Cornell Institute for Biology Teachers

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Title: **Goldenrod Gall Size as a Result of Natural Selection**

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Appropriate Level: Concepts taught in this investigation are appropriate for all high school biology students. The main write-up is for Regents Biology. There is an extension for AP or Honors level students. There is also a version directed at general or school group students.

Abstract: This investigation examines natural selection and coevolution using goldenrod *(Solidago canadensis)*, its stem gall insect *(Eurosta solidaginis)*, and associated parasites, parasitoids, and predators that feed upon the stem gall insect (i.e., *Eurytoma obtusiventris, Eurytoma gigantea*, *Mordellistena unicolor*, and birds). Through measurements of gall size and an investigation of events occurring within the galls, a correlation between gall size, frequency of predation, and type of predator can be made. An analysis of histograms and data tables charted from gall measurements and frequencies of various events leads to the conclusion that parasitic organisms select goldenrod galls within specific ranges of size. A statistical analysis using standard deviation and tests of reliability also lead to the conclusion that the forces of natural selection and coevolution are operating within this stem system.

Special Notes: Special equipment needed: Stereoscopic microscopes or hand lenses; Vernier calipers for the whole class.

Time Requirement: Three to five lab periods are required to describe the complex ecology under study, to learn to use the Vernier calipers, to measure the galls, and analyze the gall contents. (A previous period should be used to introduce the gall creatures to students via a slide presentation available through CIBT.)

Additional Teacher Information

Information with Which the Students Must be Familiar

- Evolution proceeds by natural selection.
- Different gall sizes result from genotypic variation in the gall fly and/or the goldenrod plant.
- The goldenrod gall is a primary producer and the gall insect is a primary consumer, which in turn feeds a variety of secondary consumers.
- The insect life cycle involves egg, larval, pupal, and adult stages.

Time required

In class: 3-5 lab periods (3-4 hours)

- One 45-minute lab period to become familiar with background material and to learn the techniques necessary for measuring with Vernier calipers.
- One 45-minute lab period to collect data (dissect the galls, identify the organisms, and tabulate results).
- One 45-minute lab period to construct graphs, calculate means (and selection differential when appropriate).
- Allow 30 minutes to discuss results and interpretations .

Before class: teachers' time requirements will vary

- Allow several hours to collect galls or have students bring them in for class use.
- Initially allow about one hour to become familiar with organisms for easy identification during lab.
- Allow15 minutes to set up materials for student lab work.

Students will require one evening prior to class to read the handout and answer the introductory study questions.

Materials

per team of students:

- Vernier calipers with inch and millimeter graduations and depth gauge, readings obtainable to 0.1 mm, for inside or outside measurements [for example, Ward's Natural Science Establishment (1-800-962-2660) 1994 catalog number 15W 4079 ($9.20 each) or Economy Vernier Calipers, molded in plastic, measures outside and inside depth from 0 to 160 mm, from Frey Scientific, 905 Hickory Lane, PO Box 8101, Mansfield, OH 44901-8101 (1-800-225-FREY), 1993 catalog number 990927 ($6.60 each).]

- student's straight economy dissecting forceps, 11.4 cm long with serrated tips, nickel-plated, Ward's catalog number 14W 0512 ($0.95 each)

- inexpensive kitchen paring knives or serrated steak knives

- stereoscopic microscope with 1X and 2X objectives, 10X wide field eyepiece, or hand lens

- approximately 10 goldenrod galls

In addition, a reference collection of organisms that the teacher has found in galls should be displayed for the entire class. For long term preservation, 70% ethanol may be used; otherwise, simply submerse the organisms in water.

Background Information for the Teacher

The data collected by students should indicate that natural selection tends to maintain the system as it is. Bird activity is working to make the galls smaller and wasp activity is working to make the galls larger. An interesting question to pose is "What would happen if a biocide were used and the wasps were destroyed?" Another question might be "What would happen if the woodpeckers were removed from this system?"

The date the galls used in class are collected should be carefully recorded. If galls are gathered in the autumn and dissected, the discovery of a brown pupal case most likely indicates Wasp #1. The gall fly does not pupate until spring. The discovery of a pupal case in galls collected in late March or in April could be due to the activities of Wasp #1 or it could be the gall fly. The presence of an exit tunnel would be the easiest clue to use in further identification.

CHART FOR IDENTIFICATION OF EVENTS OCCURRING WITHIN GOLDENROD GALLS

Modification from works of Uhler (1951) and Abrahamson (1977)

EVENTS	CRITERIA
Gall Fly *(Eurosta solidaginis)* present	1. exit tunnel present 2. larva present: barrel-shaped; pearly white color with black mouth hooks; 4 X 6 mm in size 3. pupa present: uniform tan in color except for dark brown mouth region; 3.5 X 7 mm in size
gall maker parasitized by wasp *(Eurytoma obtusiventris)*	1. no exit tunnel present 2. small, dark brown gall fly puparium present; 2.2 X 7.4 mm in size 3. wasp larva inside fly puparium; larva with mandibles, no mouth hooks
gall maker parasitized by wasp (*Eurytoma gigantea)*	1. no exit tunnel present 2. no puparium present 3. naked wasp larva present; larva with mandibles and no mouth hooks 4. central chamber enlarged and black fecal pellets present
attack on the gall maker or the wasps by a beetle (*Mordellistena unicolor)*	1. no exit tunnel present 2. small gall fly puparium present but empty or no puparium present 3. beetle larva present, usually not found in central cavity but more peripherally; fine sawdust also found 4. wasp larva absent, enlarged cavity filled with black fecal pellets
predation by bird on gall fly, either wasp species, or beetle	1. exterior of gall pecked open 2. central chamber empty

Tips for Teachers

- The galls may be collected at different times during the year. Galls collected in October will show much less evidence of bird predation than galls collected in the late winter or early spring. At this time, the gall population hosts many live gall flies. By the end of winter, most normal appearing galls are at least cored by birds. The percent of fall fly larvae that are consumed by insect predators does not change from winter to spring since infestation occurs during the early summer. It is bird predation that causes drastic differences.

  Large, elliptical galls should not be used other than as an illustration of a different system. These galls are the result of a moth depositing its eggs in the goldenrod plants. Large, brown larvae will be found inside the gall if it is cut open in the fall.

- Have students collect the galls themselves as a homework or extra credit assignment. If the galls are to sit for a week or two before use, be sure that air can circulate around them freely. A pile of galls kept in a box or plastic bag will quickly mold and become unusable.

- Freeze galls collected in the fall for use later in the school year. The freezing preserves the creatures in the galls and prevents the gall from becoming woody and hard to cut. The larva will still be alive.

- The instructor should practice opening the galls and identifying the organisms prior to students doing the lab. Both operations demand a certain amount of skill. Caution should be used when cutting; note that kitchen knives work better than scalpels or razor blades. Older galls are woody, as are galls that have been stored.

- The instructor should make students aware of the fact that the relationships involved in this study are highly specific. The fly only attacks certain goldenrod species and the wasps attack only this fly larva.

- The ecological importance of metamorphosis should be reviewed. The larva is important in its trophic ecology. What the adults of these various larvae feed on is not significant here.

- The exercise described here may be expanded for further study:

  a. In one additional investigation, students look at the height and diameter of goldenrod plants to determine the selective significance to the goldenrod plant of having an insect parasitize the plant. (This addition contributed by Heidi Busa, CIBT ’90.)

     i. Using a meter stick and calipers, students measure the height and stem diameter, respectively, of approximately 20 plants (10 with galls, 10 without galls).

     ii. After tabulating class data, students should be able to decide whether or not it “costs” the plant anything to “host” a parasite (and if so, what?), and extend their analysis of natural selection in this respect.

  b. In another investigation, animal behavior is examined, as students observe that the exit tunnel made by gall fly larvae tends to be in the upper hemisphere of the gall, and that predatory birds typically are able to detect the location of the gall fly’s exit tunnel and peck specifically there. (This addition contributed by Shirley Peron, CIBT ’90)

i. If this behavior study is to be done, precautions must be taken when collecting the galls to include a length of plant above the gall sufficient to show the orientation of leaves (thus indicating the top and bottom of the gall).

ii. As students examine the intact galls, they should identify the orientation of the fly's exit tunnel. Students should tally the number of tunnels in the top vs. the bottom hemisphere on their data sheet.

iii. As students examine the galls that have been bird pecked, they should tally the number of holes in the top hemisphere vs. the bottom hemisphere of the gall. Furthermore, they should tally the number of holes pecked on the exit tunnel vs. the number of holes pecked anywhere else on the gall.

iv. Results from this study should make students wonder how gall fly larvae differentiate between up and down, and how birds know where an exit tunnel is located.

Answers to questions on Reading

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

   The gall provides food and protection from predators for the developing larva.

2. Female gall flies are very selective about where they laytheir 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?

   The fly determines what species she's on by chemical cues that se receives through "taste buds" on her feet.

3. It is advantageous for the female *Eurytoma gigantaea* 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?

   Sex in wasps and their relatives is determined by whether or not the egg is fertilized: females arise from fertilized eggs and are diploid (i.e., possess 2 sets of chromosomes); males arise from unfertilized eggs and are haploid (i.e., possess only one set of chromosomes.) Since females wasps store sperm and fertilize or fail to fertilize eggs only just before they are laid, they can choose the sex of each offsring.

   Mammals, in which all individuals are diploid and in which sex is determined by means other than whether or not the egg is fertilized, cannot select the sex of their offspring.

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?

   The evolution of a new species occurs when a subpopulation within a larger species ceases to interbreed with members of the original group. The subgroup thereby becomes genetically isolated from the original group. In the northern part of the geographic range of the gall fly, females lay eggs not on tall goldenrod, but on late goldenrod. Since male gall flies seek mates on the species of goldenrod in which they themselves spent their larval life, choice of a new plant leads to genetic isolation of the new subgroup which is genetically different from the original group – it then becomes a new "race" and then, perhaps, a new "species".

Answers to questions on Introduction

1. Can you think of any other example of a predator/prey relationship in which the predator may have influenced the evolution of the prey? *Answers will vary.*

2. Using the organisms described in the introduction, construct two food chains centered around the goldenrod plant. *For example:*

   goldenrod —> goldenrod gall fly —>woodpecker

   or

   goldenrod —> goldenrod gall fly —> wasp

3. What is the difference between a parasite and a parasitoid? Give an example of each type of relationship: *Parasites do not generally kill their host organisms. The parasite-host relationship is one where the parasite harms the host organism and obtains nourishment but does not actively kill the host as in a predator-prey relationship. An example of a parasite-host relationship is the tapeworm and sheep.*

   A parasitoid first establishes the typical parasite-host relationship but at some point actively kills the host and feeds on it. An example is the wasp Eurytoma obtusiventris and goldenrod gall fly pupae.

4. Which size goldenrod gall (large, medium or small), do you think a chickadee or woodpecker might attack most often? Explain why. *Probably the woodpecker and the chickadee would attack larger size galls first since these would be more visible to the birds.*

5. Which size gall do you think the wasp, Eurytoma obtusiventris, might deposit its eggs in most often? Explain why. *The wasp,* Eurytoma obtusiventris, *would probably select smaller galls. The reason for this is that the wasp deposits its eggs directly on the fly larvae. A very large gall would make this difficult.*

6. What factors could result in the formation of different size goldenrod galls? *Gall size could be influenced by genes inherited by the goldenrod plant. Also, the fly could be encoded with the ability to produce a more or less powerful growth-stimulating chemical that it secretes into the plant.*

Answers to questions in “How to use the Vernier calipers” section:

1. Caliper #1 reads 25.0 mm.

2. Caliper #2 reads 24.8 mm.

Answers for data analysis part of the lab

1) Compiling the data: *Check the sample data page included. Numbers will vary depending on the size of the sample, time of year collected, accuracy of the student measurements, etc.*

2) Do each of the predators seem to attack the same size galls? *No. The predators seem to prefer different size galls. The wasp,* <u>Eurytoma</u> <u>gigantea,</u> *selects galls in the medium size range. The birds select larger galls.*

3-4) Graphical analysis of parasitoid’s or predator’s gall size preference: *Answers will vary with the data collected. You should find that the plot of the size of galls selected by each creature tends to form a bell-shaped distribution. It may be skewed toward one end of the graph or the other but there is a direct relationship between gall size and predator type.*

5) From your data, can you conclude that attack by the wasps (birds) is random with respect to gall size or do the wasps (birds) seem to prefer galls of certain dimensions? *Attack by wasps (birds) is not random. Wasps prefer medium to smallish galls. Birds prefer larger galls. By comparing the average size of galls selected by each, a numerical answer can be obtained. For example, according to the sample data, the average gall size attacked by Wasp #2 is 17. ± 2.7 mm and the average gall size attacked by birds is 19.6 ± 3.8.*

   Wasps prefer smaller galls than the birds because one species of wasp must insert its posterior into the chamber containing the larvae in order to deposit its eggs on the larvae. A large gall would prohibit this. Birds must spot the galls from a distance. Larger galls are more visible. Also, a larger gall may indicate a larger larva making the feast more worth the bird's toil.

6) Do you think that selection is acting on the gall fly’s ability to produce galls of a certain size? *Allow the students any reasonable answer. Research does seem to indicate that the fly possesses genes that influence gall size.*

7) 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? *Since the plant must expend energy in producing gall tissue, it would be to the plant's advantage to keep the galls small. Hence, plants genetically able to inhibit gall size would be more successful.*

Answers for AP statistical analysis section

1-3) Answers will vary depending on sample size, etc.

Annotated References

- Abrahamson, W.G. and P.O. Armbruster, and G.D. Maddox, 1983. Numerical relationships of the *Solidago altissima* stem gall insect-parasitoid guild food chain. *Oecologia* (Berlin) 58: 351-357.

  The article investigates the hypothesis that a predictable and apparent goldenrod plant resource results in stem gall insect resources that are, in turn, apparent and predictable to their enemies. The long development time provides a prolonged exposure to predators and parasitoids. It may be the reason why there is a high predictability of parasitoid guild numerical relationships. Findings suggest a well-integrated and well-controlled food chain.

- Abrahamson, W.G. and A.E. Weiss. 1997. Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies. Princeton University Press, Princeton, NJ.

  This is the definitive monograph on all aspects of the gall fly system and contains summaries of many of the observations and experiments carried out over many years by Abrahamson and his co-workers.

- Endler, J.A. 1986. Natural Selection in the Wild. Princeton University Press, Princeton.

- Fisher, R.A. 1958. The Genetical Theory of Natural Selection. 2nd ed. Longman, New York.

- Odum, E.P. 1959. Fundamentals of Ecology, 2nd ed, Saunders, Philadelphia.

- Rhodes, W.E. 1974. Solidago Galls in Outdoor Biology. *The American Biology Teacher* 36: 420-422.

  This article provides general background information on the goldenrod plant and the gall fly. A brief history of the relationship is nicely illustrated with photographs.

- Schlichter, L. 1978. Winter Predation by Black-capped Chickadees and Downy Woodpeckers on Inhabitants of the Goldenrod Ball Gall. *Canadian Field-Naturalist*. 92: 71-74.

  The goldenrod gall larva appears to be a commonly exploited winter food source for the Downy Woodpecker and the Black-capped Chickadee. Feeding preferences by the two bird species was investigated. The woodpecker fed most heavily at the forest edge while the chickadees used both the open field and the forest edge. Both left misshapen galls totally alone. It was found that most normal appearing galls were at least cored by the end of winter. Those not penetrated usually contained dead mature fly larvae or mordellid beetle larvae. This article is very useful when rounding out information for a discussion of bird predation on the gall fly larva. Many graphs help to make the information more visual.

- Uhler, L.D. 1951. Biology and Ecology of the Goldenrod Gall Fly, *Eurosta solidaginis* (Fitch). Cornell University Agricultural Experimental Station Memoir 300, pp. 51.

  The original study of the biology of *Eurosta solidaginis*.

- Weigel, B.A. and Dilks, E. l950. The Ball Gall of Goldenrod as Laboratory Material. *Turtox News* 28: 134-137.

- Weis, A.E. and W.G. Abrahamson. 1998. Just lookin' for a home. *Natural History*: Sept.

  This is a recent and very readable account of the gall--gall fly --natural enemy system. The authors outline procedures for the experiment presented in this lab and field exercise.

- Weis, A.E. and W.G. Abrahamson. 1985. Potential Selective Pressures by Parasitoids on a Plant-Herbivore Interaction. *Ecology* 66: 1261-1269.

  Using tables and figures, the authors illustrate their investigation of the evolution of the plant-herbivore relationship between *Solidago altissima* and *Eurosta solidaginis*. Previous observations had shown that galls attacked by parasites are smaller than those in which the gall maker survives. Two different mechanisms could cause this pattern: parasite attack could occur before the galls reach full size and the attacks could cause growth to cease or the attacks could occur after the gall reaches full size, but with small galls being more prone to attack. In the first case, parasite attack would diminish the cost of the gall to the plant and thus favor genotypes that attract parasites. In the second case, parasites would exert pressure on the gall maker to induce large galls. It was found through this study that the parasitoid wasp *Eurytoma gigantea* is limited to attacking small galls because of the limited reach of its ovipositor.

- Weis, A.E. and W.G. Abrahamson and K.D. McRea. 1985. Host Gall Size and Oviposition Success by the Parasitoid *Eurytoma gigantea*. *Ecological Entomology* 10: 341-348.

  Based on field information which suggests that in the natural environment the incidence of parasitism by *Eurytoma* is greater in small galls than in large galls, lab experiments were designed and carried out which demonstrated that small galls are not more frequently discovered. However, oviposition attempts on small galls are more likely to be successful. *Eurytoma* spends much time probing galls too big to penetrate which leads to a decreased foraging efficiency. A simulation model was constructed which illustrates that the gallmaker's chance of being parasitized depends on gall size, the number of parasitoids that discover the gall, and their ovipositor lengths. The article contains much useful information for answering student questions and leading a discussion of lab results.

Background Reading

Read the article “Just Lookin’ for a Home.” by (Weiss and Abrahanson, 1988) and answer the following questions.

Questions on the Reading:

1) Describe the ways in which the formation of the gall benefits the gall 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 gigantaea* 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?

GOLDENROD GALL SIZE AS A RESULT OF NATURAL SELECTION

CIBT
Student Laboratory Exercise

Objectives:

Upon completion of this lab, 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)

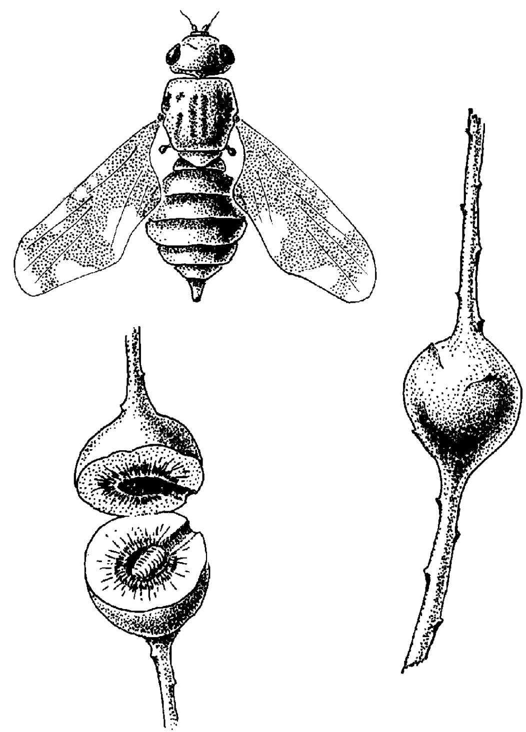
This laboratory and field exercise investigates natural selection operating as a result of predation. You will be looking at the interactions between Canada goldenrod *(Solidago canadensis)* and its stem gall insects. The term *gall* is used to describe any swelling or abnormal growth of plant tissues 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 coevolution. 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.



Top left: gall fly, ~5X magnification
Right: a ball gall on a goldenrod stem
Bottom left: cross section through a ball gall

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?

Questions on Introduction

1. In this experiment you will look at one example of how natural selection affects a population. Can you think of any other example of a predator/prey relationship in which the predator may have influenced the evolution of the prey?

2. Using the organisms described in the introduction, construct two food chains centered around the goldenrod plant.

3. What is the difference between a parasite and a parasitoid? Give an example of each type of relationship.

4. Which size goldenrod gall (large, medium, or small), do you think a chickadee or woodpecker might attack most often? Explain why.

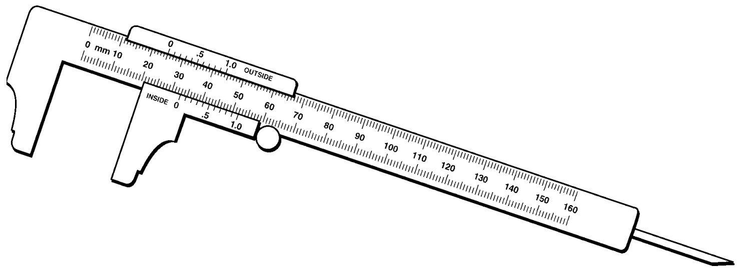
5. Which size gall do you think the wasp, *Eurytoma obtusiventris*, might deposit its eggs in most often? Explain why.

6. What factors could result in the formation of different sized goldenrod galls?

GOLDENROD GALL MEASUREMENT AND DISSECTION

HOW TO USE VERNIER CALIPERS

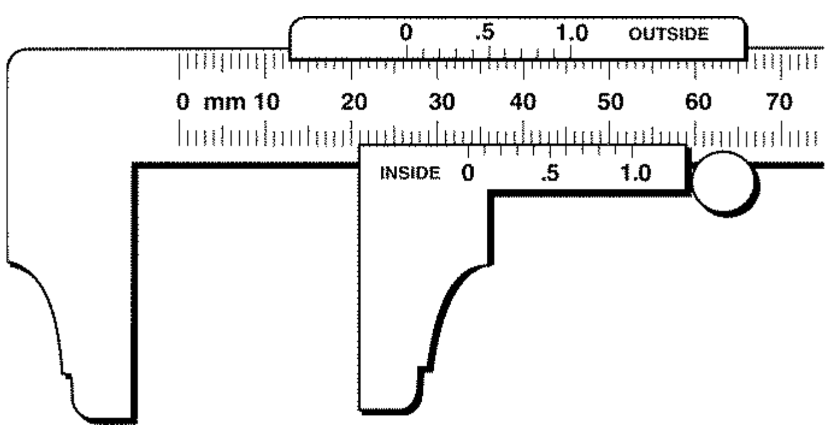
It is difficult to accurately measure the diameter of a round object such as a goldenrod gall with a ruler. Vernier calipers (shown below) are a tool for performing this job with reliability and ease.



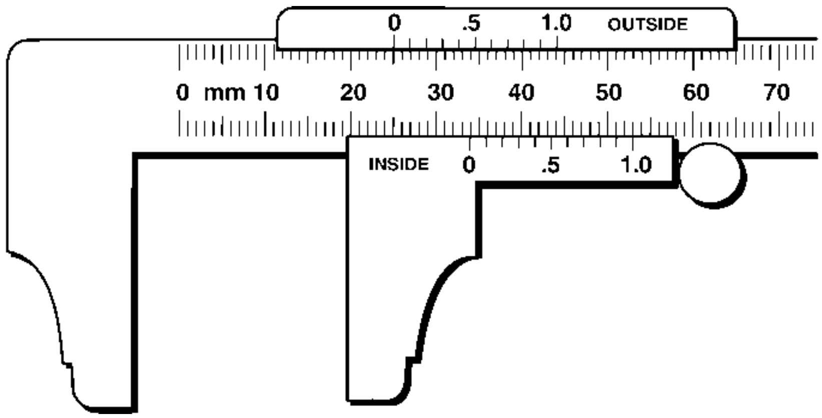
The blow-up shown below demonstrates how to read a caliper. You will see that there is a scale that reads from 0 to 160 mm. Along the top of the caliper there is another scale marked OUTSIDE that reads from 0 to 1.0. On the opposite side there is an identical scale reading from 0 to 1.0 but marked INSIDE. These “inside”/ “outside” scales slide along the length of the ruler to give you a measurement between 0 and 160 mm to the nearest 0.1 mm. Because we’re measuring the outside of the galls, use the scale marked “outside.”

After adjusting your calipers to fit the object being measured, there are two stages in obtaining a measurement. First you find where the 0 on the OUTSIDE scale is located. If it is not exactly on a line, you will know that the size is more than a certain amount but less than another. To obtain a greater degree of accuracy, you find which line on the OUTSIDE scale is the first to line up with any line on the ruler. The OUTSIDE line is then read to provide the nearest 0.1 mm.

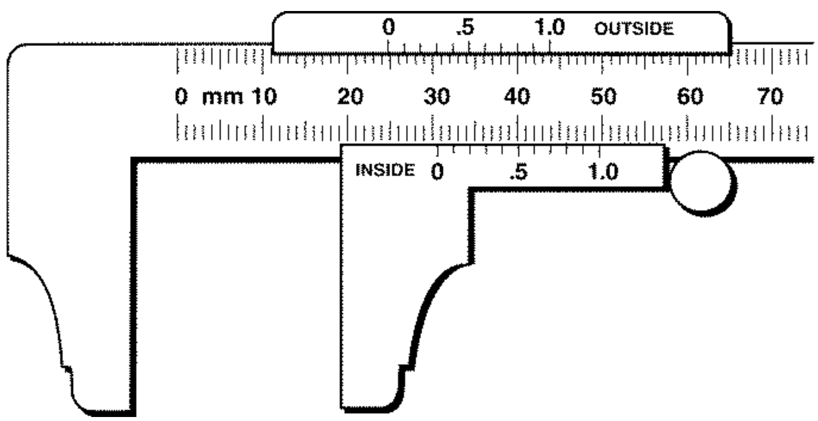
For example, on the caliper below, find where the 0 on the OUTSIDE scale is situated in reference to the ruler scale. You should see that in this picture it is between the 26 mm and 27 mm divisions. Therefore the diameter measured is greater than 26 mm but less than 27 mm. The .5 mark is the first one on the OUTSIDE scale to line up with a mark on the ruler. Thus, the reading to the nearest tenth of a mm is 26.5 mm.



1. What is the reading, to the nearest tenth of a mm, on the Vernier caliper shown below?



2. What is the measurement indicated on the caliper below?

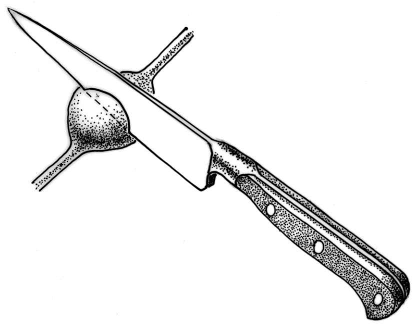


Materials Needed (per group)

- dissecting microscope or hand lenses
- goldenrod galls
- petri dishes
- forceps
- chart for identification of events occurring within goldenrod galls
- knife
- calipers
- data sheets
- cutting surface

Procedure

1. Obtain a sample of galls. 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 Table #1.



(Be cautious. It would be very easy to slip and cut your finger!)

2. Note 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.

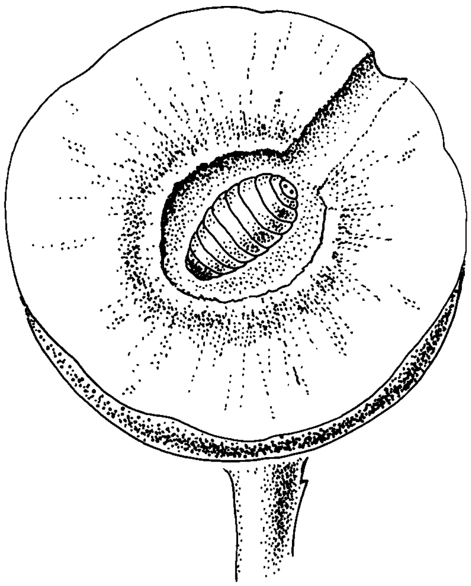
3. After measuring the gall, cut it open being careful not to squash any creature that might be inside it. Rotate the gall while cutting. Cut a groove in the gall and then pop it open.

4. Identify any organism you find inside the gall. Refer to the 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 #1.

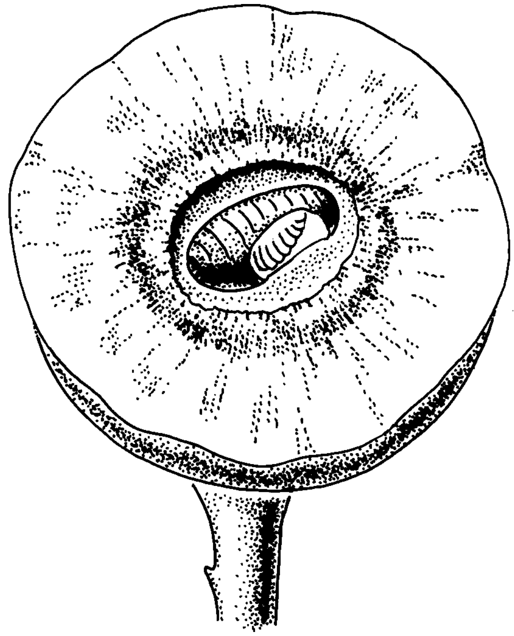
5. Proceed to another gall, first measuring it, then identifying any creature inside. Do not mix up the galls, since a correlation between each gall's size and its inhabitant are critical in this experiment!

6. Repeat this procedure for all 10 galls, then pool your data together with the rest of the class and record on Table #2.

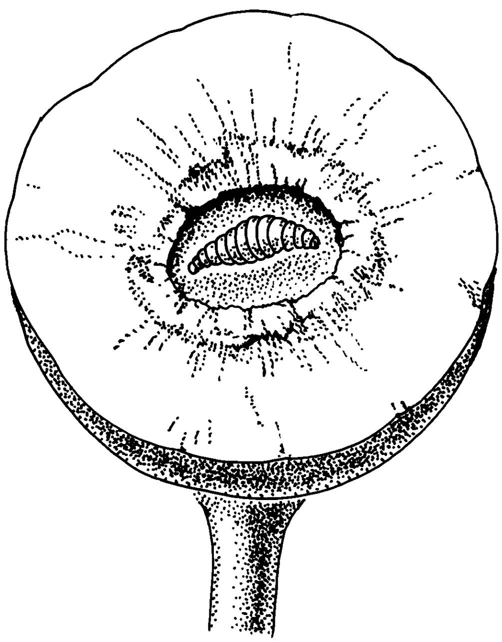
a.	Fly larva *(E. solidaginis)* present	survival (no mortality)
b.	Fly puparium present; wasp #1 (*E. obtusiventris)* responsible	mortality
c.	Wasp larvae inside; wasp #2 *(E. gigantae)* responsible	mortality
d.	Beetle larvae inside gall; beetle responsible	mortality
e.	Hole in gall wall, no larvae present; bird predation responsible	mortality
f.	Unknown-empty gall; no bird evidence	mortality
g.	Tiny hole, empty gall (if spring collection)	survival



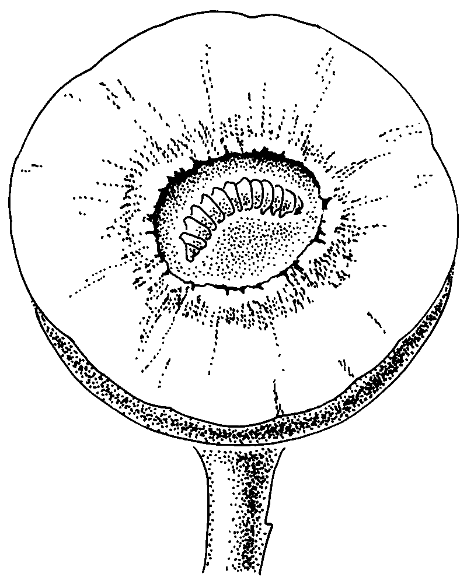
Gall fly (Eurostra solidaginis) = fleshy white/tan larva, shaped like a barrel, 4 X 6 mm in size; Note black mouth hooks. Moves when gently touched. Exit tunnel present.



Obtuse wasp = wasp #1 (Eurytoma obtusiventris) = wasp larva inside the reddish brown puparium of the gall fly, 2 X 7 mm in size. Does not move when touched. No exit tunnel.



Giant wasp = wasp #2 (Eurytoma gigantea) = either a white, fleshy, larval parasite lacking in mouth parts or no visible insect. A blackened empty chamber indicates that the gall fly larva was consumed by the giant wasp.



Beetle (Mordellistena unicolor) = slender white larva, 1 X 8 mm in size, with small appendages. Moves when touched, no exit tunnel in the gall.

Procedure for analyzing data:

1. Complete summary Table #2, which is a compilation of the findings of the entire class. Use this for all remaining questions and histograms (bar graphs).
   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 and those that died of unknown causes (wasp #1, wasp #2, beetle, birds, and unknown) and record.
   c. Calculate the average size of galls not attacked by predators or that died of unknown causes and record.

2. Examine the averages you just calculated. Is the average size of the galls that survived the same as that of total galls, or of the galls that died? Do each of the predators seem to attack the same size galls?

3. Plot six different bar graphs where the height of the bar represents the number of galls in the different graphs. The bars represent:

   (graph 1) size of the galls

   (graph 2) size of galls not attacked

   (graph 3) size of galls attacked by wasp #1

   (graph 4) size of galls attacked by wasp #2

   (graph 5) size of galls attacked by beetles (if there are enough samples to warrant)

   (graph 6) size of galls attacked by birds (if there are enough samples to warrant)

   On each graph indicate the average size of the galls. NOTE: You may want to combine data so that each bar represents a size range, e.g., 5-9 mm, 10-14 mm, etc.

4. Compare the graphs. How does the size of a particular class of galls (for example, those parasitized by wasp #1) compare to the entire range of galls? Do this for each situation. Are they evenly distributed, clustered at the extremes, or some other arrangement? Also, is the shape of the histogram about the same for each category or are they different? Why might this be so?

5. 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?
   b. Is attack by birds random with respect to gall size or do birds seem to select specific galls? Explain your findings.

6. Assume that genes present in the fly determine the size of the gall. It might be genes for a characteristic such as secretory product. The secretion could simulate plant auxins. Do you think that selection is acting on the gall fly's ability to produce galls of a certain size? Explain.

7. 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? Note that the plant must use additional energy to produce extra gall tissue.

DATA TABLE #1

Time of year galls collected ________________

(This is important because a pupal case in galls collected during the late summer, fall or winter indicates the intrusion of Wasp #1. A pupal case present in a gall collected in the spring might indicate the presence of a gall fly. Check for an exit tunnel. This would indicate the gall fly's presence.)

PRE-CUT: OBSERVATIONS				POST-CUT: CONTENTS OF GALLS				
	Diameter (mm)	Small Hole	Large Hole	Gall fly	Wasp #1	Wasp #2	Beetle	unknown
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							
gall #___	______							

DATA TABLE #2

Summary of Individual's Data

Mean Gall Size	Total Galls	Total Alive Flies	Wasp #1	Wasp #2	Beetle	Bird	Not Known	Total Dead Flies
10 mm								
11 mm								
12 mm								
13 mm								
14 mm								
15 mm								
16 mm								
17 mm								
18 mm								
19 mm								
20 mm								
21 mm								
22 mm								
23 mm								
24 mm								
25 mm								
26 mm								
27 mm								
28 mm								
29 mm								
30 mm								

Statistical Analysis

for AP classes only

In answering the questions in the data analysis section above, we relied on results from a sample of galls, but we generalized about the whole population of galls in the field. In doing so, we made the assumption that our sample of galls was representative of all the galls in the field. We can never know for sure if our assumption is true or false, but we may be able to say something about the degree of confidence we have in the assumption. For example, a sample of 100 galls is more likely to be representative of all the galls in the field than is a sample of only 10 galls. Furthermore, the reliability of our assumption depends on how variable in diameter the galls are. If all the galls in the field were very close to the same size, they could be well represented by a small sample, perhaps even as few as 10 galls. On the other hand, if the galls in the field demonstrated a great deal of variability in diameter, we'd need a larger sample in order to get an average in which we had sufficient confidence, even 100 galls might not be enough.

Scientists are always interested as much in the confidence with which they can rely on their results as they are in the results themselves, and so results are usually expressed in a way that allows us to assess their reliability. For results based on a sample, like those in this study, we must know not only the average (technically called the mean), but also the size of the sample (called the sample size). We also require some estimate of the amount of variability in the thing we're studying (usually expressed as the standard deviation). Since these measures summarize and describe our sample, they are called summary descriptive statistics.

Although calculators and computer spreadsheets may be used to calculate these statistics and save you time and effort, it's a valuable exercise to learn how to calculate these statistics by hand. You will thereby understand the statistics better and will be able better to detect erroneous values arrived at by calculator or computer. Unlike your brain, your computer can't know when its answer is ridiculous: GIGO, i.e., garbage in—garbage out. The procedure that follows will help you keep track of some cumbersome, but simple calculations.

Calculation of Means and Standard Deviations by Hand

Calculation tables #3 through #8 will help you calculate descriptive statistics to summarize each of the 6 variables you will wish to compare: all galls, surviving galls, galls attacked by wasp #1, galls attacked by wasp #2, galls attacked by beetles, and galls attacked by birds. To demonstrate that natural selection is occurring you must show that the mean size of the surviving galls or of those attacked by a particular predator is *significantly* different from the mean size of all the galls". (The word "significant" has a special meaning in science, which we will discuss later.) First, you must calculate means and standard deviations for each of the 6 distributions.

Fill in each of the columns of the tables as follows:

1. The diameter of galls observed (x). The table has some blank rows in case you observed galls smaller than 10 mm or larger than 30 mm.

2. The frequency (F) is the number of galls of each size in the sample. The sum of this column (N) is the total number of galls observed.

3. This is the frequency times the value of x (Fx). Just multiply the value in column 1 by the value in column 2. The sum of this column ($\sum Fx$) is the sum of the diameters of all galls observed. To calculate the mean (average), simply divide the sum of column 2 by the sum of column 3. In your calculations, you will refer to the mean as x_{ave}.

4. You will use columns 4 through 6 to calculate the standard deviation. Begin by subtracting the mean (x_{ave}) from the gall diameter of each row.

5. Square each of the differences you calculated in row 4.

6. Multiply each of these squared numbers by the associated frequency. You will use the sum of this column [$\sum F(x-x_{ave})^2$] to calculate a number called the variance, which is the square of the standard deviation.

CALCULATION TABLE #3: ALL GALLS

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

CALCULATION TABLE #4: SURVIVING GALLS

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

CALCULATION TABLE #5: WASP #1

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

CALCULATION TABLE #6: WASP #2

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

CALCULATION TABLE #7: BEETLES

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

CALCULATION TABLE #8: BIRDS

1 Gall Diameter x	2 Frequency F	3 Fx	4 $(x-x_{ave})$	5 $(x-x_{ave})^2$	6 $F(x-x_{ave})^2$
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
SUMS	N=	$\sum Fx=$		$\sum F(x-x_{ave})^2=$	

Calculate the mean gall size for each set of galls. We discussed how to do this above, simply by applying the formula

$$x_{ave} = \frac{\sum Fx}{N}$$

where the denominator of the fraction is the sum of column 3 in the tables and the denominator is the sum of column 2.

Calculate the standard deviation (s) of gall size for each set of galls. To do this, apply the following formula:

$$s = \sqrt{\frac{\sum F(x - x_{ave})^2}{N - 1}}$$

Look at the formula for the standard deviation. Notice that standard deviation is a good expression of the spread of the values of gall size: the more galls whose diameters are very different from the mean, the larger the numerator of the fraction and the larger the standard deviation.

Summarize the distributions of the various gall types by writing the results of your calculations in Table #9.

TABLE #9

SUMMARY DESCRIPTIVE STATISTICS FOR GALL DIAMETERS

	Total Galls	**Total Alive Flies**	**Wasp #1**	**Wasp #2**	**Beetle**	**Bird**
Sample Size N						
Mean x_{ave}						
Std. Dev. s						

Testing for Significant Differences

It is likely that your samples of the different groups of galls have different mean diameters. Are these differences due merely to the chance variations in the sampling process, or are their real differences in diameter among the different types of galls? How confident can you be that the differences you observed reflect interesting biological processes like natural selection rather than sampling errors due only to chance?

Statisticians have developed methods to help us answer such questions. Most of these methods involve tests that answer the question, "what is the likelihood that the differences we observed occurred merely by chance?" If it is very unlikely that a difference is too large to be explained by variation in the sampling process, we are justified in accepting the alternative explanation that the difference is due to something other than chance. In such a case we say that the observed difference is *significant*. But how large is too large? In biology we usually assume that, if a random sampling process could lead to a difference as big as that we observe only 5% of the time, something other than chance is operating and the difference is significant.

In your study of the effect of natural selection on the size of goldenrod galls, the appropriate test asks, "are the mean diameters of the various groups of gall killed by *Eurosta's* natural enemies different from mean size of all galls in the field?" For each predator you will ask, "is there a significant difference between the mean diameter of all galls and the mean diameter of the galls killed by this predator?" The statistical procedure most frequently used to test for a significant difference between two means is called a "t-test", named because it relies on a test statistic called Student's t. (Notice that "t" is a symbol like α or β, and should not be written in upper case or otherwise altered).