**Foundation species genotypic variation generates ecological network structure**

Biological evolution occurs in the context of complex networks of interacting species. Ecosystems are predicted to undergo both ecological and evolutionary changes in response to environmental change, such as shifting climate (Parmesan 2006). Understanding how evolutionary processes, such as natural selection, will affect the structure of ecological networks is essential for understanding ecosystem dynamics. However, no study has yet demonstrated a genetic basis to ecological network structure, which is the raw material that natural selection acts on. Here, we use both a long-term experimental common garden with genotyped individuals and a natural riparian forest of the foundation tree species, *Populus angustifolia*, to test how genetic variation contributes to the interaction network structure of a model community comprised of epiphytic lichens. We found three main results: 1) lichen communities showed significant unipartite (i.e., one mode) network structure that was similar between the common garden and natural stand, 2) genotype significantly influenced lichen species interactions within an individual tree also showing strong correlation with bark roughness, a genetically based trait known to influence bark lichen communities, and 3) bipartite (two mode) genotype-species networks, comprised of the foundation species and its associated lichen community, showed significant modular structure in both the common garden and natural stand. These results demonstrate strong support for a genetic basis to ecological network structure and the potential for selection to act in complex ecosystems. This work sets the stage for studies that address greater complexity in the evolution of biological systems and provides a framework for the discovery of evolutionarily dynamic compartments in ecosystems.

Genetic variation within species is known to be an important factor determining species interactions (e.g., Wimp et al. 2005, Crutsinger et al. 2006, Keith et al. 2010). 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. 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). Community genetics studies (Whitham barrage) have shown that genetic variation in foundation species (species that define a the composition of communities by creating locally stabile conditions and modulating resources; Ellison et al. 2005) contributes to variation in interactions with dependent communities in both terrestrial and aquatic ecosystems (Whitham, Barbour, Eel Grass, Bailey et al. 2009). These studies have primarily focused on direct interactions with a single species and have not explored more complex interactions beyond tri-trophic interactions (Smith et al. 2011), while the study of ecological networks has produced significant advances in the understanding of species interactions in complex ecosystems (MacArthur 1955; May 1972; Ulanowicz 1981; Patten and Higashi 1991). Also, studies of phylogenetic patterns in ecological networks have provided support for co-evolutionary dynamics in species interactions (Rezende et al. 2007; Rafferty and Ives 2013). As selection occurs in complex systems of many interacting species where indirect effects can play important roles (Bondavalli and Ulanowicz, Kaileen Mooney), a genetic basis of complex interaction networks is essential to our understanding of evolution in species rich ecosystems; however, no studies have yet studied the impact of genetic variation, which is integral to the process of evolution by natural selection, on network structure.

Here, we investigate how genetic variation in a foundation species determines the structure of interactions among species. Using a long-term (20 years+), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *P. angustifolia.* We focused on a model community of epiphytic lichen as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (Zytynksa et al. 2012; Lamit et al. 2014) and interactions among lichen species could be assessed directly on individual trees. In both the experimental garden and the natural stand, we discovered that genotypic variation in a *P. angustifolia* predictably influenced the structure of a multi-species interaction network and contributed to the formation of evolutionary modules comprised of tree genotypes and lichen species.

We observed significant unipartite (one-mode) network structure (Araujo et al. 2011) in the lichen species interaction networks that was similar between the experimental garden and the natural stand (Fig. 1a and 1b; Garden: z = -6.31, p = 0.0002; Natural: obs = 1.08, SES = -3.15, p = 0.002). The two networks displayed high multivariate structural similarity (Mantel R = 0.51, p = 0.029). Node level Eigen-centrality (Borrett CITE) showed strong correlation between the two stands (Fig. 1c; r = 0.7, t = 2.6135, df = 7, p = 0.035). Centrality was also highly correlated with total abundance in both networks (Fig. 1d; Garden: r = 0.77, t = 3.2427, df = 7, p = 0.014; Natural: r = 0.86, t = 4.43, df = 7, p = 0.003).

In the common garden, where the effect of environmental variation was controlled, genotype was an important factor contributing to network structure. Genotype was a significant predictor of the structure interactions on individual trees (Fig. 2a; F = 3.4213, num df = 12.000, denom df = 14.668, p-value = 0.01426). Similar to the effect of a genetically controlled trait (bark roughness) on lichen community composition (Lamit et al. 2011; Lamit et al. 2014), we found that tree individuals with similar levels of bark roughness had similar levels of lichen interactions (Fig. 2a; Mantel R = 0.08, p = 0.013), which was similar to patterns observed between bark roughness and lichen interactions in the natural stands (Fig 2b: ????)

We also examined how *P. angustifolia* genotypic differentiation might contribute to the formation of groups of tree genotypes and lichen species. Using a bipartite (two-mode) network approach in which genotype-species networks were modeled using the species maximum relativized values of each lichen species on *P. angustifolia* genotypes, we found significant modularity in the common garden stand (Fig. 3a; z = 9.64, p < 0.001). When using a similar approach in which relativized lichen abundances on individual trees in the natural stand were used, we also found significant modularity (Fig. 3b; z = 7.42, p < 0.001). Nestedness of both of these networks was significantly lower than expected under a null model (Garden: z = -2.30, p < 0.001; Natural Stand: z = -2.84, p < 0.001), most likely as a result of module formation.

These findings support the hypothesis that genotypic variation in a foundation species can contribute to the structure of a network of interacting species. Several lines of evidence support this conclusion. First, the wild stand showed significant interaction network structure (Fig. 1a and b); and both tree genotype and the genetically based tree trait, bark roughness, was a strong predictor of co-occurrence patterns (Fig. 2). Second, the common garden network (Fig. 1b) structure showed a high degree of similarity to the wild stand network structure (Fig. 1c&d). Third, tree genotype was a significant predictor of SES values (Fig. 2a), displaying significant correlation with a genetically linked trait, bark roughness, both in the common garden (Fig. 2a) and in a naturally established stand of trees (Fig. 2b). Thus, just as numerous studies have shown that plant genotype can affect species richness, abundance, diversity, and composition, for the first time this study shown both observationally and experimentally that species interaction networks also have a genetic component.

This study highlights the potential for indirect effects of genetic variation to propagate through networks of interacting species and trophic levels. Genetic effects have been hypothesized to diffuse into ecosystems; however, this overlooks the systems perspective, where positive feedbacks can amplify effects. Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. At the scale of ecosystems, trophic networks or food webs direct and control the rates of energy and nutrient flux (DeAngelis et al. 1989). One important example is Smith et al. (2011), which showed that the interactions among species across three trophic levels depended on cottonwood (*Populus* spp.) genotype. Although this study was done with lichen, these results can be generalized to other groups of organisms, although spatial scale of interactions should be considered. First, sessile organisms associated with a host species, such as coral, barnacles, endophytic fungi, epiphytic plants and intestinal endosymbionts to name a few, are communities with high potential to exhibit similar patterns of intra- and inter-host patterns resulting from host genetics (CITE). Second, communities of species with interactions that operate at a larger spatial scale might be expected to have less response to another species’ genetic variation, as diffuse co-evolutionary theory would predict (CITE). effects.

We have shown how genetic variation can contribute to the structure of a network of interacting species. This has important implications for understanding how networks of species evolve, since genetic variation is the raw material for evolution. These results point to the importance of understanding the community and ecosystem level effects of genetic variation and corroborate previous findings of the importance of plant genetics in terrestrial and aquatic ecosystems. Although this has primarily been from an ecological perspective, network theory provides not only a useful tool for systems level analysis but also a common framework for bridging disciplines. 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. These findings have implications for understanding how other networks that operate entirely or in part under principles similar to biological evolution, such as whole ecosystems.

**Methods Summary**

**Field observations in common garden and natural riparian forest stands.**

Heights and testing for height effect on composition. The study was conducted along the Weber River, UT (USA). The Weber River is cottonwood (*Populus* spp.) dominated riparian ecosystem. Although two native species, *Populus angustifolia* (James) and *Populus fremontii* (S. Watson), occur here and are known to hybridize (Eckenwalder 1996), 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 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 (0.025 km2) at the Ogden Nature Center, Ogden, UT in 1992(?). A total of thirteen genotypes replicated between 3 and 8 times each, were chosen for sampling. Genotype names follow Lamit et al. (2011). The natural 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 randomly over a 0.10 km2 area with a minimal distance of 5.56 m between trees across a range of tree core based ages from 15 to 60 years. Observations were made in the common garden in October 2010 and May 2011 and the wild stand in May 2012.

The bark lichen community in this system is comprised of fourteen species (Lamit et al. 2014); however, only 9 species were observed within our study quadrats. The lichen community included (abbreviations are given for species present in study): Xg = *Xanthomendoza galericulata,* Xm = *X. montana,* Ch = *Caloplaca holocarpa,* Cs = *Candelariella subdeflexa,* Rg = *Rinodina glauca,* Lh = *Lecanora hagenii,* Ls = *Lecanora* (unknown species)*,* Pm = *Phyciella melanchra,* Pa = *Physcia adscendens,* Pu = *Physcia undulata, Phaeophyscia orbicularis, Phaeophyscia ciliata, Melanelia subolivacea, Meanelia elegantula*, including both crustose and foliose lichen species that exhibit low inter-annual variation (Lamit et al. 2013). We were able to rapidly assess lichen interactions by quantifying thalli in closed contact as assessed using 1cm^2 cells. Species accumulation curves showed that communities in the wild and the common garden were thoroughly sampled and with very similar species richness (Supplementary Materials). On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm2 cells arrayed in a checkerboard pattern. Two adjacent 10 cm2 quadrats centered at 50 cm and 85 cm from ground level were sampled. 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 thalli in each cell are expected to be spatially independent of the other cells in the quadrat, but exposed to similar micro-environmental conditions. Bark roughness was measured on each tree following the methods of Lamit et al. (2011).

**Network modeling and analyses.**

We modeled species interaction networks using (Araujo et al. 2011) using the observations of lichen in the 1cm2 cells on individual trees of *P. angustifolia*both in a wild stand and a common garden. To explore the tree level patterns that contribute to the stand level network structure, we used established null-model based co-occurrence analysis using 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 interaction frequency to expected patterns under the null model.

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. All analyses were conducted using the programming language R version 3.0.2 (R Development Core Team 2014).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.

**References**

Robert C. Barbour, Julianne M. O'Reilly-Wapstra, David W. De Little, Gregory J. Jordan, Dorothy A. Steane, Jonathon R. Humphreys, Joseph K. Bailey, Thomas G. Whitham, and Bradley M. Potts A geographic mosaic of genetic variation within a foundation tree species and its community-level consequences Ecology 2009 90:7, 1762-1772

D. L. DeAngelis, P. J. Mulholland, A. V. Palumbo, A. D. Steinman, M. A. Huston and J. W. Elwood *Annual Review of Ecology and Systematics* Vol. 20, (1989), pp. 71-95

Eckenwalder, J.E. (1997) Chapter 1: Systematics and evolution of *Populus*. In Biology of Populus and Its Implication for Management and Conservation. Eds. R.F. Stettler, H.D. Bradshaw, Jr., P.E. Heilman, T.M. Hinckley. NRC Research Press, Ottowa, Ontario, Canada.

Fontaine, C., Guimarães, P. R., Kéfi, S., Loeuille, N., Memmott, J., van der Putten, W. H., van Veen, F. J. F. and Thébault, E. (2011), The ecological and evolutionary implications of merging different types of networks. Ecology Letters, 14: 1170–1181. doi: 10.1111/j.1461-0248.2011.01688.x

Martinsen, G. D.; T. G. Whitham; R. J. Turek and P. Keim (2001) Hybrid populations selectively filter gene introgression between species. *Evolution* 55:1325-1335.

Jordano, P., Bascompte, J. and Olesen, J.M. 2006. The ecological consequences of complex topology and nested structure in pollination webs. In: Waser, N.M. and J. Ollerton (eds.). *Specialization and generalization in plant-pollinator interactions*. University of Chicago Press, EEUU. Pages: 173-199.

Keith, A.R., Bailey, J.K. and T.G. Whitham. **2010**. A genetic basis to community repeatability and stability. *Ecology* 91:3398-3406.

Schupp, E.W., Fuentes M. 1995. Spatial patterns of seed dispersal and the unification of plant population ecology. Ecoscience 2: 267–275.

Wimp, G. M.; G. D. Martinsen; K. D. Floate; R. K. Bangert and T. G. Whitham. **2005**. Plant genetic determinants of arthropod community structure and diversity. *Evolution* 59:61-69.

Supplementary Information line (if any)

Acknowledgements

Author Contributions

Author Information (containing data deposition statement, competing interest declaration and corresponding author line)

Matthew K. Lau1, Louis J. Lamit1, Rikke R. Naesbourg1, Stuart R. Borrett2, Stephen M. Shuster1, Miguel A. Fortuna3 and Thomas G. Whitham1

1Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011-5640, USA. 2Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC, 28403-5915, USA. 3Integrative Ecology Group, Estación Biológica de Doñana, EBD-CSIC, Sevilla, Spain.

Corresponding Author: Matthew K. Lau, Harvard Forest, 324 N Main St, Petersham, MA 01366, Email: mkl48@nau.edu, Phone: 928-523-9138

**Tables**

None.

**Figure Legends**

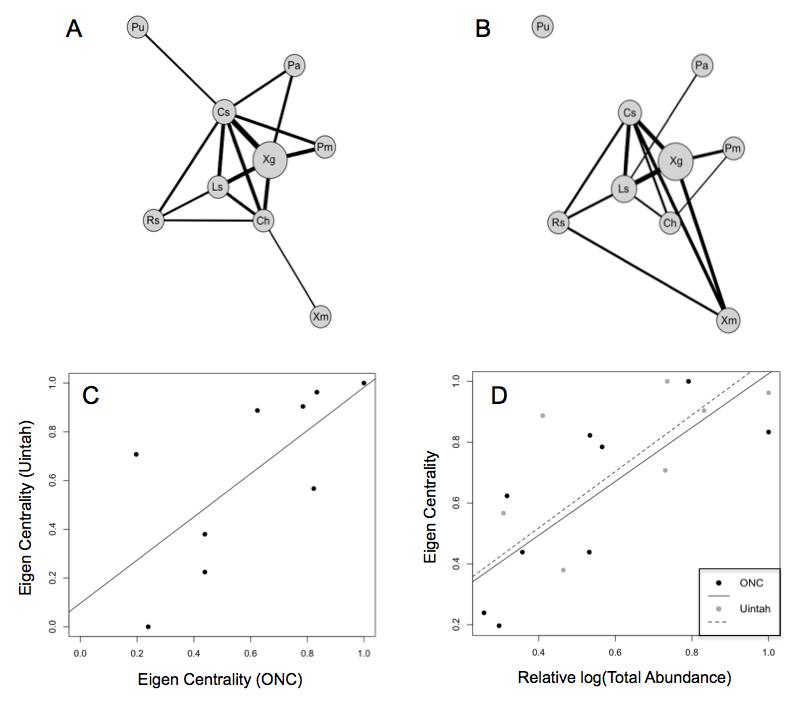
Figure 1. Significant unipartite network structure was observed for epiphytic lichens on trees of known genotype in a common garden (ONC = Ogden Nature Center, Utah, USA) (A) and individual trees in a natural stand (Uintah, Utah, USA) (B) of the foundation species, *Populus angustifolia*. Both networks are shown here with lichen species as nodes (see Methods for species names) scaled by the log of their total abundances and significant co-occurrence patterns between species shown as edges scaled by their log frequencies. The bivariate plot (C) shows the significant correlation in Eigen Centrality between the two networks. (D) The total abundance of lichen species was a significant driver of network structure for both networks.

Figure 2. Tree genotype influenced lichen co-occurrence patterns in the common garden and the natural stand through a genetically controlled tree trait. The lichen co-occurrence patterns were highly correlated with the genetically based phenotypic trait; bark roughness (i.e., the percentage of textured bark), in both the common garden and natural stand. The scatterplot (A) shows the mean (± 1SE) percent rough bark () and SES for each genotype for trees in the common garden with SES values becoming more negative (i.e., species interactions increased), indicating stronger co-occurrence patterns, as bark roughness increases. The lichen communities on individual trees in the Unitah natural stand (B) displayed a similar pattern with the SES values becoming increasingly more negative on trees with more rough bark.

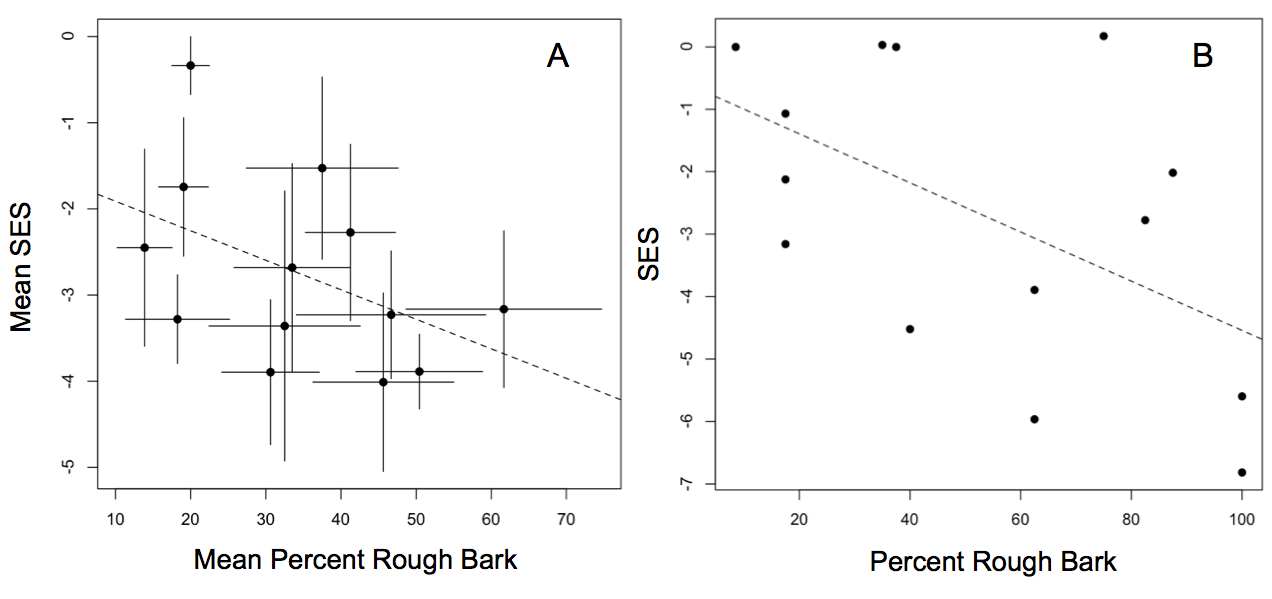
Figure 3. Bipartite networks displayed significant modularity with modules comprised of both genotypes and species. The left most set of nodes shows tree genotypes (see Methods for genotype names) for the common garden (A) or individuals in the natural stand (B) connected to lichen species on the right. Both sets of nodes are scaled by their marginal totals (i.e., total observed individuals for tree nodes and total abundance for lichen species) and arranged by ascending totals from bottom to top. Node color shows the significant module membership for both trees and lichen species with module color having no direct relationship between the two networks, as modules were determined for each network independently.

**Acknowledgments.** We thank funding sources (IGERT, FIBR, ARCS).

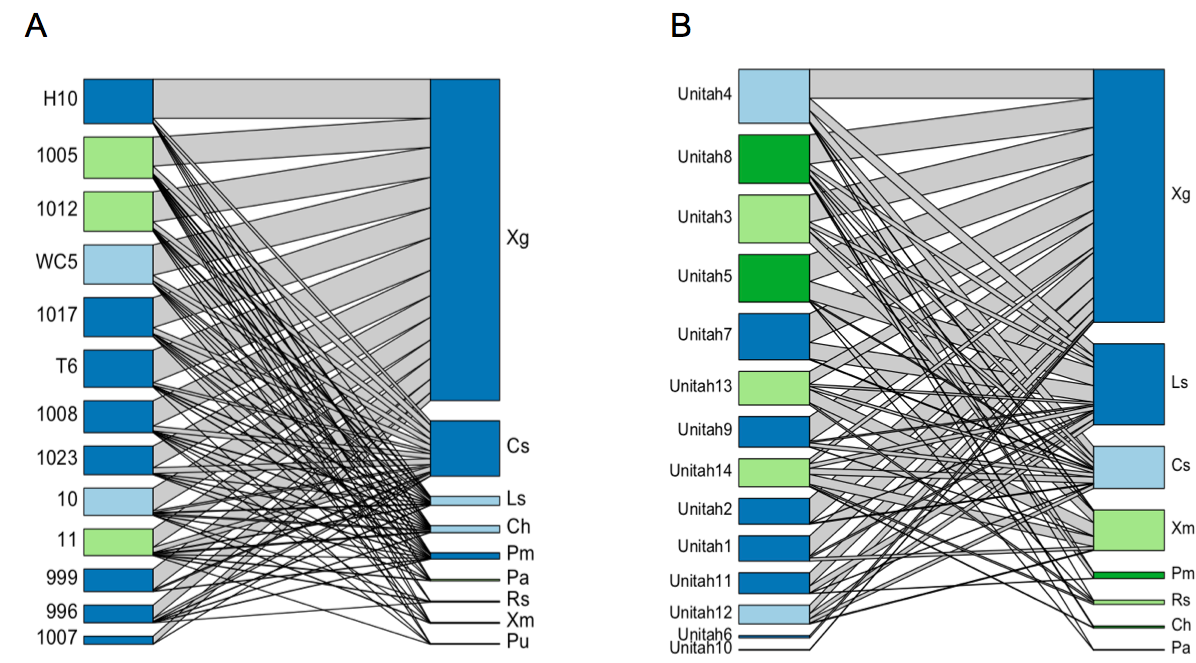
**Figure 1**.



**Figure 2.**



**Figure 3.**

****