

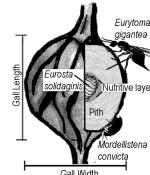
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# THE CONTINENTAL GALL SIZE PROJECT

## Procedures Manual

January 2024 – check website for updates

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The goldenrod gall fly has become a textbook example of stabilizing selection. You are joining collaborators from across North America in a two-year project to explore how the interactions among goldenrod, the gallfly, and the attacking wasps and birds varies across North America. How do such changes alter the form and intensity of natural selection? Does a shift in mean gall size affect attack rates to create a cline in optimal size?

Those new to this system might ask, what are galls? They are tumour-like plant outgrowths induced by specialized insects (or mites, nematodes, bacteria). Gallmakers are parasites that manipulate development of plant organs to gain nutrition and protection. The insect hijacks development of the plant by applying a genetically encoded stimulus. Using Richard Dawkins' term, the gall—though a mass of plant tissue—is the insect's extended phenotype.

What determines optimal gall size for the goldenrod gallfly? If the gall is too small, the fly larva within is vulnerable to attack by a parasitoid wasp. The wasp's ovipositor is only so long, so a thick gall wall prevents penetration—fatter is fitter. But larger galls are obvious to foraging birds, thus more often attacked—fattest is not fittest! Larvae inducing intermediate-sized galls have the highest survivorship. So, there is stabilizing selection on the gallfly's stimulus to produce intermediate gall sizes.

The **Continental Gall Size Project** has four components. The principal focus is on clinal variation in gall size, and its impact on selection. Specifically, does the balance between parasitoid and bird attack shift as gall size increases from north to south? The second looks at the population genetic structure of the gall fly across latitude, and across different hostplant species within latitude. The third concerns emergence phenology of the gal fly and its enemies, while the fourth relate relates gall growth to growing seasons length.

We hope you can participate in at least as the first two of these projects, which will entail a few afternoons of effort in late-winter/early-spring. The projects 3 and 4 entail repeated monitoring of field populations through the growing season. Someone in your research group can make a very valuable contribution by committing one afternoon a week through the summer.

Please visit [continentalgallproject.github.io/website/](https://continentalgallproject.github.io/website/) for information on your fellow collaborators. Also, please read the Authorship Guidelines page.

Art Weis, *University of Toronto*  
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# Project 1: Latitudinal Patterns of Selection on Gall Diameter

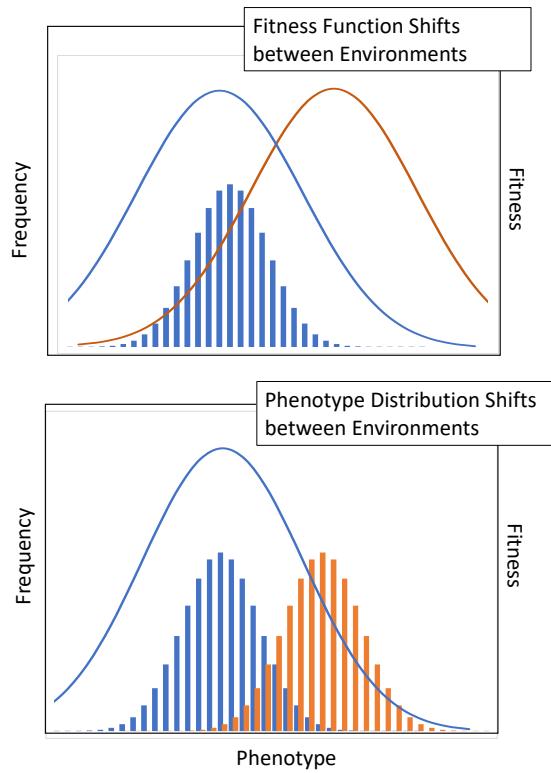
## Collection, Dissection and Data Protocols

### *The Question*

Does the selection regime on *Eurosta*'s gall size vary systematically across its geographic range? We will answer that question by measuring variation in the Lande-Arnold selection gradients across space, but also by looking into the mechanics behind this variation.

The form, direction, and intensity of natural selection can vary between environments in two non-exclusive ways.

- 1) The fitness function differs between environments in slope, curvature, symmetry, or position, relative to the distribution of phenotypic values. In the graph to the right, selection is stabilizing in the blue environment, but directional in the orange. The relationship of phenotype to fitness is different in the two environments.
- 2) The phenotypic distribution shifts in mean, variance, or skew relative to the fitness function. In the graph to the right, selection differs in the orange and blue environments even though the relationship of phenotype to fitness is the same. It is the relative abundances of the different high-and low-fitness phenotypes that switches selection between directional and stabilizing forms.



For the gall fly, does selection on gall diameter vary from north to south? If so, is it because the abundance and/or the size-dependency of parasitoid and predator attack changes the fitness function? Or is it because gall size shifts relative to fixed patterns of attack? Or both?

Or is selection unchanged due to commensurate shifts in gall size and size-dependent attack?

The following sections lay out protocols for collecting the data that will answer these questions. We give guidelines for gall collection, directions on measuring their diameters, advice on opening (dissecting) galls, identification keys to determine gallmaker fate, and instructions for data recording and submission. An appendix introduces a regression model, the Generalized Epsilon-Skew-Normal function, which estimates parameters for position, width, symmetry, and angularity of the fitness function.

### ***Scouting Galls***

Identify collection sites in the autumn. After goldenrod dies back for the winter, it becomes very difficult to identify to species. Be sure to survey the sites before first frost to determine which species is being galled. You have been sent a leaf collection kit for molecular verification of plant ID.

The gall fly is a good disperser, so any two discrete patches of goldenrod separated by less than ~50 meters can be considered as in the population. If you find clusters of patches separated by >100 meters, treat them as different populations, and keep track of their lat-long coordinates.

To look for galls, you need to look for its host plants first. Host plant *Solidago altissima* (tall goldenrod) is an open land species generally found on drier soils. It usually occurs in prairies and other grasslands, old fields, roadsides, savannas and woodlands, also occurring in forest openings. During the flowering season (August to November depending on the part of the country in which it is found), the plants are most visible. The ideal collection site will be a natural preserve/protected area, but roadsides with good parking access will work as well.



### ***Collecting Galls***

#### *Materials--*

- Garden pruning shears for clipping galls
- Paper/cloth bag for containing galls
- Paper and permanent marker for labeling sampling sites

Aim for sites where you can collect 200+ galls. We need a random sample to estimate the size distribution, but also a large enough sample to include enough galls on the tails of the distribution to allow robust estimates for fitness function parameters. If you cannot locate a site with 200+ galls, a collection with as few as 50 still contributes useful information. Several scattered populations with a total sample size of 200+ is fine, but keep track of their lat-long coordinates. If you find sites with thousands of galls, a sample size 400 will be fine; larger sample sizes will give diminishing returns on confidence intervals for the parameters of interest.

Collect the galls in mid-Spring (early-March in the far south, late-March to early April to the far north). You want to collect after Woodpecker attack is over for the winter, that is, after the birds start back foraging full time in the woodlands. But you also need to make sure you collect before the insects within the gall start to emerge.

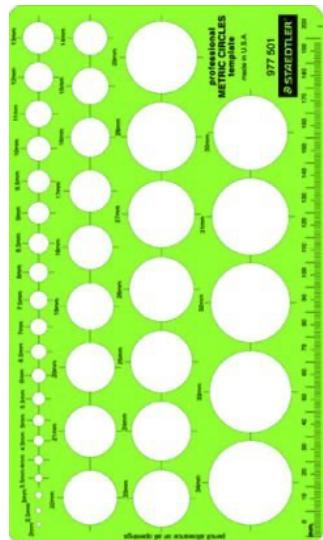
When collecting, snap the gall off the stem and place into a paper/cloth bag. If there is a time lag between collecting and processing, keep the galls in a cool place to prevent premature insect emergence. Galls kept in a fridge will last about a month before larvae dehydrate, making them more difficult to identify.

### ***Measuring and Sorting by Size***

Once the galls are collected, measure diameters. The following procedure is quick and resistant to errors.

#### ***Materials--***

- Drafting template, with circles graduated in 1 mm increments (see photo; we supply).
- 28 containers, such as plastic beer cups or paper plates, numbered from 6 to 34, corresponding to the typically smallest and largest diameter classes (you supply).
- Data sheet(s), downloaded from  
<https://continentalgallproject.github.io/website/>.



#### ***Procedure—***

- Pass each gall through the template holes. Its size is the smallest hole diameter that it can pass through without forcing.
- Place the gall into the container corresponding to its size class.
- Continue until all galls are measured. Count the number in each category and indicate on Data Sheet

### ***Dissecting, and Identifying Content***

Dissect and inspect the content of each gall. PROCESS ONE SIZE CATEGORY AT A TIME, as it minimizes opportunities for mistakes.

#### ***Opening the gall—***

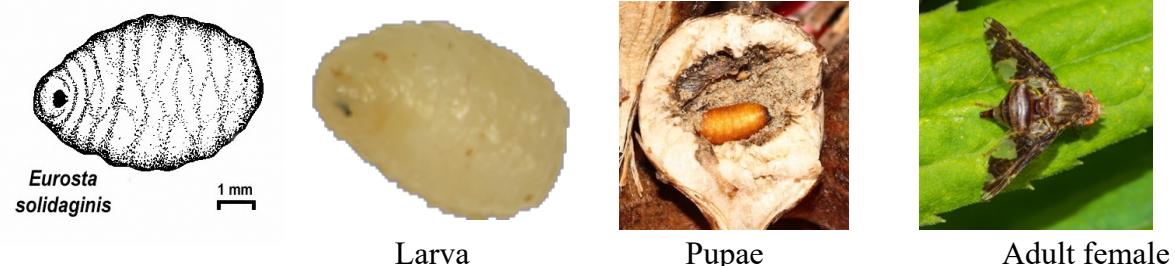
- Use ordinary garden pruning shears (you supply).
- Open the gall by cutting partway in, longitudinally (from pole to pole). Use the blade to cut nearly half-way through, then twist to pry it open and reveal the gall's central chamber, where the larvae reside.
- DO NOT cut all the way through – you will destroy the larva inside.
- DO NOT cut crosswise through the gall's equator – it will be difficult to pry open without also cutting through the central chamber and thus destroying the larva inside.

*Identification and recording occupant—*

- Using the identification key below, determine who occupies the gall, and enter the corresponding code to the appropriate column on the data sheet (see example below)
- Occupant codes are:
  - EU** — *Eurosta solidaginis*, the gallmaker
  - gig** — *Eurytoma gigantea*, external parasitoid wasp
  - obt** — *Eurytoma obtusiventris*, internal parasitoid wasp
  - mc** — *Mordelestena convicta*, inquiline beetle
  - wp** — gall empty, pecked open by downy woodpecker
  - ck** — gall empty, pecked open by black-capped chickadee
  - eld** — gall empty, Early Larval Death, no sign of occupancy
  - uk** — gall empty, but signs of occupancy/attack by another insect (i.e., frass)
- Tally the number of each occupant type for each size class at the bottom of the data sheet. Double check to make sure the numbers add up to the total.
- Transcribe tally to the excel sheet linked on the website.
- To upload your tally data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Scan the original data sheet and email to [gall.size@utoronto.ca](mailto:gall.size@utoronto.ca) with subject line “*completed data sheet*”

**Identification Guide for Gall Occupants****EU** — *Eurosta solidaginis*, the gallmaker

A cream-colored, ovoid larva, without distinct head capsule, but with black, anteriorly-directed mouth hooks. Gall central chamber ovoid, and lined with compacted, gray frass. Exit tunnel extending to just below the gall epidermis often seen.

**gig** — *Eurytoma gigantea*, external parasitoid wasp

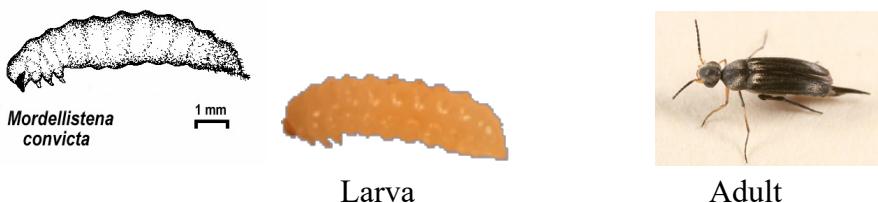
A white-colored, drop-shaped larva, with head capsule, brown mandibles, and tapered posterior end. Variable in size (Females ~5 mm long, males ~2 mm). Gall inner chamber irregular, often with brownish frass.

**obt** — *Eurytoma obtusiventris*, internal parasitoid wasp

A brownish, ovoid pupal case, ~3-5 mm long. In the fall and winter, they are already in the cocoon mode.

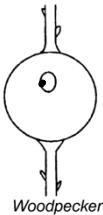
**mc** —, inquiline beetle

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.



**wp** — *Pocoides pubesneces*, Downy Woodpecker

Round, nearly cylindrical hole, ~3mm diameter, chiselled from surface into central chamber of an empty gall.

**ck** — *Poecile atricapillus*, Black-capped Chickadee

Irregular, broadly conical hole ripped from surface into central chamber of an empty gall.

**eld** — Early Larval Death

Typically, 15-30% of gallmakers die after gall initiation but before maturation. They leave galls without a discernable central chamber, although there may be voids in the surrounding tissue due to shrinkage from dehydration. Occasionally these galls contain small drops of resin, but little or no frass.

**uk** — unknown

A small percentage of galls may have no inhabitants but contain frass or show signs of tunneling. Most of these are probably an instance in which *Mordelestena* has burrowed out of the gall and into the stem.

**Data Sheet Example**

This format is the least prone to errors when recording observations.

First measure and sort galls by size class into labeled containers (e.g., beer cups, paper plates). Then tally the number of galls per size class in the TOTAL row of the table.

Next, working with one size class at time, crack open each gall and record the occupant, recording the result for each gall is the appropriate cell. Tally the counts, by size and occupant, in the table and make sure they match the TOTAL row.

Upload the table to the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.

frequency	Gall Diameter																															
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34			
24																																
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8																																
7																																
6																																
5																																
4																																
3																																
2																																
1																																
Eurosta- EU	0	0	0	0	0	1	2	1	1	0	4	7	10	6	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0		
E.gigantea- gig	0	0	1	0	1	3	3	2	1	2	3	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
E.obtusiventrис-- obt	0	0	0	0	1	0	1	1	0	0	2	3	1	3	3	2	3	1	0	0	0	0	0	0	0	0	0	0	0	0		
M.convicta-- mc	0	0	1	0	0	1	0	1	1	2	2	4	4	2	3	3	2	3	2	0	1	0	0	0	0	0	0	0	0	0		
Woodpecker-wp	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3	3	3	3	3	0	1	0	0	0	0	0	0	0	0	0		
Chickadee-ck	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0		
Early Lar Death-eld	0	1	0	0	1	1	3	2	1	1	1	2	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Unkown-uk	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
<b>TOTAL</b>	0	1	2	0	3	6	9	7	4	6	8	14	18	20	16	11	11	8	6	0	2	0	0	0	0	0	0	0	0			
Hostplant = <i>S. altissima</i>	LAT =	44.013053	LON =	-79.321744	Participant name=	Wilbur																										

Scan and email the original data sheets to [gall.size@utoronto.ca](mailto:gall.size@utoronto.ca) with subject line “completed data sheet”

## Project 2: Plant, Gallfly, and Parasitoid Phylogeography.

### Specimen collection and storage

Exploring the genetic structure of the interacting species requires specimens from throughout the geographic range. Since participants will be collecting galls across the continent for Project 1, they can contribute to Project 2 simply by preserving their insect larvae for genetic analysis.

#### ***The Question***

How have recent events (geologically speaking) influenced the distribution of the members of this tri-trophic interaction? Presumably all species were restricted to southern refugia during the Pleistocene, then expanded northward over several millennia. More recently, as European agriculture increased the intensity and frequency of soil disturbance, the quantity and quality of *Solidago* habitat has probably increased over the past few centuries. How did this history of range expansion play out? Did uneven gene flow foster the formation of geographical sub-populations? For instance, did western prairie populations diverge from populations embedded within eastern forests? Did the fly's host shift from *S. altissima* onto *S. gigantea* occur once, or independently in the northeast and western Great Lakes regions? Are the patterns of geographic differentiation among the parasitoids and inquiline congruent with the fly, and with one another?

#### ***Securing and Preserving Specimens.***

When performing the Dissections for Project 1, set aside the larvae you recover from the galls, as sorted by species (EU, gig, bt, mc).

#### ***Materials--***

- Eppendorf vials (screw capped preferred, snap cap OK; you supply).
- Label tape.
- 95% Ethyl alcohol (you supply)
- Parafilm (you supply)
- Shipping box (you supply)

#### ***Procedure--***

- Place live specimens into vials. You may put up to 5 individuals per vial, BUT only one species per vial.
- Label the vial with site code, species code, and year. 9 (e.g., KSR:gig:24)
- Wrap labeled vial tightly in parafilm.
- Pack in shipping box and send by courier to the Zhang lab at George Washington Univ., using the address on the project website.

**NOTE:** groups that cannot participate in Project 1 can still contribute specimens for the genetic analysis. So, if gall numbers in your area are too low to get a sample of >50, you can nonetheless send a smaller number of specimens for sequencing analysis in support of this effort.

Upload the table to the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.

## Projects 3 & 4: Phenology, Gall Size and Insect Size

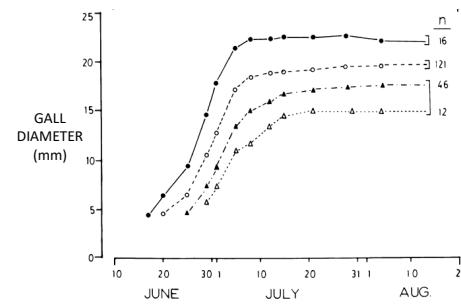
### Data & Specimen Collection and Storage

Whereas Projects 1 & 2 can be completed with several afternoon's work, 3 and 4 require a commitment over several months. Participation in the first two projects garners no obligation for the others. We break down Project 4 into components (see *Participation Options*, p. 18) and hope that those who cannot commit to the entire effort will be able to complete several of its parts.

#### *The Question(s).*

Assuming our initial observations of a cline in gall size are born out, it will be interesting to know something about the mechanisms for the size differences. Specifically, we want to know if there is also a cline in the emergence dates of the insects, in the date of gall initiation, the time for galls to reach full size, and in the host plant's flowering date.

Within a population, we know galls that start at an earlier date grow to a larger size. The working hypothesis has been that gall growth slows to a stop as plants begin the transition from vegetative to reproductive mode. If so, flies that get an early start enjoy a longer gall growth period, leading to larger diameter. As growing season shortens from south to north, it is possible that gall size decreases because of a shorter growth period. This raises the questions on the latitudinal patterns in the dates of fly emergence and gall initiation in the spring, gall growth termination in summer, and host plant flowering in autumn? Do these dates shift to afford a longer grow period, and hence larger size?



And if galls are larger toward the south, are the gallflies also larger? Within-population studies by Carol Mapes indicate no correlation between the size of the gall and the size of the emerging fly. The supposition is that although the outer gall tissue layers vary in size, the inner nutritive layer does not. Apparently, as the larva grazes on the nutritive tissue, it regrows. But, does one see a gall size-body size correlation across latitudes?

And how does a gall size cline impact body size in the parasitoid *E. gigantea*? One may expect selection to favor longer ovipositors as mean gall size increases. If this occurs, is it because body size in general increases, or, is there a shift in the allometry of ovipositor length to body size?

## Project 3--Documenting Insect Emergence Phenology.

This will document latitudinal trends in emergence dates for the gallmker and its insect enemies.

### *Monitoring Emergence*

Simply, you will cage a collection of galls, then capture the insects as they emerge over the course of Spring. ***It is important that these individuals experience the natural pace of spring warming at your latitude.*** Therefore, keep the emergence cage in an outdoors situation. A building roof or home porch or is fine.

### *Materials—*

- 100-150 galls collected from your local populations in the mid-late Spring (***in addition*** to those collected for Project 1; need not be from the same site)
- Insect Emergence Cage (we supply)
- Insect aspirator (you supply)
- Eppendorf vials (screw capped preferred, snap cap OK; you supply)
- Label tape and pencil
- 95% Ethyl alcohol (you supply)
- Parafilm (you supply)
- Shipping box (you supply)



**Emergence cage**

*Procedures—*

- Check the cage for emerged, adult insects at least three times per week.
- Aspirate insects into vials. You may put up to 5 individuals per vial, BUT only one species per vial.
- Label each vial with site code, species code emergence date, and year (e.g., **KSR:obt:15Jun:24**), using a marker that will not fade if alcohol leaks.
- Record the number of emergents per species per emergence date on the data sheet downloaded from the website (see below).
- Wrap vials in parafilm pack into shipping box, and send by courier to the Zhang lab at George Washington Univ., using the address on the project website.
- Transcribe data to the excel sheet linked on the website.
- To upload your data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Scan and email the original data sheets to [gall.size@utoronto.ca](mailto:gall.size@utoronto.ca) with subject line “*completed data sheet*”

*Data Sheet Example*

The datasheet will have columns for site, plant, date, and emerging species. Each row corresponds to date the insects were retrieved from the cage.

Upload the data to the website, following the prompts to enter info on the collection site and local investigators.

Site	Plant	Date	Eurosta	E. obt	E. gig female	E. gig male	M. convicta	other
KSR	S. alt	15-May	1	0	0	0	0	0
KSR	S. alt	18-May	3	1	0	0	0	0
KSR	S. alt	21-May	8	1	0	0	0	0
KSR	S. alt	24-May	4	2	0	0	0	0
KSR	S. alt	27-May	1	0	0	0	0	0
KSR	S. alt	30-May	0	1	0	0	0	0
KSR	S. alt	01-jun	1	0	0	0	0	0
KSR	S. alt	04-jun	0	0	0	0	0	0
KSR	S. alt	07-jun	0	0	0	0	0	0
KSR	S. alt	10-jun	0	0	0	0	1	0
KSR	S. alt	13-jun	0	0	0	0	5	0
KSR	S. alt	17-jun	0	0	0	0	2	0
KSR	S. alt	20-jun	0	0	1	0	1	1
KSR	S. alt	23-jun	0	0	3	1	0	0
KSR	S. alt	27-jun	0	0	0	1	0	0

Scan and email the original data sheets to [gall.size@utoronto.ca](mailto:gall.size@utoronto.ca) with subject line “*completed data sheet*”

THE CONTINENTAL GALL SIZE PROJECT

## Project 4 -- Gall Initiation &Growth, and Hostplant Flowering

Here you will be concerned with the phenology of the gall itself and that of its hostplant.

### ***Monitoring initiation and growth of gall, and flowering phenology of plant***

Participants will monitor plant population for gall initiation. The basic procedure is to visit a population site weekly from late-spring to mid-summer. On each visit, mark newly-formed galls, then measure their diameter. Then, once it is marked, each gall is re-measured (diameter) on subsequent visits, up to late July, when gall reach their mature size (see the growth curve figure above). Starting in August, revisit plants bi-weekly and record their flowering stage.

#### *Materials—*

- Surveyors flagging tape (you supply)
- Dial calipers (you supply)
- Clip board for data sheet (you supply)
- Datasheet, downloaded form website.

#### *Procedure--*

- Establish a path through the field site, which you will re-walk weekly.
- In early spring, mark stems along the path with newly forming galls (see below) with number flagging tape labeled with an ID number, 1 to N.
- Record date for each new gall on data sheet (see below).
- On subsequent visits, record diameter of every marked gall. Repeat until galls reach the size plateau, typically in mid-July (see the growth curve figure above). By the end you will have somewhere between 5 and 10 repeated measurements per gall.
- Starting in August, visit fields bi-weekly, and record the flowering stage for each marked plant, using the ranking scheme described below.
- In December, collect the labeled galls, measure their final diameter, dissect out and identify occupants, using the information above.
- Transcribe data to the excel sheet linked on the website.
- To upload your data file, fill out and submit the google form linked on the website, following prompts to enter host plant, latitude & longitude, and other info on the collection site and local investigators.
- Scan and email the original data sheets to [gall.size@utoronto.ca](mailto:gall.size@utoronto.ca) with subject line “completed data sheet”

#### *Tips on identifying newly-formed galls*

The new galls will appear as slight swellings at the base of the plant's terminal bud (see photo). They are slightly paler than the stem below and the bud above. You can verify by touch. Gently pinch the base of the bud. A new gall feels larger and more solid than the base of an ungalled bud. You may also see a series of pin holes on the leaves just below the new gall. These are the leaves that wrapped the bud back when the gallfly deposited the egg; the holes are the

oviposition punctures. If you have doubts, mark the stem anyway; if it is a “false positive” you can exclude it later. Note that some of these “false positives” will be galls induced by the Goldenrod Bunch Gall Midge (*Rhopalomyia solidaginis*). As the bunch galls develop in the bud they will very obviously not be ball galls on the stem.

As the spring progresses, you may encounter galls along the transects that you missed on previous dates...this always happens. Do not include these in the sample.



### Newly-formed Gall

*Note—the brown spots seen in this photo are not typical*

### **Scoring Plant Flowering Phenology**

As the summer comes to an end, field sites should be revisited bi-weekly to score the hostplant's flowering phenology. Please start before you see the first plant in flower to avoid truncated data. The most practical way to score phenology for this project is to use this scale, from 0 to 4.

- 0 → Terminal bud in vegetative state, no visible flower buds**



- 1 → Flower buds visible, but not open**



- 2 → From 1 to 25% of flower buds opened**



3 → >25% of flowers buds opened



4 → At least some flowers senescing



## Project 4 Data Sheet

Site	Host	GallNum	InitDate	FinalDiam	Occupant	22-May	03-Jun	10-Jun	17-Jun	24-Jun	01-Jul	08-Jul	15-Jul	22-Jul	29-Jul	05-Aug	12-Aug	19-Aug	02-Sep	16-Sep	30-Sep	07-Oct	
KRS	Salt	1	22-May	25.3	EU	4.9	9.8	16.8	22.4	24.7	24.9	24.8	24.9	22.5	23.2	24.0	23.9	0	0	0	2	3	4
KRS	Salt	2	03-Jun	24.1	EU	na	5.0	10.1	17.5	22.5	23.2	24.0	23.9	23.2	23.2	23.3	0	0	1	2	3	4	
KRS	Salt	3	03-Jun	23.3	na	na	5.1	9.9	18.2	23.2	23.6	23.2	23.3	23.3	23.3	23.3	0	0	1	3	4	4	
KRS	Salt	4	03-Jun	20.0	obt	na	4.9	9.7	16.3	19.9	20.1	20.2	20.1	20.1	20.1	20.1	0	0	1	2	2	3	
KRS	Salt	5	03-Jun	24.1	EU	na	5.5	12.0	19.0	23.3	23.8	23.6	23.6	23.6	23.6	23.6	0	1	1	2	4	4	
KRS	Salt	6	03-Jun	19.1	na	na	5.3	11.5	17.5	18.0	19.0	18.9	19.0	19.0	19.0	19.0	0	0	1	2	2	2	
KRS	Salt	7	03-Jun	18.3	obt	na	5.3	10.9	16.8	17.2	18.5	18.5	18.4	18.4	18.4	18.4	0	0	1	2	2	2	
KRS	Salt	8	10-Jun	17.6	gig	na	na	5.0	10.0	14.3	17.1	17.2	17.1	17.1	17.1	17.1	0	0	1	2	2	3	
KRS	Salt	9	10-Jun	17.6	eld	na	na	4.9	9.9	13.2	16.1	17.3	17.2	17.2	17.2	17.2	0	1	1	3	4	4	
KRS	Salt	10	10-Jun	17.1	gig	na	na	5.9	12.0	14.1	15.9	16.9	17.0	17.0	17.0	17.0	0	0	0	2	2	3	
KRS	Salt	11	17-Jun	15.9	gig	na	na	5.4	8.1	11.0	15.1	16.0	16.0	16.0	16.0	16.0	0	0	1	2	2	3	
KRS	Salt	12	17-Jun	14.8	eld	na	na	5.2	8.3	10.1	14.9	15.1	15.1	15.1	15.1	15.1	0	0	1	2	3	4	

Gall ID  
 Initiation date,  
 final size,  
 content  
 (optional)

Gall diameter  
 over time  
 (optional)

Flowering  
 Phenology  
 Score  
 (optional)

***Participation Options for Project 4***

We hope many of you will opt in to this fourth part of Continental Gall Size Project. We also realize it requires a considerable time commitment--revisiting sites weekly in the summer then biweekly into the fall semester. But even if you can complete one or two of the three components, you will greatly improve the outcome.

Option 1: Complete all three components.

This entails the weekly marking of galls in late-spring, early summer, re-measuring galls through mid-summer, monitoring plant flowering stage bi-weekly in the fall, then in October, collecting galls, measuring final diameters, and identifying the occupant. In other words, collect data for all columns seen on the sample data sheet.

Option 2: Gall initiation date and final size.

Here, new galls are marked and initiation date recorded in mid-spring. Then in October, the marked galls are collected, measured, and occupants identified. These are the yellow columns on sample data sheet.

Option 3: Plant Flowering Phenology

If time does not permit gathering spring/summer data on galls, data on plant flowering is still useful. Simply mark 25-30 goldenrod stems with galls at the start of August. Revisit these bi-weekly and record flowering score. These are the green columns on the sample data sheet.

Option 4: initiation and flowering

This combines options 2 and 3—the yellow and green columns on the sample data sheet.

## APPENDIX: The Generalized Epsilon-Skew-Normal function to detect the “*shape of selection*”

A key goal for this project is to determine if geographic variation in selection intensity is explained by variation in attack frequency and in attack size-dependence. Fitting an appropriate fitness function for populations throughout the range can be illuminating in this regard.

In older analyses we used a Gaussian curve to characterize the gall size fitness function. On reflection, this choice introduces potential biases when estimating the functional relationship of larval survival to gall size. Specifically, the inherent symmetry of the Gaussian curve imposes the supposition that protection from parasitoid attack increases with size in the same fashion as the escape from bird attack decreases with size. This seems unlikely, given the different behavioral and morphological mechanism that mediate size-dependent attack in these enemies.

The Generalized Epsilon-Skew-Normal function can relieve this bias. The regression routine fits two gaussian-like functions to the data...one on either side of the function peak. The function's parameters include terms for skew and kurtosis to capture asymmetry and angularity. Model selection criteria can indicate when these terms improve fit.

The **Generalized Epsilon-Skew-Normal** to estimate the “Shape of Selection”

- Unimodal relationship around optimal diameter,  $D$ .
- Detects asymmetry about the optimum

$$W_i = \begin{cases} \left[ a + \exp\left\{-\frac{d-D}{\omega(1-s(1-D))}\right\} \right]^k & \text{for } d \leq D \\ \left[ a + \exp\left\{-\frac{d-D}{\omega(1+s(1-D))}\right\} \right]^k & \text{for } d > D \end{cases}$$

where...

$d$  = gall diameter (independent variable)

$D$  = peak position (optimal diameter)

$\omega$  = function width

$s$  = skew (left-right symmetry)

$k$  = kurtosis (curvy vs. angular)

Examples of the **Generalized Epsilon-Skew-Normal** function

