahamson and his collaborators. His book on the subject (W.G. Abrahamson & A.E. Weis, *Evolutionary Ecology Across Three Trophic Levels*) is on reserve in the Science library, just off the Merrill entrance lobby. There are also reprints of articles by him and others in the laboratory and on e-reserve for your perusal.

Questions to think about:

The size of the gall depends on the interactions between the substances secreted by the gall fly larvae that induce gall formation (i.e., the kinds and amounts of plant-hormone-like compounds) and how the plant tissues react to these substances. We assume that there are hereditary differences between gall fly larvae in the amounts and maybe kinds of plant-hormone-like compounds secreted, and that these are manifested in the differences in gall sizes. Thus, if the size of the gall affects the resistance of the gall fly larvae to attack by one or more of its predators, then we would expect natural selection to favor the gall flies whose larvae elicited the formation of a gall of the optimal size for survival.

Are galls that contain live gall fly larva of a different size than those galls where the gall fly has been taken by a predator? Do gall sizes differ for the different species of predators (e.g., wasp, beetle, or bird)? To answer these questions, you will need to calculate means and variances for the different classes of interactions and assess the statistical significance of these differences. We will provide details of how to do such statistical tests during the second week; read the section on statistics for gall analysis later in this handout in preparation for next week. In this first week, you will generate histogram plots of the number of galls in different size categories. The gall size distribution for one class of interaction (e.g., predation by the beetle larva, *Mordellistena convicta*) can then be visually compared with the size distribution for another interaction (e.g., predation by birds). In this way, you can determine if different predators attack galls of different sizes and, if so, determine the overall selection on gall size for your population.

Protocol:

During the first part of lab, we will take a mini-field trip to a fallow field in the Amherst College bird sanctuary or to a site in Hadley. We will collect and label galls in the field and record relevant information about their location (e.g., distance of the host plant from the forest edge, height of the gall off the ground). Back in the laboratory we will measure gall size, examine gall contents, plot histograms of the size measurements, and discuss testable hypotheses about how selection is affecting gall size. During the second week of the lab, we will use statistics to test our hypotheses and perhaps develop new ideas for future studies.

You may work in pairs or groups larger than two, but **every student should collect and analyze at least five galls**. Use the accompanying key and the specimens presented on the front desk to properly categorize your gall on your data sheet. Place your data sheet in the pile at the front bench at the end of the lab. We will compile the data from all lab sections for next week.

Key for determining the inhabitants of galls and their fates

1	Gall with hole	2
1a	Gall without hole	3
2	Hole large (3-8 mm), conical & irregular	Bird attack
2a	Hole small (< 2 mm) and circular	<i>Eurytoma gigantea</i> (emerged)
3	Gall empty	4
3a	Gall with insect larva or pupa inside (larva may be tunneling)	5
4	Gall chamber contains frass	killed by unknown insect
4a	Gall chamber brown, but without frass	early larval death

5	Pupal case	6
5a	Cream colored larva in chamber or tunneling in pith region	7
6	Small brown pupal case (4-6 mm) in chamber <i>Eurytoma obtusiventris</i> (in pupal shell of <i>E. solidaginis</i>)	
6a	Large brown pupal case (7-12 mm) in chamber	pupated <i>Eurostra solidaginis</i>
7.	Cylindrical larva; 4-5 mm long & < 1 mm wide; three legs; hairs on posterior segments; galls have irregular brown tunnels through pithy region; larva usually in tunnels.....	<i>Mordellistena convicta</i>
7a	Larva globular in shape; always in chamber	8
8	Larva drop-shaped; distinct white head capsule with darkened sharp mandibles; brown spiracles apparent under microscope; highly variable in size (2-5 mm); gall chamber frequently contains brown frass.....	<i>Eurytoma gigantea</i>
8a	Larva ovoid; no distinct head capsule; mandibles modified to anteriorly directed mouth hooks; general appearance of a maggot; gall chamber lined with tightly pressed gray frass, exit hole excavated up to but not through gall epidermis	<i>Eurosta solidaginis</i>

Week 2: Data analysis, hypothesis testing, and writing a report

Objectives:

- Review hypotheses regarding natural selection in the goldenrod-gall fly system
- Learn the importance of statistical data analyses to evaluate hypotheses
- Understand and apply Kolmogorov-Smirnov tests to compare the size (or height up the stem) distributions of galls from the different interaction classes

Introduction:

The purpose of the gall fly lab is to examine whether natural selection is acting on gall size (or height up the stem) as a result of predation by wasps, beetles, and birds, and whether different kinds of predators attack different sizes (or heights) of galls. To study these questions, we collected several hundred galls, measured them, and checked for the presence of different predators.

Hypotheses and predictions:

Before we analyze our data, we should have some questions in mind. Several hundred galls are a lot of data to sift through, and our work will be easier if we ask questions and make some hypotheses and predictions about how natural selection may operate on gall size (or height). For example, what selective pressures may favor small gall size? Large gall size? A gall being lower down on the stem? Higher up the stem? Birds are much larger than the wasp or beetle predators, and being warm-blooded, they have much higher metabolic rates. Perhaps birds prefer to attack larger galls with the expectation that larger galls will contain larger, juicier fly larvae. On the other hand, the wasps are small and female *Eurytoma gigantea* lay their eggs in galls by inserting their

ovipositor (long egg-laying probe) through the wall of the gall, into the central cavity of the gall chamber in which the fly larva resides. Thus, for *E. gigantea*, there may be an upper limit to gall size, beyond which a female wasp's ovipositor cannot penetrate the wall to reach the fly larvae. If so, predation by female *E. gigantea* should be restricted to smaller gall sizes. These are just two of the many hypotheses we can test by looking for associations between gall size and parasite/predator attacks. Many more testable hypotheses will come from discussions in the lab. Below are some questions that should be readily addressed using our data.

Some hypotheses about gall sizes or location:

Within-year comparisons:

- *Eurytoma obtusiventris* lays its eggs in the gall fly embryo while it is still on or near the surface of the goldenrod stem. Might this aspect of this parasitic wasp's life cycle impact the size or distance up the stem of the goldenrod gall?
- The predator wasp *Eurytoma gigantea* has a long ovipositor, so that it can penetrate the wall of the gall to lay its eggs. Do gall flies attacked by *Eurytoma gigantea* also tend to be in galls that are smaller than the average diameter for non-predated galls?
- Do galls of certain sizes disproportionately fall prey to beetle predation? Beetles lay eggs on the outside of the formed gall. One might therefore expect that beetles would attack smaller galls which would require less effort to reach the gall fly larva.
- Do insects and birds attack galls of different sizes?
- Birds use vision to hunt their prey. Large galls might therefore be more apparent to them. Do the bird attacks tend to occur on the larger galls? Similarly, one might also hypothesize that large galls might be preferred by birds if they house larger gall fly larvae.
- Do chickadees, less specialized for boring into galls, prefer smaller galls than woodpeckers?
- We might expect bird attacks to be associated with the height of the gall above the ground because birds like to feed from elevated perches. Do bird attacks, in fact, depend on gall height above the ground?
- Is the distance from trees correlated with bird predation?
- Is the tendency for galls to be near the ground or upright correlated with gall size or any of the measured sources of mortality?
- Are double gall stems more likely to be attacked by birds than single gall stems?
- Does the distribution of galls with viable fly larvae differ from the distribution of galls in which the larvae did not survive? If a difference occurs and if variation in gall size is heritable, what does the difference tell you about natural selection?
- Are gall flies dying of causes other than birds or insects different in size from the viable population of *Eurosta*?
- Is there a difference in gall size between the live *Eurosta* (the ones that will survive to reproduce next year) and the population of galls that existed before selection?
- Is there a difference in gall size, height up the stem or various rates of predation/parasitism in galls obtained from the Amherst College bird sanctuary or the field across from Ideal Movers as compared to the more fragmented Hampshire Mall site?

Between-year comparisons:

- The larvae in galls are probably a less preferred food item than insects that do not require so much work to catch, such as insects living under loose scales of bark. Indeed, one of the major evolutionary benefits to the fly from gall stimulation may be deterrence of bird predators, which may choose to ignore galls unless other food is scarce. When winters are harsh, the most accessible food items may be rare because birds require more food. This scarcity of food abundance may drive birds to invest the extra time and energy necessary to break into galls. If true, we would expect that more galls would be attacked by birds during cold winters than during warm winters.
- On the other hand, since predation on gall flies by insects happens in the spring and early summer, differences in the selection pressure that insects exert across years may be independent of weather patterns. Are there differences between years in the intensity of selection by insect predators, or is insect predation relatively constant across years?
- The flies that survived last year are the parents of this winter's crop of fly larvae. Is there a difference in gall size between the flies that survived last year (e.g., last year's galls that contained live larvae when we collected them) and this year? Likewise, is there a difference in the variation of gall size between years?
- Does the proportion of *Eurosta* surviving to reproduce vary across years?

Analyzing your data:

If you are interested in knowing whether birds prefer large galls, you will want to compare the gall sizes attacked by birds with the gall sizes containing healthy fly larvae. One way to visualize these data is in a **histogram** or **frequency distribution** plot. In a histogram, the height of each bar represents the frequency of observations in a particular category. For this example, the x-axis is gall size (the range will vary from year to year, but the increment is always 1 mm), and the y-axis is the number of galls in each 1-mm category.

By comparing frequency distributions for the different predators, we can obtain insight into patterns in our data. For example, do the different frequency distributions have peaks at the same gall size category? These peaks, or **modes**, are simply the size of gall that occurs most frequently in your sample. If the modes of two distributions are similar, then it is likely that the two distributions have the same average gall size; if the modes are very different, then it is likely that average gall sizes will differ, too.

In addition, examine the shape of your distribution (e.g., the gall sizes on both sides of the mode). Do the number of observations become smaller quite rapidly on one side of the mode as opposed to the other – that is, does the histogram have a long tail? Such a difference in tail length is termed **skew**, and may be indicative of **directional selection** (i.e., more predation on certain gall sizes than on others). On the other hand, the distribution of values around the mode may be symmetrical. A symmetrical distribution may be indicative of **stabilizing selection**, or selection against the extremes; this might occur when one predator prefers large galls, whereas the other predator prefers small galls. If this were so, few live fly larvae may be found in large and small galls, whereas many live fly larvae may be found in medium-sized galls. Finally, if the distribution(s) are symmetrical, are they very broad, or narrow? A broad (**platykurtic**) distribution may be a sign that little selection is acting on gall size. In contrast, a narrow (**leptokurtic**) distribution with a very pronounced peak may be a sign of very intense stabilizing selection.

Choosing a statistical test:

The histograms you created are very useful for seeing patterns in your data, such as whether two histograms have different modal gall sizes, or differ in the variation and range of values around the mode. However, the histograms do not tell you whether any differences you see are greater than those expected by chance. To determine whether differences between two histograms are greater than expected on the basis of chance (i.e., statistically significant), we need to conduct statistical tests.

The Kolmogorov-Smirnov Two-Sample Test:

The Kolmogorov-Smirnov two-sample test is a nonparametric procedure that tests for differences between two distributions. The null hypothesis for this test is that the two samples are distributed identically (i.e., taken from the same population). Therefore, the test is sensitive to differences between the two distributions in location (mode, mean, and median), dispersion (variance, range of values), skewness, etc.

The two-sample test measures the agreement between two cumulative distributions. If two samples have, in fact, been drawn from the same distribution, then the cumulative distributions of both samples should be close to each other, inasmuch as they both should show only random deviations from the overall population distribution. If the cumulative distributions of the two samples are "too far apart" at any point, this suggests that the two samples come from different populations. A statistical test allows us to calculate **P**, the probability that the observed difference is due to chance alone. If the probability (P) is small (typically ≤ 0.05), then we reject the null hypothesis and conclude that the distributions differ significantly. That is, scientists consider a result statistically significant if there is less than or equal to a 5% probability of getting that result by chance alone. If the null hypothesis is rejected, then it is likely that some variable in your experiment had an effect on the data collected. On the other hand, if the probability is greater than 0.05 then the null hypothesis cannot be rejected, and we conclude that the two distributions are not different.

In general, testing hypotheses in science is a matter of disproving them: you can never be absolutely certain or "prove" that a hypothesis is correct. If repeated attempts to disprove it fail then you accept it as probably correct...until some observations come along that disprove it.

An example using the Kolmogorov-Smirnov test:

The data in Table 1 on the next page are the diameters (in mm) of galls that were collected for two categories: (1) galls with live gall fly larva and (2) galls that were attacked by birds. To perform the Kolmogorov-Smirnov test, proceed as follows.

1. Arrange the data in two columns as in Table 1, which tallies each gall and its diameter (in a column of increasing gall diameter) for the two different gall samples.
2. In a second table (as in Table 2 on the next page), determine the *cumulative frequencies* (F_1 and F_2) for the gall sizes in the two samples. For example, in column (3) of Table 2, note that there are 2 measurements for sample 1 that are in the first bin (i.e., the 16-17 mm category). In contrast, there are no such measurements for sample 2 in column (4). For each subsequent entry in columns (3) and (4), respectively, the number of galls of that size is added to the previous total number of galls, to generate the cumulative frequency. Note that the final entry in each column must equal the sample size (n_1 and n_2 , respectively).

3. Compute the **relative cumulative frequencies** by dividing the cumulative frequencies in columns (3) and (4) by their sample sizes (n_1 and n_2). Enter these values into columns (5) and (6), respectively. Note that the final entry in each of these columns must equal 1.00.

Table 1. Example raw data for the size distribution of galls with live fly larvae and those galls attacked by birds.

Sample 1 Live gall fly larvae ($n_1 = 15$)	Sample 2 Attacked by birds ($n_2 = 10$)
17	20
17	21
18	22
19	23
19	24
20	26
20	26
21	27
21	27
21	27
22	
22	
22	
23	
25	

Table 2. Calculation of the Kolmogorov-Smirnov two-sample test using the raw data from Table 1.

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Lower size limit	Upper size limit	F_1	F_2	F_1/n_1	F_2/n_2	$d = [F_1/n_1 - F_2/n_2]$
16.1 mm	17 mm	2	0	0.133	0.000	0.133
17.1 mm	18 mm	3	0	0.200	0.000	0.200
18.1 mm	19 mm	5	0	0.333	0.000	0.333
19.1 mm	20 mm	7	1	0.467	0.100	0.367
20.1 mm	21 mm	10	2	0.667	0.200	0.467
21.1 mm	22 mm	13	3	0.867	0.300	0.567
22.1 mm	23 mm	14	4	0.933	0.400	0.533
23.1 mm	24 mm	14	5	0.933	0.500	0.433
24.1 mm	25 mm	15	5	1.000	0.500	0.500
25.1 mm	26 mm	15	7	1.000	0.700	0.300
26.1 mm	27 mm	15	10	1.000	1.000	0.000

4. Compute d , the absolute value of the difference between the relative cumulative frequencies in columns (5) and (6). Enter this in column (7).
5. Locate the largest difference, D , in column (7). For this example, D is 0.567.
6. Calculate the critical value for the Kolmogorov-Smirnov two-sample test (D_{crit}) using the formulas in Table 3.

Table 3. Critical values of D for Kolmogorov-Smirnov two-sample test (large samples, two-tailed)*

Level of significance	Critical value of D	Level of significance	Critical value of D
0.10	$1.22\sqrt{\frac{n_1+n_2}{n_1n_2}}$	0.01	$1.63\sqrt{\frac{n_1+n_2}{n_1n_2}}$
0.05	$1.36\sqrt{\frac{n_1+n_2}{n_1n_2}}$	0.005	$1.73\sqrt{\frac{n_1+n_2}{n_1n_2}}$
0.025	$1.48\sqrt{\frac{n_1+n_2}{n_1n_2}}$	0.001	$1.95\sqrt{\frac{n_1+n_2}{n_1n_2}}$

*Table from Siegel & Casterlin, Nonparametric Statistics for the Behavioral Sciences

7. Compare D (from step 5) to the critical value of D obtained in step 6. In this example, the critical value for D is 0.555 (for an alpha of 0.05). Since your observed D (0.567) is larger than the critical value computed from the expression in Table 3, you can reject the null hypothesis at the level of significance associated with that expression. Thus, we conclude that birds attack larger galls at higher frequency than expected by chance.
8. Note that a significant K-S test only tells you that the two distributions differ. It does not tell you HOW they differ (e.g., which way the two distributions are shifted relative to one

another). **Thus, you should also compute the means and medians for your two sample sets prior to evaluating your original hypothesis.**

A note about tails:

In order to determine whether your trends are significant (i.e., whether $P \leq 0.05$), you need to know whether you are running a one-tailed or two-tailed test. When you have a clear expectation about the direction of the outcome and an outcome in the other direction would not make sense, you can use a one-tailed test. When an outcome in either direction makes sense, you should use a two-tailed test. If your test is one tailed but your table for assessing statistical significance is two-tailed, you should divide the P value given by the table in half; thus, if the critical values in a two-tailed table indicates that $P = 0.10$, and your test is one-tailed, your $P = 0.05$. Conversely, if you are running a two-tailed test and using a one-tailed table, you should double the P value that you obtain from the table.

The tails in this discussion refer to the distribution of outcomes. Any test will have one tail of the distribution that corresponds to one of the two categories of outcomes and another tail for the other category. (e.g., one category might correspond to birds preferentially attacking larger-than-average galls, and the other category might correspond to birds preferentially attacking smaller-than-average galls.

Summary of some statistical tests for evaluating questions:

To determine whether the various sources of mortality occur disproportionately on galls of certain sizes use a Kolmogorov-Smirnov test (see the above example).

To compare differences in frequencies, use a G test of independence with a Williams’s correction.

The G test of independence tests the goodness of fit of the observed frequencies to their expected frequencies. In the example below, we want to compare the number of galls that were attacked by birds in two years (2000 and 2001). Such data can be displayed in the form of a 2×2 table or a **contingency table** (see Table 4).

Table 4. Contingency table of the number of galls attacked by birds in two years.

Year	No. galls attacked	No. galls not attacked	Total no. galls Σ
2000	30 (<i>a</i>)	270 (<i>b</i>)	300 (<i>a+b</i>)
2001	10 (<i>c</i>)	490 (<i>d</i>)	500 (<i>c+d</i>)
Total No. galls Σ	40 (<i>a+c</i>)	760 (<i>b+d</i>)	800 (<i>a+b+c+d</i>)

The G test enables you to test whether the frequency of gall attacks is different between the two years. The null hypothesis for this test is that the two years are identical (i.e., that there is no difference in the frequency of bird attacks between years). Said another way, the null hypothesis assumes that the two years are in fact not distinct and were drawn from the same population. To calculate the expected number of attacks under the null hypothesis, pool both years into a single population. For example, combining data for both years gives a total population of 800 galls. Of these 800 galls, 40 were attacked by birds ($40/800=0.05$) and 760 ($760/800=0.95$) were not attacked. Thus, for a population of 300 galls (e.g., year 2000) we would expect that 5% of them or

$300 \times 0.05 = 15$ galls would be attacked by birds. Similarly, in 2001 we would expect a 5% attack rate ($500 \times 0.05 = 25$ galls). Calculate the G test using the procedure on the following page.

Calculate the following quantities:

$$(A) a \ln a + b \ln b + c \ln c + d \ln d$$

$$[= 4671.9]$$

$$(B) (a+b) \ln (a+b) + (c+d) \ln (c+d) + (a+c) \ln (a+c) + (b+d) \ln (b+d)$$

$$[= 10007.3]$$

$$(C) n \ln n \quad (n = \text{sample size})$$

$$[= 5347.7]$$

2. Compute G as the following:

$$G = 2 \times (\text{quantity A} - \text{quantity B} + \text{quantity C})$$

$$\text{For this example: } [G = 24.54]$$

3. Use the William's correction formula (for a 2×2 table) to obtain q .

$$q = 1 + \frac{\left(\frac{n}{a+b} + \frac{n}{c+d} - 1 \right) \left(\frac{n}{a+c} + \frac{n}{b+d} - 1 \right)}{6n}$$

$$\text{For this example: } [q = 1.01]$$

4. Calculate the adjusted G_{adj} by dividing the G value obtained in step 2 by q .

$$\text{For this example: } G/q = 24.54/1.01 = 24.21].$$

5. Compare G_{adj} with the critical value of Chi-square for one degree of freedom ($\chi^2_{(0.05, 1)} = 3.841$). Because G_{adj} is greater than the critical value ($24.21 > 3.841$), we can reject our null hypothesis and conclude that the frequency of bird attack in the two years is statistically different.

To compare differences in means, use an analysis of variance if your data meet parametric assumptions, or a Kruskal-Wallis test if they do not. We will show you how to use these tests if you need to use them.

To compare differences in variances (or standard deviations), use an F test if your data meet parametric assumptions. If not, we will need to decide on an appropriate test for your data.

Writing the Scientific Report

The statistics you calculated are useful as you describe what you observed in an objective fashion. This isn't the end, however. When dealing with scientific questions, most of your effort is spent trying to explain why you obtained the results that you did, and how your results test or extend existing theory. The last part of this two week lab will, then, be about writing a report in a scientific format.

Objectives:

- Review hypotheses and results of statistical tests regarding natural selection in the goldenrod-gall fly system
- Understand the basics of scientific writing, and apply that understanding to class data gathered from this experimental system

Writing a paper in Scientific Format:

Formal scientific papers are generally divided into 5 sections: Introduction, Materials & Methods, Results, Discussion and Literature Cited. Although published articles may deviate from this structure for a variety of reasons, it is important for budding science authors to learn how to write in this format. In particular, you need to know (1) the appropriate section for each part of your presentation, and (2) how to effectively develop the presentation within each section. We therefore require that you write your paper in this five-section format. The purpose and contents of each section are described below. Analyze the structure of a paper that has been published in this format before and during your writing of the report. Good models can be found in recent issues of *Behavioral Ecology and Sociobiology* or *Ecology*, which can be found on the open metal shelves in the Science library (off the entrance lobby to Merrill). You can also refer to the reprints that have been put on e-reserve.

We request that students work in pairs to write the reports, generating one report for each pair. If this arrangement presents a problem, please talk with your lab instructor about it. Your reports should address specific concepts and questions regarding natural selection in the goldenrod gall fly system. Each report must include a detailed analysis of the experimental evidence that supports (or refutes) a particular hypothesis (or hypotheses). Each pair of students should first choose a specific question that they can address with the 2008 data and/or data from past years. Then, at least one hypothesis must be generated that seeks to answer that question, and this hypothesis should be assessed using the appropriate data and statistical test. Although the analysis of one hypothesis is the minimum requirement, students may assess more than one hypothesis if they so desire.

To test each hypothesis, you should compare the patterns of selection in at least two specific data sets. The data sets can be selected from 2008 alone. Alternatively, you may choose to address a question in which you compare data from two or more years. Use the questions found in the statistics section of this handout to focus and guide you (or come up with your own questions to address!).

Writing a good scientific paper requires concise and clear writing. Try to lead the reader by the nose: What was the point of the study, and why should anyone care? How, where, and when did you do the study? What did you find out? What do the findings mean, and how do they relate to past and future work? Estimated lengths of the reports are from 5 to 9 double-spaced typewritten pages. Keep in mind that a concise, well-reasoned 5-page paper is preferable to a bulky 9-page paper that contains the same amount of information

Required Sections of the Goldenrod Gall Lab Report

Title (1 pt). Make the title informative and specific. The title identifies the important contents of the paper and orients the reader by specifying the major findings. A vague or inaccurate title can waste a reader's time by suggesting, erroneously, that the paper contains certain information. Likewise, a good paper burdened with a bad title may never catch the eye of the interest of many of its intended readers. Titles should be *specific*, for example:

VAGUE: A Look at Fungal Toxins

SPECIFIC: The Role of Fungal Toxins in Plant Disease

VAGUE: Ecological Studies of Some Northern Lakes

SPECIFIC: Seasonal Algal Succession in Three North Temperate Lakes

The **Introduction (10 pts)** sets the stage for your scientific argument. A good introduction “hooks” the reader, interesting them in the study and in the questions being explored. It should place your work in a broader context and give readers an appreciation for the purpose of your study (e.g., what is natural selection and what features of the goldenrod gall fly system make it an appropriate system to use in studying natural selection?). The introduction must also have a clear statement of your specific experimental question, and hypotheses and predictions should be explicitly stated.

You are not expected to have expansive knowledge of previous work on this topic, but you should cite at least two published studies in the scientific literature that provide background for your question. Note: it may be easiest to compose the “broader context” part of the introduction after you have drafted the Materials & Methods, Results, and Discussion sections.

The **Materials & Methods (7 pts)** section provides a textual (e.g., in prose format) summary of the procedures and materials that you used for the study. Enough detail should be given so that the reader could repeat the experiment. For example, where was your study site (field, laboratory, both)? How did you collect data (e.g., what types of variables were measured & with what equipment)? How did you record (or pool) data? For each hypothesis, mention which statistical test you used to evaluate the hypothesis and why.

Although many of the methods are given in your lab handout, you should summarize this section in full. Doing so should help you recognize the parts of your presentation that belong in the Methods as opposed to the Results or Introduction.

In the **Results (10 pts)** section, present and summarize your data and report the results of your statistical analyses. If you have developed your Introduction well (by explicitly stating your question and hypotheses), the reasons why you are presenting each result should be clear to the reader. Be sure to report the means and medians of the data sets that you analyzed using a K-S test and/or the frequencies of the data sets that you examined using a G test; note any trends in the data that may have directed you to the particular question that you asked.

In addition to a textual account of the results, the data should be organized into one or more tables and figures, which show the trends and relationships (or lack of trends and relationships). We should not see copies of the raw data. These files will generally have inappropriate abbreviations and extraneous information that would clutter your paper. Part of the reason for writing a lab report is to learn not only what material to include, but what material to exclude.

Use the papers published in scientific journals as your guide whenever you have uncertainties about what to include. Tables and figures should be numbered consecutively, and clearly labeled (table titles, table headings, figure legends, axes, etc.). *Take special care* to indicate the units of measurement of your variables. In addition, each figure must have a complete caption *briefly* identifying important components, explaining the method used to generate the data, and any other relevant information. A table or figure (and their captions) should *stand independent* of the paper, allowing the reader to understand what was done without referring back to the text. The tables and figures can be inserted near the point in the text where they are first mentioned, or they can be grouped at the end of the paper.

The text of the results section should mention each major result and refer the reader to the figure or table that shows the particular result being described, e.g., by typing in "(Figure 5)." at the end of the sentence. Take care to present your tables and figures in a way that complements the textual presentation. Also, describe the results of the statistical tests in this section, including the calculated and critical values and the P values. These values should be reported in parentheses at the end of sentences that describe the differences, trends or lack thereof. For example: ($D_{calculated} = 0.92$, $D_{critical(0.05)} = 1.44$, $P > 0.05$). Include printouts of the K-S and/or G test calculations in an Appendix. Finally, do not interpret your results in the Results section – that comes in the next section!

The **Discussion (10 pts)** section is where you interpret your results and relate them to the general topic, previous results, and the hypotheses you were testing. Begin by briefly re-stating for the reader the overall question and the specific hypotheses that you tested. Then, evaluate your hypotheses and make conclusions. Do your results support or refute your predictions? Are your results statistically significant? Do you believe that your hypothesis effectively tested your original question, or do you now believe that your hypothesis was deficient and needs revision? Were there any sources of potential error in your study? How do your results compare with similar questions raised in the literature (cite these studies here)? What new questions occur to you now that you have performed your study and how might you test them?

The **Literature Cited (3 pts)** section lists those books and articles that you actually cited in your report. Note: you must cite at least two published references in your lab report. The references should be arranged alphabetically by author. The format of the references differs for different journals. We suggest that you use the following formats, which are fairly generic:

For a journal article,

First author's surname, initials, Second author's surname, initials, {and so on for all authors} Year. Title of article. Name of journal volume of journal: first page of article-last page of article.

For an article within a book,

Author's surname, initials, Second author's surname, initials, {and so on for all authors}. Year. Title of article, pp. first page of article-last page of article. In editor's surname initials, Second editor's surname, initials, {and so on for all editors} [ed.]. Title of book. Volume {if any}, number {if any}, publishing company: city.

For a book.

Author's (or editor's) surname, initials, Second author's surname, initials, {and so on for all authors or editors}. Year. Title of book. Volume number. Publishing company: city.

In-text citations.

Do not use direct quotations in your paper. Rather, describe or paraphrase the results of other authors and cite their work when you do so. When you refer to another's work or another's ideas in your report, you should give credit as illustrated below.

Experiments by Temeles and Roberts (1993) showed that rufous hummingbirds (*Selasphorus rufus*) spent more time feeding as flower length increased.

or

Rufous hummingbirds (*Selasphorus rufus*) spent more time feeding as flower length increased (Temeles and Roberts 1993).

The complete reference to the person's work would be included in your Literature Cited section so that the reader can examine your source material. If you are citing a paper that you have not read yourself, you cite it in the following way:

Brooks (1984, cited by Wolff 1985) proposed that females may benefit from infanticide because infanticide eliminates individuals that would otherwise...

References and additional resources:

The following is a list of relevant papers on the goldenrod-gall system that are available on e-reserve. As mentioned earlier, you should read at least two of these references (but are strongly encouraged to read more) and incorporate them as citations in your lab report. You are also welcome to search the scientific literature for any other references that are relevant to your study.

Weis AE and WG Abrahamson (1998) Just lookin' for a home. (goldenrod gall fly). *Natural History*, 107:60-63.

Abrahamson WB, McCrea KD, Weis AE (1989) Variation in selection pressures on the goldenrod gall fly and the competitive interactions of natural enemies. *Oecologia* 79:15-22.

Confer JL, Orloff J (1990) Spatial distribution of the goldenrod ball gall insects. *The Great Lakes Entomologist* 23:33-37.

Confer JL, Paicos P (1985) Downy woodpecker predations at goldenrod galls. *Journal of Field Ornithology* 56:56-64.

Mecum LK (1994) Downy woodpecker (*Picoides pubescens*) predation on the goldenrod gallmaker *Eurosta solidaginis*. Master's thesis, Bucknell University.

Weis AE, Abrahamson WG (1985) Potential selective pressures by parasitoids on a plant-herbivore interaction. *Ecology* 66:1261-1269.

Weis AE, Abrahamson WG, Andersen MC (1992) Variable selection on *Eurosta's* gall size. I: the extent and nature of variation in phenotypic selection. *Evolution* 46:1674-1697.

Ramløv H, Lee RE Jr. Extreme resistance to desiccation in overwintering larvae of the gall fly *Eurosta solidaginis* (Diptera, tephritidae). *Exp Biol*. 2000 Feb;203(Pt 4):783-9.

Smith PT, Krager K, Cronin JT, Kambhampati S. Mitochondrial DNA variation among host races of *Eurosta solidaginis* Fitch (Diptera: Tephritidae). *Mol Phylogenet Evol.* 2002 Nov;25(2):372-6. Erratum in: *Mol Phylogenet Evol.* 2003 D

Lee RE Jr, Hankison SJ. Acquisition of freezing tolerance in early autumn and seasonal changes in gall water content influence inoculative freezing of gall fly larvae, *Eurosta solidaginis* (Diptera, Tephritidae). *J Insect Physiol.* 2003 Apr;49(4):385-93.

Abrahamson WG, Blair CP, Eubanks MD, Morehead SA. Sequential radiation of unrelated organisms: the gall fly *Eurosta solidaginis* and the tumbling flower beetle *Mordellistena convicta*. *Evol Biol.* 2003 Sep;16(5):781-9.

Craig TP, Itami JK, Horner JD. Geographic variation in the evolution and coevolution of a tritrophic interaction. *Evolution Int J Org Evolution.* 2007 May;61(5):1137-52.

Craig TP, Itami JK, Craig JV. Host plant genotype influences survival of hybrids between *Eurosta solidaginis* host races. *Evolution Int J Org Evolution.* 2007 Nov;61(11):2607-13. Epub 2007 Aug 23.

Crawford KM, Crutsinger GM, Sanders NJ. Host-plant genotypic diversity mediates the distribution of an ecosystem engineer. *Ecology.* 2007 Aug;88(8):2114-20.

Irwin JT, Lee RE Jr. Mild winter temperatures reduce survival and potential fecundity of the goldenrod gall fly, *Eurosta solidaginis* (Diptera: Tephritidae). *Journal of Insect Physiology.* 2000 May; 46(5): 655-661.

Carango P, McCrea KD, Abrahamson WG, and Chernin MI Induction of a 58,000 dalton protein during goldenrod gall formation. *Biochemical and Biophysical Research Communications.* 1988 May; 152(3):1348-1352.

Style (4 pts) Good writing is important. Is the lab report clearly written and organized, with all required material in the correct sections? Does each paragraph have a topic sentence? Did you use proper grammar? Has the report been proofed for correct spelling and typos? Did you use the proper binomial nomenclature for the scientific names of organisms?

Final Note: We encourage students to discuss results and ideas with your classmates and/or lab instructors. However, we remind you that the written lab reports are to be prepared independently by each pair of students, with no sharing of paper or electronic lab reports with other students in Bio 18 – current as well as students from previous years.

The lab report is due next week, at the beginning of your regularly-scheduled lab section (e.g., Feb. 19, 20, 21 or 22).

Blank Page