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

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**Abstract**

**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) Do plant genotype-herbivore-parasitoid networks exhibit a compartmentalized structure? (2) Which processes drive the dissimilarity in network structure among different compartments? (3) Which plant and herbivore traits determine this compartmentalized 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 (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 scale 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.

**Do plant genotype-herbivore-parasitoid networks exhibit a compartmentalized structure?**

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, standardizing them 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 value *Q*. This modularity value is calculated as,

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where *m* is half the total number of observed links in the network, *Aij* is the weighted, bipartite matrix and *Kij* is the matrix of expected weights (Dormann & 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).

**Which processes drive the dissimilarity in network structure among different compartments?**

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 is amenable for both qualitative (i.e., presence/absence data) and quantitative networks given that an appropriate dissimilarity index is chosen (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 determine this compartmentalized structure?**

*I need to make more progress on the results section for this before I write up the methods. This is where I think I should focus on variation in susceptibility to different galling insects.*

**Results**

**Do plant genotype-herbivore-parasitoid networks exhibit a compartmentalized structure?**

We found that our willow genotype-herbivore-parasitoid network structure was subdivided into five different compartments (*Q* = 0.33, *Z* = 2.41, *P* = 0.008; Fig. 1). The modularity of our observed network was 33% higher than we would expect from a randomized network with the same number of compartments. Moreover, our observed modularity was 18% higher than the average modularity of the networks generated by our null model (*Q* = 0.28 ± 0.02 SD). Each of the five compartments consisted of an average of 5 willow genotypes (range = 1-8), 2 gall species (range = 1-3), and 3 parasitoid species (range = 1-4)(Fig. 1). Three gall species and four parasitoid species were subdivided into distinct compartments. Nevertheless, two of the gall species and four of the parasitoids participated in multiple modules, suggesting that gall susceptibility to different parasitoid species depended on the compartment identity of the willow genotype (Fig. 1).

**Which processes drive the dissimilarity in network structure among different compartments?**

Compartment identity corresponded with strong differences in overall herbivore-parasitoid network structure (*F*4,20 = 9.78, *P* < 0.001; Fig. 2). Specifically, average network dissimilarity among compartments (mean = 0.80 ± 0.09 SD) was 78% higher than within compartments (mean = 0.45 ± 0.24 SD)(Fig. 2). Compartment identity was also associated with differences in species turnover (*F*4,20 = 9.63, *P* < 0.001) and interaction (*F*4,20 = 14.59, *P* = 0.005) components of network dissimilarity. Species turnover, however, was the predominant component, contributing to 82% of the dissimilarity among compartments in overall network structure.

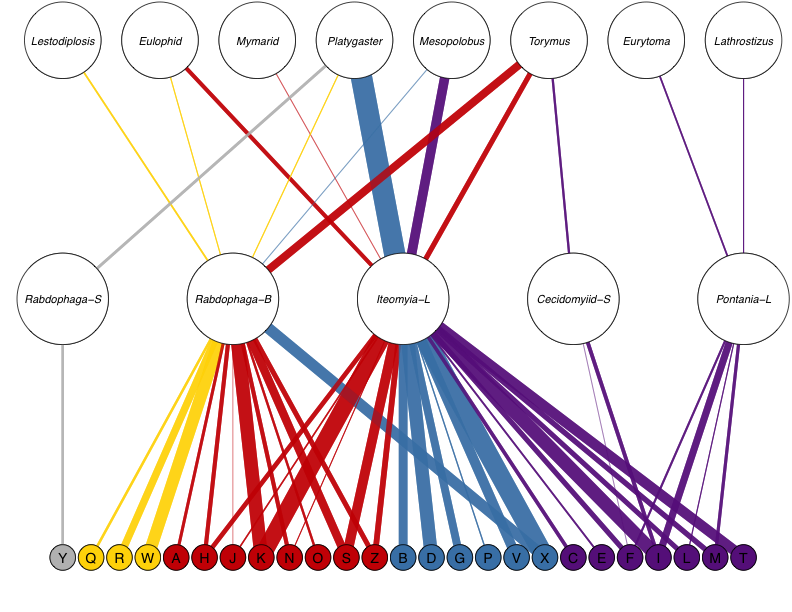
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**Figure Legends**

**Figure 1.** 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 2.** Nonmetric multidimensional scaling (NMDS) ordination of herbivore-parasitoid network dissimilarities among 25 genotypes of *Salix hookeriana* (stress = 0.12). The position of each point corresponds to the herbivore-parasitoid network associated with a particular willow genotype. The shapes and colors of each point correspond to the compartment identity of the willow genotype.

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