**Plant genetic variation shapes the architecture of herbivore-parasitoid networks**

Matthew A. Barbour1, Jordi Bascompte2, Joshua R. Nicholson1, Riitta Julkunen-Tiitto3, Erik S. Jules4, and Gregory M. Crutsinger1

1Department of Zoology, University of British Columbia, #4200-6270 University Blvd., Vancouver, B.C., V6T 1Z4, Canada

2Estación Biológica de Doñana, CSIC, C/ Américo Vespucio s/n, 41092 Sevilla. España

3Department of Biology, University of Eastern Finland, PO Box 111, FI-80101, Joensuu, Finland

4Department of Biological Sciences, Humboldt State University, 1 Harpst St., Arcata, California, 95521, USA

\*Author for correspondence, email: barbour@zoology.ubc.ca

**Abstract**

Theory predicts that a modular network structure (*i.e.*, groups of species that frequently interact with each other) and increased connectance (*i.e.*, number of links between species over the number of possible links) promotes food web stability and persistence. However, interactions within food webs ultimately occur between individuals, not species, and it remains unclear how genetic and phenotypic variation within species contributes to network properties that confer stability to food webs. We address this knowledge gap by examining how genetic and phenotypic variation within a dominant host-plant, the willow *Salix hookeriana,* influenced its associated herbivore-parasitoid interaction network. Specifically, we surveyed 26 unique willow genotypes growing in a common garden for their insect induced-galls (herbivores) and reared their associated parasitoids to build a quantitative food web of herbivore-parasitoid interactions for each willow genotype. Additionally, we measured gall morphology, as well as a suite of host-plant architectural (*e.g.*, height, branching complexity) and leaf quality (*e.g.*, phenolic chemistry, trichomes, leaf C:N) traits to elucidate the underlying mechanisms by which willow genetic variation affects herbivore-parasitoid assemblages. We found that willow genotypes varied more than three-fold in the richness and abundance of both galls and parasitoids, resulting in variation in herbivore-parasitoid network connectance. Herbivore-parasitoid network composition also varied among host-plant genotypes, leading to the formation of distinct network clusters, or modules. Moreover, we found that heritable plant traits affected both gall density and gall size, which in turn, shaped differences in herbivore-parasitoid network structure. Together, our results indicate that genetic variation can play a key role in the assembly of food webs and that incorporating individual variation will further our understanding of factors governing the structure and dynamics of food webs.

**Introduction**

Here, we provide empirical evidence that genetic variation within a dominant plant species can structure ecological networks by altering the strength of interactions among its associated species. Using a large common garden experiment consisting of 26 genotypes of a dominant plant species, *Salix hookeriana*, we built quantitative interaction networks for each genotype’s associated gall-inducing insects (herbivores) and their parasitoids to address the following questions: (1) How does plant genotype shape the architecture of herbivore-parasitoid networks? (2) Which plant and herbivore traits underlie changes in network structure?

**Materials & Methods**

### Natural History

Our focal plant species, *Salix hookeriana* (Coastal willow), is a dioecious and deciduous shrub (< 8 m), generally restricted to less than 100 m elevation, and commonly occurs in meadows, floodplains, and coastal dunes from northern California to Alaska, USA (Argus 2013). As with other willows, *S. hookeriana* is an ideal system for studying the effects of host-plant genetics on herbivore-parasitoid interaction networks for two main reasons. First, willows display considerable genetic (Brunsfeld, Soltis, & Soltis, 1991) and phenotypic variation (Argus, 2013; Nichols-Orians, Fritz, & Clausen, 1993), which corresponds to variation in susceptibility to different species of herbivorous insects (Barbour et al. 2014, in review; Fritz & Price 1988; Roche & Fritz 1994). Second, previous work has shown that willow genotype may mediate the strength of pairwise trophic interactions between herbivores and their natural enemies (Price’s work, Craig’s work, Fritz’s work, Barbour et al. 2014, in review).

Our focal herbivore community consisted of all the species that induce closed galls on the leaves, buds, or shoots of *S. hookeriana*. These herbivores included four species of gall midges (Family Cecidomyiidae) and a leaf galling sawfly *Pontania californica* (Family: Tenthredinidae). Gall midges included the leaf galler *Iteomyia salicisverruca* (hereafter *Iteomyia*), bud galler *Rabdophaga salicisbrassicoides*, shoot galler *Rabdophaga salicisbattatus*, and an undescribed shoot gall (Cecidomyiid sp. A) that occurs at the apex of willow shoots (Plate 1, supplement)(for details on their biology see Caltagirone 1964, Gagné 1989, Russo 2006).

The closed morphology of these galls restricts the natural enemy community to eight species of insects that can successfully locate and attack herbivore larva within these galls. These eight species consist of seven parasitoid wasps (Chalcidoidea = 5 sp.; Platygastroidea = 1 sp.; Ichneumonoidea = 1 sp.) and one predatory Cecidomyiid midge (*Lestodiplosis septemmaculata*)(Table S1 contains details on the biology of this natural enemy community). Gall size influences the probability of parasitoid attack as larger galls are increasingly more difficult for parasitoids to access larva because they become limited by the length of their ovipositor. Therefore, we focused on gall size as a functional trait that potentially mediates herbivore-parasitoids interactions (further details in *Quantitative herbivore-parasitoid networks* section).

**Common garden**

To isolate the effects of plant genetic variation on herbivore-parasitoid network structure, we used a common garden experiment consisting of 26 different genotypes of *S. hookeriana* (13 males; 13 females), located at Humboldt Bay National Wildlife Refuge (HBNWR) (40°40'53"N, 124°12'4"W) near Loleta, California, USA. This garden was planted in February 2009 with 25 clonal replicates (i.e., stem cuttings) of each willow genotype (locally collected from around Humboldt Bay) in a completely randomized design in two hectares of a former cattle pasture at HBNWR. Willows in our garden begin flowering in February and reach their peak growth in early August. During this study, willows had reached 2-4 m in height. Further details on the genotyping and planting of the common garden are available in Barbour et al. (2014, In review).

**Quantitative herbivore-parasitoid networks**

To build a quantitative herbivore-parasitoid network for each willow genotype, we collected all galls occurring on a haphazardly selected basal branch from about 5 randomly chosen replicates of each genotype (N = 146 trees, range = 4-9 trees per genotype). In order to control our sampling to shoot densities, we estimated the number of shoots surveyed based on an allometric equation using the stem diameter of the sampled basal branch (details in supplementary materials). We collected all galls in September 2012 when gall larva were in late instars of their development or had already spun cocoons within the gall. All galls were placed into 30 mL plastic transport vials and maintained at room temperature in the lab for four months. We then measured gall size to the nearest 0.01 mm (maximum diameter perpendicular to plant tissue orientation), dissected them, and determined gall survival or parasitoid species identity. We omitted galls for which we could not reliably determine the cause of mortality from further analysis.

**How does plant genotype shape the architecture of herbivore-parasitoid networks?**

To determine if the willow genotype-herbivore-parasitoid network exhibited a compartmentalized structure, we first pooled all observed herbivore-parasitoid links for each plant genotype, controlled by the number of shoots surveyed. This resulted in a weighted, bipartite network where willow genotype comprised one set of nodes, and unique herbivore-parasitoid links made up the second set of nodes (Fig. 1)(FIGURE with the drawings). This departs from a typical bipartite network in ecology (e.g. seeds-dispersers, plants-pollinators, herbivores-parasitoids), but it represents this tri-trophic interaction in a bipartite network.

To identify different compartments in our weighted, bipartite network, we used the QuanBiMo algorithm (Dorman and Strauss 2014). Conceptually, this algorithm identifies compartments based on a hierarchical representation of link weights between nodes of a network and uses simulated annealing to optimally allocate nodes to different compartments to maximize the modularity. This modularity value (*Q*) is calculated as,

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where *m* is half of the total number of observed links in the network, *Aij* is the weighted bipartite matrix and *Kij* is the matrix of expected weights (Dormann and Strauss 2013). The compartment to which a species *i* or *j* is assigned is *ci,cj*. The indicator function δ(*ci,cj*) = 1 if *ci* = *cj* and 0 if *ci* ≠ *cj*. *Q* ranges from 0, which means the community has no more links within compartments than expected by random chance, to a maximum value of 1, which indicates increasing support for the division of a network into compartments. Since *Q* is determined by an optimality function and is susceptible to being trapped at local optima, we repeated this calculation 100 times and used the iteration with the highest *Q* value to identify the compartments within our network.

Since the magnitude of *Q* is influenced by the number of nodes in the network, the number of links between nodes, and the total number of links observed (Dormann & Strauss, 2013; Thébault, 2012), we used a conservative null model to examine whether the degree of compartmentalization we observed was significantly different from what we would expect by chance. Specifically, we used a swapping algorithm that randomized the observed interaction values in the bipartite matrix while preserving both the row and columns totals and the number of realized interactions (i.e., connectance) from the original network. Both the modularity and null model analysis were conducted with the *bipartite* package in R (Dormann R citation; R Development Core Team 2014).

We measured dissimilarity between networks by analyzing differences in the composition of their links (i.e., species interactions) (Poisot et al. 2012). These differences in link composition can then be partitioned into species turnover and interaction components. Differences due to species turnover arise from the gain/loss of species altering link composition, whereas differences due to interactions occur when species switch the partners with which they are interacting despite having the same species composition. This framework works for both qualitative (i.e., presence/absence data) and quantitative networks given that an appropriate dissimilarity index is used (Poisot et al. 2012). This network dissimilarity data can then be analyzed in the traditional framework used for community composition data (Legendre’s Numerical Ecology book).

To test whether different compartments corresponded to dissimilarity in herbivore-parasitoid network structure, we used permutational ANOVA (PERMANOVA with *vegan* package in R) on pairwise network dissimilarities (Bray-Curtis index). We then partitioned the relative contribution of species turnover and interaction components (with *betalink* package in R) to understand the processes driving network dissimilarity among compartments.

**Which plant and herbivore traits underlie changes in network structure?**

Therefore, to make the connection between plant genotype and gall density, we first analyzed the broad-sense heritability of resistance to different gall species. Then, we used linear mixed-effect models to identify the plant traits associated with resistance to different gall species. We have measured 40 different plant traits, ranging from leaf quality to plant architecture traits, associated with *S. hookeriana* all of which exhibit heritable variation (range *H2* = ). In addition, we used broad-sense heritability analyses to determine the amount of genetic variance contributing to gall size and then linear mixed-effect models to identify the plant traits associated with gall size. We only conducted these analyses when we identified significant broad-sense heritability in gall density or gall size.

After identifying the plant traits contributing to variation in gall density and gall size, we sought to tease apart the factors contributing to variation in gall-parasitoid attack. *A priori* we predicted that gall density, gall size, as well as plant morphology (architecture and trichome density) would contribute to variation in the strength of gall-parasitoid interactions. So, we used generalized linear and additive mixed effect models to tease apart the contributions of these factors to parasitoid attack rates.

All analyses were conducted in R using the packages *lme4*, *gamm4,* and *RLRsim*.

**Results**

**How does plant genotype shape the architecture of herbivore-parasitoid networks?**

*Herbivore Community*

We found that *S. hookeriana* displayed heritable variation in susceptibility to galling insects in terms of both the frequency of gall attack and gall size. Specifically, attack rates from four of the five gall species varied between 22.8- and 70.2-fold among willowgenotypes, resulting in genotype explaining 23% of the variance in gall community composition (*H2* = 0.23, *F* = 2.62, *P* = 0.001). In particular though, the heritable variation in susceptibility was highest for *Iteomyia* (*H2* = 0.36, *RLRT* = 27.78, *P* < 0.001), which was also the dominant herbivore in this network (44% of total galls). Willow genotype also influenced variation in the size of *Iteomyia* galls (*H2* = 0.13, *RLRT* = 3.68, *P* = 0.022), ranging in size from 4.8-11.0 mm among the most disparate genotypes (2.3-fold variation).

*Herbivore-Parasitoid Network*

We also found that *S. hookeriana* genotypes differentially contributed to herbivore-parasitoid network architecture, departing substantially from the symmetric contribution assumed by most food web models. We documented herbivore-parasitoid interactions on 25 of 26 genotypes, but only 9 of these genotypes made above average contributions to the frequency of herbivore-parasitoid interactions (Figure 2A). Willow genotype also explained 17% of the dissimilarity in herbivore-parasitoid network structure (*H2* = 0.17, F = 2.17, *P* = 0.001). The dissimilarity in network structure among genotypes was predominantly driven by species turnover (66%), and in particular, attack from three different parasitoid species on *Iteomyia* (63% of total observed herbivore-parasitoid interactions). Parasitism from the egg, endoparasitoid *Platygaster* on *Iteomyia* varied 34.9-fold among willow genotypes (*H2* = 0.31, *RLRT* = 21.61, *P* < 0.001), while attack from the larval, ectoparasitoids *Mesopolobus* (*H2* = 0.11, *RLRT* = 3.77, *P* = 0.024) and *Torymus* (*H2* = 0.25, *RLRT* = 14.75, *P* < 0.001) on *Iteomyia* varied 10.5- and 5.7-fold among willow genotypes, respectively.

The asymmetric contributions of willow genotype to herbivore-parasitoid network structure resulted in a compartmentalized network architecture (*Q* = 0.33, *Z* = 2.41, *P* = 0.008; Fig. 2B). The modularity algorithm we used detected five distinct compartments. We found that *Iteomyia* participated in three of these compartments, where each unique compartment corresponded to attack from a different dominant parasitoid species (i.e., *Platygaster*, *Mesopolobus*, or *Torymus*). In other words, *Iteomyia*’s susceptibility to each of the three dominant parasitoid species depended on the compartment identity of the willow genotype. *R. salicisbrassicoides* experienced a similar shift as *Iteomyia* in its source of parasitism, although the strength of these interactions were comparatively weak. In contrast, the three other gall species in our system each participated in a single, but not necessarily distinct, compartment. The sawfly *Pontania* had a distinct parasitoid community from the gall midges, which is likely a result of their distinct evolutionary histories. For both *R. salicisbattatus* and Cecidomyiid sp. A, we only detected a single associated parasitoid species each, but this may simply be a reflection of their relatively low abundances (5% and 4% of total galls, respectively).

**Which plant and herbivore traits underlie changes in network structure?**

*Herbivore Community*

We found that the gall community was influenced both by leaf quality and plant architecture traits. In particular, both *Iteomyia* and *R. salicisbrassicoides* were found at higher densities on shorter willows with higher leaf C:N (*Iteomyia*, *R2* = 0.17, *F*2,119 = 12.14, *P* < 0.001; *R. salicisbrassicoides*, *R2* = 0.15, *F*2,120 = 10.97, P < 0.001). *Pontania* was found at higher densities on smaller willows with low leaf trichome density, but higher concentrations of flavones (*R*2 = 0.17, *F*3,106 = 7.38, *P* < 0.001). Cecidomyiid sp. A was positively associated with higher concentrations of flavanones and flavanonols (*R2* = 0.10, *F*1,131 = 15.21, *P* < 0.001). *Iteomyia* gall size was positively associated with higher concentrations of salicylates and flavones (*R2* = 0.14, *F*2,75 = 5.88, *P* = 0.004).

*Herbivore-Parasitoid Network*

We found that the dominant interactions in the herbivore-parasitoid network were shaped by an interaction between density-dependent attack and gall size. Specifically, we found that the probability of *Platygaster* parasitizing *Iteomyia* increased at higher gall densities, but only for small gall sizes (***Χ2*** = 20.61, P < 0.001). In contrast to *Platygaster*, *Mesopolobus*’ probability of attacking *Iteomyia* was highest for intermediate sized galls at low densities (***Χ2*** = 18.10, P = 0.003). Gall size did not appear to influence the likelihood of *Torymus* attack, but as with *Mesopolobus*, we found that the probability of parasitism was higher at low gall densities (***Χ2*** = 10.82, *P* = 0.001). In contrast to the dominant herbivore-parasitoid interactions, the probability of observing these weaker links was independent of both gall density and gall size (Supplement Table).

**Discussion**

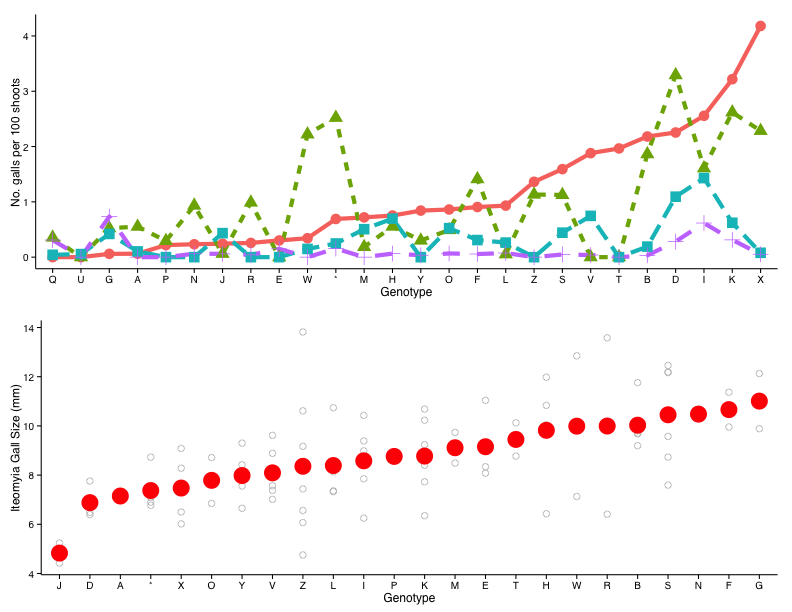
**Figure Legends**

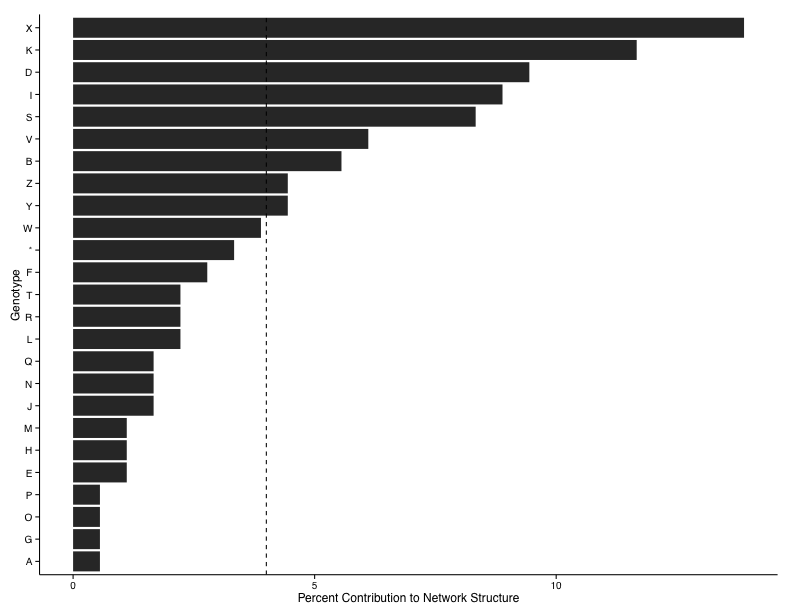
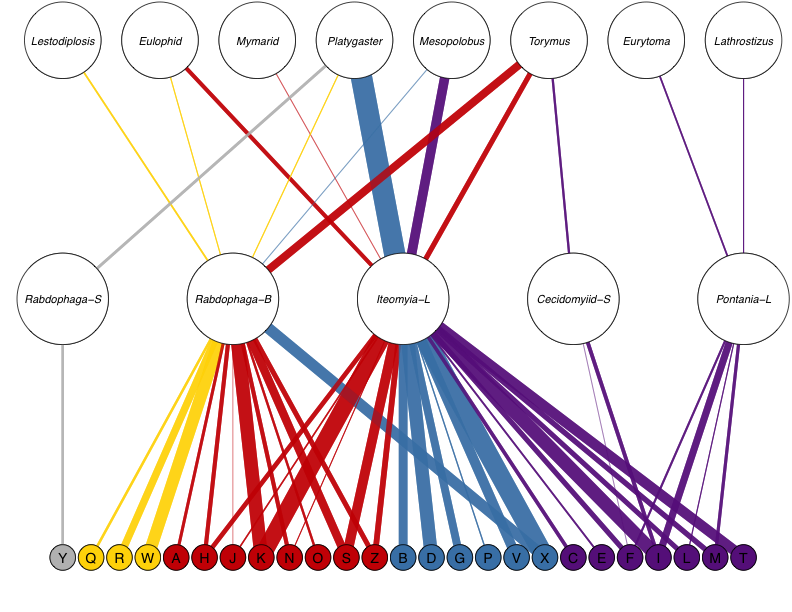
**Figure 1.** Variation in gall density and gall size among different genotypes of *Salix hookeriana* measured in a common garden experiment. Orange = *Iteomyia*, Green = *R. salicisbrassicoides*, Blue = *Pontania*, Purple = Cecidomyiid sp. A.

**Figure 2A.** Percent contribution of different genotypes of *Salix hookeriana* to the total number of herbivore-parasitoid interactions (controlled for sampling effort).

**Figure 2B.** Quantitative, tripartite network of interactions among 25 genotypes of *Salix hookeriana*, five species of its associated galling insects, and the eight species of natural enemies that attack these galls. The width of each link is proportional to the observed density of each interaction. Each color corresponds to a different compartment. Note that each genotype was associated with a different compartment and has been filled in with in the appropriate color. However, galls and parasitoids can participate in multiple modules, so the color of their corresponding node was kept white. Each gall species was also labeled with the type of damage it causes to the plant (-S = stem, -B = bud, -L = leaf).

**Figure 3.** Factors determining parasitoid attack on *Iteomyia salicisverruca*.

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