Genotypic variation in foundation trees generates ecological network structure

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ABSTRACT

Biological evolution is argued to occur in the context of complex networks of interacting species in which natural selection defines the structure of ecological networks[?, ?, 1–3]. However, fundamental to this evolutionary process is the discovery of a genetic basis to ecological network structure, which remains unknown. Here, we use both a long-term experimental common garden[?] with genotyped individuals and a natural riparian forest of the foundation tree species[4] *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 a natural stand, 2) individual tree genotype significantly influenced lichen species interactions, which was strongly correlated with bark roughness, a genetically based trait known to influence epiphytic lichen[5], 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.

Introduction

Evolution occurs in the context of complex networks of interacting species. In ecological communities, community dynamics depend on key interactions[6] that occur in species interaction networks, such as: trophic[7] and mutualistic[8] interaction networks. Phylogenetic patterns in ecological networks support the importance of evolutionary processes in shaping species interactions[1, 9].

Community genetics studies[10] have shown that genetic variation in foundation species[4] (species, such as trees, that largely define the composition of communities by creating locally stabile conditions and modulating resources; contributes to variation in interactions with dependent communities in both terrestrial and aquatic ecosystems[11]. more specifically, genetic variation affects diverse chemical, phenological, morphological and other traits producing a multivariate phenotype[12] that makes each individual unique so that different communities assemble on different genotypes resulting in different species interaction[13, 14]. although, some empirical studies have shown that genetic variation in a single species can impact a tri-trophic interaction[15]; currently no studies have yet investigated the impact of intraspecific genotypic variation on network structure, which is integral to the process of evolution by natural selection.

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Here, we investigate how genetic variation in a foundation tree species determines the structure of a network 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 9 epiphytic lichens species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation[5, 16]. In addition, the ecology of lichen, with local interactions and slow population turnover rates, allowed us to assess interactions among lichen species rapidly on individual trees.

Methods

Field observations in common garden and natural riparian forest stands

The study was conducted along the Weber River, UT (USA), which is a 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, 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 microenvironment associated with each individual and spatial autocorrelation. Asexually propagated clones of genotyped *P. angustifolia* individuals4 were obtained from wild collections and planted randomly in a single field (0.025 km^2) 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 were previously published[4]. Observations were made in the common garden in October 2010 and May 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. We conducted sampling of the stand in May 2012. A total of 14 trees were chosen randomly over a 0.10 km^2 area with a minimal distance of 5.56 m between trees across a range of tree core based ages from 15 to 60 years.

Bark and Lichen Community Observations

The bark lichen community in this system is comprised of fourteen species; 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[4]. We were able to rapidly assess lichen interactions by quantifying thalli in closed contact as assessed using 1 cm^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 cm^2 cells arrayed in a checkerboard pattern. Two adjacent 10 cm^2 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 cm^2. 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[5].

Network modeling and analyses

We used the observations of lichen in the 1cm^2 cells on individual trees of *P. angustifolia* both in the common garden and the natural stand. Uni-partite networks were generated using an analytical procedure that removes non-significant interactions between species[17]. We used a null model based approach for all other analyses of network structure. A conservative null model that constrained both the row and column marginal totals was used in order to account for the effects of variation in species' total abundances24. From a total of 5000 null matrices, a standardized score was calculated for each statistic ($z = \frac{x_{obs} - \bar{x}_{sim}}{sd_{sim}}$), including the C-score[23], nestedness[24] and modularity[25]. Here, we follow the convention of the co-occurrence literature and

⁶⁶ refer to the standardized C-Score as the Standardized Effect Size (SES)[?].

⁶⁷ A correlation test with Pearson's *r* was used to test for the correlation between the wild and common garden networks. A

⁶⁸ Welch Analysis of Variance (ANOVA), which relaxes the assumption of homogeneity of variance, was used to test for the

⁶⁹ effects of genotype on tree scale SES values. A permutation based Mantel Test was used to test for the effect of bark roughness

⁷⁰ on SES values in the common garden. All analyses were conducted using the programming language R[?].

Results

⁷¹

⁷² In both the experimental garden and the natural stand, we discovered that genotypic variation in a *P. angustifolia* predictably

⁷³ influenced the structure of the lichen species interaction network and contributed to the formation of evolutionary modules

⁷⁴ comprised of tree genotypes and the lichen community. We observed significant unipartite (one-mode) network structure[17] in

⁷⁵ the lichen species interaction networks that was similar between the experimental garden and the natural stand (Fig. 1a and

⁷⁶ 1b; Garden: z = -6.31, p = 0.0002; Natural: z = -3.15, p = 0.002). The two networks displayed high multivariate structural

⁷⁷ similarity (Mantel R = 0.51, p = 0.029). Node level eigen-centrality[18], a measure of species importance that integrates indirect

⁷⁸ connections, showed strong correlation between the two stands (Fig. 1c; r = 0.7, t = 2.6135, df = 7, p = 0.035). Centrality was

⁷⁹ 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

⁸⁰ = 0.86, t = 4.43, df = 7, p = 0.003). In combination, the similarity of both the whole and node level network structure between

⁸¹ the common garden and the wild indicates that the common garden environment captures much of the natural variation that

⁸² exists in nature and accurately reflects natural processes.

⁸³ In the common garden, where the effect of environmental variation was controlled, genotype was an important factor

⁸⁴ contributing to network structure. Genotype was a significant predictor of interactions on individual trees (Fig. 2a; F = 3.4213,

⁸⁵ num df = 12.000, denom df = 14.668, p-value = 0.01426). Similar to the effect of a genetically controlled trait (bark roughness)

⁸⁶ on a dominant lichen[4], we found that individual tree genotypes with similar levels of bark roughness had similar levels of lichen

⁸⁷ interactions (Fig. 2a; Mantel R = 0.08, p = 0.013), which was similar to the correlation observed between bark roughness and

⁸⁸ lichen interactions in the natural stands (Fig 2b: r = -0.53, p = 0.050).

⁸⁹ We also examined how *P. angustifolia* genotypic differentiation contributes to the formation of groups of tree genotypes

⁹⁰ and lichen species and found significant modular structure. Using a bipartite (two-mode) network approach in which genotype-

⁹¹ species networks were modeled using the species maximum relativized values of each lichen species across all *P. angustifolia*

⁹² genotypes, we found significant modularity in the common garden stand (Fig. 3a; z = 9.64, p ¡ 0.001). When using the same

⁹³ analyses on individual trees in the natural stand, we also found significant modularity (Fig. 3b; z = 7.42, p ¡ 0.001). Furthermore,

⁹⁴ nestedness of both of these networks was significantly lower than expected under a null model (Garden: z = -2.30, p ¡ 0.001;

⁹⁵ Natural Stand: z = -2.84, p ¡ 0.001), most likely as a result of module formation.

Discussion

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. 2a). Second, the common garden network (Fig. 1b) structure showed a high degree of similarity to the wild stand network structure (Fig. 1c and 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). Last, both of the bipartite genotype-species networks in the common garden and natural stand displayed significant modularity, suggesting that genotypic variation is leading to the formation of evolutionarily dynamic compartments within the community. Thus, just as numerous studies have shown that plant genotype can affect species richness, abundance, diversity, and composition and previous work has demonstrated that evolutionary processes shape ecological networks[2,3], our study includes genetics in an empirical investigation that combines both experimental common garden findings along with studies in the wild that are in close agreement.

Our results point to the importance of understanding the community level effects of genetic variation and corroborate previous findings of the importance of plant genetics in shaping ecosystems[9]. This study highlights the potential for indirect effects of genetic variation to propagate through networks of interacting species and trophic levels. 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[19]. One important example[?], showed that the interactions among species across three trophic levels depended on cottonwood (*Populus* spp.) genotype. Although our study was conducted with a community of lichens, these results should be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level[?,20], although spatial scale of interactions should be considered[21]. As heritable variation is the raw material for natural selection to act upon, a genetic basis for interaction network structure indicates evolutionary dynamics should be considered at the community level and that conserving genetic variation is important to consider in efforts to restore or preserve complex species interactions and the associated ecosystem functions[22] that they provide.

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Acknowledgments

Author contributions statement

M.L. and L.L. conceived the study, M.L. and L.L. conducted the field work, R.N. assisted in lichen identifications, M.L. wrote the first draft of the manuscript, S.B. and T.W. contributed substantively to the conceptual development, T.W. established the common garden. All authors contributed to revisions of the manuscript.
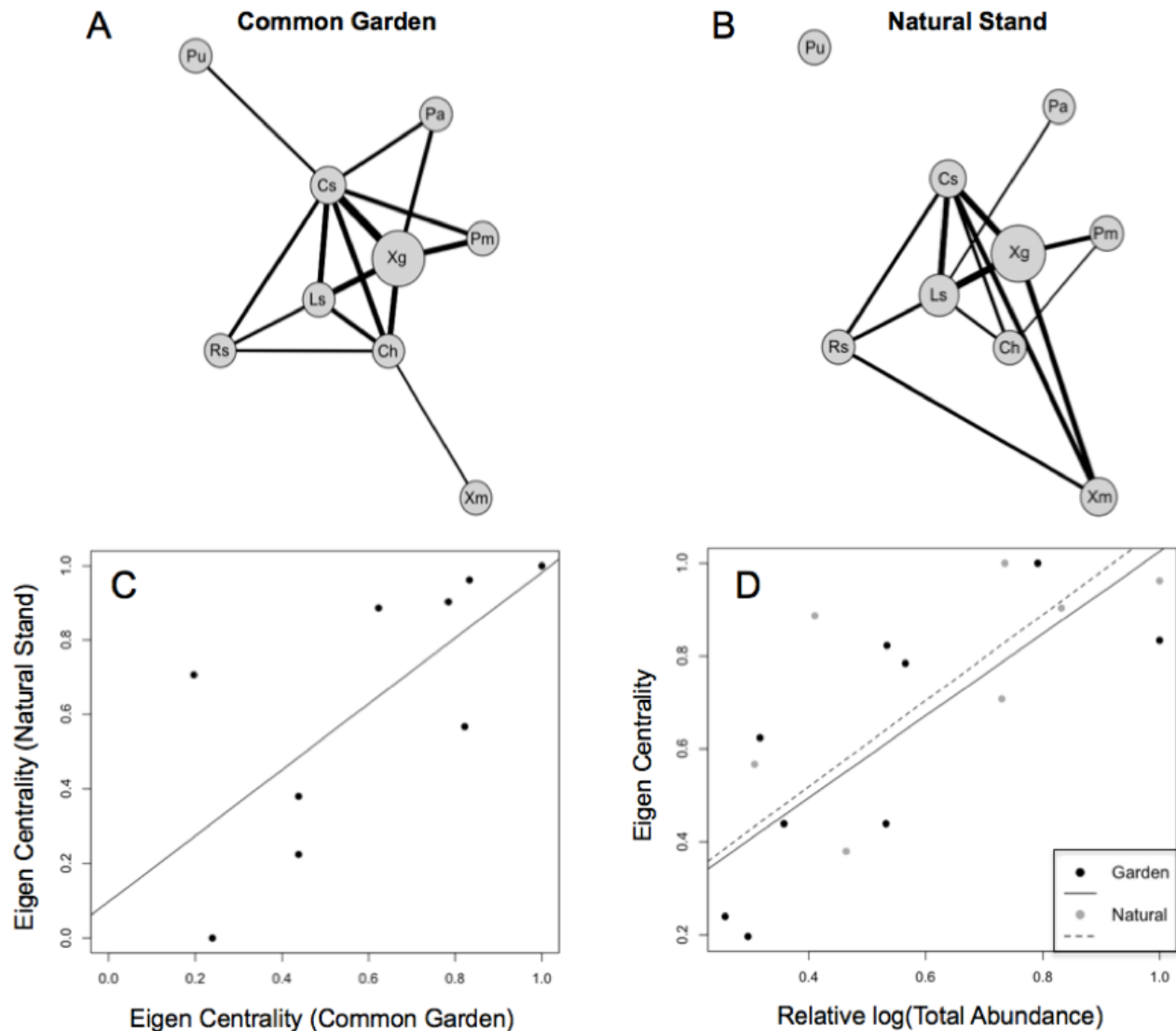
Additional information

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 complete 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.
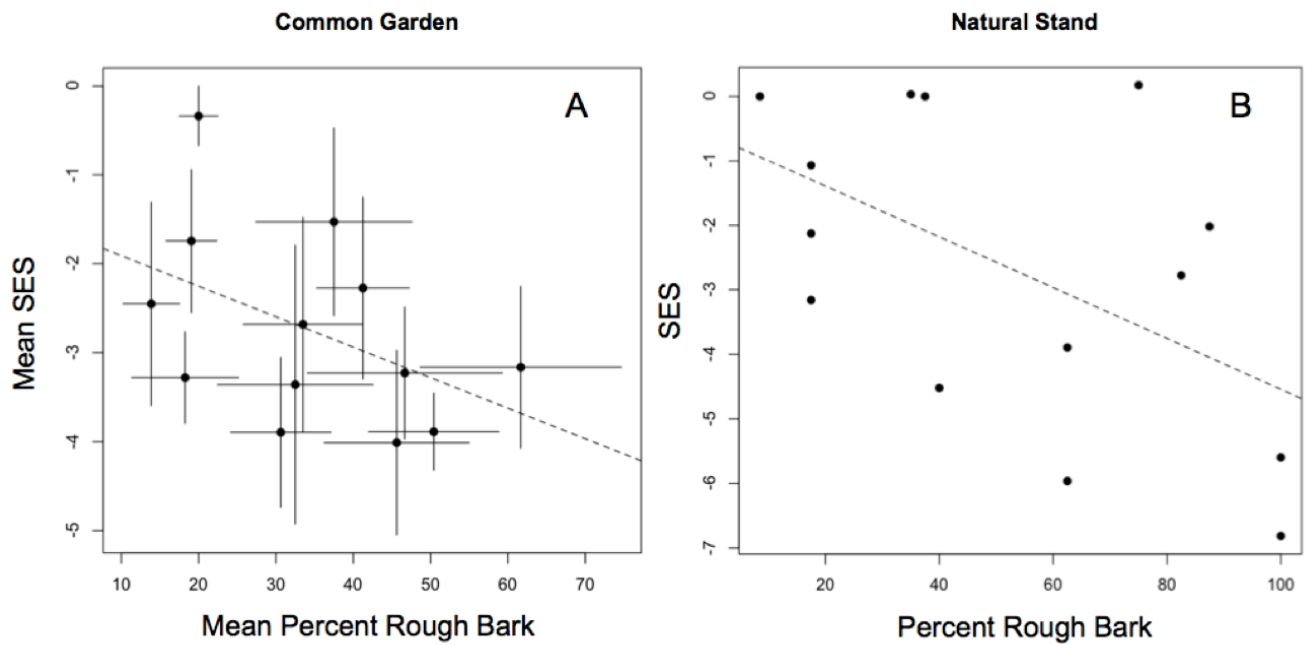
Figure 2. Tree genotype influenced lichen co-occurrence patterns in the common garden and the natural stand through a genetically controlled tree trait. The lichen co-occurrence patterns were highly correlated with the genetically based phenotypic trait; bark roughness (i.e., the percentage of textured bark), in both the common garden and natural stand. The scatterplot (A) shows the mean ($\pm$ 1 SE) percent rough bark (broadsense heritability, $H^2 = 0.36$, $\chi^2 = 9.214$, $p = 0.002$) 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.
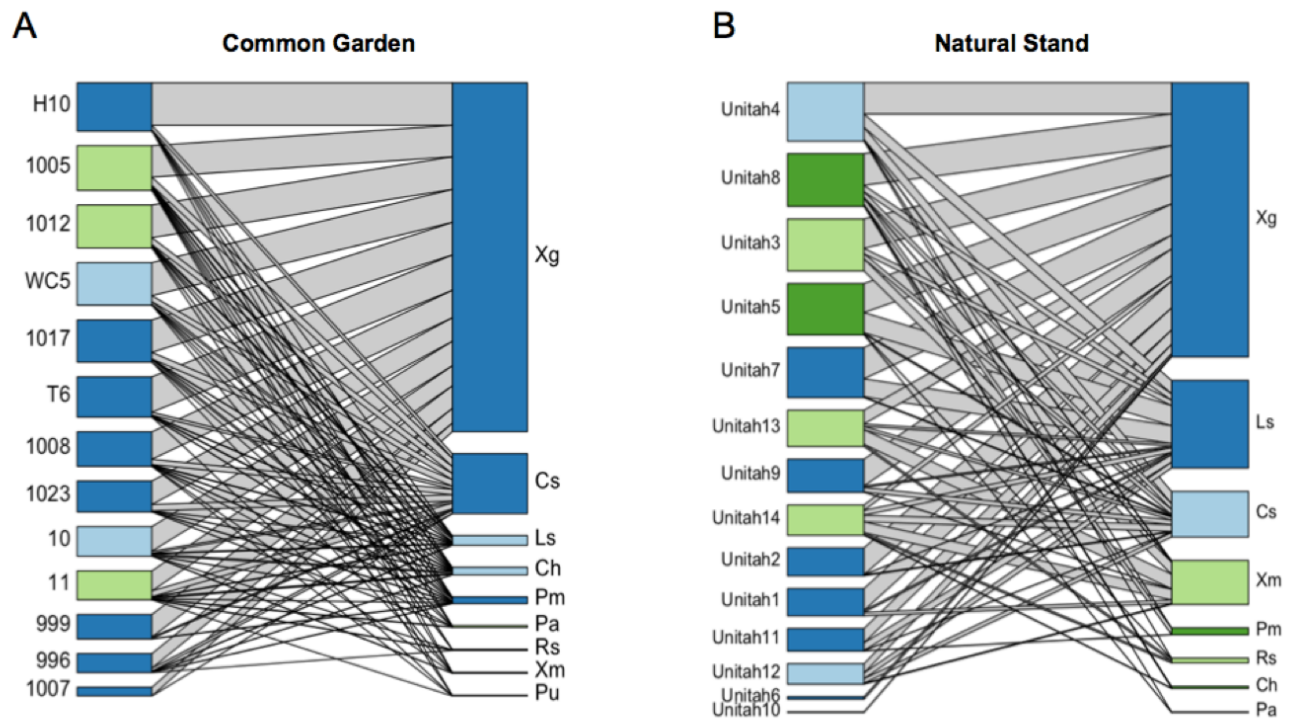
Figure 3. Bipartite networks displayed significant modularity with modules comprised of both genotypes and species. The left most set of nodes shows tree genotypes (see Methods for genotype names) for the common garden (A) or individuals in the natural stand (B) connected to lichen species on the right. 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.