**Genetically based interactions of a foundation plant species and an insect drive community interaction network structure through leaf senescence.**

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**Abstract.** Multiple studies have demonstrated that genetic variation in a single species can structure entire communities and tri-trophic interactions; however, few studies have examined evolutionary dynamics in complex ecological networks. Here, we use a long-term experimental common garden to test for the effect that genotypic variation in a foundation species (*Populus angustifolia* James) in its interactions with a common insect (*Pemphigus betae*) can affect a larger community network of arthropods. These findings support the interacting foundation species hypothesis in which the genetics-based interactions of a few highly interactive species can define whole communities of organisms. While other studies have shown such effects on community richness, abundance and composition, here we examine their community networks. Three main results emerged: i) Genotypic variation in leaf senescence contributed to uni-partite (one mode) leaf modifier network structure by altering inter- and intra-tree co-occurrence pattern, ii) leaf senescence also increased the modular (i.e., compartmental) structure of bipartite (two-mode) networks of tree genotype and the leaf modifier community, and iii) senescence of leaves was related to genetically based susceptibility to *P. betae*, which produced dispersion of leaf modifiers. These results demonstrate how genetic variance in foundation plant species can affect the structure of ecological networks though its interactions with a common herbivore. The formation of genotype-species modules mirrors the patterns of network structure observed in species-species networks of plants and herbivores. Together, these findings demonstrate how community-level genetic effects contribute to ecological patterns and provide a potential mechanism for evolutionary dynamics in complex ecosystems.

**Introduction**

Understanding the interplay between ecological and evolutionary dynamics in the context of complex multi-species communities is a major research frontier. There is now a significant body of research in the fields of community genetics and co-evolutionary biology that have shown how genetic variation in a single species can contribute to the composition, diversity and stability of whole communities (Whitham et al. 2006) and how species interactions drive the evolution of biological diversity (Thompson 2009). In addition, multiple studies have demonstrated a genetic basis to tri-trophic interactions (Bailey et al. 2006; Smith et al. 2011), landscape level variation in these interactions (Bernhardsson et al. 2013) and the general importance of Interspecific Indirect Genetic Effects (IIGE; Shuster et al. 2006; Allan et al. 2012). More recently, the application of network theory to ecological and evolutionary research has enabled the study of structure and dynamics beyond isolated species pairs (May 1972; Proulx et al. 2005; Borrett et al. 2014), and phylogenetic analyses of bipartite (i.e., two-mode) plant-mutualist networks (e.g., plants and pollinators or seed dispersers) support the hypothesis that evolutionary dynamics contribute to interaction network structure (Rezende et al. 2007a,b; Rafferty and Ives 2013). Collectively, this work has paved the way for the study of the genetic basis of more complex networks of interactions among multiple interacting species.

Genetic variation in foundation species is likely to contribute to the structure of ecological interaction networks. Genetic variation in plant species, such as cottonwoods (Keith et al. 2010) and evening primrose (Johnson and Agrawal 2005), has been shown to affect insect community composition and stability. In addition, plant genetic similarity has also been shown to affect the similarity of the communities of arthropods associated with cottonwoods (Bangert et al. 2006), eucalypts (Barbour et al. 2009), and epiphytes and invertebrate communities living on the tropical tree, *Brosimum alicastrum* (Zytynska et al. 2011). In parallel, plant-herbivore networks have been observed to form distinct modules of multiple species, which tend to interact more with each other than other species in the community, which is hypothesized as a mechanism for minimizing the negative impacts of bio-trophic interactions (Thébault and Fontaine 2010; Fontaine et al. 2011).

Because genetic variation leads to distinct but overlapping communities of associated species, it is possible that these compositional effects will lead to a modularization of arthropods and other groups of interacting species. To test the overarching hypothesis that plant genetics can fundamentally structure community interaction networks through interacting foundation species, we examine an aphid gall-forming herbivore (*Pemphigus betae*) that previous research has shown to create artificial resource sinks on the leaves of its host tree, *P. angustifolia* (Compson et al. 2011). This, in turn affects a diverse community of insects, fungi, birds and inquilines (Dickson and Whitham 1996). Although the creation of artificial resource sinks has demonstrated advantages for the insect in enhancing resource availability, it can also trigger premature leaf senescence resulting in the death of most or all of the aphids in the gall (Williams and Whitham 1986), and is therefore a potentially important tree trait determining an IIGE. , and because previous research has demonstrated both the community-wide importance and genetic basis to the interaction between *P. angustifolia* and *P. betae* (Bailey et al. 2006; Keith et al. 2010; Smith et al. 2011), as plant-herbivore interaction networks tend to form modular networks (Thebault and Fontaine 2010),

Here, we use a long-term experimental common garden with trees of known genotype to explore how genetic variation in a foundation species can alter the interaction network of an associated community of leaf modifying arthropods in part through a genetically based early leaf senescence response to *P. betae*. We hypothesize that genetically based variation in traits (e.g., sink-source relationships and premature leaf senescence) among *P. angustifolia* genotypes plays a fundamental role in defining the structure of the network of an associated community. We analyze both the unipartite (one-mode) of leaf modifiers (i.e., ???) and the bipartite (two-mode) network structure between tree genotype and leaf modifiers to test the hypothesis that early leaf senescence will alter interaction network structure relative to healthy leaves and that both will be genotype dependent. Specifically, we predict the structure of the unipartite and bipartite networks differ between senescing and healthy leaves and that bipartite network modularity will increase as a result of leaf senescence. Discerning the genetic basis to interaction network structure has important implications for evolutionary and ecological dynamics in natural ecosystems.

**Methods**

*Study System and Leaf Modifier Observations*

The common garden was planted along the Weber River, UT (USA), which is a cottonwood (*Populus* spp.) dominated riparian ecosystem. Asexually propagated clones of *P. angustifolia* individualscollected from wild stands were planted randomly in a single field at the base of Weber Canyon, Utah (GPS: 41.132805, -111.902227) in 1989 to isolate the effect of tree genotype from the effect of localized microenvironment conditions. All trees were verified to be nearly pure *P. angustifolia* and categorized into genotypes using co-dominant, RFLP markers (Martinsen et al. 2001).

Leaves for modifier community observations were collected from individual trees on August 7-9, 2012. We chose 4-5 replicate trees from each of 7 genotypes randomly within the common garden. Leaves were classified as either senescing or healthy based on color with senescing leaves being almost entirely chlorotic and yellow indicating significant re-allocation of resources as compared to green, “healthy” leaves. All leaves were collected between 1 and 2 m height primarily within ~0.25 m depth from the outer edge of the canopy around the entire tree, both of which were done in order to minimize the influence ontogenetic and micro-environmental effects on the leaf modifier community. For healthy leaves, 20 shoots between 1 and 2 years of age based on bud scarring were collected and 50 leaves were selected randomly from among these shoots. Because senescing leaves were generally at lower densities spread among green leaves, we conducted timed sampling (5 min.) of senescing leaves from the same region of the canopy as the green, healthy leaves. Thus, different proportions of healthy and senescing leaves were sampled and in order to account for different sampling intensities, all analyses are conducted on percent or relativized values.

Leaf modifiers were categorized based on morphology and known species in the garden and followed the methods of Bangert et al. (2008), and the presence of each modifier was assessed on individual leaves. Leaf modifiers were predominantly herbivores. We also observed a predator or *P. betae* and two leaf modifier categories, aborted *P. betae* and aborted *Phylacolpa* sp., were included in the dataset. Although these two leaf modifiers are morphological variants of the same species, they represent a change in the interaction between these two species and *P. angustifolia*. In addition, their abundances were generally low and did not largely contribute to network structure (see Fig. 1). Counts of the total number of galls of *P. betae* were also quantified for each leaf.

*Network Modeling and Structural Analyses*

Uni-partite networks were modeled using the methods of Araujo et al. (2011). This method uses an analytically based null model of pairwise co-occurrence patterns to test for significant, non-random pairwise patterns between species. We also performed an overall test of the significance of uni-partite network structure with a null model based co-occurrence analysis that employed a conservative randomization algorithm that constrained marginal totals (i.e., leaf modifier abundances and total number of individuals on trees) for the presence of each leaf modifier at the scale of the entire tree. We used Stone and Robert’s (1990) average checkerboard score, C-Score, and 5000 simulated null communities to generate a p-value and Standardized Effect Size (SES = ), and, thus, lower values of SES indicate lower checkerboards or dispersion of species pairs than expected under the null model (Gotelli 2001).

The contribution of intra-tree patterns to network structure was assessed in two ways. First, SES values were quantified using the leaf scale observations of occurrences of leaf modifiers for each tree, which were then used in the analyses of the effect of tree genotype. Second, as both patterns operating simultaneously could generate SES values that would be near zero even though leaf modifiers are exhibiting strong co-occurrence patterns, we conducted a Multivariate Co-occurrence Analysis (MVCA; Lau 2014), which separated out the patterns of clustering and dispersion of leaf modifiers on individual trees. To do this, the co-occurrences and checkers (i.e., one species occurs in the absence of another) for all unique species pairs were counted and relativized to the maximum count for each pair. The relativization was performed to reduce the effect of highly abundant species pairs.

A bipartite (i.e., two-mode) network approach was also used to analyze the effect of genetic variation of the interaction between the foundation species and the leaf modifying community. Genotypic averages of leaf modifiers were used to generate a tree genotype, leaf modifier interaction network for the leaf modifier community on both senescing and healthy leaves. These averages were then relativized by the maximum observation for each leaf modifier and are used here to estimate the expected relative frequency of interaction between each genotype with each leaf modifier. We then quantified the modularity, which measures the degree to which the network is comprised of groups of nodes, for both of these networks using the methods of Newman (2004).

*Statistical Tests and Software*

In addition to the co-occurrence based analyses described above we conducted statistical analyses of both univariate and multivariate data. Restricted Area Maximum Likelihood (REML) was conducted to test for the effect of tree genotype on single variables. *Pemphigus betae* count data were square root transformed to conform prior to analyses. We used Permutation Analysis of Variance (PerMANVOA; Anderson 2001) to test for the effect of genotype and leaf type on leaf modifier composition and the co-occurrence patterns for the MVCA. Mantel tests were performed to compare the similarity between both unipartite and bipartite networks. We used Bray-Curtis dissimilarity for distance-based analyses. For both the PerMANOVA and Mantel tests we used 5000 permutations of the data to generate the empirical test statistic distribution. Senescing and healthy leaves were collected from the same trees in a paired structure that needed to be accounted for statistically. For univariate variables, we analyzed the difference between healthy and senescing leaves. For the PerMANOVA where tree type was included as a factor in the analysis, the permutation was stratified in order to account for the non-independence of healthy and senescent samples from the same tree. All analyses were performed in R version 3.0.2 (R Development Core Team 2014) using the *vegan*, *ecodist* and *bipartite* packages.

**Results**

*Genotypic variation in leaf senescence contributes to network structure*

Leaf modifiers displayed significant network structure that was directed by genotypic variation in leaf senescence. Stand level (i.e., inter-tree) co-occurrence patterns of leaf modifiers were significantly clustered on both healthy (SES = -4.71, p = 0.021) and senescent (SES = -3.71, p = 0.0006) leaves. The structure of the healthy and senescent leaf modifier networks (Fig. 1) showed non-significant multivariate correlation (Mantel R = 0.04, p = 0.61). Both genotype and leaf type (i.e., senescing or healthy) affected composition, but the interaction was not significant (Table 1).

Genotypes differed in the effect of leaf senescence on intra-tree leaf modifier co-occurrence patterns. Genotypes did not differ in the effect of senescence on SES values for leaf modifiers at the scale of individual leaves (X2=1.84 p=0.17); however, the MVCP analysis revealed that this was due to contrasting co-occurrence patterns among pairs of species. The effect of leaf senescence was significant for both leaf modifier co-occurrences (i.e., clustering) and checkers (i.e., dispersion), while tree genotype only had a significant effect on leaf modifier checkers (Table 2), suggesting that genotypes primarily differed in the dispersive effect of senescence on leaf modifiers.

Genotypic variation contributed to bipartite network structure that shifted as a result of leaf senescence. The senescent leaf bipartite network displayed low similarity to the healthy bipartite network (Mantel R = -0.11, p = 0.537), i.e., they were very different in the interation networks they supported. A major structural difference was that modularity increased from senescent (0.17) to healthy (0.23) leaves with several genotypes shifting their module membership as a result of senescence (Fig. 2).

*Genetically based susceptibility to P. betae is linked to leaf senescence*

Early senescence of leaves was driven by genetically based susceptibility to *P. betae*.Leaf mod percent leaves colonized was 32% lower on healthy leaves (REML: *X*2 = 3.97, p = 0.046). Genotype was a significant predictor of the difference between percent leaves colonized leaves (REML: *X*2 = 4.42, p = 0.035), percent of leaves with single (*X*2=3.77, p = 0.052) and double (*X*2=5.38, p = 0.020) *P. betae* galls (Fig. 3).

**Discussion**

**I think there are several issues that are best developed in separate subsections. Are these the main issues you see? Others?**

1. **Mechanistic basis of an interacting foundations species hypothesis –** sink-source relationships and premature leaf abscission
2. **Genetics of network structure -**
3. **Senescence relationships with community succession –** our findings show how the presence of one organisms can change the quality of the resource for subsequent organisms and how this relates to larger scales of succession in which foundation plant species in early succession create the conditions for subsequent communities via their modification of the soil environment and other aspects that favor a new community.

These results support our interacting foundation species hypothesis Keith et al. 2010; Busby et al. 2014), in which the genetics-based interactions of two or more species can define the richness, abundance and composition of a much larger community. Our findings expand upon their findings to include the structure of a network of associated species. This extension is important because richness, abundance and composition are descriptive statistics, but do not directly address the potential underlying mechanisms. Our mechanistic, genetics-based network analysis is based upon species interactions and the underlying sink-source/induced premature leaf abscission mechanisms that can drive these higher order patterns. Three lines of evidence support this conclusion. First, genotype and leaf senescence influenced the co-occurrence patterns of leaf modifiers both within and among trees with the senescence of leaves leading to greater dispersion of leaf modifiers within trees. Second, the tree genotypes and leaf modifier bipartite network structure showed low similarity between senescing and healthy leaves with the healthy network exhibiting a higher level of modularity. Third, genotypes varied in their senescence response to *P. betae*. Together these results indicate that the tree trait, leaf senescence, is genetically based and contributes to the structure of a leaf modifier interaction network.

The finding of a modular structure in a genotype-leaf modifier network mirrors research in at the higher scale of plant-herbivore networks. Thebault and Fontaine (2010) compared two sets of ecological interaction networks, plant-mutualists and plant-herbivores, and found that plant-herbivore networks tended to be modular in contrast to the nested structure more often found in plant-mutualist networks (Bascompte et al. 2006). They hypothesized that this modular structure diminishes the fitness impacts of biotrophy imposed by the herbivores by breaking up herbivore species into groups.

Similarly, we found that genetically based variation in senescence contributed to the formation of modules, most likely as a response to the negative impacts of biotrophy from leaf modifiers and *P. betae* in particular. Previous research by Williams and Whitham (1986) found that the number of galls on a single leaf increased the probability of abscission and leaf galling reduced the chlorophyll content of leaves. In further support of early abscission being a response of the tree to the parasitic effects of the aphid at the scale of individual leaves, Compson et al. (2011) traced the movement of carbon isotopes into galled leaves *P. angustifolia* from non-galled leaves on adjacent shoots.

Foundation species are defined by their influence on a large proportion of the community by modulating resources and creating locally stabile conditions (Ellison et al. 2005). It is well established that trait variation with a genetic basis determines interactions between species (Wade 2007). We have shown here that the genetically based effect of leaf senescence increases the dispersion of leaf modifiers, and thus alters the potential rates of interactions among leaf modifiers by decreasing the probability that two or more leaf modifiers will occur on the same leaf. Thus, the interaction between *P. angustifolia* and *P. betae* is amplifying the IIGE to a broader swath of leaf modifier community with the potential to affect higher trophic levels, as we have already observed here with the decrease of the interaction between *P. betae* and its predator (Fig. 1; LM4 = *Phoridae*).

The IIGE amplification through the leaf modifier interaction network also has the potential to contribute to a feedback loop via its impacts on leaf decomposition and soil nutrient levels. Schweitzer et al. (2005) found that the soil microbial community and decomposition rates under *Populus* *angustifolia* varied among genotypes. This study also found that the microbial community composition also shifted in response to tree genetics. Because of the genetic basis of leaf senescence and the potential effects that early senescence of leaves can have on nutrient content of leaves, the IIGE on the leaf modifier community, which by definition alters the shape of the leaves, is likely contribute to an established plant-soil feedback (Smith et al. 2011; Pregitzer et al. 2013), and potentially contribute to variation in tree fitness and the evolution of interaction network structure as a result of shifting *P. angustifolia* genotypic frequencies.

The genetic basis of network structure provides a useful perspective for understanding evolutionary dynamics in complex ecosystems, especially in the context of changing environments. Climate change is a prime example, as it is predicted to induce strong ecological and evolutionary shifts in terrestrial ecosystems (Parmesan 2006), and phenological traits of tree species, including senescence related traits such as leaf coloring, are already exhibiting strong relationships with shifting climatic conditions (Way 2011). As we have demonstrated a genetic basis to he interaction network structure, climate change driven selection on phenological traits in foundation species is likely to have strong community-wide impacts via networks of interacting species through direct and indirect interactions and feedbacks. In particular, resolving the structure of bipartite genotype-species networks provides a useful framework with which to resolve potential IIGEs in ecosystems and the impacts of selection on foundation species and their associated communities.

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**Tables**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | *df* | *SS* | *MS* | *F* | *R2* | *P* |
| Genotype | 6 | 2.1047 | 0.35078 | 1.9044 | 0.14618 | **0.001** |
| Leaf Type | 1 | 1.856 | 1.85604 | 10.0764 | 0.12891 | **0.001** |
| Interaction | 6 | 1.2274 | 0.20456 | 1.1106 | 0.08525 | 0.317 |
| Residuals | 50 | 9.2099 | 0.1842 |  | 0.63967 |  |
| Total | 63 | 14.398 |  |  | 1 |  |

Table 1. PerMANOVA table showing the significant main effects of *P. angustifolia* genotype and leaf type (senescing or healthy) on the leaf modifier community composition. **Bold** values are statistically significant (< 0.05).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Co-occurrences** | |  |  |  |  |  |
|  | *df* | *SS* | *MS* | *F* | *R2* | *P* |
| Genotype | 6 | 3.0573 | 0.50954 | 1.5246 | 0.13518 | 0.202 |
| Leaf Type | 1 | 0.7211 | 0.72113 | 2.1577 | 0.03189 | **0.022** |
| Interaction | 6 | 2.1263 | 0.35438 | 1.0603 | 0.09402 | 0.444 |
| Residuals | 50 | 16.7109 | 0.33422 |  | 0.73891 |  |
| Total | 63 | 22.6155 |  |  | 1 |  |
|  |  |  |  |  |  |  |
| **Checkers** |  |  |  |  |  |  |
|  | *df* | *SS* | *MS* | *F* | *R2* | *P* |
| Genotype | 6 | 1.6215 | 0.27025 | 1.9324 | 0.145 | **0.001** |
| Leaf Type | 1 | 1.61 | 1.61 | 11.5123 | 0.14397 | **0.001** |
| Interaction | 6 | 0.959 | 0.15983 | 1.1429 | 0.08575 | 0.349 |
| Residuals | 50 | 6.9925 | 0.13985 |  | 0.62528 |  |
| Total | 63 | 11.183 |  |  | 1 |  |

Table 2. PerMANOVA tables for the multivariate co-occurrences and checkerboards for all unique species pairs. **Bold** values are statistically significant (< 0.05).

|  |  |
| --- | --- |
| Label | Leaf Modifier |
| LM1 | Leaf edge herbivory |
| LM2 | Scraper |
| LM3 | Leaf cutter |
| LM4 | *P. betae* predator (*Phoridae*) |
| LM5 | Aborted *P. betae* lesion |
| LM6 | Intra-leaf herbivory |
| LM7 | Thysanoptera |
| LM8 | *Anacampsis niveopulvella* |
| LM9 | *Cicadellidae* |
| LM10 | tip.miner = *Geometridae* 1 |
| LM11 | edge.miner = *Geometridae* 2 |
| LM12 | mid.miner = *Gemoetridae* 3 |
| LM13 | Aborted *Phylacolpa* |
| LM14 | *Phylacolpa* |
| LM15 | *P. betae* |

Table 3. Leaf modifiers associated with labels used in figures.

**Figure Legends**

Figure 1. Unipartite network plots showing significant pairwise leaf scale occurrences of the leaf modifying species (see Table 3) found on the senescing and healthy leaves of *P. angustifolia*. Nodes show each leaf modifier scaled by the log of its total abundance and edges show the log of the frequency of occurrence. Note that node positions are the same for all species between the two networks and only species with at least one significant edge in either network are plotted here.

Figure 2. Bipartite networks for the senescing and healthy leaves comprised of tree genotype (left mode) and leaf modifier (right mode). Nodes are scaled by the marginal totals for genotypes and leaf modifiers and edges are scaled by the mean relative frequency of each leaf modifier on each genotype. Node color indicates module membership, which was computed separately for each network.

Figure 3. Barplot of the mean (+- 1 SE) percent differences in *P. betae* gall density across all leaves and on individual leaves for each genotype. Genotypes varied significantly in their senescence effects (Healthy – Senescent) on *P. betae* up to a gall density of two galls per leaf (i.e., double galls).

Figure 1

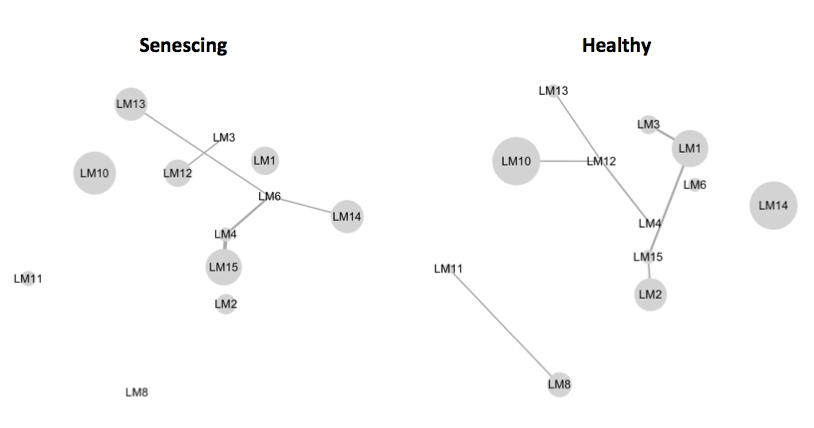


Figure 2.

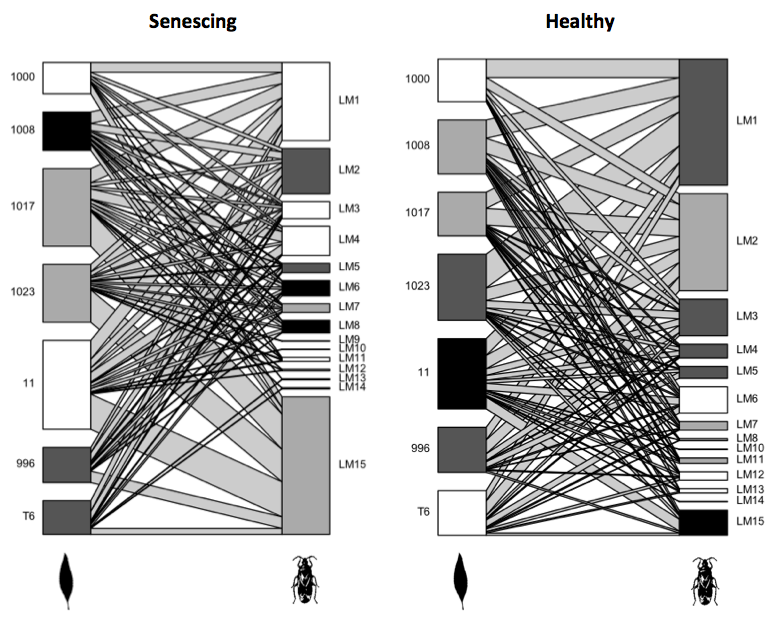


Figure 3

