

# Genotypic variation in a foundation tree results in heritable ecological network structure of lignicolous lichen

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1 Biological evolution occurs in the context of complex ecosystems of  
2 interacting species whereby natural selection defines the structure  
3 of ecological networks. Fundamental to understanding evolutionary  
4 processes is elucidating the genetic basis to ecological network  
5 structure, which is defined by interactions among species. Although  
6 previous work has demonstrated that genotypic variation in foundation  
7 species contributes to interaction network structure, we are not  
8 aware of a study that has quantified the genetic contribution to network  
9 structure or shown network structure to be a heritable trait. To  
10 examine this, in a 20+ year common garden we observed interactions  
11 among nine epiphytic lichen species associated with genotypes of  
12 (*Populus angustifolia*), a foundation species of riparian ecosystems.  
13 We constructed signed, weighted, directed interaction networks for  
14 the lichens and conducted genetic analyses of whole network similarity,  
15 degree and centralization. We found three primary results.  
16 First, using multiple metrics, tree genotype significantly predicted  
17 lichen network structure; i.e., clonal replicates of the same genotype  
18 tended to support more similar lichen networks than different genotypes.  
19 Second, broad sense heritability estimates show that plant  
20 genotype explains network similarity ( $H^2 = 0.41$ ), network degree  
21 ( $H^2 = 0.32$ ) and network centralization ( $H^2 = 0.33$ ). Third, one of  
22 the examined tree traits, bark roughness, was also heritable ( $H^2 = 0.32$ )  
23 and significantly correlated with lichen network similarity ( $R^2 = 0.26$ ), supporting a mechanistic pathway from variation in a heritable  
24 tree trait and the genetically based variation in lichen network  
25 structure that selection can act upon. We conclude that tree genotype  
26 can influence not only the relative abundances of organisms but also the interaction network structure of associated organisms.  
27 Given that variation in network structure can have consequences for  
28 the dynamics of communities through altering system-wide stability  
29 and resilience and modulating perturbations, these results have im-  
30 portant implications for the evolutionary dynamics of ecosystems.

networks | heritability | community | genetics | lichen | cottonwood |  
*Populus* | common garden

1 Evolution occurs in the context of complex ecolog-  
2 ical networks. Community genetics studies have  
3 shown that genetic variation in foundation species,  
4 which have large effects on communities and ecosystems by modulating and stabilizing local conditions  
5 (1), plays a significant role in defining distinct communities of interacting organisms: such as, endophytes,  
6 pathogens, lichens, arthropods, and soil microbes (2–

4). Multiple studies have now demonstrated that genetic variation influences numerous functional traits (e.g., phytochemical, phenological, morphological) that in combination results in a multivariate functional trait phenotype (5) in which individual plant genotypes support different communities and ecosystem processes (6, 7). The importance of genetic variation in structuring ecological systems was reviewed

## Significance Statement

Evolution occurs in the context of ecosystems comprised of complex ecological networks. Research at the interface of ecology and evolution has primarily focused on pairwise interactions among species and have rarely included a genetic component to network structure. Here, we used a 20+ year common garden experiment to reveal the effect that genotypic variation can have on networks of lichens that colonize the bark of a foundation tree species. We found that lichen interaction network structure is genetically based and primarily driven by bark roughness. These findings demonstrate the importance of genetic variation and evolutionary dynamics in shaping ecological networks as evolved traits. In particular, this study points to the importance of assessing the effect of foundation species genetics on the structure of species interactions that can generate heritable network variation that selection can act upon.

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.

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(8), and not only were many instances of strong genetic effects found in many ecosystems but the effect of intraspecific variation was at times greater than inter-specific variation. There is now evidence to support that selection, acting on this heritable variation, tends to occur among groups of species (9) and that genetic variation and phylogenetic relatedness contribute to variation in community assembly (10) and species interactions (6, 11, 12), which shape the structure of ecological interaction networks (13–15).

In this community-level context, the “genetic similarity rule” provides a useful framework we can apply to interaction networks at the nexus of ecological and evolutionary dynamics. In a study combining experimental common gardens and landscape-scale observations of interactions between *Populus* spp. (cottonwoods) and arthropods, (16) observed that individuals genotypes that are more genetically similar will tend to have similar phytochemical traits and thus tend to have similar interactions with other species than individuals that are less similar. However, studies in the network ecology literature generally do not include a genetic component (17) and community genetics studies have primarily focused on community composition in terms of the abundance of species (8). Some studies have examined the effects of genetic variation on trophic chains in plant-associated communities (including *Populus*, *Solidago*, *Oenothera*, *Salix*) (18–22) and generally found that increasing genotypic diversity leads to increased trophic complexity. Only two other studies, that we are aware of, have explicitly examined the effect of genotypic variation on the structure of interaction networks between tree individuals and associated herbivores (23, 24) and both found that genotypic diversity generates increased network modularity (i.e., compartmentalization). However, both of these studies were examining networks at the scale of forest stands, rather than networks associated with individual trees; therefore, neither was able to observe replicated networks in order to statistically test for genetic effects on network structure and quantify the genetic component (i.e. heritable variation) in network structure.

Network theory and evidence from empirical studies in ecology have demonstrated that indirect effects can lead to self-organization, producing sign-changing, amplifying and/or dampening effects (25, 26). The development of interspecific indirect ge-

netic effects (IIGE) theory (27) in evolutionary biology points to the importance of studying the genetic basis of interaction network structure because genetic based differences in network structure among individuals can be acted upon by natural selection when there are fitness consequences of different networks of IIGEs that can result in community evolution (28). For example, although the analysis was of abundances rather than interaction networks, (29, 30) found that the mycorrhizal communities on the roots of drought tolerant and intolerant trees are dominated by different orders of ectomycorrhizal fungal mutualists that also differ in the benefits they provide that enhance tree performance. Because drought tolerant genotypes are three times more likely to survive record droughts, selection acts both on the tree and its fungal community and with increased drought the community phenotype has changed over time. Also, in an antagonistic interaction context, (2) found that with the addition of a damaging leaf pathogen to cottonwoods in a common garden, the impacts of these strong interactors results in a different and diminished community of arthropods relative to control trees. Thus, selection acting on the tree may alter the network structure of associated communities in which different networks of communities are most likely to survive pathogen outbreaks.

Regardless of whether the IIGE is unilateral (i.e., tree affects the community) or reciprocal (i.e., the community also affects the relative fitness of the tree), selection on tree, community or both can change network structure (28) and thereby alter community dynamics. Evolutionary applications of network theory have demonstrated that indirect effects of interactions among species can lead to network structures that amplify or dampen the effects of selection (31). Networks that form a star-like structure in which there is a central species or core group of species that interact with other, peripheral species, can amplify selection events. Empirically, network analysis of the structure of bipartite (i.e., two-mode) mutualistic networks has shown in multiple cases that nestedness, or the degree to which species tend to interact with similar subsets of the community, tends to promote stability and resilience to disturbances (32)

#### Spandrels

Here, we investigate how genetic variation in a foundation tree species determines the structure of a network of interactions among a community of

tree associated lichen species. Previous studies have examined aspects of networks (33). Here we examine the genetic basis of network structure on a community of sessile lignicolous (i.e., bark) lichens on cottonwood trees. Using a long-term (20+ years), common garden experiment with clonally replicated *Populus angustifolia* individuals of known genetic identity. We focused on a community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (34, 35). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover rates, facilitated the assessment of interactions among lichen species on individual trees. manuscript We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (23, 24, 36, 37). If correct, we expect to find that network structure is genetically based, or, in other words, plant genotypes will support different and heritable interaction networks. Applying a probability-theory based network modeling approach, we constructed a set of interaction network models for the lichens associated with individual trees. Using these models, we then examined the genetic basis of the structure of these ecological networks via several network metrics that measures different aspects of network structure at the scale of individual species (i.e., nodes) or the entire network observed on each tree genotype. In particular, we focus the metric of centrality for individual species and centralization for whole networks, which measures how much a species is connected in the network relative to other species. Based on previous community genetics theory, particularly the community similarity rule (16), we hypothesize that trees will co-vary in functional phenotypic traits such as bark roughness and chemical composition and trees of the same genotype will tend to have similar traits leading to similarities in lichen network structure. This work is important because it provides a mechanistic basis for understanding how community network theory is intimately associated with the evolutionary process and how human alterations of the environment (e.g., climate change, invasive species, pollution) may have cascading, indirect effects that alter network structure and evolution.

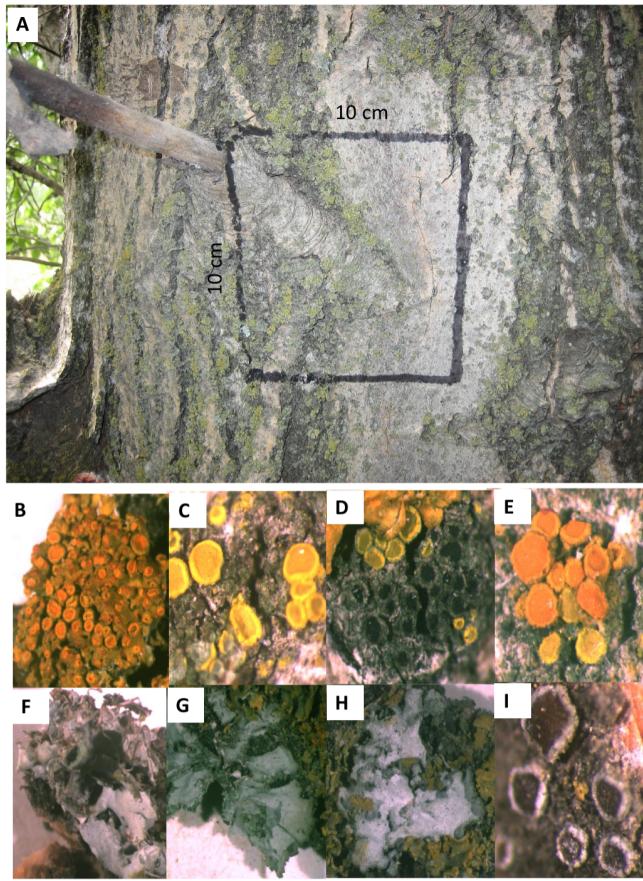
## Materials and Methods

**Study System.** 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 advanced generation backcrosses of *P. angustifolia* were sampled. Bark lichens have been extensively studied in this system and provide an ideal system in which to observe and model lichen interaction networks, as their sessile nature permits accurate identification of individuals (38).

A long-term, common garden experiment was used to isolate the effect of tree genotype from the effect of the localized microenvironment associated with each individual and spatial autocorrelation. Established in 1992, asexually propagated clones of genotyped *P. angustifolia* individuals were obtained from wild collections and planted in fully randomized design at the Ogden Nature Center, Ogden, UT. From the population of established individuals in the common garden, we chose a total of ten genotypes, replicated between 3 and 8 times each, for sampling.

**Bark Lichen and Trait Observations.** On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm<sup>2</sup> cells arrayed in a checkerboard pattern. Given the small size and sessile nature of lichens, we were able to rapidly assess lichen interactions by quantifying thalli in close contact. Sampling was restricted to the northern aspect of the trunk to maximize the abundance of lichen and control for the effect of trunk aspect. Two adjacent 100 cm<sup>2</sup> quadrats centered at 50 cm and 95 cm from ground level were sampled (Fig 1 A and B). The observed lichen community included (abbreviations are given for species present in study): Xg = *Xanthomendoza galericulata*, Xm = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Candeliella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were not observed in the present study but are known to occur in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia sublivacea*, *Meanelia elegans*.

The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of  $0.12 \pm 0.001$  cm<sup>2</sup> (1 S.E.) (see Supporting Information). Based on the median thallus size, we expected thalli observed in each cell to generally be spatially independent of thalli present in other cells but exposed to similar micro-environmental conditions created by the bark and the location of the sampling area on an individual tree. Therefore, we were confident in treating the cell-wise observations in quadrats



**Fig. 1.** The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). (A) Lichens were sampled within a fixed area ( $100 \text{ cm}^2$ ) on individual trees at two heights, 50cm and 95cm from the ground. (B-I) Close-up photos show the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Physciella melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (B-D) and R.R. Naesbourn (E-I).

as independent with respect to lichen-lichen interactions. We quantified the texture of the bark in the quadrat is the percent of  $1 \text{ cm}^2$  cells with rough bark. In addition to bark roughness, we also measured several bark chemistry traits by taking bark samples immediately adjacent to each quadrat using the methods of (38): including, the concentration of condensed tannins, pH and carbon and nitrogen concentrations and pH.

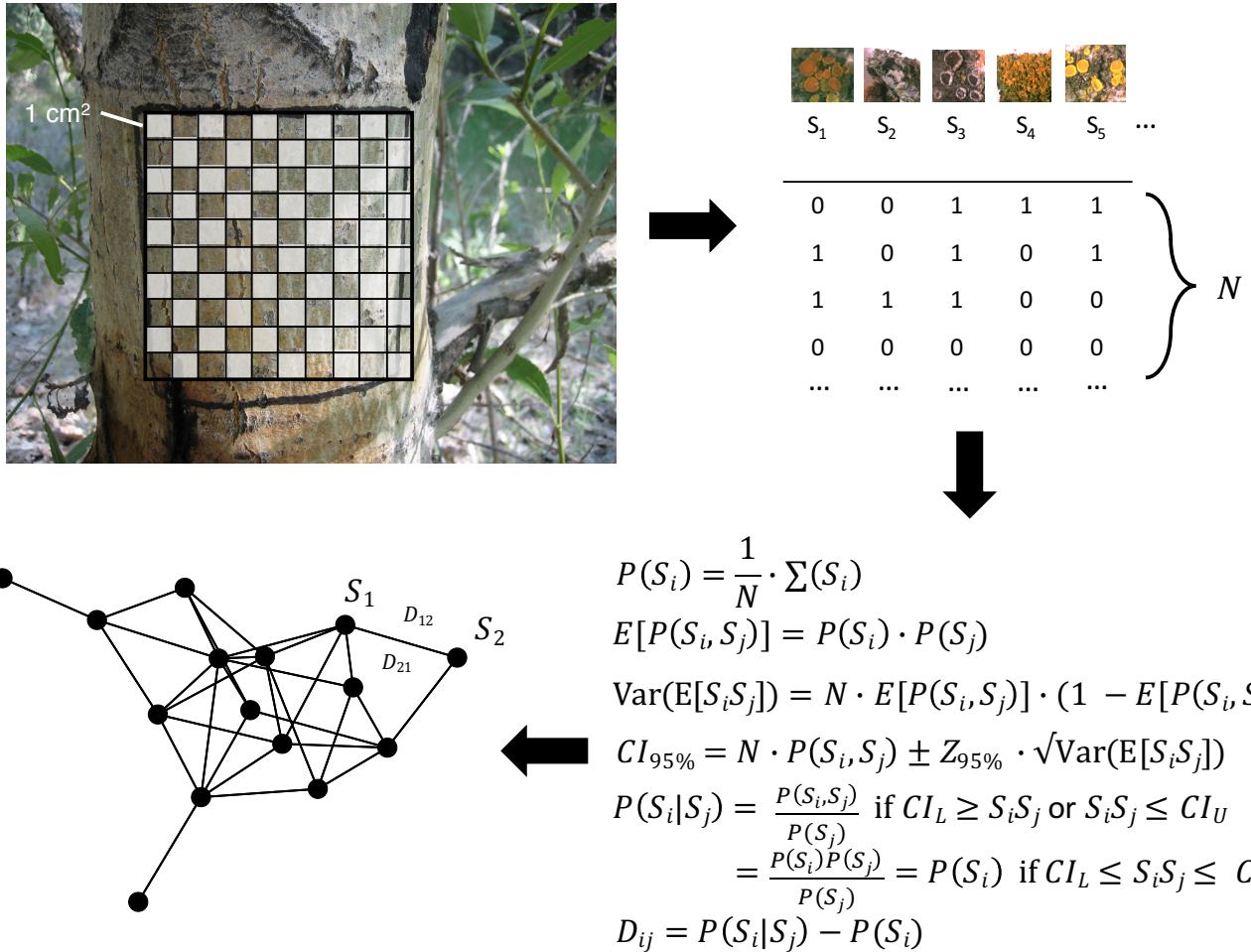
**Lichen Network Modeling and Analysis.** For each tree, repeated observations of lichen were made in order to construct replicated interaction networks for each genotype. We conducted a modified sampling procedure originally developed by (39) with the addition that we quantified the presence of lichen in the  $1 \text{ cm}^2$  cells on individual trees of *P. angustifolia*. Unipartite networks were generated using the conditional probabilities of each species pair, i.e. the probability of observing one species given an observation of another species  $P(S_i|S_j)$ , based on the method developed by (40). To calculate conditional

probabilities, we quantified the individual probabilities of species occurrences  $P(S_i)$  and the joint probability of co-occurrences  $P(S_i, S_j)$  using the frequencies of each species and their co-occurrences. We were then able to calculate the conditional probabilities of each species pair as  $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$ , based on the axioms of probability. This yielded a matrix that could possibly be asymmetric, i.e.  $P(S_i|S_j)$  does not have to be equal to  $P(S_j|S_i)$ . Another important property of this matrix is that the diagonal,  $P(S_i|S_i)$ , was equal to one for all species present and zero for species that were not observed in any cell.

We then applied an analytical procedure to remove non-significant links between species. This procedure determines if the joint probability of a species pair (i.e.  $P(S_i, S_j)$ ) is different from zero (Fig. 2). Here, a confidence interval  $CI_{95\%}$  is calculated as  $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$ , where the expected frequency of co-occurrences  $E(S_i S_j)$  is the total number of cells surveyed ( $N$ ) times the independent probabilities of each species  $P(S_i) * P(S_j)$ ,  $Z_{95\%}$  is the Z-score for 95% from a Z-distribution and the expected variance of  $E(S_i S_j)$  is the total number of cells times the expected probability of  $S_i S_j$  and its compliment (i.e.  $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$ ). If the observed number of co-occurrence falls outside of the confidence interval, the joint probability  $P(S_i, S_j)$  is determined to be equal to the product of the individual probabilities (i.e.  $P(S_i)P(S_j)$ ), and the conditional probability reduces to the individual probability of that species  $P(S_i)$ . Therefore, unless the co-occurrence of a species pair falls outside the confidence interval, the probability that the observation of one species given the other is no different than simply observing that species alone. This enables us to remove links from a given network by re-scaling the resulting conditional probabilities by subtracting the individual probabilities from the conditional probabilities (i.e. how different the conditional probability is from the independent probability), which makes any species with a non-significant conditional probability zero.

The resulting matrix ( $\mathbf{D} = D_{ij}$ ) can be interpreted as one species' impact on another with zero being no effect and values less than or greater than zero being negative and positive effects, respectively. Here, we will refer to  $\mathbf{D}$  as a signed, weighted interaction matrix. As such,  $\mathbf{D}$  has the properties that it can be asymmetric (i.e.  $D_{ij}$  does not necessarily equal  $D_{ji}$ ) and it scales between -1 and 1, and, therefore, does not have the mathematical properties of a probabilistic network (?). Also, as the method does not track individuals within species and interactions such as competitive exclusion or facilitation within species would result in the same species being observed. Therefore, the results of intra-specific interactions always results in the same species being observed and a resulting  $D_{ii} = 0$ .

**Network Metrics.** To quantify the structural variation of lichen networks we calculated several metrics at both the



**Fig. 2.** Lichen interaction networks were constructed by conducting field observations in 1 cm<sup>2</sup> cells within a 100 cm<sup>2</sup> grid on each tree using a checkerboard pattern (grey cells). Thus, a set of  $N$  total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (40), we calculated the conditional probabilities,  $P(S_i | S_j)$ , for all species pairs and removed (i.e. set equal to zero) species pairs whose joint probabilities,  $P(S_i S_j)$ , were not significant using a confidence interval based comparison of their observed co-occurrence frequency,  $S_i S_j$ , to that expected due to chance alone,  $E[P(S_i S_j)] = P(S_i) P(S_j)$ , and  $P(S_i S_j)$  reduces to  $P(S_i)$ , the observed individual probability of species  $S_i$ . In the context of these networks, asymmetry and positive/negative valued connections are distinct quantities. In-coming and out-going connections can be interpreted as “influenced by” and “influenced”, respectively; while positive and negative should be seen as one species increasing or decreasing, respectively, the probability of another species’ occurrence.

node and whole-network level. For individual nodes (i.e. species) in each network, we calculated both the degree and the Freeman’s centrality (41). We also calculated two similar global network metrics: degree and centralization. The first was network degree, which is a count of the total number of links in a network. As the networks contained not only positive and negative connections, as well as directional connections (both in-coming and out-going), we calculated the same network metrics for all combinations of these types of connections in each network. Although there are many more possible network metrics that could have been examined, we chose to focus on a restricted set for the sake of clarity. Also, degree and centrality form the basis of many other network metrics. To calculate separate metrics for positive and negative links, we applied methods for calculating the centrality accounting for the sign differences (42) using the `signnet`

package (43).

**Statistical Analyses, Software and Data.** We used a combination of parametric and non-parametric, permutation based frequentist statistical analyses to test for the effects of genetic variation on lichen communities and their interaction networks. To assess the effect of genotype on univariate responses, we used additive, random effects models with Restricted Maximum Likelihood (REML). We used a combination of Least Squares Regression, Analysis of Variance (ANOVA) and correlation tests to quantify and test for the relationship among other variables. Bark roughness, lichen cover and species richness were square-root transformed to meet the assumptions of homogeneity of variance and normality for these tests.

For multivariate response variables, such as lichen community composition and network structure, we used dis-

tance based multivariate statistical approaches, including Permutational Analysis of Variance (PERMANOVA) and Mantel tests. To quantify the similarity of lichen networks among individual trees, we calculated the pairwise Euclidean distance of the  $\mathbf{D}$  interaction matrices among all pairs of trees.

For visualization of multivariate patterns, we used Non-metric Multi-Dimensional Scaling (NMDS) (44) to produce dimensionally reduced ordinations of these multivariate responses and fitted vectors for continuous predictor variables to the ordinated values (45). Using random initial configurations with a maximum of 500 iterations and a change in stress threshold of less than  $10^{-12}$ . Final configurations has the lowest stress with at most a stress level of 0.10.

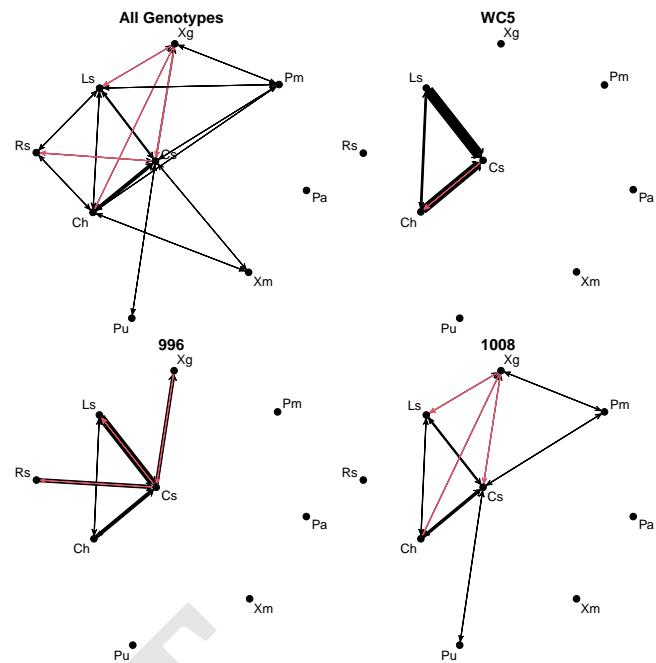
For each network, we also calculated metrics that measure different structural aspects. Although there are many other metrics, for the sake of simplicity we focus on a subset that represent several interesting features of network structure (see (17)). We calculated the number of interactions or “links” in each network, which provides a measure of the size of the network (23, 46). We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network (41). In a network with a low level of centralization species have similar amount of interaction in the network, while a network with a high level of centralization tends to have one or small number of species that interact with other species. We used a related function to calculate the centrality of each species (i.e. node level centrality) in each network as well.

For all tests where genotype was used as a predictor, we quantified the heritability of the response variable. Because the trees in the garden were clonal replicates of each genotype, we calculated broad-sense heritability, which is the genotypic variance divided by the total phenotypic variance (47). This can be interpreted as a measure of the phenotypic variance due to genotypic variation. We also apply this to the community genetics context as the variance in *extended* phenotypic variance due to genotypic variation (48). For the multivariate analyses, where we employ PERMANOVA, we followed the methods of (27) to adjust the degrees of freedom for unbalanced genotype replicates.

All code and data for the project are openly available online. Code and data are available at [github.com/ecgen/comgen](https://github.com/ecgen/comgen). The project is also archived via Zenodo at [zenodo.com/doi/XXXXXXX](https://zenodo.com/doi/XXXXXXX). All analyses were conducted using the programming language R version 3.6.1 (R Development Core Team 2019).

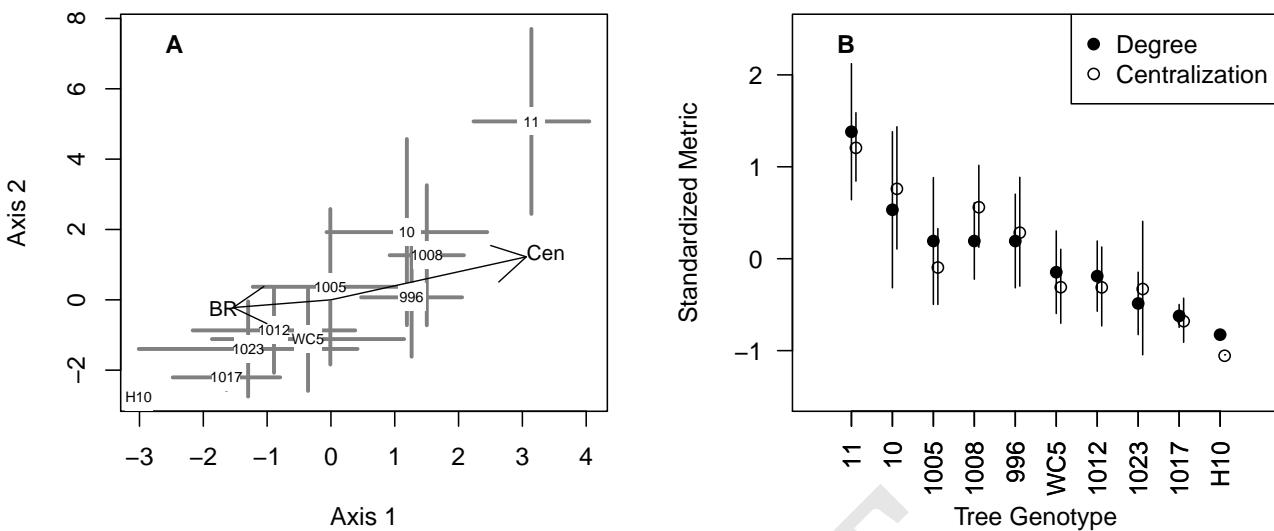
## Results

Tree genotype influenced lichen network structure and multiple lichen network metrics were heritable. Tree genotype significantly predicted the struc-



**Fig. 3.** Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes (996, WC5 and 1008) showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive) of interactions are shown as edges between species (abbreviated by the first letter of the genus and specific epithet), which are scaled by their magnitude. The sign of the interaction is indicative of greater (positive) or lesser (negative) paired occurrences than expected relative to the overall frequency of occurrence of each species. Ecologically, the links in the network are likely the product of multiple types of interactions (e.g. mutualism, parasitism, competition, facilitation) that could vary over both space and time.

tural similarity of lichen networks (PERMANOVA: Pseudo- $F_{9,27} = 3.58$ ,  $H^2 = 0.41$ ,  $p\text{-value} = 0.0537$ ) (Fig. 4). Overall network level metrics responded significantly to tree genotype (Table 1), including network degree ( $RLRT = 3.52$ ,  $H^2 = 0.32$ ,  $p\text{-value} = 0.0255$ ) and centralization including both in-coming and out-going links ( $RLRT = 4.04$ ,  $H^2 = 0.33$ ,  $p\text{-value} = 0.0184$ ) or when separated into in-coming only ( $RLRT = 3.9852$ ,  $H^2 = 0.3309$ ,  $p\text{-value} = 0.0190$ ) or out-going only ( $RLRT = 3.8615$ ,  $H^2 = 0.3193$ ,  $p\text{-value} = 0.0205$ ). Metrics including only positive links also showed a significant effect of tree genotype, including positive degree ( $RLRT = 3.6925$ ,  $H^2 = 0.3242$ ,  $p\text{-value} = 0.0229$ ), positive in-going centralization ( $RLRT = 4.4812$ ,  $H^2 = 0.3487$ ,  $p\text{-value} = 0.0142$ ). Metrics calculated with negative links were not significant, including degree (negative) ( $RLRT = 0.0327$ ,  $H^2 = 0.0318$ ,  $p\text{-value} = 0.3859$ ) and both in-coming (negative) ( $RLRT = 0.3304$ ,  $H^2 = 0.1057$ ,  $p\text{-value} = 0.2508$ ) and out-going centralization (negative) ( $RLRT = 0.0862$ ,  $H^2 = 0.0513$ ,  $p\text{-value} = 0.3446$ ).



**Fig. 4.** The similarity of lichen networks varied among tree genotypes. A. The plot shows genotype centroids of NMDS ordinated ( $R^2 = 0.999$ , stress = 0.008) lichen network similarity ( $\pm 1$  S.E.). Genotype centroids that are closer together tend to have more similar lichen network structure. Arrows showing the direction (arrowhead) and magnitude (length) of the vectors of correlation between bark roughness (BR) and network centralization (Cen) and the ordinated network similarity. B. Plot showing the standardized ( $\frac{x-\bar{x}}{\sigma}$ ) means ( $\pm 1$  S.E.) for the two of the genetically based lichen network metrics: overall degree (i.e. total number of links) and centralization, which is a measure of the dominance of one species in the network.

response	df	statistic	H2	p-value
Lichen Network Similarity	9	3.5821	0.41	0.0537
Degree		3.5175	0.32	0.0255
Degree (positive)		3.6925	0.32	0.0229
Degree (negative)		0.0327	0.03	0.3859
Centralization		4.0444	0.33	0.0184
Centralization In-Degree		4.4812	0.35	0.0142
Centralization In-Degree (positive)		3.9852	0.33	0.0190
Centralization In-Degree (negative)		0.3304	0.11	0.2508
Centralization Out-Degree		3.8615	0.32	0.0205
Centralization Out-Degree (positive)		3.5585	0.31	0.0248
Centralization Out-Degree (negative)		0.0862	0.05	0.3446

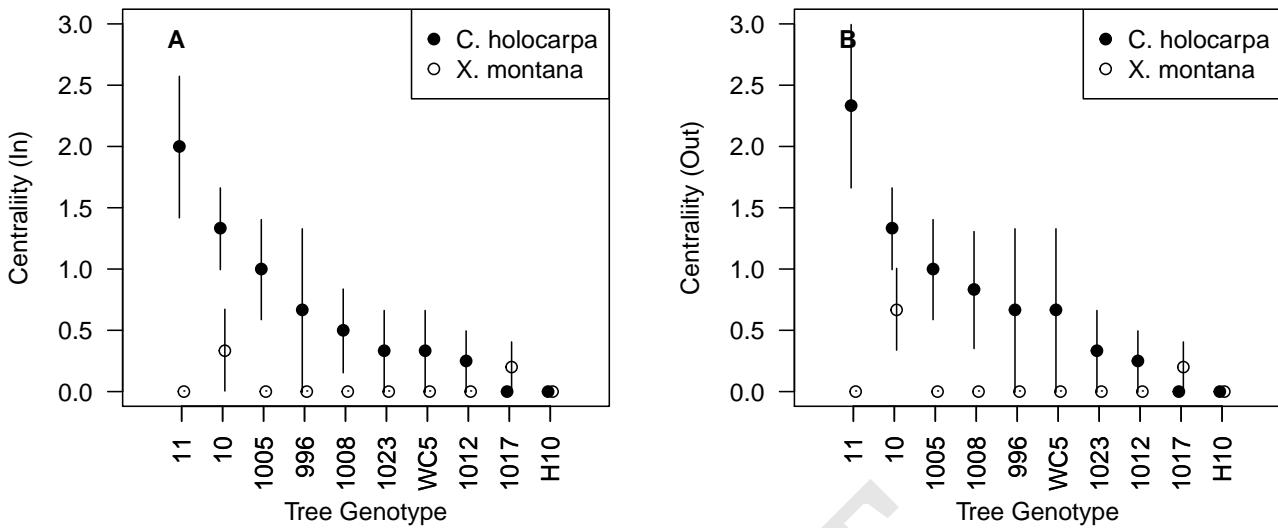
**Table 1. Genotypic effects on the associated lichen network structure.**

The genetic response of network centralization was driven by variation in *Caloplaca holocarpa*. Centralization varied significantly among species ( $F_{8,324} = 7.99$ ,  $R^2 = 0.16$ ,  $p\text{-value} < 0.0001$ ). *Caloplaca holocarpa* centrality was the main species to exhibit a significant response to tree genotype in terms of positive centrality for both the in-coming ( $RLRT = 3.61$ ,  $H^2 = 0.32$ ,  $p\text{-value} = 0.0240$ ) and out-going ( $RLRT = 3.13$ ,  $H^2 = 0.30$ ,  $p\text{-value} = 0.0327$ ) perspectives, but not for either negative centrality metrics in-coming ( $RLRT = 0$ ,  $H^2 = 0$ ,  $p\text{-value} = 1$ ) or out-going ( $RLRT = 0$ ,  $H^2 = 0$ ,  $p\text{-value} = 0.4543$ ). None of

the other species' centralities showed a genotypic response (Supplementary Table 3) with the exception of *X. montana* ( $RLRT = 2.92$ ,  $H^2 = 0.32$ ,  $p\text{-value} = 0.0375$ ); however, the centrality of *X. montana* was much lower overall relative to *C. holocarpa* and the variation in *X. montana* centrality was restricted to two genotypes (Fig. 5).

#### Add transformations of variables to methods.

Genotype indirectly influenced lichen network centralization via the genetically based variation in bark roughness. The percent cover of rough bark ( $RLRT = 4.8526$ ,  $H^2 = 0.3221$ ,  $p\text{-value} = 0.0113$ ) and condensed tannins ( $RLRT = 3.0522$ ,  $H^2 = 0.3205$ ,  $p\text{-value} = 0.0343$ ) both displayed significant responses to tree genotype. None of the other bark traits, pH ( $RLRT = 0.00$ ,  $H^2 = 0.00$ ,  $p\text{-value} = 1.0000$ ) or carbon-nitrogen ratio ( $RLRT = 0.0000$ ,  $H^2 = 0.0000$ ,  $p\text{-value} = 1.0000$ ), showed a significant response to tree genotype and none other than bark roughness was correlated with network similarity (Table 2); therefore, we focused our subsequent analyses on the indirect effect of genotype on lichen network structure via bark roughness. We found that bark roughness was significantly correlated with network similarity (PERMANOVA: Pseudo- $F_{1,32} = 13.029$ ,



**Fig. 5.** Dot-plots showing the mean (dot) and  $\pm 1$  SE of in-degree (A) and out-degree (B) centrality for two species, *C. holocarpa* and *X. montana*. *Caloplaca holocarpa* centrality was highly variable among genotypes. *Xanthomendoza montana* centrality, both in- and out-degree, was only non-zero for two genotypes, and only out-degree centrality displayed a significant response to genotype.

$R^2 = 0.26$ ,  $p\text{-value} = 0.0096$ ) and other lichen net<sub>453</sub> work metrics, including negative correlations with<sub>454</sub> overall network degree ( $df = 35$ ,  $t = -2.13$ ,  $r = -0.34$ ,<sub>455</sub>  $p\text{-value} = 0.04$ ) and centralization ( $df = 35$ ,  $t = -2.52$ ,<sub>456</sub>  $r = -0.39$ ,  $p\text{-value} = 0.02$ ). In other words, trees with<sub>457</sub> more similar levels of bark roughness tended to have<sub>458</sub> lichen interaction networks with similar structure.<sub>459</sub> To quantify the genetic bases of this effect of bark<sub>460</sub> roughness on network structure, we used the residual<sub>461</sub> values from regressions of network degree and cen-<sub>462</sub> tralization in tests of the effect of tree genotype and<sub>463</sub> found no significant effect of tree genotype for either<sub>464</sub> degree ( $RLRT = 0.00$ ,  $H^2 = 0.00$ ,  $p\text{-value} = 1.0000$ )<sub>465</sub> or centralization ( $RLRT = 0.00$ ,  $H^2 = 0.00$ ,  $p\text{-value}$ <sub>466</sub> = 1.0000), suggesting that the observed relationship<sub>467</sub> between bark roughness and lichen network structure<sub>468</sub> was largely genetically based (Fig. 6).

	df	SumOfSqs	R2	F	Pr(>F)
Bark Roughness	1	20850.09	0.26	12.9234	0.0101
Condensed Tannins	1	5993.66	0.07	3.7150	0.0813
pH	1	1273.19	0.02	0.7892	0.3712
Carbon:Nitrogen Ratio	1	3896.18	0.05	2.4150	0.1890
Residual	32	51627.33	0.64		
Total	36	80993.59	1.00		

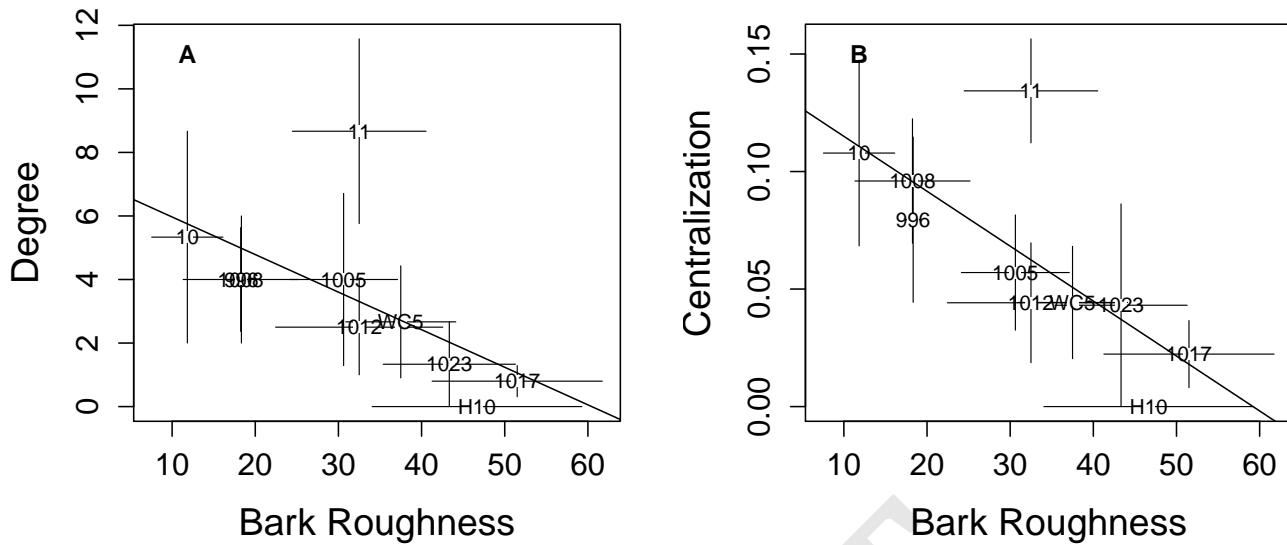
**Table 2. PERMANOVA Pseudo-F Table of lichen net-  
work similarity response to bark traits.**

## Discussion

We found that tree genotype influenced lichen net-<sub>460</sub> work structure in the experimental cottonwood forest.<sub>461</sub> Network similarity and metrics of network structure<sub>462</sub> tended to be more similar on trees of the same geno-<sub>463</sub> type. Generally, this genetic effect was manifested in<sub>464</sub> positive interactions and largely driven by *C. holocarpa*.<sub>465</sub> The genetically based trait, bark roughness,<sub>466</sub> was the only trait observed to effect network vari-<sub>467</sub> ation, largely via shifts in positive in-coming and<sub>468</sub> out-going interactions. Bark roughness has been<sub>469</sub> demonstrated previously to be under strong genetic<sub>470</sub> control (49), and bark roughness has also previously<sub>471</sub> been shown to be an important tree trait influencing<sub>472</sub> bark lichens (39); however this is the first demon-<sub>473</sub> stration of a link from genes to lichen network structure.<sub>474</sub> As such these results have important implications for<sub>475</sub> the potential influence of genetically based variation<sub>476</sub> in ecosystems with networks of interacting species.<sub>477</sub>

## Implications of the Heritability of Interaction Net- work Structure.

The patterns of interaction in individual-<sub>474</sub> based networks depart from these the-<sub>475</sub> oretical benchmarks, revealing the role of<sub>476</sub> variability in space, time, traits, and pref-



**Fig. 6.** Bivariate plots of the negative relationship between bark roughness and two network metrics: A) degree and B) centralization. Each plot displays the genotype mean ( $\pm 1$  S.E.) for both variables and a least-squares regression line calculated using the genotype means. Generally, as roughness increased the number of interactions (degree) and dominance of those interactions decreased.

478      erences in shaping ecological interactions  
479      (Araújo et al. 2008).

480      Space and time create templates for ecolog-  
481      ical interactions (Cantor et al. 2018) that  
482      favor departures from homogeneous and  
483      abundance-based network patterns. The  
484      spatial configuration of an environment may  
485      foster the rise of modules of interacting in-  
486      dividuals (Fortuna et al. 2009, Tur et al.  
487      2015). Similarly, temporal variation in the  
488      availability of partners affects the network  
489      structure on different time scales (Dátillo  
490      et al. 2014b, Valverde et al. 2016). For  
491      example, networks describing interactions  
492      among individual insects and different plant  
493      species show temporal modularity, with dif-  
494      ferent individuals sharing pollen resources  
495      at different times in the flowering season  
496      (Tur et al. 2015). Space and time may  
497      therefore promote spatiotemporal variation  
498      in the network structure by affecting the  
499      likelihood of potential interactions. Even  
500      so, the macroscopic properties of individual-  
501      based networks may show structural con-  
502      stancy. For example, networks of inter-  
503      actions among protective ant species and

504      individual plants show daily turnover in ant  
505      species, while maintaining nestedness and  
506      average levels of reciprocal specialization  
507      (Dátillo et al. 2014b).

508      Space and time set the scales in which  
509      individual-based networks occur, but the  
510      interaction patterns are further modified  
511      by variation in individual traits. For ex-  
512      ample, the numbers of individual honey-  
513      bees (*Apis mellifera*, Apidae) visiting this-  
514      tle (*Cirsium arvense*, Asteraceae) flowers  
515      increase with the number of flower heads  
516      and the height of the inflorescences on  
517      individual plants (Dupont et al. 2011).  
518      Network description of intraspecific var-  
519      iation in dietary niches uncovers subtle as-  
520      sociations between traits and resource use  
521      that go beyond the number of resources  
522      used. For example, in a monomorphic  
523      population of three-spine sticklebacks (*Gas-*  
524      *terosteus aculeatus*, Gasterosteidae), differ-  
525      ences in trait combinations (e.g., body and  
526      snout shapes) were associated with dietary  
527      modules, i.e., groups of individuals feed-  
528      ing on similar prey (Araújo et al. 2008).  
529      Similarly, networks describing diet overlap

530 among thick-billed murres (*Uria lomvia*,  
531 *Alcidae*) revealed sexbased dietary groups  
532 (Provencher et al. 2013). Network analyses  
533 can therefore reveal how patterns of interaction  
534 across individuals are associated with  
535 variation in individual traits.

536 A disconnected network structure implies  
537 that the dynamical consequences of eco-  
538 logical interactions are limited to pairs  
539 or to small groups of interacting species  
540 (e.g., Guimarães et al. 2007). In con-  
541 trast, a connected network structure im-  
542 plies that trophic cascades, trait-based cas-  
543 cades, and eco-evolutionary feedbacks can  
544 propagate across multiple species in the  
545 community. For example, indirect effects  
546 generated by the decline in sea otters in  
547 the Aleutian Archipelago propagated across  
548 trophic chains, resulting in a dietary shift  
549 in bald eagles (Anthony et al. 2008). In  
550 fact, most of the classic empirical exam-  
551 ples of keystone species shaping ecological  
552 communities due to indirect effects show  
553 species with either (a) generalist diets or  
554 (b) generalism at the species level emerging  
555 due to intrapopulation variation in traits or  
556 preferences (Estes et al. 2003, Paine 1966).  
557 From a network perspective, at least some  
558 keystone species can be viewed as highly  
559 connected species that shift the community-  
560 level dynamics by promoting connectedness  
561 in ecological networks.

562 Highly connected species may also connect  
563 modules generated by trait variation across  
564 potentially interacting species (Donatti et  
565 al. 2011, Schleuning et al. 2014). High trait  
566 variation across species may lead to the for-  
567 mation of modules due to specialization  
568 (Poisot et al. 2013), intervalty (Guimerà et  
569 al. 2010, Stouffer et al. 2006), interspecific  
570 competition (Dalerum et al. 2016, Valverde  
571 et al. 2020), trade-offs in resource use (Pin-  
572 heiro et al. 2019), and the combination of  
573 multiple ecological and evolutionary factors  
574 (Rezende et al. 2009). However, the impact  
575 of trait variation on species interactions is  
576 not straightforward, being dependent on  
577 the rules connecting traits of interacting  
578 species (Gibert DeLong 2017, Gravel et al.

579 2016). If trait variation hinders the emer-  
580 gence of highly connected species, loosely  
581 connected networks should emerge in which  
582 some modules may present internal nested  
583 patterns due to differences in species abun-  
584 dance and forbidden links (Lewinsohn et  
585 al. 2006, Pinheiro et al. 2019, Vacher et al.  
586 2008). If, however, some highly connected  
587 species are able to interact with partners  
588 with disparate traits, then different modules  
589 will be connected to each other through the  
590 pathways created by these highly connected  
591 species (Olesen et al. 2007, Schleuning et  
592 al. 2014). Therefore, the presence of highly  
593 connected species shapes the cohesiveness  
594 of ecological communities, markedly affect-  
595 ing different aspects of their network struc-  
596 ture.

597 We found significant heritability of lichen inter-  
598 action network structure, and, in line with the ge-  
599 netic similarity rule, networks observed on trees of  
600 the same genotype tended to be structurally sim-  
601 ilar. This is the first study that we are aware of  
602 to examine the heritability of network structure in  
603 a real ecosystem. Previous work in the evolution  
604 of ecological networks have primarily focused on  
605 macro-evolutionary dynamics (13? ? ) or have been  
606 simulation based individual-level models that inte-  
607 grate intraspecific variation to the species level (?  
608 ), even though recent syntheses ave pointed to the  
609 importance of processes operating across scales of  
610 organization (? ). There are several important func-  
611 tional ramifications of genetically based variation  
612 in network structure. First, intra-specific diversity  
613 could be creating lichen interaction modules with  
614 different dynamics. When communities are com-  
615 prised of individuals whose habitat is primarily the  
616 determined by another organism, these communities  
617 inherently form modules within the larger ecosystem,  
618 as they tend to interact more with each other than  
619 with other individuals (? ? ? ). Our study demon-  
620 strates that the environmental differences determined  
621 by the genetic variation within a single species can  
622 create differences that not only impacts community  
623 composition, as repeatedly demonstrated in other  
624 community genetics studies (8? ), but also the struc-  
625 ture of interactions among individuals within these  
626 modules. Some network structures are likely to be  
627 more stable, either in response to disturbance or via

628 self-organized dynamics. For example, centralized  
629 networks, although more efficient, are theorized to  
630 be more susceptible to targeted attacks on the center  
631 of the network. For example, consider a forest with  
632 two genotypes that support lichen communities that  
633 are similar in total abundances of each species but  
634 differ in terms of the structure. Extensions of game  
635 theory to evolutionary biology have demonstrated  
636 that network structure can lead to variation in evo-  
637 lutionary dynamics. Some structures tend toward  
638 dominance and dampening of selection, while others  
639 lead to amplification of selection. One class of net-  
640 works that are theorized to have amplifying effects  
641 on networks have "star" shapes with one or a few  
642 species at the center and radiating interactions out  
643 from the central core (?). This is structurally what  
644 we have observed with the networks that tend to  
645 occur on some of the genotypes in our study, i.e.  
646 the more centralized networks. It is possible that  
647 these more centralized networks could function as  
648 hot-spots of evolutionary dynamics resulting from  
649 the amplifying effect the network structure fostered  
650 on that tree genotype.

651 Second, even if the composition of the communi-  
652 ties is the same among individuals and genotypes,  
653 interactions may not be. We didn't observe compo-  
654 sitional differences using the same data from which the  
655 lichen networks were derived. If we only had our com-  
656 position dataset from this study, we would have con-  
657 cluded no response of the lichen community to tree  
658 genotype, even though the underlying interactions  
659 among lichen species does vary among genotypes.  
660 As such differences in network structure could occur  
661 without observable differences in species richness or  
662 community composition, which have been the pri-  
663 mary focus of almost all previous community genetics  
664 studies (8). Community composition of lichen has  
665 previously been observed to be different among tree  
666 genotype in the same experimental garden (38, 39),  
667 the difference between the present study is likely a re-  
668 sult of differences in sampling method and the choice  
669 of genotypes leading to overall higher abundances of  
670 observed lichens to assure the possibility of observing  
671 xlichen interactions. The previous study also used a  
672 visual estimation method, unlike the current study,  
673 which observed lichen at the scale of 1 cm<sup>2</sup> cells,  
674 which could over-estimate cover depending on the  
frequency at which actual thallus size was less than<sup>674</sup>  
1 cm<sup>2</sup>, as well as both the northern and southern<sup>675</sup>

676 aspects of each tree. These differences do not negate  
677 the findings of either study. The present study's  
678 finding of differences in network structure without  
679 significant compositional differences points to the  
680 importance of quantifying how network structure  
681 changes in response to genetic variation in order to  
682 fully understand evolutionary dynamics in complex  
683 communities. Having not observed a compositional  
684 effect of tree genotype without measuring the net-  
685 work structure could lead to the conclusion of no  
686 genetic effect on the community, even though im-  
687 portant variation in network structure are leading  
688 to evolutionary dynamics. It is possible that these  
689 underlying differences in interactions among lichen  
690 could lead to differences in community composition  
691 at a future point in time via there effects on species  
692 abundances (27); however, this is not needed for  
693 evolutionary dynamics to play out via selection that  
694 leads to shifts in trait distributions without shifting  
695 species abundance distributions, which is possible  
696 under stabilizing, disruptive and directional selection  
697 (47). Thus, it is imperative that further community  
698 genetics research assess or at least be aware of the  
699 potential effects of variation in interactions and not  
700 just observe species abundances.  
701

**702 Evolutionary Implications of a Genetic Basis to  
703 Network Structure.** A genetic basis to network struc-  
704 ture suggests that some amount of interaction net-  
705 work complexity is determined and therefore could  
706 be predicted. Variation in space and time create  
707 variation in ecological networks that influences evo-  
708 lutionary dynamics via shifts in ecological dynamics,  
709 such as population demographics (?). Given that  
710 ecosystems are comprised of hundreds and thousands  
711 of species, each having a multitude of interactions,  
712 the potential to find traction for making predictions  
713 in the context of ecological, let alone evolutionary, dy-  
714 namics seems daunting. The promise of predictability  
715 lies in the presence of assymetries in ecosystems that  
716 contribute to the occurrence of foundation species,  
717 such as hierarchy and nestedness created by body  
718 size differences or life-history strategies, has been  
719 widely observed (1). The second part is that heri-  
720 tability (i.e., genetic determination) means that there  
721 is structure in the spatial or temporal variation that  
722 is created by individuals of foundation species whose  
723 traits are in part determined by underlying trait  
724 differences. Although this variation is inherently  
725 a function of both genetics and environmental ef-

fects (47), the community and network level effects are also a function of the scale of the interaction (27). Self-organization of the dynamics within these communities also points to a mechanism for strong community and ecosystem effects of genetic variation, contributing to what may seem unlikely magnitudes of genetic effects as has been observed in some systems, such as forest in riparian ecosystems (16).

Ecological network studies have focused on asymmetry and the quantification of its structure in communities, with qualitative discussion of the impacts on evolutionary dynamics (36, 37, 54, 55). More specific predictions, within a quantitative framework, can be found in applications of evolutionary game theory, and although developed at the population scale, such theory can apply to communities. One seemingly useful direction from evolutionary network developments from game theory is the classification of networks into two general categories, rooted and cyclic, in which rooted networks have interactions in which evolutionary effects emanate from one or multiple origins but these effects do not have connections back to the origins, whereas cyclic networks contain feedbacks to one or more origins. Although it did not explicitly define it in this context, the previous work of (17) developed the perspective that the structure of the network in the context of a foundation species, such as cottonwoods in which there are demonstrable community level genetic effects, is inherently created when trait variation among genotypes of a foundation species has ecological effects on associated species.

This builds on many previous studies demonstrating that the community level effects vary among multiple genotypes. It is not clear what potential there is for feedbacks there are to the origins (e.g. the cottonwood genotypes) from the community, and as such it cannot be determined whether these networks are cyclic or rooted. In terrestrial ecosystems, lichen play important ecological roles, such as substrate stabilization and nutrient fixation (?). Crustose lichen in tropical forest ecosystems can constitute significant proportions of the standing biomass (?). In some systems lignicolous lichens can have demonstrable effects on the availability of nutrients for the trees that they are associated with (?), although none of these ecosystem functions have been measured for the lichen in the system in this study. Elucidating the absence and/or presence of and quantifying such

feedbacks would allow for the determination of the cyclic nature and potential evolutionary dynamics of this system. The presence of feedbacks would provide the potential for non-linear dynamics in which evolutionary effects are damped or amplified by the structure of the network. For example, a star structure in which there is a primary or core set of central species with feedbacks from the radiating species has been demonstrated to be a structure that amplifies evolutionary dynamics (31). If such feedbacks do not exist, and these sub-networks of the lichen and tree genotypes are likely to be multi-rooted networks. Such a structure is theorized to generally promote diversification as variation arising from the shifting distribution of the “roots”, i.e. genotypes; however, loss of genotype/root diversity could lead to fixation of a single genotype in the population and a decrease in community-wide diversity. However, such feedbacks to tree fitness are not necessary for evolutionary dynamics to play out within the lichen networks through the effects of altered network structure on lichen interaction outcomes, such as competitive exclusion leading to selection. Also, since lichen are multi-species complexes, there is high potential for evolutionary dynamics to shift within the context of the lichen symbiosis; and there is substantial evidence that lichen have served as the “cradle of symbiotic fungal diversification” (?) and recent research has shown significant network structure of endolichenic fungi and lichen collected from across North America (?).

**Implications for Interspecific Indirect Genetic Effects (IIGEs).** Interspecific indirect genetic effects (IIGE) theory provides a quantitative framework within which to approach evolutionary theory at higher levels of biological organization: from populations to communities and ecosystems. To date, this theory has focused on modeling the strong effects of foundation species (7, 27), but it has not yet integrated developments in the ecological or evolutionary network theory literature. This is to say that it has not developed a way to examine complex interactions among species; however, previous studies have demonstrated this network context is likely to be important, as altering the structure of interaction networks provides a means for genetic effects to be damped or magnified within the system of interacting species. For example, (24) showed that the genetics based interactions of aphid resis-

tant and aphid susceptible trees resulted in different interaction networks of their associated arthropod communities composed of 139 species. At the scale of ecosystems, trophic networks or food webs direct and control the rates of energy and nutrient flux (50). Furthermore, in a predator-prey-plant study, Smith (20), showed that the interactions among species across trophic levels depended on plant genotype. Also, work by (51–53) observed consistent patterns of centralized interactions of species modules focused around hubs of plant-fungal interactions. In other words, a small number of plant and fungal symbionts tended to have disproportionate numbers of interactions with other species and likely are the drivers in determining community assembly, structure and dynamics.

The results of the current study provides clear empirical evidence that variation in network structure can be genetically based (i.e. heritable) and points to the need to expand IIGEs encompass the structure of interaction networks. Although such a synthesis necessitates a much greater effort than can be afforded in this paper, it is possible to point to several productive pathways forward. In terms of interaction networks, foundation species are relatively central within the system of interactions, that is their direct and/or indirect effects are greater than other species. So, when the more centralized (foundation) species have genetically based interactions, genetic effects will tend to be magnified in the community. Here, we found that even though more abundant or more centralized (i.e. “important”) species were present in the community, their effects were not the main component responding to genetic effects. Considering the impact of network structure would be a productive path forward for the theoretical development and application of the IIGE concept. These results also provide insights into the dynamics of real ecosystems and the potential inter-play of ecology and evolution with regard to empirical and theoretical support for neutral theory. Empirical (?) and theoretical work (?) have provided evidence for the applicability of functional equivalence of species in communities; and recently, models have also been developed that can reconstruct interactions networks with similar structural characteristics to those observed in real ecosystems (?). The findings of our study support the possibility that, although communities and their interaction networks may assemble as a result of

conditions that are entirely agnostic to functional variation, if there are strong ecological asymmetries, such as the case in foundation species systems, then spatial and temporal variation in network structure will arise as a result of trait variation within the foundation species. Taking even the extreme case, even when such genetically based variation is the result of “neutral” evolutionary processes (e.g., genetic drift), as long as resulting genetic diversity produces ecological relevant phenotypic diversity, then network structure will still have a genetic basis. Given that ecosystems with large asymmetries seem to be the rule rather than the exception for ecosystems (1? ? ? ? ?), the results of our study are likely to be broadly applicable and provides further support for the conclusion that the community context of evolutionary dynamics at either micro- or macro-evolutionary scales (?) can not be safely ignored. As genetic variation is inherent to biological systems, it would now seem that the adage “you can’t be neutral on a moving train” might well apply to ecosystems that are comprised of interacting species.

**Applicability to Other Systems.** In attempting to apply these findings to other systems, it is important to consider the spatial and temporal scaling of genetic effects. In the present study, the sessile nature of lichens means that individuals, and potentially multiple generations, live their entire lives on a single tree. As such, our study examines one scaling of a genetic effect, in which the phenotype of a single tree individual (i.e., tree genotype) has complete influence on the community with little to no effect of other tree individuals in the population. The extreme from this would be where the associated community moved among and interacted with not only other community members but also multiple tree individuals at a high rate, as would be the case with free-living animals (e.g. flying insects). In the latter case, the effect of tree genetics would then be the integral effect of all the tree individuals in the population, and, all other factors being equal, any one tree genotype would have a lower effect on associated community. In reality, ecosystems are a mixture of species of different body sizes and life-histories, and, as such, vary in the degree to which they interact with other organisms, which is the basis of the theory of the geographic mosaic of co-evolution (36, 57). It is now important to consider how the impacts of genetic effects on the network structure of sub-groups, such as lichens,

may or may not propagate through the ecosystem to more mobile organisms. As developed previously, the degree to which a genetic effect influences the community is a function of the fidelity of the genetic effect (i.e., heritability) and both the frequency and the intensity of the interaction (27). One possible path forward is for future work to extend the many previous community genetics studies that have focused on sessile organisms, such as galling insects (11, 18, 24, 58?), to quantify the frequency of these interactions in the context of the larger community. This would provide an estimate of the relative impact of these focal, often termed foundation, species. In addition, community genetics theory has only considered first order interactions, i.e., between two organisms (7, 27, 28). Given that network structure could be influenced by genetic effects, as evidenced by the present study, assessing higher order interactions could provide a path forward for theoretical advances that could help with identifying important characteristics of sub-groups to focus on in empirical studies.

Although our study was conducted with a community of lichens, these results can be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level (7, 56). However, there are important points to consider when extending the observed genetically based response of the lichen networks to other systems. As bark lichen individuals do not move, but grow in a primarily two dimensional plane, these communities and their interactions occur in the highly localized context of the tree's bark surface. Lichen individuals are also many orders of magnitude smaller than the tree individual in this system (38). For these reasons, the genetic effects on these communities is not damped by the movement of individuals and the mixing of the effect of different tree genotypes on the lichen community, as might occur for more mobile species (e.g. insects and birds). Relatedly, we only examined lichen in this study, and other species whose distributions, abundances and/or interactions vary in their response to tree genotype, such as animals that may also impact lichen communities, could be playing a role that we did not examine. For example, an analysis of the multivariate correlations of different components of the community in this system demonstrated significant patterns of genetic co-responses to tree genotype,

supporting the non-mutually exclusive possibilities of shared responses to tree genotype or tree genotypic effects on interactions among these sub-communities (4). As such, although we can not rule out the possibility that other unmeasured tree traits or organisms correlated with bark roughness are underlying the observed patterns, substantial research supports the importance of genetically based tree traits for communities and ecosystems (8), and in particular bark roughness for bark lichen communities (38, 39, 49).

**Conclusion.** In the face of the high degree of complexity and potential context dependency of ecological processes, the current study points to the utility of considering the spatial and temporal scales of interactions, as discussed to some in previous studies (16, 59, 60). In the present study, we found that community assembly processes, such as environmental filtering and species interactions, are genetically based. This is likely due, in part, to the large difference in the differences in size and longevity of the lichen and cottonwood individuals with the trees determining the environment in which the lichen occur. As developed elsewhere, the importance of foundation species, their interactions and the hierarchical structures that they create in communities (1? ?) suggests that future work would be aided by determining these modules within the biotic community that include species with similar differences in body-size and time-scales. Also, 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 their associated ecosystem functions (61). Such findings will bring us closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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1. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3(9):479–486.
2. Busby PE, et al. (2015) Genetics-based interactions among plants, pathogens, and herbivores define arthropod community structure. *Ecology* 96(7):1974–1984.

3. Barbour RC, et al. (2009) A geographic mosaic of genetic variation within a foundation species and its community-level consequences. *Ecology* 90(7):1762–1772.
4. Lamit LJ, et al. (2015) Tree genotype mediates covariance among communities from microbes to lichens and arthropods. *Journal of Ecology* 103(4):840–850.
5. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia* 170:695–707.
6. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364(1523):1607–16.
7. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes.
8. Des Roches S, et al. (2018) The ecological importance of intraspecific variation. *Nature Ecology and Evolution* 2(1):57–64.
9. Wade MJ (2007) The co-evolutionary genetics of ecological communities.
10. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming decade. *New Phytologist*.
11. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nature reviews. Genetics* 7:510–523.
12. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 366(1569):1425–37.
13. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co-extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
14. Guimarães PR, et al. (2007) Interaction Intimacy Affects Structure and Coevolutionary Dynamics in Mutualistic Networks. *Current Biology* 17(20):1797–1803.
15. Gómez JM, Abdelaziz M, Camacho JPM, Muñoz-Pajares AJ, Perfectti F (2009) Local adaptation and maladaptation to pollinators in a generalist geographic mosaic. *Ecology Letters* 12(7):672–682.
16. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure. *Molecular ecology* 15:1379–1391.
17. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics: opportunities for synthesis. *Ecosphere* 8(8):e01900.
18. Bailey JK, Wooley SC, Lindroth RL, Whitham TG (2005) Importance of species interactions to community heritability: a genetic basis to trophic-level interactions. *Ecology Letters* 8(0):051120262725008.
19. Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology*.
20. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic interactions and selection: trees, aphids and birds. *Journal of evolutionary biology* 24(2):422–9.
21. Smith DS, et al. (2015) Introduced elk alter traits of a native plant and its plant-associated arthropod community. *Acta Oecologica* 67:8–16.
22. Barbour MA, et al. (2016) Genetic specificity of a plant-insect food web: Implications for linking genetic variation to network complexity. *Proceedings of the National Academy of Sciences of the United States of America* 113(8):2128–2133.
23. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foundation species generates network structure that may drive community dynamics and evolution. *Ecology* 97(3):15–0600.
24. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation species affect community diversity, stability and network structure. *Proceedings of the Royal Society B: Biological Sciences* 284(1854):20162703.
25. Newman MEJ (2006) Modularity and community structure in networks. *Proceedings of the National Academy of Sciences of the United States of America* 103(23):8577–82.
26. Sole R, Bascompte J (2006) *Self-Organization in Complex Ecosystems*. (Princeton University Press, Princeton, N.J.).
27. Shuster SM, Lonsdorf EV, Wimp GM, Bailey JK, Whitham TG (2006) COMMUNITY HERITABILITY MEASURES THE EVOLUTIONARY CONSEQUENCES OF INDIRECT GENETIC EFFECTS ON COMMUNITY STRUCTURE. *Evolution* 60(5):991.
28. Whitham TG, Allan GJ, Cooper HF, Shuster SM (2020) Intraspecific Genetic Variation and Species Interactions Contribute to Community Evolution. *Annual Review of Ecology, Evolution, and Systematics* 51(1).
29. Gehring C, et al. (2014) Plant genetics and interspecific competitive interactions determine ectomycorrhizal fungal community responses to climate change. *Molecular Ecology* 23(6):1379–1391.
30. Gehring CA, Shultz CM, Flores-Rentería L, Whipple AV, Whitham TG (2017) Tree genetics defines fungal partner communities that may confer drought tolerance. *Proceedings of the National Academy of Sciences*.
31. Lieberman E, Hauert C, Nowak MA (2005) Evolutionary dynamics on graphs. *Nature* 433(7023):312–316.
32. Rohr RP, Saavedra S, Bascompte J (2014) On the structural stability of mutualistic systems. *Science* 345(6195).
33. Barbour MA, et al. (2019) Trait plasticity is more important than genetic variation in determining species richness of associated communities. *Journal of Ecology* 107(1):350–360.
34. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecological Economics* 71:80–88.
35. Zytynska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 366:1329–1336.
36. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499.
37. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance. *Science* 312:431–433.
38. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influences a dominant bark lichen. *Fungal Ecology* 4(1):103–109.
39. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly across multiple environments. *Ecology* 96(4):960–971.
- 1021 Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence networks to assess the impacts of climate change. *Ecography* 34:897–908.
- 1022 Butts CT (2019) *sna: Tools for Social Network Analysis*. R package version 2.5.
- 1023 Everett MG, Borgatti SP (2014) Networks containing negative ties. *Social Networks* 38(1):111–120.
- 1024 Schöch D (2020) *signnet: An R package to analyze signed networks*.
- 1025 Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software* 22(7):1–19.
- 1026 Oksanen J, et al. (2019) *vegan: Community Ecology Package*. R package version 2.5-6.
- 1027 Borrett SR, Lau MK (2014) *enaR: An R package for Ecosystem Network Analysis*. *Methods in Ecology and Evolution* 5(11):1206–1213.
- 1028 Conner K, Hartl DL (2004) *A Primer of Ecological Genetics: a textbook*. (Sinauer Associates, Inc., Sunderland, Massachusetts), 1st edition, p. All.
- 1029 Dawkins R (year?) *The extended phenotype : the long reach of the gene*. p. 468.
- 1030 Bdeir R, et al. (2017) Quantitative trait locus mapping of Populus bark features and stem diameter. *BMC Plant Biology*.
- 1031 Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Social Networks* 28:466–484.
- 1032 Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommunity level.
- 1033 Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs in plant-root fungal biomes. *Journal of the Royal Society Interface*.
- 1034 Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus networks. *Nature Communications*.
- 1035 Diaz-Castelazo C, et al. (2010) Changes of a mutualistic network over time: Reanalysis over a 10-year period. *Ecology*.
- 1036 Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. *Ecology letters* 14(9):877–85.
- 1037 Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and evolution. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 366(1569):1322–8.
- 1038 Thompson JN, Schwid C, Guimaraes PR, Friberg M (2013) Diversification through multiltrait evolution in a coevolving interaction. *Proceedings of the National Academy of Sciences*.
- 1039 Crutsinger GM, et al. (2014) Testing a 'genes-to-ecosystems' approach to understanding aquatic-terrestrial linkages. *Molecular Ecology* 23(23):5888–5903.
- 1040 Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to body size yields high degree of intervality. *Journal of theoretical biology* 271(1):106–113.
- 1041 Zytnska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2).
- 1042 Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological networks to habitat loss. *Ecology letters* 16:844–52.

## Supplementary Materials

### Tables.

		df	SS	R2	F	p-value
1146	geno	9.00	44078.13	0.54	3.58	0.05
1147	Residual	27.00	36915.46	0.46		
	Total	36.00	80993.59	1.00		

**Table 1. PERMANOVA Pseudo-F Table of lichen network similarity to genotype.**

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	response	df	statistic	H2	p-value
Lichen Network Similarity		9	3.5821	0.41	0.0537
Average Mutual Information			3.5235	0.31	0.0254
Centralization			4.0444	0.33	0.0184
Centralization In-Degree			4.4812	0.35	0.0142
Centralization Out-Degree			3.8615	0.32	0.0205
Centralization In-Degree (positive)			3.9852	0.33	0.0190
Centralization In-Degree (negative)			0.3304	0.11	0.2508
Centralization Out-Degree (positive)			3.5585	0.31	0.0248
Centralization Out-Degree (negative)			0.0862	0.05	0.3446
Degree			3.5175	0.32	0.0255
Degree (positive)			3.6925	0.32	0.0229
Degree (negative)			0.0327	0.03	0.3859
Percent Lichen Cover			0.0000	0.00	1.0000
Lichen Species Diversity			0.0000	0.00	0.4543
Lichen Species Richness			0.0000	0.00	0.4543
Lichen Species Evenness			0.0000	0.00	0.4543
Percent Rough Bark			4.8526	0.32	0.0113
pH			0.0000	0.00	1.0000
Carbon-Nitrogen Ratio			0.0000	0.00	1.0000
Condensed Tannins			3.0522	0.32	0.0343
BR-L Residuals			0.0000	0.00	1.0000
BR-Cen Residuals			0.0000	0.00	1.0000

**Table 2. Genotypic effects on tree traits and bark lichen.**

lichen species	mean	statistic	H2	p-value
Positive				
In-Degree				
<i>X. galericulata</i>	0.2703	0	0	1
<i>C. subdeflexa</i>	0.8919	2.1926	0.2158	0.0595
<i>L. spp.</i>	0.4324	0	0	1
<i>C. holocarpa</i>	0.5946	3.6146	0.3241	0.024
<i>X. montana</i>	0.0541	0	0	0.4543
<i>P. melanra</i>	0.1351	0	0	1
<i>P. adscendens</i>	0			
<i>P. undulata</i>	0.027	0	0	0.4543
R. sp.	0.1351	2.049	0.2613	0.0656
Out-Degree				
<i>X. galericulata</i>	0.027	0	0	0.4543
<i>C. subdeflexa</i>	0.6757	0	0	1
<i>L. spp.</i>	0.5946	0.0061	0.0126	0.4247
<i>C. holocarpa</i>	0.7027	3.1318	0.2981	0.0327
<i>X. montana</i>	0.0811	2.9228	0.3163	0.0375
<i>P. melanra</i>	0.1351	0	0	1
<i>P. adscendens</i>	0			
<i>P. undulata</i>	0.027	0	0	0.4543
R. sp.	0.2973	0.1505	0.0612	0.3119
Negative				
In-Degree				
<i>X. galericulata</i>	0			
<i>C. subdeflexa</i>	0.1892	0	0	0.4543
<i>L. spp.</i>	0.1892	0.0015	0.0057	0.4398
<i>C. holocarpa</i>	0.1351	0	0	1
<i>X. montana</i>	0.027	0.0377	0.0394	0.3807
<i>P. melanra</i>	0			
<i>P. adscendens</i>	0			
<i>P. undulata</i>	0			
R. sp.	0.1622	0	0	1
Out-Degree				
<i>X. galericulata</i>	0.2432	0	0	1
<i>C. subdeflexa</i>	0.4054	0	0	0.4543
<i>L. spp.</i>	0.027	0	0	0.4543
<i>C. holocarpa</i>	0.027	0	0	0.4543
<i>X. montana</i>	0			
<i>P. melanra</i>	0			
<i>P. adscendens</i>	0			
<i>P. undulata</i>	0			
R. sp.	0			

**Table 3. REML tests of the effect of tree genotype on lichen species centrality.**

	BR	CT	pH	CN	PC	SR	SE	SD	L	Cen	Df	SumOfSqs	R2	F	Pr(>F)	
BR									-0.34	-0.39	geno	9.0000	1.5049	0.2001	0.7507	0.8878
CT					-0.34				0.34		Residual	27.0000	6.0143	0.7999		
pH											Total	36.0000	7.5193	1.0000		
CN																
PC																
SR									0.76	0.47						
SE									0.85	0.45						
SD									0.59	0.33						
L										0.88						
Cen																

**Table 4. Matrix of correlations among tree traits, lichen community metrics and network metrics**

**Table 5. Pseudo-F Table of lichen community similarity PERMANOVA.**

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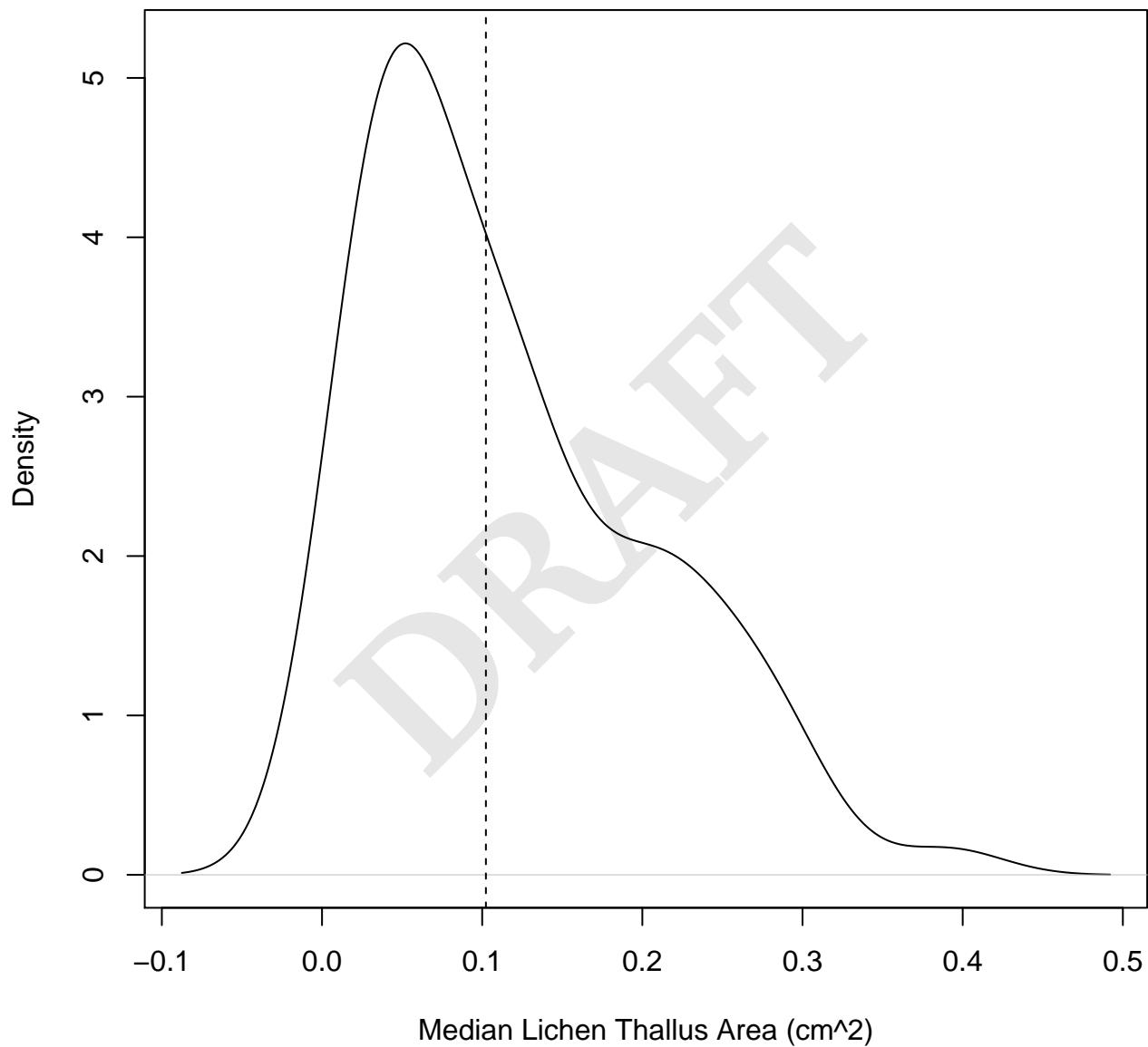
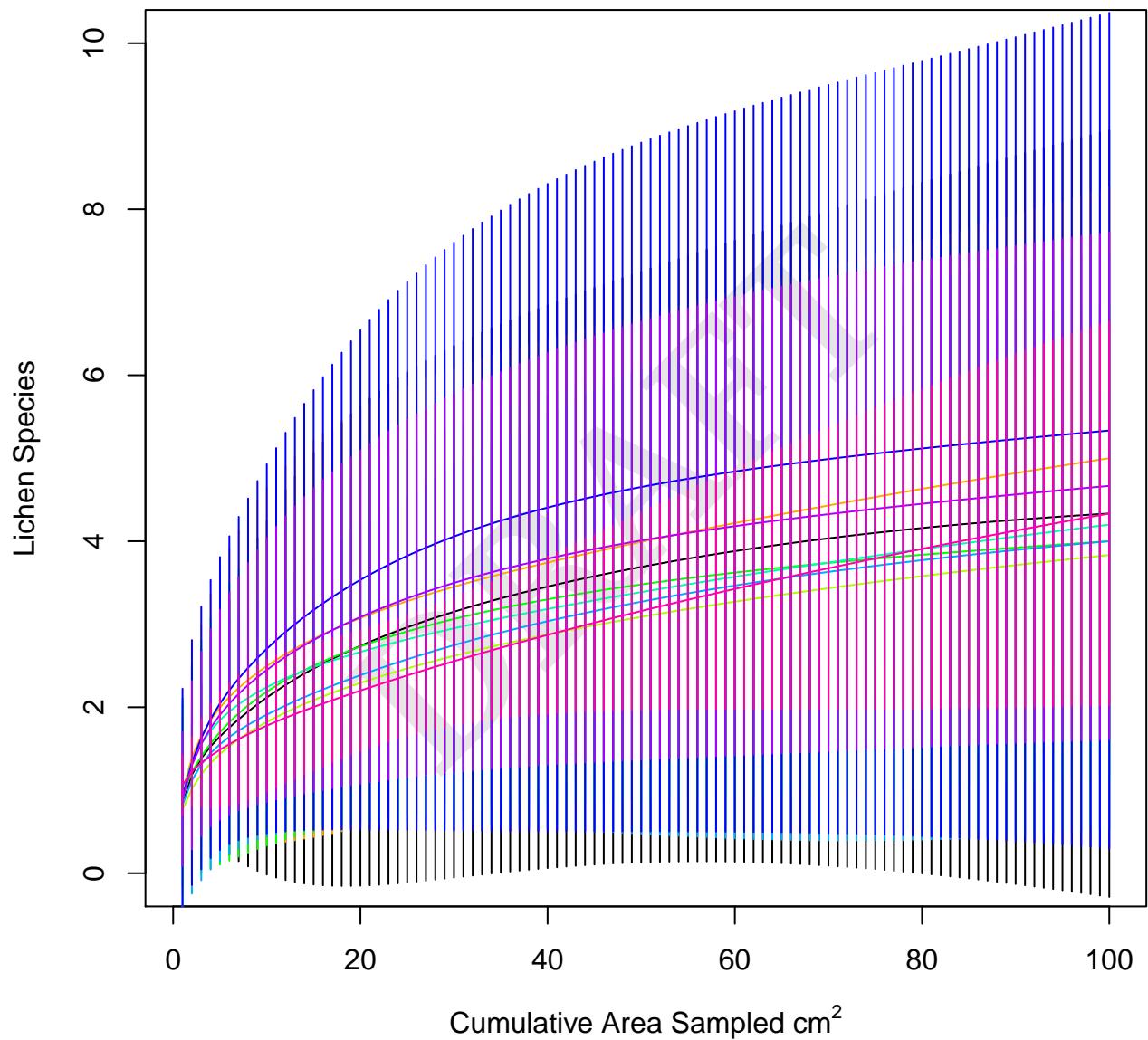


Fig. 1



**Fig. 2.** Species-area curve by genotype.

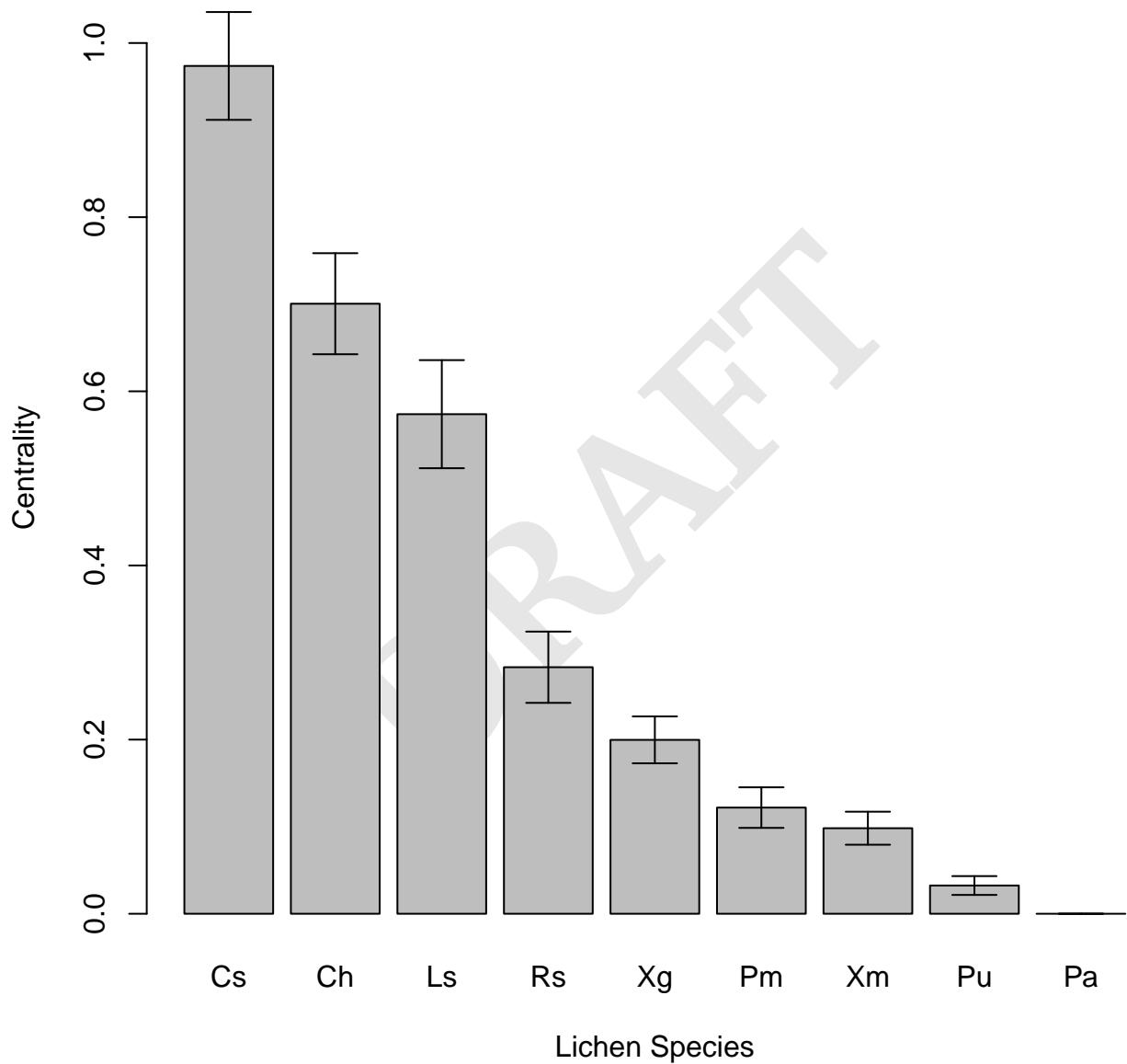


Fig. 3