**Host-plant genetic variation structures gall-parasitoid networks**

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**Abstract [word count: 0 (pre-April 15)]**

**Introduction [word count: 65 (pre-April 15)]**

*Focal Research Questions:*

1. *Does host-plant genotype alter herbivore-parasitoid network structure? – i.e. is the density of herbivore-parasitoid interactions affected by host-plant genotype? If so, is the resulting genotype-herbivore-parasitoid network modular or nested?*
2. *Is variation in network structure among host-plant genotypes due to species turnover or rewiring of trophic interactions?*
3. *What are the direct and indirect mechanisms by which host-plant traits shape network structure? – includes plant traits, gall densities, gall size.*

**Methods [word counts: 613 (pre-April 15), 866 (April 15), 1076 (April 16), 1213 (April 18)]**

### Natural History

*Salix hookeriana* – Coastal willow (*S. hookeriana*) is a deciduous shrub (< 8 m) that occurs along the Pacific coast ranging from northern California to Alaska. This willow species grows primarily in meadows, floodplains, and coastal dunes, and is generally restricted to less than 100 m elevation (Argus, 2013). As with other willows, *S. hookeriana* is dioecious and reproduces both sexually (wind and insect pollination; e.g., Sacchi and Price 1988) and asexually through vegetative growth (e.g., Argus, 2013). The genus *Salix* has been a model system for examining the role of host-plant genetics in shaping plant-insect interactions (Robert S. Fritz & Price, 1988; Hochwender & Fritz, 2004) for a few reasons. First, willows support a diverse community of arthropods that include many different feeding guilds, such as gallers, leaf miners, leaf chewers, xylem and phloem feeders (Gagné 1989, Roche and Fritz 1997, Nyman et al. 1998, Sipura 1999, Sipura et al. 2002, Hochwender and Fritz 2004). Second, there can be considerable genetic variation within willow populations (Brunsfeld, Soltis, & Soltis, 1991), with different genotypes displaying extensive phenotypic differences (Argus, 2013; Nichols-Orians, Fritz, & Clausen, 1993). Finally, preference and performance of individual herbivore species has been linked to some willow traits, including leaf phenolic chemistry, trichome density, water and nutrient content, and shoot length (Dalin & Björkman, 2003; R. S. Fritz, Gaud, Sacchi, & Price, 1987; Matsuda & Matsuo, 1985; Matsuki & MacLean Jr., 1994; Wennström, Hjulström, Hjältén, & Julkunen-Tiitto, 2010).

*Gall community* – Willows host a diverse community of galling insects from three different arthropod families (Tenthredinidae, Cecidomyiidae, and Eriophyidae) that induce both open and closed galls that may occur on the leaves, petioles, and stems of *S. hookeriana*. Larva within open galls are partially exposed to the environment outside of the plant tissue and therefore may be vulnerable to attack from generalist predators. We focused on five galling insect species in this study that form closed galls (i.e. no openings to external environment) on *Salix hookeriana*. Specifically, four gall midges (Family Cecidomyiidae) and the leaf galling sawfly *Pontania californica* (Family: Tenthredinidae). Members of Cecidomyiidae included the leaf galler *Iteomyia salicisverruca* (hereafter *Iteomyia*), bud galler *Rabdophaga salicisbrassicoides*, stem galler *Rabdophaga salicisbattatus*, apical stem galler Cecidomyiid sp. 1. Details on the biology of these galling midges and sawfly species are discussed by Gagné (1989), Russo (2006), Caltagirone (1964); however, we point out some of the more relevant details here. In late April – early May, *Iteomyia* galls begin to appear. *Iteomyia* induces smooth, large galls that often contain multiple larva. Each gall is characterized by one or more slightly bent projections that extend below the leaf, each of which is correlated with a larval chamber, allowing you to count the number of larvae per gall. The larval chambers are located near the base of the galls where the galls are attached to the host leaf. Galls actually bulge on the dorsal surface of leaves, but the majority of gall growth occurs on the underside of leaves. By late August, *Iteomyia* larva are fully grown and they have spun cocoons within the galls, therefore they appear to overwinter inside the galls. *Rabdophaga salicisbrassicoides* induces open or closed rosette bud galls. These galls begin to appear in \_\_\_\_. Larva appear to be fully developed by late August. *Rabdophaga salicisbattatus* induces stem galls that contain multiple larva. *Rabdophaga salicisbattatus* appears to have a similar phenology as *R. salicisbrassicoides*. An unknown species of Cecidomyiid gall (Cecidomyiid sp. 1) induces galls at the apical meristem of growing shoot tips that fold one of the leaves so that it is parallel to the shoot. It also begins to appear the same time as other *Rabdophaga* species in this system. *Pontania* californica induces round-ovoid, smooth galls that bear tiny, wartlike scales and contain a single larva. At our site, they are multivoltine, and are the first gall species to emerge in April, complete their development, and emerge again in late June?.

**Common garden**

In February 2009, we established a common garden experiment consisting of 27 different genotypes of *S. hookeriana* (‘willow’ hereafter) at Humboldt Bay National Wildlife Refuge (HBNWR) (40°40'53"N, 124°12'4"W) near Loleta, California, USA. We haphazardly chose willow individuals from around Humboldt Bay and subsequently genotyped them using microsatellite markers (see *Molecular Methods* below). We propagated clonal replicates of each genotype using 75 cm cuttings that had been soaked in water for two weeks and planted directly into the ground in two hectares of a former cattle pasture at HBNWR. We planted cuttings in a completely randomized design with 25 replicates per genotype (27 genotypes × 25 replicates = 675 trees total), and cuttings spaced 3 m apart in a 45 m × 135 m grid. Each cutting was surrounded by a 1 × 1 m square of heavy-duty weed cloth to prevent vegetation growth in the immediate area. A 2.5 m tall fence was built around the experiment to exclude deer. Willows in our garden begin flowering in February and reach their peak growth in late July to early August. During this study, willows had reached 2-3 m in height.

## Molecular methods

To confirm that clones were genetically unique, we genotyped each willow using 2 microsatellite loci, SB80 and SB194 (Barker, Pahlich, Trybush, Edwards, & Karp, 2003). Polymerase chain reaction (PCR) amplifications were performed in 10 µl reaction volumes containing 5 ng DNA, 1 pmol each of forward and reverse primers, 0.5 pmol M13 IRD-labeled primer, 200 µM dNTP (New England Biolabs, Ipswitch, MA, USA), 1 X Paq5000 PCR buffer (Agilent Technologies Canada Inc., Toronto, ON), 1 U Paq5000 DNA polymerase (Agilent Technologies Canada Inc.) and 2.0 mM MgSO4. Cycling conditions were 94 °C/2 min; 35 cycles of 94 °C/40 s, 54 °C/1 min, 72 °C/1 min; 72°C/10 min. The PCR products were analyzed on a LiCor 4200 automatic sequencer using 5.5% polyacrylamide gels (KBplus, LiCor Biotechnology) and scored using RFLPscan (LiCor Biotechnology, Lincoln, NE) due to their tetraploidy. Of the 27 genotypes collected, 26 were unique and were used in this study (Table S1).

### Gall Surveys and Sampling

We collected galls in September of 2012 when gall larva were in late instars of their development or had already spun cocoons within the gall. The timing of collection was important because if galls were collected too early, we would not be able to reliably sample the parasitoid community. I collected all galls occurring on a haphazardly sampled basal branch from X - Y replicates of each genotype (N = , mean = ). All galls were placed into 30 mL plastic transport tubes and maintained at room temperature. After four months, I measured gall size, dissected them, and sorted insect larva, pupa and adults to morphospecies. To estimate gall densities per shoot, I recorded the diameter of each basal branch sampled. I then recorded the number of shoots occuring on a measured basal branch of X different plants (one replicate of each genotype) and created an allometric equation to estimate the number of shoots sampled on each replicate plant. The allometric equation was INSERT EQUATION, R^2 and P-value.

***Analyzing Network Patterns***

We used generalized linear models (GLiM) with the estimated number of shoots sampled as an offset to test for differences in the following responses: density of gall-parasitoid interactions, and proportion of galls parasitized among genotypes. We fit separate models for each interaction, summed the test statistics, and used resampling to test the significance of this multivariate test statistic (Warton & Hudson, 2004; Warton, 2011). We also evaluated which interactions were driving differences in gall-parasitoid networks by examining which models for each interaction were significant.

To analyze genotype-gall-parasitoid network architecture architecture, we pooled replicate samples for each host-plant genotype and calculated the frequency of all observed gall-parasitoid links occurring on each host-plant genotype. This resulted in a weighted, bipartite network where host-plant genotype comprised one set of nodes, and gall-parasitoid links made up the second set of nodes. This departs from a typical bipartite network in ecology (e.g. seeds-dispersers, plants-pollinators, hosts-parasitoids), but it represents this tri-trophic interaction in a bipartite network. We then standardized these frequencies by dividing the observed number of links by the estimated number of shoots sampled for each genotype. Since modularity and nestedness analyses require integer frequencies, we rounded the standardize estimate of interaction frequency to the nearest integer. We used modularity analysis to test whether certain genotypes were more connected to particular gall-parasitoid interactions, thereby forming strongly interacting groups or modules. Conceptually, these modules are identified using algorithms that randomly partitions the network into different sets of interacting nodes until the grouping with highest modularity value *Q* is found (i.e. the most strongly interacting group structure). The value *Q* reflects the extent to which links are formed within modules instead of between modules (i.e. the most strongly interacting group structure). For our analyses, we used the QuaBiMo algorithm to detect these modules and calculate *Q* for our weighted, bipartite network (Dormann & Strauss, 2013). Since *Q* is influenced by the number of species in the network, the number of links between species, and the total number of interactions observed (Dormann & Strauss, 2013; Thébault, 2012), we used three different null models to examine whether the observed patterns were significantly different from what you would expect from a random network. These three null models provide complimentary information on the processes shaping the structure of weighted, bipartite networks (Dormann, Fründ, Blüthgen, & Gruber, 2009). Following Dormann et al. (2009), we used null model I-III. Null model I uses the Patefield algorithm to randomize the observed interaction values in the matrix while preserving the row and column totals of the network. Null model III is a swapping algorithm employed on a network generate by null model I, but it additionally constrains the connectance to the same value observed in the original web. Null model II shuffles the observed interaction values so while the row and column totals

***Analyzing Network Mechanisms***

*A priori* we identified three possible mechanisms that may influence gall-parasitoid interactions: gall density, gall size, and tree architecture (plant size, height, and foliage density). Using the full data set, we first examined whether there was an effect of gall size on the probability of gall-parasitoid interactions, for which we used GLiMs.

We first examined correlations among these traits, specifically between gall density and gall size as well as gall density and the variance in gall size.

We will use GLiMs on significant gall-parasitoid interaction models to examine how gall-parasitoid interactions are affected by gall density, gall size, and tree architecture. We will use AICc to compare possible all possible models.

Similarly, we will use general linear models (GLMs) to examine the effect of plant genotype on gall density and gall size.

*Using the approach advocated by (De Cáceres, Legendre, & He, 2013), I will examine whether genotypes vary in the composition and size structure of their galling insect communities (using distance-based redundancy analysis). I will then use ordinations to visualize these differences and whether they correspond with different modules (or genotypes that vary in their contribution to nestedness).* To identify different gall size classes, I will use logistic regression to identify different gall size classes that correspond to different parasitoid communities.

To examine which plant traits account for variation in gall community composition and size structure, I will use mean trait values for genotype traits and see how they explain this variation.

**Results [word count: 0 (pre-April 15)]**

*How does gall-parasitoid network structure vary among host-plant genotypes?*

*Network size—*

*Network connectance—*

*Proportion of parasitized galls—* Parasitoid attack rates on *I. salicisverruca* galls varied from 6% - 71% among genotypes, whereas parasitoid attack rates on other gall species did not vary.

*Modularity of genotype-gall-parasitoid networks—*

*Nestedness of genotype-gall-parasitoid networks—*

*Dissimilarity in gall-parasitoid interaction networks among genotypes and contribution of species turnover to this dissimilarity—* On average, gall-parasitoid networks were 49% dissimilar from each other among genotypes. Of this dissimilarity, 71% could be attributed to species turnover. In other words, differences in gall-parasitoid network structure among genotypes were primarily due to the addition of new gall or parasitoid species rather than a “rewiring” of these trophic interactions. When we considered the presence/absence of species interactions, gall-paristoid networks were an average of X% dissimilar.

*Density of gall-parasitoid interactions –*

**Table 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gall species | Range | df | Chi-square | *P* |
| *Iteomyia salicisverruca* | 6% - 71% | 19 | 45.17 | <0.001 |
| *Rabdophaga salicisbrassicoides* | 5% - 38% | 14 | 12.17 | 0.593 |
| *Rabdophaga salicisbattaus* | -- | -- | -- | -- |
| Cecidomyiidae sp. A | 17% - 43% | 1 | 1.04 | 0.562 |
| *Pontania californica* | 11% - 40% | 6 | 2.43 | 0.876 |

*Notes*: removed any genotypes with zeros in any of the columns (although the results are robust to this removal). Not sufficient data for Rabdophaga salicisbattaus, however, if I allow one of the columns to be zeros, it is significant, but I don’t believe it…

1) Does the susceptibility of herbivores to parasitoids depend on the plant genotype harboring them? The answer would be yes if most of the differences among the 26 herbivore-parasitoid networks associated to the plant genotypes were due to a rewiring of the interactions between co-occurring herbivore and parasitoid species. The Poisot et al. framework will help us to answer this question because it tells us how much of the differences among networks are due to species turnover and how much is due to the rewiring of the interactions between co-occurring species. We should use qualitative values in here since we are only interested in quantifying changes in who-interacts-with-whom.

2) Do some herbivore-parasitoid interactions tend to be associated to certain plant genotypes? That is, are there some specificity in the interactions that the plant genotypes harbor? To answer this question we would build a bipartite network, as you said, with the 26 plant genotypes as the first set of nodes and the N unique herbivore-parasitoid interactions as the second one. A link here would indicate that a given plant genotype harbors a given herbivore-parasitoid interaction and its weight would then be its frequency. Then, we would apply a modularity algorithm for weighted bipartite networks (as you said, the recently published QuaBiMo). Miguel reviewed this paper and is aware of the details.  He can help with any doubts you may have regarding implementation.  At first I doubted since I am more familiar with Guimerà's algorithm, but Miguel told me this is an extension for bipartite, weighted networks.  It seems this is  nice way to go.

In both cases, we would pool all of the samples together at the genotype level (by aggregating data from the 5 replicates per plant genotype).

***Writing from proposal***

1. The vast majority of community genetics research has focused on community composition (Whitham et al. 2012), thereby neglecting the more complex network of species interactions occuring between community members. Those studies that have moved beyond compositional descriptions and pairwise interactions have focused on simple food chains or aggregated species together at different trophic levels (Fritz 1995, Stiling and Rossi 1996, Bailey et al. 2006, Mooney and Agrawal 2008, Abdala-Roberts and Mooney 2012). However, research on ecological networks (Ings et al. 2009) suggests that the architecture of species interactions within a food web may have profound consequences on food web dynamics (Thébault and Fontaine 2010, Stouffer and Bascompte 2011). My second chapter proposes to bridge research in community genetics and ecological networks to examine how genetic variation within a single host-plant species influences the structure of associated herbivore-parasitoid food webs.

# Chapter 2: Consequences of genetic variation within the willow *Salix hookeriana* in structuring herbivore-parasitoid networks

## Background

Food webs represent a network of who eats whom in an ecological community. Typically, nodes within a food web network represent species, and links between nodes represent the presence/absence of a feeding interaction. One of the general properties of species-level food web networks is that they tend to be organized into compartments, or modules (Girvan and Newman 2002, Krause et al. 2003, Rezende et al. 2009, Allesina and Pascual 2009). These modules represents groups of species that interact with each other more frequently than would be expected by random chance (Newman 2006). Recent work suggests that compartmentalization in food webs buffers the propogation of species extinctions throughout the network, thereby increasing the resilience of the food web to perturbations (Stouffer and Bascompte 2011). However, a persistent issue in food web research is the fact that species *per se* do not interact, but it is individuals within species that interact with each other (Ings et al. 2009, Gómez and Perfectti 2012). Since network structure has a fundamental influence on network dynamics (Allesina and Pascual 2008, Thébault and Fontaine 2010, Stouffer and Bascompte 2011), our understanding of food web dynamics may be hindered by not knowing the underlying structure of species-level networks.

Aside from studying the consequences of network structure on dynamics, a lot of recent research has focused on examining the role of traits in establishing links within ecological networks. However, much of the work that has been done has focused on interspecific trait variation because network models have been interested in predicting species-level network structure (Petchey et al. 2008, Rohr et al. 2010, Eklöf et al. 2013). Consequently, we know little about how intraspecific trait variation structures interactions in more complex food webs.

In this chapter, I examine whether host-parasitoid food webs exhibit a compartmentalized structure below the species level. In addition, I examine how intraspecific trait variation influences the structure of this interaction network. I propose to do this by examining the structure of willow genotype-gall-parasitoid networks in a common garden of 26 willow clones (described in Ch. 1). This approach requires data on quantitative interaction strengths (van Veen et al. 2006). Galling insects are ideal herbivores for studying quantitative food web structure, because they are often host to a diverse community of parasitoids (Hawkins 1988), as well as being relatively straightforward to collect, rear, and determine survival or identity of the attacking parasitoid. Willows provide an ideal study system because they host a diverse community of galling insects (Gagné 1989, Nyman et al. 2007) and their is evidence that genetic variation within willows influences the organization of the galling insect community (Fritz and Price 1988, Hochwender and Fritz 2004) as well as parasitoid attack rates (Fritz 1995, Fritz et al. 2003).

## Hypotheses and Predictions

**(H1)**: I hypothesize that willow genotype-gall and gall-parasitoid networks will exhibit modular structure (Fig. 3). **(H2)**: I also hypothesize that gall-parasitoid networks will exhibit modular structure (Fig. 3). **(H3)**: I hypothesize that plant genotype may shape the frequency of gall-parasitoid interactions in three fundamentally different ways: (1) variation in galling insect densities, (2) variation in gall size, or (3) variation in plant architecture. I expect the first mechanism to act primarily on egg-parasitoids, because they attack before galls have begun to develop. I predict the second mechanism to act primarily on late instar-parasitoids, which must oviposit through well-developed gall tissue. For these two mechanisms, I predict that leaf phenolic chemistry and/or C:N content will be associated with these mechanisms. This prediction stems from my observations that the abundance of the most common galling insect in the garden, *Iteomyia salicisverruca*, is positively correlated with leaf C:N. In addition, leaf phenolic chemistry has been shown to be important for one of the gall-inducing sawfly species at the study site (*Pontania californica*, (Roininen et al. 1999, Nyman and Julkunen-Tiitto 2000)). In regard to the third mechanism, I have two alternative predictions. First, galls may experience more frequent attack in plants with greater foliage density because these plants are associated with higher parasitoid densities (results Ch. 1; Langellotto and Denno (2004)). In contrast, more complex plant architecture may reduce parasitoid foraging efficiency (Lawton 1983) and consequently reduce parasitism rates on galling insects.

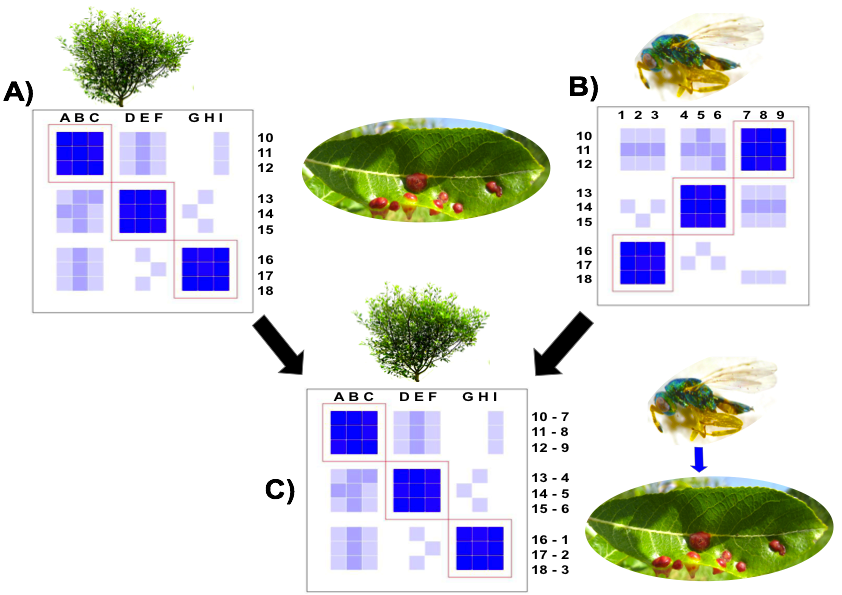


Figure 3: Conceptual diagram of how genetic variation within the willow *Salix hookeriana* may influence genotype-gall-parasitoid network structure (H1 & H2). Increasingly dark blue coloration indicates increasing interaction strength. Red boxes indicate modules, or groups of genotypes/species that interact with each other more frequently than expected by random chance. (A) Hypothetical quantitative bipartite network of genotype-gall interactions. Letters A - I correspond to unique plant genotypes. Numbers 10 - 18 correspond to galling-inducing herbivore species. (B) Bipartite network of gall-parasitoid interactions. Numbers 1 - 9 correspond to different parasitoid species. (C) Bipartite network of willow genotype and gall-parasitoid interactions.

## Methods

To test these hypotheses, I took advantage of the same willow common garden described in Chapter 1 as well as the same plant trait measurements.

### Gall Surveys and Sampling

In August and September of 2012, I collected all galls occurring on a haphazardly sampled basal branch for 5 replicates of each genotype. All galls were placed into 30 mL plastic transport tubes and maintained at room temperature. After four months, I measured gall size, dissected them, and sorted insect larva and adults to morphospecies. To account for the number of shoots sampled for galls, I recorded the diameter of each sampled basal branch. I then recorded the number of shoots occuring on a measured basal branch of 26 different plants (one replicate of each genotype) and created an allometric equation to estimate the number of shoots sampled on each replicate plant.

### Network Structure

To examine willow genotype-gall network structure, I propose to use two methods. First, to examine whether there is more variation among than within genotypes in gall community composition, I will use 9,999 iterations of PERMANOVA on euclidean distances of my site-by-species matrix of gall densities, with genotype nested within gender. Second, I will use the mean gall densities observed on each willow clone to build a quantitative bipartite network (Fig. 3a). I will then use modularity analysis to examine whether certain plant genotypes and gall species formed distinct compartments or modules. Modularity analysis uses an algorithm to search for sets of nodes (e.g., species or individuals) in a network that interact with each other more frequently than would be expected by random chance (Newman 2006). I plan to use the QuaBiMo algorithm developed by Dormann and Strauss (2013), to evaluate the modularity, *Q*, of the network. Since modularity relies upon an optimization routine, I will run 100 iterations and retain the highest value of modularity. This value of modularity is dependent on the number of species in the network, the number of links between species, and the total number of observed interactions; therefore, I will use a null model to evaluate whether the network is more modular than I would expect by random chance.

To examine gall-parasitoid network structure, I propose to use three different approaches. First, I will build a quantitative bipartite network based on pooled observations of parasitoid rearings from each gall species. I will then use the same modularity analysis proposed above to examine whether gall and parasitoid networks form distinct modules at the species level (Fig. 3b). Second, using the method described by Poisot et al. (2012), I will calculate the dissimilarity in quantitative gall-parasitoid networks among individual trees. Each cell of the gall-parasitoid interaction matrix will correspond to the percentage of larva parasitized by a specific parasitoid species:

Interaction strength for each matrix cell = (count of parasitoid sp. *i*) / (count of parasitoid sp. *i* + count of other parasitoids attacking gall + count of surviving gall larva).

I will then use PERMANOVA, to examine whether there is more variation among than within genotypes in gall-parasitoid network structure. Finally, I will use mean frequencies of observed gall-parasitoid interactions on each willow clone to create a genotype-by-parasitism interaction matrix and use modularity analysis to examine whether certain genotypes are associated with certain gall-parasitoid interactions (Fig. 3c).

### Intraspecific trait variation

To examine which plant traits influence galling insect densities, I will conduct separate multiple linear regressions using all leaf phenolic principal components, SLA, leaf water content and leaf C:N as explanatory variables. I will also conduct this same analysis to understand which plant traits influence gall sizes.

I have *a priori* hypotheses about how the relative importance of gall density, gall size, and plant architecture in shaping gall-parasitoid interactions will likely depend on whether parasitoids attack eggs or late-instar larva; therefore, I will analyze each gall-parasitoid interaction with separate logistic regression models. For each regression, my response variable will be the frequency of a particular gall-parasitoid interaction on each willow clone, with the corresponding mean gall density, gall size, and plant architecture traits.

## Progress & Timeline for Chapter 2

Thus far, I have finished field and lab data collection. I am in the process of analyzing the willow genotype-gall and gall-parasitoid interaction networks. I plan to submit this research for publication in February, 2014.