Detecting Selection in a Natural Population

Preparation for Laboratory: Tutorial 3: An Evolutionary Play - submit answers to questions
Additional Background: Freeman, " Natural Selection" pp. 440-442

Methods

By collecting galls, measuring the distribution of gall sizes (phenotypes) and determining how distributions change after mortality agents act, you will be able to estimate the strength and form of selection on gall size for these populations of goldenrod and fly. You will also be able to compare the rates of mortality due to different agents, which will help you interpret why selection has acted in the way it has. Finally, you will be able to compare our class results with published data to determine how consistent selection might be in different localities and times.

Sampling Galls

Study site – The Brown Family Environmental Center at Kenyon College has several sites where agriculture was abandoned in 1994. Corn fields were located adjacent to the Kokosing River and behind the old farm house. After cessation of cultivation, these fields were colonized by typical, early successional plants such as goldenrods, asters, thistle, mullein, a variety of invasive weeds, and poison ivy. Woody growth is becoming established where we have minimized our management: ash-leaved maple, sycamore, cottonwood, multiflora rose, and oaks are among the thirty species scattered in the fields along the Kokosing River. Behind the farmhouse, prairie grasses were seeded in 1997. In spring, 2001, the new prairie was burned to inhibit woody growth and to promote the prairie grasses. Goldenrod is thriving in both prairie and old field (though habitat differences might influence the goldenrod-gall interaction).

Gall collection – When collecting a sample of individuals from a population, it is important to consider carefully how to avoid bias when selecting individuals for examination. Ideally, we want to choose individuals from a population randomly (e.g., the condition of one individual could not be predicted from any of the previous collections). Achieving true random samples is not always practical, but we should be careful to avoid systematic bias (such as noticing and selecting preferentially the largest galls). What other potential biases can you think of? The following technique should give us a reliable sample:

> Locate the end of a transect at the edge of the field to be sampled. The starting and ending points of these transects were established by the instructors by laying out a long measuring tape and then using a random numbers table to set the beginning point.
>
> Visually examine the top 1/3 of goldenrod stems to locate ball galls. Collect all ball galls from goldenrod within 0.5 meter of the measuring tape, until you have collected ten galls. Make sure you do not miss small galls! **To collect a gall, break the stem below the ball gall - keep all the stem above the gall ball as we will want to note the presence and condition of co-occurring rosette galls.**

After collecting ten galls (or after one hour of searching, whichever comes first), take your

samples to the laboratory in the BFEC education building. There, you will measure and evaluate your galls.

For each gall:

- Measure the diameter at the **widest point** with calipers. The measurement should be made with an accuracy of 0.1 millimeter. Be sure that the caliper dial is correctly set to 0.0 before making your measurement (otherwise it is easy to introduce a systematic error into your share of the data).
- Measure the stem diameter with an accuracy of 0.1 millimeter at a point 1 inch below the gall.
- Measure the gall wall thickness at **narrowest point** with an accuracy of 0.1 millimeter.
- Note whether a double ball gall was present. When double galls are present enter the diameter, wall thickness and fate for both of the galls. Use separate lines.
- Note whether a rosette gall was present.
- Examine the gall to characterize its fate (see below). Enter the fate into your data sheet on the same row as the gall diameter.

Carefully cut open the gall and examine the contents. You will need to remove layers, starting at one side of the gall. Watch for the open chamber, then examine the inside of the chamber. Examples of galls will be in the laboratory.

If a cream-colored, fat larva is present in the gall, that fly has survived all the mortality agents discussed above and will almost certainly survive to emerge later in the spring. All other galls were induced by a fly that did not survive.

Categorize the failed galls according to the presence of

- a wasp larva (*Erytoma gigantea*) or lumen with dark brown frass
- a wasp pupa (*Erytoma obtusiventris*)
- a beetle
- no open chamber (Early Death!)
- unknown (no living organism)

After you and your partner have finished examining your galls, give your data sheet to the instructor or lab TA. Do not leave the lab until your data sheet has been turned in. Make sure all information is entered on the data sheet, including your names.

Assignment: The following paper will be used as a reference when writing your first paper. You should read it carefully before coming to lab next week.

Weis, Arthur E. and Warren G. Abrahmson. 1985. Potential Selective Pressures by Parasitoids on a Plant-Herbivore Interaction. Ecology: 66(4) 1261-1269.

You can obtain a copy of this paper by using the link provided on the biology 109 resource page,

which can be accessed from the biology homepage at:

http://biology.kenyon.edu/

The guide "How to Read a Scientific Paper" provided in this manual may help you move through this paper. We will briefly discuss this paper before you begin analysis of the data you have collected. If you do not understand portions of the paper write down your questions so they can be discussed in class next week.

Solidago sp. Ball Gall Data Sheet

Transect #:_____ Students_____

Site: BFEC at Kenyon College Date:_____

Gall Diameter 0.1 mm	Stem Diameter 0.1 mm*	Wall Thickness 0.1 mm**	Double Ball Gall? Y/N	Rosette Gall Y/N	Fly Larva Y/N	Gall Fate[1]

Site: Ramser Arboretum Date:_____

Gall Diameter 0.1 mm	Stem Diameter 0.1 mm*	Wall Thickness 0.1 mm **	Double Ball Gall? Y/N	Rosette Gall Y/N	Fly Larva Y/N	Gall Fate[1]

* Measure stem approximately 1 inch below gall **Measure thickness at narrowest point

[1] Gall Fate

F = Fly larvae usually oval; no distinct head capsule; anteriorly directed mouth hooks, grey frass may fill capsule
G = *E. gigantea* larvae drop-shaped; distinct white head capsule and dark mandibles; brown frass may be present
O = *E. obtusiventris* larva: present as a brown pupal case
B = Beetle larva have 3 pairs of prolegs, gall has signs of tunneling
E = Early Death - no larva or frass present, center cavity **very small** or not present
U = Unknown cause of death but a central cavity is clearly present

Goldenrod Gall Analysis

Preparation for Laboratory: read Weis & Abrahmson. (1985) access the paper at the Biology 109 Resource Page

The data that were collected by all the Biology 109 students has been entered into a Minitab file. It is located at:

p:data/biology/biol109/goldenrod

The worksheet contains the following columns:

C1 **Site** the location where the galls were collected.
B = Brown Family Environmental Center
R = Ramser Arboretum

C2 **Galldiam** - measurements of the diameter of the gall at its widest point

C3 **Stemdiam** - measurements of the stem diameter taken 1 inch below the gall

C4 **Thickness** - measurement of the gall wall thickness taken from the outer edge of the lumen to the exterior surface of the gall. These were taken at the **narrowest point** for all galls that contained living insects or had distinct lumens.

C5 **Rosette** - this column contains codes to identify plants that had both ball galls and rosette galls.

C6 **Fate** - this column contains codes to identify the fate of the gall contents.
b = beetle larvae (*M. unicolor*) present
e = galls with no central lumen (early death)
f = living fly larva present
g = living wasp present (*E. gigantea*)
o = pupal case present (*E. obtusiventris*)
u = unknown - galls with lumens but no living organism (fate unknown)

Beyond the worksheet – some basic questions

Look at the data on gall diameter, stem diameter, and wall thickness to determine if there are any unusual outliers and if it is normally distributed. Do not include these histograms in your paper!

The galls come from two sites. Can differences in gall size be explained by their source?
What statistical test should you use to answer this type of question?

- Determine if stem diameters varied between the two sites (stem diameter below the gall was established before the fly infected the plant).
- Determine if gall diameter varied between sites (do not try to separate effects of parasites or predators).
- Determine if the thickness of gall walls varied between the two sites.

If plants from the two sites appear to be similar in basic plant size, then data from the sites can be aggregated to test hypotheses about the interaction between flies and their consumers. If not, then you may need to do some more sorting of the data.

Can differences in gall size be explained by the fate of the gall fly?
Remember that there are six categories of fate: galls with flies, beetles, 2 types of wasp, early larval death or unknown cause of death. **What statistical test is appropriate?**

Is there selection for gall size? Are gall flies that stimulate the production of large galls more apt to survive?
The ideal experimental design would be to measure the size of galls before selection occurs, but this is not possible because the parasites have already infected the galls. Based on the work of Weis and Abrahamson (1985) we know that parasitic attack does not affect the mature size of the galls, so we can infer the size of the galls before the selection event, which leads to the death of the fly. Galls in which early larval death has occurred are not suitable for parasites and predators and should be eliminated from the data set. The diameters of the remaining galls represent the **preselection pool** (galls suitable for attacks by parasites and predators). The preselection pool contains all galls with living organisms in them.

Organizing the data for analysis: you will need to create several additional columns before you continue data analysis. To make the organization of these columns easier they have already been named. As you manipulate the data make sure that you put the correct data in the named columns.

C7 **Diam/live** - Create a column of gall diameters for galls in which flies survived long enough to create a central lumen. This is done by omitting the diameters of galls coded for early death (e). With early death, flies do not have sufficient time to fully stimulate gall growth. Keep in mind the fact that at the point you examined the galls selection had already occurred. Only galls with live flies are suitable for selection. Diam/live contains data on all the galls that were suitable for selection.

C8 **Fate/live** - this column is like C6 except it lacks a code for early death. Galls without lumens have been eliminated.

Manip ➤ Copy Columns ➤ Copy from columns (*Enter C2 C6*) ➤ To columns (*Enter C7 C8*) ➤ **Omit Rows** ➤ Omit rows with text column (*Enter C6*) equal to (*Enter "e"*)

The above command copies all the gall diameters and fates into **Diam/live, Fate/live** but excludes any rows with the code **e**. Scroll to check the data went where you thought.

C9 **Diam/fly** - this column will contain the diameter data for galls with **surviving flies**. Create it as above using the galldiam data (C2) and the codes in the fate column. In this instance you will store the data in C9 and chose the option ➤ **Use rows** with text column (*Enter C6*) equal to (*Enter "f")* then chose ➤ **Omit rows** ➤ **Omit no rows** .

Check to make sure you have copied the correct data. Is the first value in your new data set the same as the first value with an f next to it in the original data set?

What is the average size and variance in size of galls suitable for attack by parasites and predators? You will need to determine the mean and standard deviation of the diameter of galls that had a central lumen. These data are in C7 "diam/live". (Remember some of these galls have already been attacked.)

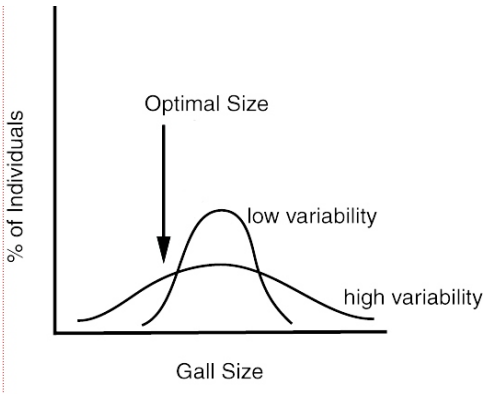
Reminder: calculate "variance" by squaring the standard deviation.

What is the average size and variance in size of galls when flies survived the period of attack by predators or parasites?
To answer this question, you will need to obtain mean and standard deviation for gall diameters where galls were occupied by fly larvae as observed by our class. These data are in C9 "Diam/fly".

Were galls suitable for attack similar in diameter to those in which flies survived? If not, in what direction was the change?
If the diameter of all the galls with living organisms prior to the selection event and the diameters of galls with surviving flies are the same, no selection occurred in our system. **You will need to think carefully about what test is appropriate here.** You will be comparing the gall diameters "suitable for selection" to those of "surviving flies." Are these independent groups? What test allows you to compare a mean to an expected value? What would be an appropriate expected value to use?

If the mean gall sizes of the two groups are different, is the intensity of selection similar to that reported in the literature?
Selection intensity is defined on page 1264 of Weis and Abrahamson (1985). **To determine the selection intensity** calculate the difference in mean gall diameter for galls in the "preselection group" (suitable for selection) and in the "postselection group" (surviving flies). This difference is then divided by the standard deviation for "preselection" galls.

Why is the difference scaled by standard deviation? The reasoning is as follows. Assume you have two populations of galls that differ in variability but neither is at their optimal size. If the preselection population is extremely variable, a relatively large proportion of individuals probably are close to the optimal size (and fewer individuals must die to shift the average). If the preselection population has very low variability (so that by definition, most of the individuals are at the "preselection" not the optimal size) then many more individuals will have to die to shift the average.



Did variation in gall diameter get smaller after mortality, as predicted by a hypothesis of strong directional selection?

To answer this question you need to use the variances in gall diameter that you calculated previously. In order to determine whether a difference in variances might be due to chance, you need to calculate an index of difference – just as you have done for T-tests and Chi-square tests. For evaluating differences in variance, your index of difference is the ratio of the larger variance to the smaller variance (the index is called "F"). The critical value for F depends on both the sample size for the numerator (the group generating the larger variance) and the denominator (the group generating the smaller variance). Calculate F for your data, and compare the value of this index of difference to the appropriate critical value, given in the table below:

Critical values of F (for P = 0.05)

		d.f. for numerator			
		20	**30**	**60**	**120**
d.f. for denominator	**20**	2.12	2.04	1.95	1.90
	30	1.93	1.84	1.74	1.68
	60	1.75	1.65	1.53	1.47
	120	1.66	1.55	1.43	1.35

Based on your F ratio is there evidence selection is reducing variance in gall size is occurring?

Did the fates of flies in galls vary systematically with size?

As was done in the Weis and Abrahamson (1985) paper you should create a graph that illustrates the percent mortality caused by wasps and beetles and a graph that illustrates the percent survivorship of flies based on gall size. Preselection diameters have been coded into several categories (diamcode). You can use cross tabulation of diamcode with fate/live, ✓ the **row percent** box to obtain the data necessary for the figures. We will not apply a statistical test to these data.

At this point create two new columns of data one containing the **Fate** data for the BFEC and one containing the **Fate** data for the Ramser Arboretum. You can create them in one step using the **stack/unstack** command found under **manip**.

C10 **fate-BFEC** - fate of galls at the BFEC

C11 **fate-Ramser** - fate of galls at the Ramser Arboretum

C12 **Diamcode** - categories of gall diameters that can be used to examine the relationship between gall size and fate over a reasonable number of size categories. The values are the midpoint of the 3mm range for each group. This was done by your instructor using the **code** command.

Did the fates of flies differ in the two sampling sites within Knox County?
As a first step to answering this question, tally the fate data in the unstacked columns (C11, C12) with a ✓ by row percent. These data should be organized as a table for your paper. Then do the appropriate statistical test to determine if the fates are different at the two sites. **Hint:** This is distributional data. **What statistical test is appropriate?** Use the stacked data (C1, C6) for this test.

Your Assignment:

You are to communicate your results in the form of a scientific paper. This format probably is unlike papers you have written for other classes, and is unlike a typical laboratory report. One model for a scientific paper is the article by Weis and Abrahamson (1985). Guidelines for writing a scientific paper are in your laboratory manual, pages 7 – 16 or online at the Biology 109 Resource page. ***You should read and use these guidelines.*** You are expected to produce a near-final draft that is as complete as possible.

- The near-final draft is due in laboratory during the week of **October 14-17** (see syllabus).
- Instructors will read the draft and suggest revisions for the final manuscript during individual sessions the week of October 21-24.
- Final drafts are due the week of October 28-31.

Although Weis and Abrahamson (1985) is given as a general model, please do not think that your paper must be as long. Be especially careful that you do not intentionally or inadvertently plagiarize from this paper (for example, sections from the "introduction" and "natural history" might be very tempting for you to copy or paraphrase). If you have questions about the definition of plagiarism you should read the Student Handbook on the subject.

Notes for your Methods section:

The location and basic characteristics of study sites should be given in your methods.

Location:	
BFEC	0.9 km southwest of Gambier, adjacent to the Kokosing River, Knox County
Ramser Arboretum	1.5 km east of Jelloway, Knox County
Field area:	
BFEC	2.2 hectares
Ramser Arboretum	5.2 hectares

Solidago sp. was the dominant plant flowering in both areas, and the densities were approximately equal.

Suggestions for your Results section:

A figure is not appropriate for reporting every result. For example, when a test fails to reject the null hypothesis you can be more concise by reporting this result in the text (means, variation, and statistical test results may be included in the text). Similarly, if you are reporting a single figure (such as selection intensity) or ratio (such as an F ratio), these are effectively reported within the text of the results (no figure is needed).

A figure is very effective if you are comparing patterns among three or more groups, especially when you want to indicate variation using 95% confidence intervals or standard errors. If you have a pattern created by multiple groups (as supported by a significant analysis of variance), then a figure is appropriate.

Tabulations (counts) of data over multiple groups usually are better presented in tabular form. For example, in comparing gall fates in the two study sites, a table may be better than a figure. Choosing between using tables or figures is sometimes a matter of style or preference; if you have difficulty deciding please check with your instructor or with a course teaching assistant.

Your results section should include answers to the questions posed above. You may add questions, if you wish.

Literature Cited

Weis, Arthur E., and Warren G. Abrahamson. 1985. Potential selective pressures by parasitoids on a plant-herbivore interaction. Ecology 66: 1261-1269.