**Title:** Genetic variation in a foundation tree species drives the ecological network structure of a dependent community.

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**Introduction**

* + Understanding the influence of genetic variation on the interactions of associated community members
  + Interaction network modeling can be used to understand complex relationships among species, building on the co-occurrence literature
  + Two scales of co-occurrence, stand = sorting (patterns among trees) and tree = interactions (patterns within a tree)
  + Three sources of variation (age, microsite, phenotype)
  + Epiphytic lichen are an ideal model system
  + Previous work has shown compositional affects of plant genotype

As future ecosystems are predicted to undergo major shifts in response to climate change, it is imperative to resolve the evolutionary dynamics of species interactions in the context of complex networks. In ecological communities plant population dynamics depend on animal herbivore, pollinator and seed disperser interaction networks (Jordano et al. 2006, Schupp and Fuentes 1995, Fontaine et al. 2011). At the scale of ecosystems, trophic networks or food webs direct and control the rates of energy and nutrient flux (DeAngelis et al. 1989).

Recent work has shown genetic variation in plant species contributes to variation in interactions with dependent communities. A large body of work on plant associated arthropod communities has shown that genetic variation in a can influence community composition, diversity and stability (e.g., Wimp et al. 2005, Crutsinger et al. 2006, Keith et al. 2010); and, of particular relevance to plant-pollinator networks ecology, Burkle et al. (2013) found that floral visitation varied significantly among genotypes of *Solidago altissima.* In addition to arthropods, research in other systems, such as plant-plant (Lamit et al. 2011) and plant-fungal (Barbour et al. 2009, Busby et al. 2013), have also demonstrated community level impacts of genetic variation in dominant plant species.

Although these studies have demonstrated the effect of genetic variation on interactions between plants and their associated species, few studies have expanded our understanding of the effect of genetics on the complex interactions within these communities. Bailey et al. (2006) showed that the interactions between three species across three trophic levels depended on cottonwood (*Populus* spp.) genotype.

In order to better understand how genetic variation can contribute to interactions among species, we used the epiphytic lichen community associated with *Populus angustifolia* (narrowleaf cottonwood), as it is comprised of species that live in close association with an individual tree and can be easily identified in the field. Previous studies in this system have shown that the composition of this community varies in response to tree genetic variation (Lamit et al. 2013). Building on these findings, we set out to determine if genetic variation in a foundation tree species can contribute to predictable variation in the interactions among associated species. To do this we modeled species dependency networks (Araujo et al. 2011) and quantified the co-occurrence patterns on individual trees of *P. angustifolia*both in a wild stand and a common garden with trees of known genetics. We hypothesized that stand level structure could arise from three main sources of variation: 1) substrate (i.e. tree) age, 2) direct and indirect influence of variation among trees and 3) genetically based variation in tree traits. As heritable variation is the raw material for natural selection, a genetic basis for interaction network structure has implications for the evolution of complex interactions among species.

**Methods**

*Study System and Sampling*

The study was conducted along the Weber River, UT (USA). The Weber River is cottonwood (*Populus* spp.) dominated riparian ecosystem. There are two native species *Populus angustifolia* (James) and *Populus fremontii* (S. Watson), which are both widely distributed throughout western North America and known to hybridize (Eckenwalder 1996). For the purposes of this study, only pure or highly advanced backcrosses of *P.* *angustifolia* were sampled in order to avoid the effect of the hybridization between these two species.

A stand of *P. angustifolia* near the city of Uintah, UT (GPS: N41.13903, W110.94400) was used for the wild stand survey. A total of 14 trees were chosen haphazardly over a 0.10 km2 area with a minimal distance of 5.56 m between trees. Only trees with *P. angustiolfia* morphology were sampled. All trees were geospatially referenced using an average of 10 points taken with a Garman Handheld GPS using (Garman, Inc.).

A common garden setting was used to isolate the effect of tree genotype from the effect of the localized micro-environment associated with each individual and spatial autocorrelation. Asexually propagated clones of genotyped *P. angustifolia* individuals (Martinsen et al. 2001) were obtained from wild collections and planted randomly in a single field (5.2 km2) at the Ogden Nature Center, Ogden, UT in 199?. Although some replanting of replicates occurred, the trees in the garden are either the same age or within three years of each other. A total of thirteen genotypes replicated between 3 and 8 times each, were chosen for sampling.

*Lichen Co-occurrence and Tree Trait Sampling*

To establish co-occurrence patterns among the lichen species, fine scale distribution data were collected for epiphytic lichen associated with *P. angustifolia* in both the wild stand in May 2012 and the common garden in October 2010 and May 2011. Previous work in this system has shown that there is a total of fourteen species, *Xanthomendoza galericulata, X. montana, Caloplaca holocarpa, Candelariella subdeflexa, Rinodina glauca, Lecanora hagenii, Lecanora* (unknown species)*, Phyciella melanchra, Physcia adscendens, Physcia undulata, Phaeophyscia orbicularis, Phaeophyscia ciliata, Melanelia subolivacea, Meanelia elegantula*, including both crustose and foliose lichen species (Lamit et al. 2013). All of these species could be identified in the field with the exception of *Physica* and *Physciella* species, as distinguishing these species was difficult in the field due to the often small thallus size. Given this and the general tendency for these species to function similariy, we combined them into a generic category, which we designated Phys. On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm2 cells arrayed in a checkerboard pattern inside each of two adjacent 10 cm by 10 cm quadrats. centered at 50 cm and 85 cm from ground level. The checkerboard sampling pattern was chosen to isolate each cell based on an average thallus size of 1 cm2. Samples were restricted to the northern aspect of the trunk to maximize the abundance of lichen and control for the effect of aspect.

The bark roughness of each tree was observed within the same quadrats used to sample the co-occurrence patterns on each tree in both the wild stand and the common garden. Rough bark was defined as any disruption of the outermost layer of tissue, which in early stages of growth is typically smooth and white (Lamit et al. 2011). The presence of rough bark was visually assessed within every 1 cm2 cell (100 cm2 total). Thus, the percent of rough bark was equal to the total number of cells containing rough bark.

In the wild stand, two additional tree traits were observed: canopy cover and tree age. Percent canopy cover was quantified using a spherical densiometer (Forestry Suppliers, Inc.). Densiometer readings were taken at 1 m distance from the trunk on the northern side of each tree. Tree age data was obtained from Lamit et al. 2013. Briefly, tree cores were taken at a height of 1 m using a increment borer (Forestry Suppliers, Inc.). Annual growth rings were counted at 200X magnification using a dissection microscope (Zeiss).

*Network and Null-Model Based Co-occurrence Modeling*

Stand level dependence network models were generated for both the wild stand and the garden using the methods of Araujo et al. (2011). This method uses conditional probability to compare the observed to the expected frequency of co-occurrences between all pairs of species. The method produces a matrix of statistically significant dependency values for each dataset. Here it is important to note that each dependency value is the probability of co-occurrence relative to the probability of ooccurrence across the entire sample, and, therefore, it can produce two different values for each species pair. The result is a matrix that defines a network of significant relationships between species based on the degree to which they occur with or without another species.

To explore the tree level patterns that contribute to the stand level network structure, we conducted null-model based co-occurrence analysis. We used Stone and Roberts (1991) C-score (*C-Score* = , where *r* is the total number of occurrences for a species and *S* is the number of co-occurrences of species *i* and *j* for all *n* species). A Standardized Effect Size (*SES* = ) was then calculated for each tree by standardizing the observed C-score with C-scores for co-occurrence matrices generated by permuting the matrices so that species totals were preserved (Gotelli 2002). Thus, the SES is a measure of the observed co-occurrence patterns relative to co-occurrence patterns due to chance.

*Statistical Analyses and Software*

We used a combination of univariate and multivariate statistical tests. A correlation test with Pearson’s r was used to test for the correlation between the wild and common garden networks. Linear regression and Analysis of Variance (ANOVA) were used to test for the effects of tree age, canopy cover, bark roughness and genotype on SES values. A permutation based Mantel Test was used to test for the effect of spatial distance on SES values (Mantel 1957). A vector analysis was used to assess the relationship between community composition and SES values in the common garden. Last, to resolve the indirect effect of tree age on SES via increasing bark roughness with tree age, we built, fit and tested a Structural Equation Model (SEM) (Grace 2006) with direct effects only from tree age to percent rough bark and from percent rough bark to SES, and the direct effect of tree age on SES was not included in the model.

All modeling and analyses were conducted using the R statistical programming language (v3.0, R Development Core Team 2013). The methods of Araujo et al. (2011) were implemented *de novo* (see Supplementary Materials) and the *sna* package was used for network analyses and plotting (Butts et al. 2010). The *vegan* package was used for all co-occurrence modeling and multivariate analyses. To conduct the SEM we used the model fitting and test procedures in the *sem* package (Fox et al. 2013).

**Results**

The lichen community exhibited network structure that was similar between the wild stand and the common garden. Significant species dependency network structure was observed for both the wild stand and the common garden (Fig. 1a and b). Dependency values that were non-zero in either network were highly correlated (r = 0.64, P << 0.0001; Fig. 1c). The most notable difference between the two networks was the increase chance in the connections of *Lecanora* sp. (Fig. 1d). correlated

In the wild, roughness was the primary driver of network structure, age indirectly influenced network structure through roughness and microsite did not contribute to network structure.

In the garden, tree genotype was the main driver of network structure.

**Discussion**

* + Hypothesis of genetics supported
  + Network patterns
  + Community assembly
  + Genetics of interaction networks
  + Implications and Future Research

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Summary:

* Intro
  + Understanding the influence of genetic variation on the interactions of associated community members
  + Interaction network modeling can be used to understand complex relationships among species, building on the co-occurrence literature
  + Two scales of co-occurrence, stand = sorting (patterns among trees) and tree = interactions (patterns within a tree)
  + Three sources of variation (age, microsite, phenotype)
  + Epiphytic lichen are an ideal model system
  + Previous work has shown compositional affects of plant genotype
  + Two main questions:

1. Do lichen show significant patterns co-occurrence network structure?
2. How does tree genetics influence the network structure?
   1. Substrate age
   2. Microsite (spatial autocorrelation)
   3. Genetically based traits

* Methods
  + Site Description
  + Garden Description
  + Co-occurrence sampling
  + Lichen identification
  + Tree age, microsite, tree traits
  + Network and co-occurrence modeling
  + SEM
  + Statistical analyses
* Results
  + The wild and garden stands showed significant co-occurrence network structure that was highly correlated.
  + In the wild, roughness was the primary driver of network structure, age indirectly influenced network structure through roughness and microsite did not contribute to network structure
  + In the garden, tree genotype was the main driver of network structure.
* Discussion
  + Hypothesis of genetics supported
  + Network patterns
  + Community assembly
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  + Implications and Future Research