Objectives:

Upon completion of these modules, you should be able to:

- make measurements using Vernier calipers.
- examine gall contents through a dissecting microscope or a hand lens and both identify the organisms within the gall (if any are present) and describe the events which took place.
- record data in appropriate tables.
- calculate means and construct histograms (frequency distributions) from data collected.
- relate insect life history describing the events involved in complete metamorphosis.
- describe how differential survival of gall flies leads to evolution (gall size).
- analyze findings to determine whether the data demonstrate that different exploiters tend to attack different size galls; use standard deviations as a part of the analysis (extended version, only).

This laboratory and field exercise investigates natural selection operating as a result of predation. You will be looking at the interactions between Canada goldenrod (Solidago canadensis) and its stem gall insects. The term gall is used to describe any swelling or abnormal growth of plant tissues caused by an external stimulus. Many different organisms (bacteria, fungi, insects) can cause plant galls to form. Among the most common gall types are those initiated by insect larvae.

The goldenrod plant and goldenrod gall insects represent a type of species interaction that can lead to co-evolution. Changes in one species will eventually result in changes in another. Adaptations resulting from their interactions become progressively more refined and specialized.

The most common goldenrod gall is the ball gall. It is caused by a species of fly called Eurosta solidaginis (shown on next page). In the spring, the female flies lay their eggs in the terminal buds of developing goldenrod plants. Larvae hatch from the eggs and tunnel into the dividing meristematic tissue of the plant stem. While tunneling, the larvae secrete compounds that are believed to act like plant growth hormones. The plant therefore produces more stem tissue in the area occupied by the larvae, which results in the formation of a spherical gall. The larvae remain in the gall throughout the fall and winter, pupating in the spring. In May, the pupae mature into adults and emerge from the galls.

Eurytoma obtusiventris is a parasitoid wasp that attacks the gall fly egg shortly after it is laid.
among the young, developing leaves of the plant. The development of the wasp larva inside the gall fly embryo is delayed until late summer, by which time the fly larva has hatched and grown to nearly its full size. Then the wasp larva develops quickly, eventually consuming the fly larva. Due to the presence of the wasp larva, the fly pupates prematurely (in August rather than the following May), and the wasp larva then overwinters in the fly pupal case, pupating and emerging as an adult wasp in the spring. Because the wasp initially parasitizes the fly, then kills and eats it, the wasp is classified as a parasitoid. A "true" parasite does not typically kill and then feed on its host.

Another species of wasp, *Eurytoma gigantea*, also preys upon the gall fly. During July, the female wasp inserts her eggs into the chamber in the goldenrod gall where the fly larva is developing. The wasp larvae hatching from these eggs eat the fly larvae before the fly pupates. The wasp larva then overwinters inside the gall, pupates in the spring, and emerges as an adult.

Other common predators of gall fly larvae are a beetle (*Mordellistema convicta*), the downy woodpecker, and the black-capped chickadee. Beetle larvae tunnel through the gall to reach the fly larvae. The birds peck their way through gall tissue to reach the larvae leaving a large hole.

By collecting and dissecting goldenrod galls and identifying their inhabitants, we can draw a picture of the interactions between goldenrod, gall flies, and their parasites, parasitoids, and predators. There is some evidence that the size of the goldenrod gall is genetically determined by the fly. With this in mind, we can ask the following question: does the size of the gall have anything to do with the chances of fly larvae surviving to form mature adults? This is a way of asking whether natural selection, in the form of predation on fly larvae, determines the size of the goldenrod gall produced by infection by these fly larvae. One could make several testable hypotheses, e.g., wasps preferentially look for meals in small galls while birds prefer large galls. If this were true, there might be selection for flies producing galls of some optimal size, small enough to avoid detection by birds but large enough to make it hard for the wasp to reach the fly larvae with her ovipositor. How could you analyze your data to try to answer this question?

One could also determine how natural selection might be affecting this interaction. A large sample of fully-formed galls can be used to represent the population of gall flies before predation. This works because the gall remains after the fly larva has been consumed. Dissection of the galls will reveal how many still contain living gall-formers. This sample represents the population after selection. These data will allow an analysis of the question of whether predation on the gall fly acts as a selective force on gall size. In other words, does the size of the gall have anything to do
with the chances of fly larvae surviving to form a mature adult?
Introduction

In this exercise, you will first just observe a number of galls and attempt to explain what caused them. You will record your observations and your inferences in Table 1, then you will discuss them with your group and complete the table.

In the next module, you will measure the galls and record your data in Table 2.

Materials You Will Need:

Galls, hand lenses and/or stereomicroscope

Procedure I Brainstorming: Observation and Inferences

1. Study the galls and make observations (color, texture, markings)
2. How are the galls similar? How do they differ?
3. Are there holes in the galls? If yes please describe them.
4. What is a possible explanation (inference) about where the galls come from?
5. What can you infer about what made the holes?
6. Record observation and Inferences on Table I: Brainstorming

Time of year galls collected ________________
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<thead>
<tr>
<th>Individually Brainstorming</th>
<th>Group Thinking</th>
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Materials Needed (per group)

- Dissection microscope or hand lenses
- Knife
- Goldenrod galls (5-10)
- Calipers
- Petri dishes
- Data sheets
- Forceps
- Cutting surface
- Dichotomous Key
- Sharpie marker
- Chart for Identification of Events Occurring Within Goldenrod Galls (optional)

Procedure

1. Obtain a sample of galls. Use a marker to number each gall from 1 to the last one. For the first gall, use the calipers and measure the diameter around the gall's equator at the widest point. Record this number in the appropriate place on Data Table #2. Rotate the gall 90 degrees and take a second measurement. Record in Data Table #2. Calculate the average diameter and record it. Repeat for each gall.

2. Note the presence or absence of holes in the gall and place a check in the appropriate place on Table 1 to indicate whether they are large or small.

3. After measuring the gall, cut it open being careful not to squash any creature that might be inside it. Rotate the gall while cutting. Cut a groove in the gall and then pop it open.

4. Identify any organism you find inside the gall. Refer to the dichotomous key, diagrams and descriptions on the following pages. (Use a dissecting microscope or hand lens to get a better view of the organism and the inside of the gall.) Can you explain how that organism came to be there? Record your findings in the appropriate place on Table #2. Do not mix up the galls, since a correlation between each galls’ size and its’ inhabitants are critical in this lab!
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<tr>
<th>PRE-CUT: OBSERVATIONS</th>
<th>POST-CUT: CONTENTS OF GALLS</th>
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<td>Diameter (mm)</td>
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Gall Lab Dichotomous Key

1. A. Gall contains a larva (feeding stage without protective cocoon) Go to Step 2

1. B. Gall contains a reddish brown pupa (non feeding stage between the larva and adult in within a protective cocoon) Obtuse Wasp=Wasp #1 (Eurytoma obtusiventris)

2. A. White barrel shaped (Fat) larva (4X6mm) with black mouth hooks, can move Gall fly (Eurosta solidaginis)

2. B. White slender shaped larva (1X8 mm) Go to Step 3

3. A. Slender Larva lacking mouth parts Giant Wasp =Wasp #2 (Eurytoma gigantea)

3. B. Slender larva with small appendages (1X8 mm), can move Beetle (Mordellistena unicolor)

1. Describe the ways in which the formation of the gall benefits the fly larva.

2. Female gall flies are very selective about where they lay their eggs, laying only on Solidago altissima even though other, closely related species of goldenrod may grow nearby. How does the fly know the proper plant on which to lay her eggs?

3. It is advantageous for the female Eurytoma gigantae to place their female offspring on larger galls. How do wasps determine which offspring will be female and which male? Can mammals determine the sex of their offspring? Why or why not?

4. Why do the authors of the article think they are observing the evolution of a new species of gall fly in New England and the northern great Plains?

5. Use your computer or ipad to locate two journal articles that are more current in the goldenrod gall research. List the name of the article, the journal, the date and a brief description of the article.

6. Using the lab background information and the research article to define/explain the following terms and describe their connection with goldenrod galls. Give examples where appropriate.
   a. Symbiosis
   b. Parasitism
   c. Parasitoid
   d. Protein
   e. Hormone
   f. Enzyme
   g. Diameter
   h. Gall
   i. Natural Selection
   j. Diversity
   k. Predator–Prey Interaction
   l. Metamorphosis

7. Referring to the “backyard biology section” of the Weiss and Abrahansan article, create a chart that will help you organize and “score” each collected specimen. Remember your first column is the independent variable.
8. Below is a list of organisms included in this goldenrod article. Match the scientific names with the common names and use your resources to obtain the scientific names for those not identified in the reading.

___ Eurytoma gigantean a. beetle
___ Eurosta solidaginis b. gall fly
___ Solidago altissima c. parasitoid wasp (lays eggs into fly egg)
___ ________________ d. downy woodpecker
___ ________________ e. black-capped chickadee
___ Mordelistera convicta f. parasitoid wasp
___ Eurytom obtusiventris g. tall goldenrod

9. Obtain a sheet of plain white paper and create a food web. Use the specimens collected in the experiment and tape or glue them to their appropriate location (niche) and remember to include a title and label all structures.

10. Describe one experiment in the article. Identify each of the following factors in your description:

   Problem/purpose
   Hypothesis
   Experiment
       Independent variable
       Dependent variable
       Control group
   Data collected (include units)
   Results/Conclusions: support or refute hypothesis?
Procedures for Analyzing Data:

1. Complete Summary Table #4, which is a compilation of the findings of the entire class or the Excel file that comes with this lab. Use this for all remaining questions and histograms. (Reminder: histograms and bar graphs are not the same thing. Bar graphs are used for independent variables like names while the independent variables on histograms are numbers or measurements.)

2. Calculating averages (optional, depending on the level of students):
   a) Calculate the average size of all the galls sampled and record.
   b) Calculate the average size of galls attacked by each type of predator (wasp #1, wasp #2, beetle, birds, and unknown). Record.
      a. Example, galls attacked by Wasp #1: If you have 2 galls at 14 mm, 4 galls at 17 mm, 1 gall at 21 mm, and 5 galls at 22 mm, here is how to set up the equation:
         i. \( \frac{(2 \times 14 \text{mm}) + (4 \times 17 \text{mm}) + (1 \times 21 \text{mm}) + (5 \times 22 \text{mm})}{12 \text{ galls total}} = 18.92 \text{ mm/gall} \)
         This is the average size of all galls attacked by Wasp #1.
   c) Calculate the average size of galls that contained live gall fly larva. Record.

3. Examine the averages you just calculated. Do each of the consumers attack the same size galls?

4. Plot 4 different bar graphs where the height of the bar represents the number of galls in the different graphs, the bars represent:
   (1) size of all of the galls opened by the class
   (2) size of galls that contain live gall flies
   (3) size of galls attacked by wasp #1 and size of galls attacked by wasp #2 (both on the same graph)
   (4) size of galls attacked by beetles and size of galls attacked by birds ....if there are enough samples to warrant (again, both on the same graph)
On each graph indicate the average size of the galls. NOTE: You may want to combine data so that each column represents a size range, eg. 9 - 11 mm, 12-14 mm, etc. Determine which axis is the independent and which is the dependent variable and choose an appropriate scale. **All graphs are to be done in pencil.**

### DATA TABLE #3
**Summary of Individual’s Data**

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<th>Total Galls</th>
<th>Total Alive Flies</th>
<th>Wasp #1</th>
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**Conclusions**

**Answer all questions in the space provided or on a separate sheet of paper.**

1. Compare the graphs and answer the questions listed below for each consumer.
   a. How does the size of a particular class of galls (for example, those parasitized by wasp #1) compare to the entire class set of galls? Use data from the lab to support your claim.

   Range of all galls: _______________
   Gall larvae: _______________
   Wasp #1: _______________
   Wasp #2: _______________
   Beetle: _______________
   Bird: _______________
b. Are the sizes of galls that were attacked evenly distributed (bell curve), clustered at the extremes (at either end of the graph), or do you see some other pattern?

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c. Describe the shape of each histogram. Is the shape about the same for each category of organism (wasp #1, wasp #2, etc) or are they different? Why might this be so?

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2. From your data…

a. Can you conclude that attack by the wasps is random with respect to gall size or do the wasps seem to prefer galls of certain dimensions? Support your statement using data from this lab. If you observe a pattern, propose a hypothesis about the reasons that the pattern may exist. In other words, what might the benefit be to the wasp to attack that particular size range of galls instead of random sizes?

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b. Is attack by birds random with respect to gall size, or do birds seem to select specific sizes of galls? Explain your findings and give supporting evidence. Based on what you know about birds, propose a sound scientific reason for any pattern that you see, i.e. why do the birds seem to prefer or not prefer specific sizes?

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3. According to the article “Just Lookin’ For a Home”, the size of the gall is determined by genes present in the fly. Genes code for particular products. The genes of interest in this system appear to code for a secretory product that simulates plant growth hormones causing the goldenrod to produce more or less gall tissue. What might be the advantage to the gallfly of the plant producing a larger or smaller gall? Do you think that natural selection is acting on the gallfly’s ability to produce galls of a certain size? Explain.
4. Gall flies parasitize the goldenrod plant, causing it to produce gall tissue. Since the plant must use additional energy to produce extra gall tissue, what would be in the “best interest” of the plant? If genes in the goldenrod influence the size of the gall produced in the presence of a fly larva, how might natural selection act on the plant's response to the gall fly?

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5. Based on your answers to questions 6 and 7, what would you expect to see if you came back in 20 years and sampled the goldenrod gall population in the same area? Explain.

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6. Discuss one other system or relationship in which competing interests affect how the participants react to each other. Be specific and give supporting evidence.

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