

Genotypic variation in a foundation tree alters ecological network structure of an associated community

Matthew K. Lau^{a,b,1}, Louis J. Lamit^b, Rikke R. Naesbourg^c, Stuart R. Borrett^d, Matthew A. Bowker^e, and Thomas G. Whitham^a

^aDepartment of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011, USA; ^bHarvard Forest, Harvard University, 324 N Main St, Petersham, MA 01366, USA; ^cDepartment of Biology, Syracuse University, 107 College Place Syracuse, NY 13244, USA; ^eUniversity of California Berkeley, Berkeley, CA, USA; ^fDepartment of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC, 28403, USA; ^gSchool of Forestry, Northern Arizona University, Flagstaff, AZ 86011, USA

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1 **Biological evolution occurs in the context of complex networks of**
2 **interacting species in which natural selection defines the structure**
3 **of ecological networks. Fundamental to this evolutionary process**
4 **is the discovery of a genetic basis to ecological network structure.**
5 **Although previous work has demonstrated that tree genotype con-**
6 **tributes to interaction network structure at the scale of forest stands,**
7 **the contribution of tree genetics to localized interaction networks**
8 **at the scale of individual trees has not yet been explored. To test**
9 **the degree to which tree genetics can contribute to network struc-**
10 **ture we conducted quantitative modeling of interaction networks. We**
11 **constructed networks of epiphytic lichen associated with individual**
12 **trees that were a part of a long-term experimental common garden**
13 **of genotypes of (*Populus angustifolia*), a foundation species. We**
14 **found three main results. First, tree genotype significantly predicted**
15 **lichen network similarity, i.e. trees of the same genotype had more**
16 **similar lichen networks. Second, positive interactions of one lichen**
17 **species, *Caloplaca holocarpa* drove the genetically based variation**
18 **in network structure. Third, bark roughness was both predicted by**
19 **tree genotype and correlated with lichen network similarity. We con-**
20 **clude that tree genotype can influence not only the relative abun-**
21 **dances of organisms but also the interaction network structure of**
22 **associated organisms. Given that variation in network structure can**
23 **have consequences for the dynamics of communities through alter-**
24 **ing the stability of the system and modulating or amplifying perturba-**
25 **tions, these results have important implications for the evolutionary**
26 **dynamics of ecosystems.**

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

1 **E**volution occurs in the context of complex ecolog-
2 **ical networks. Initially, evolution in a community**
3 **context was focused on examples of highly co-evolved**
4 **pairs of species (e.g. Darwin's famous prediction of**
5 **the Sphinx Moth and Christmas Orchid) (?). How-**
6 **ever, studies of diffuse co-evolution (*sensu* (?)) (?**
7 **? ?), geographic mosaics of co-evolution (1) and**
8 **community genetics (2) have provided an in-road for**
9 **ecological network approaches (3? ?) to illuminate**
10 **a more complex perspective of the interface between**
11 **ecological and evolutionary dynamics. There is now**
12 **evidence to support that selection tends to occur**
13 **among groups of species (? ? ?) favoring the de-**
14 **velopment of small webs (? ? ?) and that genetic**

15 variation and phylogenetic relatedness contributes
16 to variation in community assembly (4) and species
17 interactions (2, 5, 6), which shapes the ecological
18 interaction networks (7).

19 Community genetics studies (8) have shown that
20 genetic variation in foundation species (9) plays a
21 significant role in defining distinct communities of in-

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 analyses. Here, we use a long-term common garden experiment to reveal the effect that genotypic variation can have on networks of lichens that occur on the bark of a foundation tree species. We found that lichen interaction network structure is genetically based and primarily driven by a tree trait, 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 interactions, given that interaction network structure has systems-level properties that could affect the response of these communities to selection.

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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¹Dr. Matthew K. Lau. E-mail: matthewklau@fas.harvard.edu

teracting organisms: such as, endophytes, pathogens, lichens, arthropods, and soil microbes. Multiple studies have now demonstrated that genetic variation influences numerous functional traits (e.g., phytochemical, phenological, morphological) produces a multivariate phenotype (10) that contributes to variation in associated communities (5). The importance of genetic variation in structuring ecological systems was recently reviewed (11), 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.

Additional work has provided support for the hypothesis that not only does composition vary among genetically distinct genotypes of foundation species but that it also impacts the structure of species interactions. However, studies in the network ecology literature generally do not include a genetic component (?). And, community genetics studies have primarily focused on community composition in terms of the abundance of species (11). Multiple studies from different plant-associated communities (including *Populus*, *Solidago*, *Oenothera*, *Salix*) have examined the effect of genetic variation on trophic interactions (12?–15) and generally found that increasing genotypic diversity leads to increased trophic complexity. Similarly, two other studies have examined the effect of genotypic variation on the structure of interactions between tree individuals and the associated community (3, 16) and both found that genotypic diversity generates increased network modularity (i.e. compartmentalization).

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 (17). Here we examine the genetic basis of network structure on a community of sessile lignicolous (i.e. bark) lichen on cottonwood trees. Using a long-term (20+ years), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *Populus angustifolia*. We focused on a model community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (18, 19). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover

rates, allowed us to assess interactions among lichen species on individual trees. We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (1, 3, 16, 20). If correct, we expect to find that network structure is genetically based in which different plant genotypes support different interaction networks and that these interactions networks can function as indicators of ecological dynamics important for conserving biodiversity. 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. Based on previous community genetics studies, particularly (?) which proposed the community similarity rule, we hypothesize that trees will vary in some phenotypic traits and those trees of the same genotype will tend to have similar traits leading to similarities in lichen network structure.

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 (21).

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 thirteen 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 cm² 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 10 cm² quadrats centered at 50 cm and 85 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 melanochra*, 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 subolivacea*, *Meanelia elegantula*.

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 ± 0.001 cm² (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 as independent with respect to lichen-lichen interactions.

We also measured several bark traits for each tree: including, bark roughness, condensed tannin, carbon and nitrogen concentrations and pH. **ADD METHODS FROM JAMIE.**

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 quantified the presence of lichen in the 1 cm² 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 (22). 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 (S_{ii}) 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 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 how one species impacts another with zero being no effect and values less than or greater than zero interpreted as negative and positive effects, respectively. Here, we will refer to this matrix (\mathbf{D}) as an interaction matrix with the properties that it can be asymmetric (i.e. P_{ij} does not necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e. a species does not influence it's own probability of being observed).

Network Metrics. To quantify the structural variation of lichen networks we calculated several metrics at both the node and whole-network level. For individual nodes (i.e. species) in each network, we calculated both the degree Eq. (1) and the centrality. 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.

ADD EQUATIONS FOR METRICS

$$\sum x_i \quad [1]$$

- Node degree
- Node centrality
- Network degree



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). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground (A and B, respectively). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

- 229 • Centralization
- 230 • In vs out
- 231 • Pos vs neg

232 To calculate separate metrics for positive and negative
 233 links, we applied methods for calculating the centrality
 234 accounting for the sign differences (?? and Borgatti 2014).
 235 We used the `signnet` package version ????, which is available at ????.
 236

237 **Statistical Analyses, Software and Data.** We used a com-
 238 bination of parametric and non-parametric, permutation
 239 based frequentist statistical analyses to test for the effects
 240 of genetic variation on lichen communities and their inter-
 241 action networks. To assess the effect of genotype on uni-
 242 variate responses, we used additive, random effects models
 243 with Restricted Maximum Likelihood (REML). We used
 244 a combination of Least Squares Regression, Analysis of
 245 Variance (ANOVA) and correlation tests to quantify and
 246 test for the relationship among other variables. Bark
 247 roughness, lichen cover and species richness were square-
 248 root transformed to meet the assumptions of homogeneity
 249 of variance and normality for these tests.

For multivariate response variables, such as lichen com-
 250 munity composition and network structure, we used dis-
 251 tance based multivariate statistical approaches, including
 252 Permutational Analysis of Variance (PERMANOVA) and
 253 Mantel tests. To quantify the similarity of lichen net-
 254 works among individual trees, we calculated the pairwise
 255 Euclidean distance of the **D** interaction matrices among
 256 all pairs of trees.
 257

For visualization of multivariate patterns, we used
 258 Non-metric Multi-Dimensional Scaling (NMDS) (??) to
 259 produce dimensionally reduced ordinations of these multi-
 260 variate responses and fitted vectors for continuous predic-
 261 tor variables to the ordinated values (??). Using random
 262 initial configurations with a maximum of 500 iterations
 263 and a change in stress threshold of less than 10^{-12} . Final
 264 configurations has the lowest stress with at most a stress
 265 level of 0.10.
 266

For each network, we also calculated metrics that mea-
 267 sure different structural aspects. Although there are many
 268 other metrics, for the sake of simplicity we focus on a
 269 subset that represent several interesting features of net-
 270 work structure (see (23)). We calculated the number of
 271 interactions or “links” in each network, which provides a
 272



Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 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 (22), 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.

measure of the size of the network (24?). We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network (?). 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. The modularity of each network was also quantified using a weighted algorithm (?), which measures the degree to which a given network is divided into groups of species more connected to each other than other species. As with the other response variables, the number of links was log-transformed and both modularity and centralization scores were fourth-root transformed to meet variance and normality assumptions.

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 (?). 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 (2? ?). For the multivariate analyses, where we employ PERMANOVA, we followed the methods of (?) 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. The project is also archived via Zenodo

307 at zenodo.com/doiXXXXXX. All analyses were conducted
 308 using the programming language R version 3.6.1 (R De-
 309 velopment Core Team 2019).

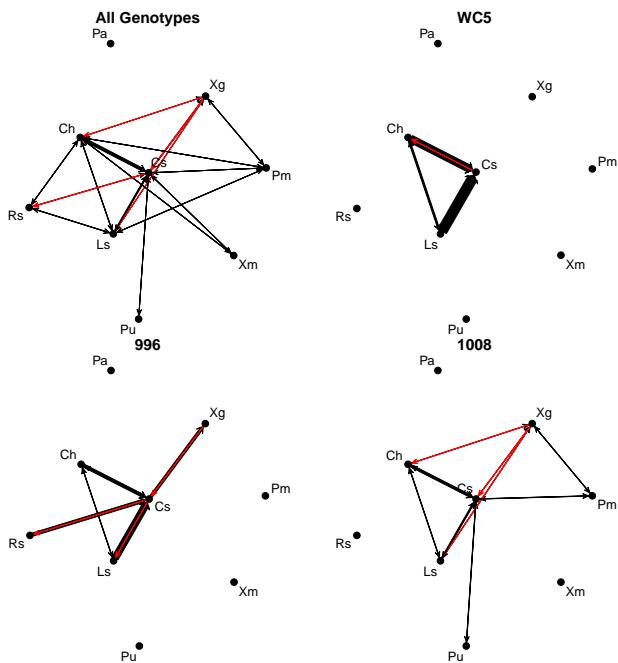


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive) 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.

310 Results

311 Tree genotype influenced lichen network structure.
 312 Tree genotype significantly predicted the struc-
 313 tural similarity of lichen networks (PERMANOVA:
 314 Pseudo- $F_{9,27} = 3.58$, $H^2 = 0.41$, $p\text{-value} = 0.0537$)
 315 (Fig. 4). Overall network level metrics responded
 316 significantly to tree genotype (Table 1), including net-
 317 work degree ($RLRT = 3.52$, $H^2 = 0.32$, $p\text{-value} =$
 318 0.0255) and centralization including both in-coming
 319 and out-going links ($RLRT = 4.04$, $H^2 = 0.33$, $p\text{-}$
 320 value = 0.0184) or when separated into in-coming
 321 only ($RLRT = 3.9852$, $H^2 = 0.3309$, $p\text{-value} =$
 322 0.0190) or out-going only ($RLRT = 3.8615$, $H^2 =$
 323 0.3193, $p\text{-value} = 0.0205$). Metrics including only
 324 positive links also showed a significant effect of tree
 325 genotype, including positive degree ($RLRT = 3.6925$,
 326 $H^2 = 0.3242$, $p\text{-value} = 0.0229$), positive in-going
 327 centralization ($RLRT = 4.4812$, $H^2 = 0.3487$, $p\text{-}$
 328 value = 0.0142) Metrics calculated with negative
 329 links were not significant, including degree (negative)
 330 ($RLRT = 0.0327$, $H^2 = 0.0318$, $p\text{-value} = 0.3859$)
 331 and both in-coming (negative) ($RLRT = 0.3304$, H^2
 332 = 0.1057, $p\text{-value} = 0.2508$) and out-going centraliza-
 333 tion (negative) ($RLRT = 0.0862$, $H^2 = 0.0513$,
 334 $p\text{-value} = 0.3446$).

	response	statistic	H^2	p-value
Lichen Network Similarity	3.5821	0.4130	0.0537	
Degree	3.5175	0.3156	0.0255	
Degree (positive)	3.6925	0.3242	0.0229	
Degree (negative)	0.0327	0.0318	0.3859	
Centralization	4.0444	0.3305	0.0184	
Centralization In-Degree	4.4812	0.3487	0.0142	
Centralization In-Degree (positive)	3.9852	0.3309	0.0190	
Centralization In-Degree (negative)	0.3304	0.1057	0.2508	
Centralization Out-Degree	3.8615	0.3193	0.0205	
Centralization Out-Degree (positive)	3.5585	0.3119	0.0248	
Centralization Out-Degree (negative)	0.0862	0.0513	0.3446	

**Table 1. Genotypic effects on the associated lichen net-
 work structure.**

The genetic response of network centralization was
 335 driven by variation in *Caloplaca holocarpa*. Central-
 336 ity varied significantly among species ($F_{8,324} = 7.99$,
 337 $R^2 = 0.16$, $p\text{-value} << 0.0001$). *Caloplaca holocarpa*
 338 centrality was the main species to exhibit a signifi-
 339 cant response to tree genotype in terms of positive
 340 centralization for both the in-coming ($RLRT = 3.61$, H^2
 341 = 0.32, $p\text{-value} = 0.0240$) and out-going ($RLRT =$
 342 3.13, $H^2 = 0.30$, $p\text{-value} = 0.0327$) perspectives, but
 343 not for either negative centrality metrics in-coming
 344 ($RLRT = 0$, $H^2 = 0$, $p\text{-value} = 1$) or out-going
 345 ($RLRT = 0$, $H^2 = 0$, $p\text{-value} = 0.4543$). None of
 346 the other species' centralities showed a genotypic re-
 347 sponse (Supplementary Table 6) with the exception
 348 of *X. montana* ($RLRT = 2.92$, $H^2 = 0.32$, $p\text{-value} =$
 349 0.0375); however, the centrality of *X. montana* was
 350 much lower overall relative to *C. holocarpa* and the
 351 variation in *X. montana* centrality was restricted to
 352 two genotypes (Fig. 5).

Genotype indirectly influenced lichen network cen-
 354 tralization via the genetically based variation in bark
 355 roughness. The percent of rough bark was the only
 356 tree trait that displayed a significant response to
 357 genotype ($RLRT = 4.8526$, $H^2 = 0.3221$, $p\text{-value} =$
 358 0.0113). None of the other bark traits, condensed
 359 tannins ($RLRT = 0.0007$, $H^2 = 0.0041$, $p\text{-value} =$
 360 0.4439), pH ($RLRT = 0.00$, $H^2 = 0.00$, $p\text{-value} =$
 361 0.0000) or carbon-nitrogen Ratio ($RLRT = 0.0000$,
 362 $H^2 = 0.0000$, $p\text{-value} = 1.0000$), showed a significant

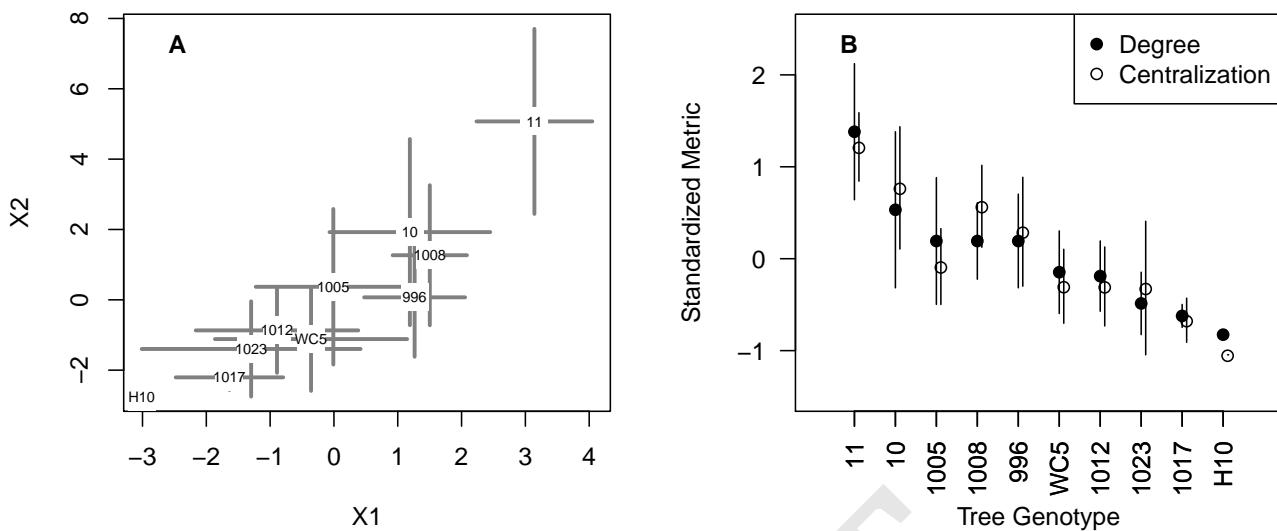


Fig. 4. The similarity of lichen networks varied among tree genotypes. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Genotype centroids that are closer together tend to have more similar lichen network structure. B. Plot showing the standardized ($\frac{x - \bar{x}}{\sigma}$) means (± 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 to tree genotype and none other than bark roughness were correlated with network similarity (Supplementary Table 5); therefore, we focused our analysis on bark roughness. We found that bark roughness was significantly correlated with network similarity (PERMANOVA: Pseudo- $F_{1,32} = 13.029$, $R^2 = 0.26$, *p-value* = 0.0096) and other lichen network metrics, including negative correlations with overall network degree ($df = 35$, $t = -2.13$, $r = -0.34$, *p-value* = 0.04) and centralization ($df = 35$, $t = -2.52$, $r = -0.39$, *p-value* = 0.02). To determine how much of the effect of bark roughness was genetically based, we used the residual values from regressions of network degree and centralization in tests of the effect of tree genotype and found no significant effect of tree genotype for either degree ($RLRT = 0.00$, $H^2 = 0.00$, *p-value* = 1.0000) or centralization ($RLRT = 0.00$, $H^2 = 0.00$, *p-value* = 1.0000), suggesting that the observed relationship between bark roughness and lichen network structure was largely genetically based (Fig. 6).

Discussion

We found that tree genotype influenced lichen network structure in the experimental cottonwood forest. Network similarity and metrics of network structure

tended to be more similar on trees of the same genotype. Generally, this genetic effect was manifested in positive interactions and largely driven by *C. holocarpa*. Bark roughness was the primary genetically based trait driving network structure. The genetically based trait, bark roughness, was the main driver of network variation. Not only was bark roughness the only trait observed to be genetically based, it was correlated with network structure and residual variation from this correlation was not explained by tree genotype. These results have important implications for the potential influence of genetically based variation in ecosystems with networks of interacting species.

Differences in distributions below the quadrat scale are leading to shifting patterns of interactions among lichen species, largely increased positive incoming and out-going interactions. It could also be that some other variable correlated with bark roughness is altering the quality (i.e. how) the lichen species are interacting, that is as opposed to simply the "quantity" of interactions. Bark roughness effect was negative, possibly serving the role that other lichen play in facilitating the success of new propagule attachment and the growth of establishing thalli. This is supported by the patterns overall

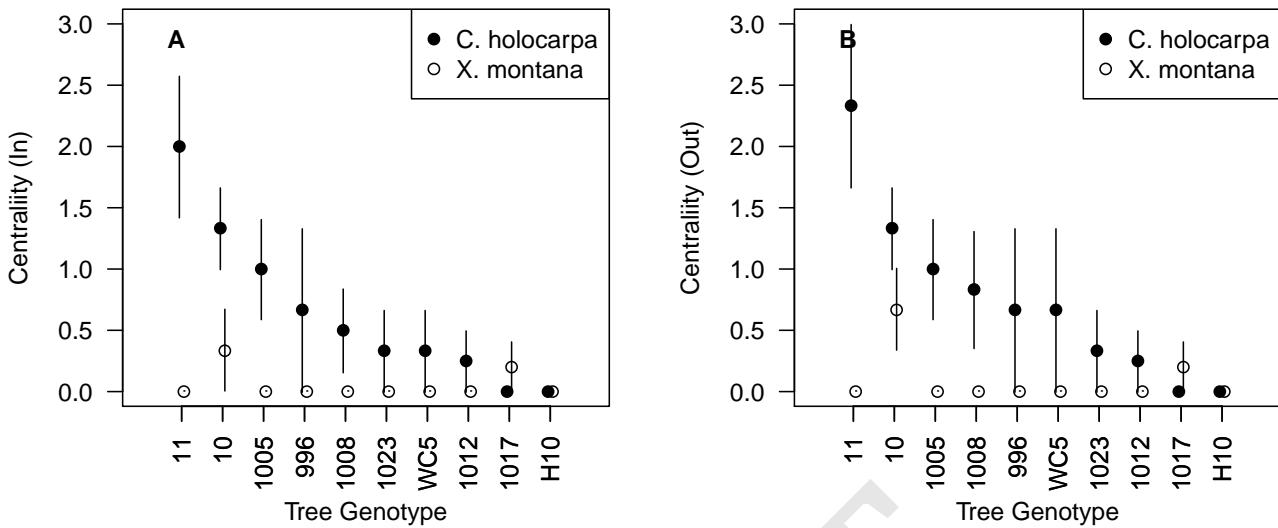


Fig. 5. Dot-plots showing the mean (dot) and ± 1 SE of in-degree (A) and out-degree (B) centrality for two species, *C. holocarpa* and *X. montana*. *Caloplaca holocarpa* centrality was highly variable among genotypes. *Xanthomendoza montana* centrality, both in- and out-degree, was only non-zero for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

being positive, including *C. holocarpa*'s centrality being positive both in and out. We don't know specific microscopic dynamics, such as photobionts, mycobionts, endolichenic fungi and bacteria, but variation in these underlying interactions could also be playing a role. Also, bark roughness had previously been shown to be an important tree trait influencing bark lichens (21) that is under strong genetic control (25).

There are important functional ramifications of genetically based variation in network structure. First, even if the composition of the communities is the same among individuals and genotypes, interactions may not be. We didn't observe compositional differences using the same data from which the lichen networks were derived. If we only had our composition dataset from this study, we would have concluded no response of the lichen community to tree genotype, even though the underlying interactions among lichen species does vary among genotypes. Community composition of lichen has previously been observed to be different among tree genotype in the same experimental garden, though this was observed with a larger sampling of total area and quadrats per tree. Regardless, this could result in a situation in which abundance based investigations of community-level genetic effects may miss important variation in the interactions among individuals.

in these communities, leading to an underestimate of genetic effects in ecosystems. It is possible that these underlying differences in interactions among lichen could lead to differences in community composition at a future point in time, however, this is not needed for evolutionary dynamics to play out.

Second, following on the previous point, genetic diversity could be influencing the stability of communities through the effects on the structure of interactions. Some network structures are likely to be more stable, either in response to disturbance or via self-organized dynamics. For example, centralized networks, although more efficient, are theorized to be more susceptible to targeted attacks on the center of the network. For example, consider a forest with two genotypes that support lichen communities that are similar in total abundances of each species but differ in terms of the structure. Extensions of game theory to evolutionary biology have demonstrated that network structure can lead to variation in evolutionary dynamics. Some structures tend toward dominance and dampening of selection, while others lead to amplification of selection (Newman). One class of networks that are theorized to have amplifying effects on networks have "star" shapes with one or few species at the center and radiating interactions out from the central core (Leiberman). This is struc-

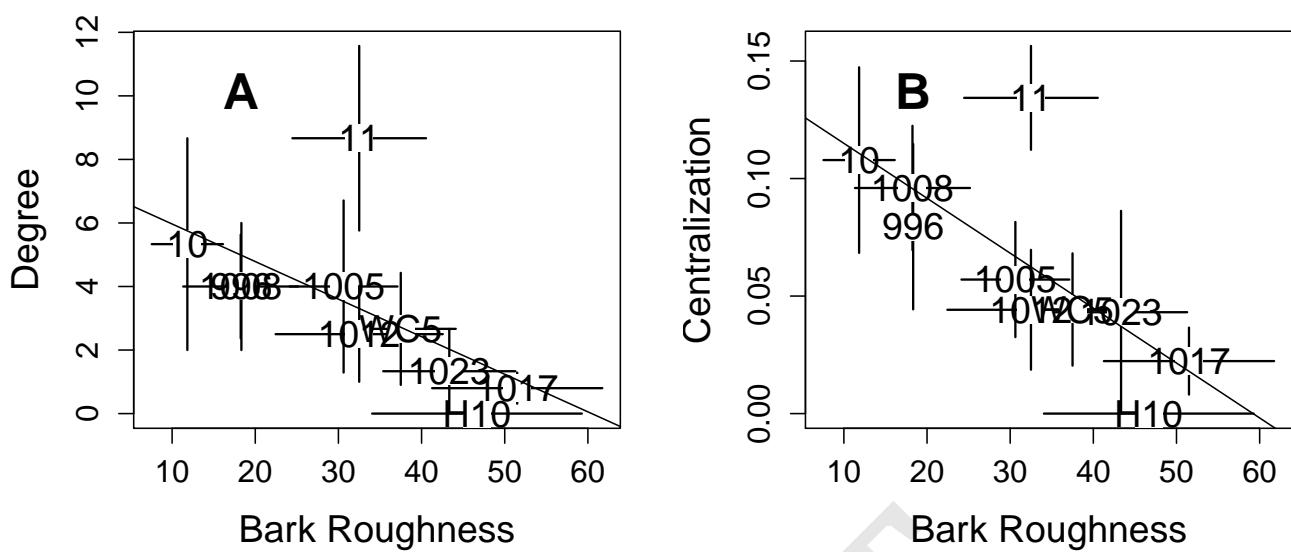


Fig. 6. Bivariate plots of the relationship between bark roughness and three network metrics: A) degree and B) centralization. Each plot displays the genotype mean \pm 1 S.E. for both variables and a least-squares regression calculated using the genotype means.

turally what we have observed with the networks that tend to occur on some of the genotypes in our study, i.e. the more centralized networks. It is possible that these more centralized networks could function as hot-spots of evolutionary dynamics resulting from the amplifying effect the network structure fostered on that tree genotype.

Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. For example, (3) showed that the genetics based interactions of aphid resistant 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 (26). Furthermore, in a predator-prey-plant study, Smith (13), showed that the interactions among species across trophic levels depended on plant genotype. Also, work by (27–29) 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.

There are several important points to consider with regard to the generalization of the observed genetically based response of the lichen networks. Body size and sessile nature of lichen important to observing genotype responses. As bark lichen individuals do not move, but grow in a largely two dimensional plane, these communities and their interactions occur in the highly localized context of the tree's bark surface. Lichen individuals are many orders of magnitude smaller than the tree individual and the life-span of a tree is many times that of a lichen. For these reasons, any genetic effects on these communities is not dampened 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). We only looked at lichen, other species whose distribution, abundance or interactions respond to tree genotype, such as epiphytic plants (e.g. moss and liverworts), algae or insects, could be playing a role. Other traits could also be playing a role, such as traits that are correlated with bark roughness, such as micro-aspect, albedo, moisture, etc.

- Barbour 2018 Trait plasticity is more important than genetic variation in determining species richness of associated communities. Synthesis: These results indicate that trait plasticity can be

523 a dominant driver of above- and below-ground
524 biodiversity.

- 525 Barbour 2015 Multiple plant traits shape the
526 genetic basis of herbivore community assembly.
527 Synthesis: Taken together, our results support
528 that the genetic basis of herbivore community
529 assembly occurs through a suite of plant traits
530 for different herbivore species and feeding guilds.
- 531 Busby 2019 Contingency rules for pathogen com-
532 petition and antagonism in a genetically based,
533 plant defense hierarchy. Synthesis: Our results
534 point to a *Populus* defense hierarchy with re-
535 sistance genes on top, followed by pathogen
536 competition, and finally pathogen antagonism
537 by endophytes. We expect these rules will help
538 to explain the variation in pathogen antagonism
539 that is currently attributed to context depen-
540 dency.
- 541 Barker 2019 Linking plant genes to insect com-
542 munities: Identifying the genetic bases of plant
543 traits and community composition. Synthesis:
544 These findings support the concept that partic-
545 ular plant traits are the mechanistic link between
546 plant genes and the composition of associated
547 insect communities.
- 548 Cooper 2018 Genotypic variation in phenological
549 plasticity: Reciprocal common gardens reveal
550 adaptive responses to warmer springs but not to
551 fall frost. Synthesis: Trees transferred to warmer
552 climates generally showed small to moderate
553 shifts in an adaptive direction, a hopeful result
554 for climate change. Trees experiencing cooler
555 climates exhibited large, non-adaptive changes,
556 suggesting smaller transfer distances for assisted
557 migration.
- 558 Whipple 2018 Epigenetic inheritance across the
559 landscape. Synthesis: Transgenerational epi-
560 genetic variation may alter the interpretation
561 of landscape genomic studies that rely upon
562 phenotypic analyses, but should have less influ-
563 ence on landscape genomic approaches that rely
564 upon outlier analyses or genome-environment
565 associations.

566 Although our study was conducted with a commu-
567 nity of lichens, these results should be generalized
568 to other groups of diverse organisms around the
569 world that also exhibit significant genetic signals at
570 the community level (30, 31). In the face of the
571 high degree of complexity and potential context de-
572 pendence of ecological processes, the current study
573 points to the utility of considering the spatial and
574 temporal scales of interactions, as discussed to some
575 in previous studies (32–34). In the present study,
576 we found that community assembly processes, such
577 as environmental filtering and species interactions,
578 are genetically based. This is likely due, in part,
579 to the large difference in the differences in size and
580 longevity of the lichen and cottonwood individuals
581 with the trees determining the environment in which
582 the lichen occur. We suggest that future work would
583 be aided by determining these modules within the
584 biotic community that include species with similar
585 differences in body-size and time-scales. As heritable
586 variation is the raw material for natural selection
587 to act upon, a genetic basis for interaction network
588 structure indicates evolutionary dynamics should be
589 considered at the community level and that con-
590 serving genetic variation is important to consider in
591 efforts to restore or preserve complex species interac-
592 tions and their associated ecosystem functions (35).
593 With such findings, it appears that we are closer to
594 understanding the evolutionary drivers of Darwin’s
595 entangled bank and the interconnectedness of species
596 in complex communities.

597 Future work should consider the potential influ-
598 ence on evolutionary dynamics of the associated com-
599 munities. The network of interactions of species that
600 are strongly influenced by a foundation species, could
601 amplify the effects of genotype, this serves as a means
602 for genetic effects to increase rather than diffuse
603 through an ecosystem either through space or over
604 time, as has been proposed in the construction of the
605 genetic diffusion hypothesis. Altered abundances can
606 lead to differences in interactions Genotype effects
607 on abundances of individual abundances may cancel
608 out. Specifically for asexually reproducing species,
609 such as many lichen are, shifting interaction frequen-
610 cies could lead to evolutionary outcomes, given the
611 potential to take-up symbionts and genetic material
612 from thalli that they come into contact with. Alter-
613 ing interaction frequencies could differences in the
614 frequencies the exchange of genetic materials among
615 lichen that could then be passed on to vegetative
616 and possibly sexually produced reproductive propa-
617 gation. The larger scale (stand or region) effects of

618 these "evolutionary units" on each tree would de- 695
619 pend on the connectivity and rate of movement of
620 propagules among trees per the geographic mosaic
621 of co-evolution hypothesis (1, 36).

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- 629 1. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499.
- 630 2. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes
631 to ecosystems. *Nature reviews. Genetics* 7:510–523.
- 632 3. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation
633 species affect community diversity, stability and network structure. *Proceedings of the Royal
634 Society B: Biological Sciences* 284(1854):20162703.
- 635 4. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming
636 decade. *New Phytologist*.
- 637 5. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant ge-
638 netic factors across levels of organization. *Philosophical transactions of the Royal Society of
639 London. Series B, Biological sciences* 364(1523):1607–16.
- 640 6. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure.
641 *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*
642 366(1569):1425–37.
- 643 7. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co-
644 extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
- 645 8. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly
646 across multiple environments. *Ecology* 96(4):960–971.
- 647 9. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and
648 dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 3(9):479–486.
- 649 10. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic,
650 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype
651 in a foundation tree species. *Oecologia* 170:695–707.
- 652 11. Des Roches S, et al. (2018) The ecological importance of intraspecific variation. *Nature
653 Ecology and Evolution* 2(1):57–64.
- 654 12. Johnson MTJ (2008) Bottom-up effects of plant genotype on aphids, ants, and predators.
655 *Ecology*.
- 656 13. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter-
657 actions and selection: trees, aphids and birds. *Journal of evolutionary biology* 24(2):422–9.
- 658 14. Smith DS, et al. (2015) Introduced elk alter traits of a native plant and its plant-associated
659 arthropod community. *Acta Oecologica* 67:8–16.
- 660 15. Barbour MA, et al. (2016) Genetic specificity of a plant-insect food web: Implications for
661 linking genetic variation to network complexity. *Proceedings of the National Academy of
662 Sciences of the United States of America* 113(8):2128–2133.
- 663 16. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foun-
664 dation species generates network structure that may drive community dynamics and evolu-
665 tion. *Ecology* 97(3):15–0600.
- 666 17. Barbour MA, et al. (2019) Trait plasticity is more important than genetic variation in determin-
667 ing species richness of associated communities. *Journal of Ecology* 107(1):350–360.
- 668 18. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecological
669 Economics* 71:80–88.
- 670 19. Zytynska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree
671 species influences the associated epiphytic plant and invertebrate communities in a com-
672 plex forest ecosystem. *Philosophical transactions of the Royal Society of London. Series B,
673 Biological sciences* 366:1329–1336.
- 674 20. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate
675 Biodiversity Maintenance<10.1126/science.1123412. *Science* 312:431–433.
- 676 21. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influ-
677 ences a dominant bark lichen. *Fungal Ecology* 4(1):103–109.
- 678 22. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net-
679 works to assess the impacts of climate change. *EcoGraphy* 34:897–908.
- 680 23. Lau MK, Borrett SR, Baisier B, Gotelli NJ, Ellison AM (2017) Ecological network metrics:
681 opportunities for synthesis. *Ecosphere* 8(8):e01900.
- 682 24. Lau MK, Borrett SR, Hines DE, Singh P (2015) enaR: Tools for Ecological Network Analysis.
- 683 25. Bdeir R, et al. (2017) Quantitative trait locus mapping of *Populus* bark features and stem
684 diameter. *BMC Plant Biology*.
- 685 26. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Social Networks*
686 28:466–484.
- 687 27. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommu-
688 nity level.
- 689 28. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs
690 in plant-root fungal biomes. *Journal of the Royal Society Interface*.
- 691 29. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus
692 networks. *Nature Communications*.

30. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and
693 evolution. *Philosophical transactions of the Royal Society of London. Series B, Biological
694 sciences* 366(1569):1322–8.
31. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes. 696
32. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure.
697 *Molecular ecology* 15:1379–1391.
33. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to
698 body size yields high degree of intervality. *Journal of theoretical biology* 271(1):106–113.
34. Zytynska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2). 701
35. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net-
702 works to habitat loss. *Ecology letters* 16:844–52.
36. Barbour RC, et al. (2009) A geographic mosaic of genetic variation within a foundation tree
704 species and its community-level consequences. *Ecology* 90(7):1762–1772. 705

Supplementary Materials

Tables.

		df	SS	R2	F	p-value
706	geno	9.0000	44078.1327	0.5442	3.5821	0.0537
707	Residual	27.0000	36915.4605	0.4558		
	Total	36.0000	80993.5932	1.0000		

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

DRAFT

response	statistic	H2	p-value
Lichen Network Similarity	3.5821	0.4130	0.0537
Average Mutual Information	3.5235	0.3101	0.0254
Centralization	4.0444	0.3305	0.0184
Centralization In-Degree	4.4812	0.3487	0.0142
Centralization Out-Degree	3.8615	0.3193	0.0205
Centralization In-Degree (positive)	3.9852	0.3309	0.0190
Centralization In-Degree (negative)	0.3304	0.1057	0.2508
Centralization Out-Degree (positive)	3.5585	0.3119	0.0248
Centralization Out-Degree (negative)	0.0862	0.0513	0.3446
Number of Network Links (Degree)	3.5175	0.3156	0.0255
Degree (positive)	3.6925	0.3242	0.0229
Degree (negative)	0.0327	0.0318	0.3859
Percent Lichen Cover	0.0000	0.0000	1.0000
Lichen Species Diversity	0.0000	0.0000	0.4543
Lichen Species Richness	0.0000	0.0000	0.4543
Lichen Species Evenness	0.0000	0.0000	1.0000
Percent Rough Bark	4.8526	0.3221	0.0113
pH	0.0000	0.0000	1.0000
Carbon-Nitrogen (CN) Ratio	0.0000	0.0000	1.0000
Condensed Tannins (CT)	0.0007	0.0041	0.4439
BR-L Residuals	0.0000	0.0000	1.0000
BR-Cen Residuals	0.0000	0.0000	1.0000

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

	r	R2	estimate	SE	t	p-value
br_L	-0.34	0.11	-0.07	0.03	-2.13	0.04
br_Cen	-0.39	0.15	-0.00	0.00	-2.52	0.02
ct_L	0.34	0.11	0.57	0.27	2.13	0.04
ct_Cen	0.08	0.01	0.00	0.00	0.46	0.65
ph_L	0.08	0.01	0.67	1.41	0.48	0.64
ph_Cen	0.13	0.02	0.02	0.02	0.78	0.44
cn_L	0.06	0.00	50.19	145.84	0.34	0.73
cn_Cen	0.16	0.03	2.14	2.18	0.98	0.33

Table 3. Tests of the correlation between tree bark traits and lichen network structure

	df	SS	R2	F	p-value
geno	9.0000	44078.1327	0.5442	3.5821	0.0537
Residual	27.0000	36915.4605	0.4558		
Total	36.0000	80993.5932	1.0000		

Table 4. PERMANOVA Pseudo-F Table of lichen network similarity to genotype.

	Df	SumOfSqs	R2	F	Pr(>F)
BR	1.0000	21021.8765	0.2595	13.0299	0.0096
CT	1.0000	2349.3142	0.0290	1.4562	0.2016
pH	1.0000	2098.8999	0.0259	1.3010	0.2899
CN	1.0000	3896.1757	0.0481	2.4150	0.1890
Residual	32.0000	51627.3270	0.6374		
Total	36.0000	80993.5932	1.0000		

Table 5. PERMANOVA Pseudo-F Table of lichen network similarity response to bark traits.

lichen species	mean	statistic	H2	p-value
Positive				
<i>In-Degree</i>				
X. galericulata	0.2703	0	0	1
C. subdeflexa	0.8919	2.1926	0.2158	0.0595
L. spp.	0.4324	0	0	1
C. holocarpa	0.5946	3.6146	0.3241	0.024
X. montana	0.0541	0	0	0.4543
P. melanachra	0.1351	0	0	1
P. adscendens	0			
P. undulata	0.027	0	0	0.4543
R. sp.	0.1351	2.049	0.2613	0.0656
<i>Out-Degree</i>				
X. galericulata	0.027	0	0	0.4543
C. subdeflexa	0.6757	0	0	1
L. spp.	0.5946	0.0061	0.0126	0.4246
C. holocarpa	0.7027	3.1318	0.2981	0.0327
X. montana	0.0811	2.9228	0.3163	0.0375
P. melanachra	0.1351	0	0	1
P. adscendens	0			
P. undulata	0.027	0	0	0.4543
R. sp.	0.2973	0.1505	0.0612	0.3119
Negative				
<i>In-Degree</i>				
X. galericulata	0			
C. subdeflexa	0.1892	0	0	0.4543
L. spp.	0.1892	0.0015	0.0057	0.4398
C. holocarpa	0.1351	0	0	1
X. montana	0.027	0.0377	0.0394	0.3807
P. melanachra	0			
P. adscendens	0			
P. undulata	0			
R. sp.	0.1622	0	0	1
<i>Out-Degree</i>				
X. galericulata	0.2432	0	0	1
C. subdeflexa	0.4054	0	0	0.4543
L. spp.	0.027	0	0	0.4543
C. holocarpa	0.027	0	0	0.4543
X. montana	0			
P. melanachra	0			
P. adscendens	0			
P. undulata	0			
R. sp.	0			

Table 6. REML tests of the effect of tree genotype on lichen species centrality.

	BR	CT	pH	CN	PC	SR	SE	SD	L	Cen
BR									-0.34	-0.39
CT								-0.34		0.34
pH										
CN										
PC								0.49		-0.46
SR									0.76	0.47
SE									0.85	0.45
SD									0.59	0.33
L										0.88
Cen										

Table 7. Matrix of correlations among tree traits, lichen community metrics and network metrics

	Df	SumOfSqs	R2	F	Pr(>F)
geno	9.0000	1.5049	0.2001	0.7507	0.8878
Residual	27.0000	6.0143	0.7999		
Total	36.0000	7.5193	1.0000		

Figures.

**Table 8. Pseudo-F Table of lichen community similarity
PERMANOVA.**

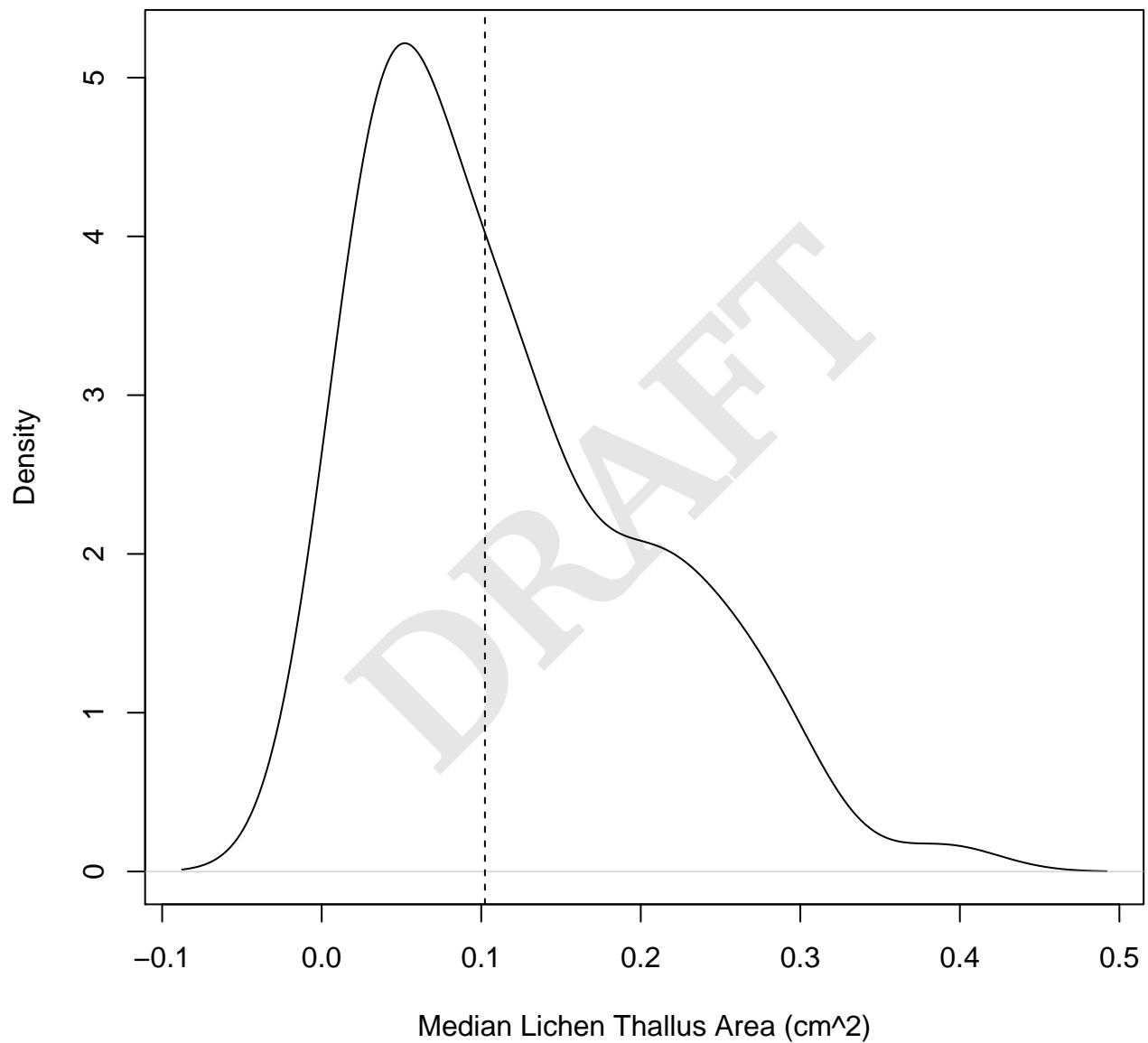


Fig. 1

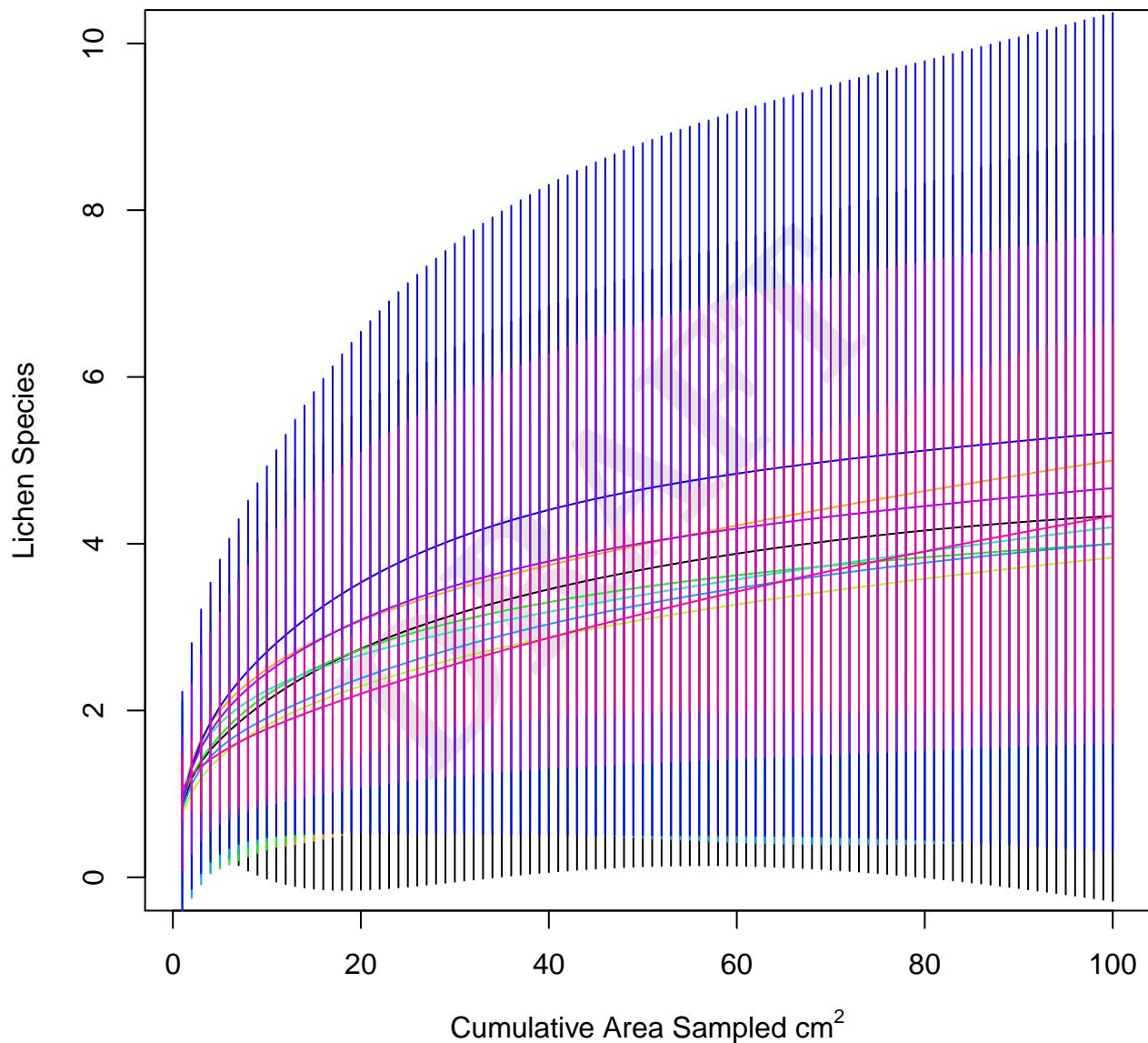


Fig. 2. Species area curve by genotype.

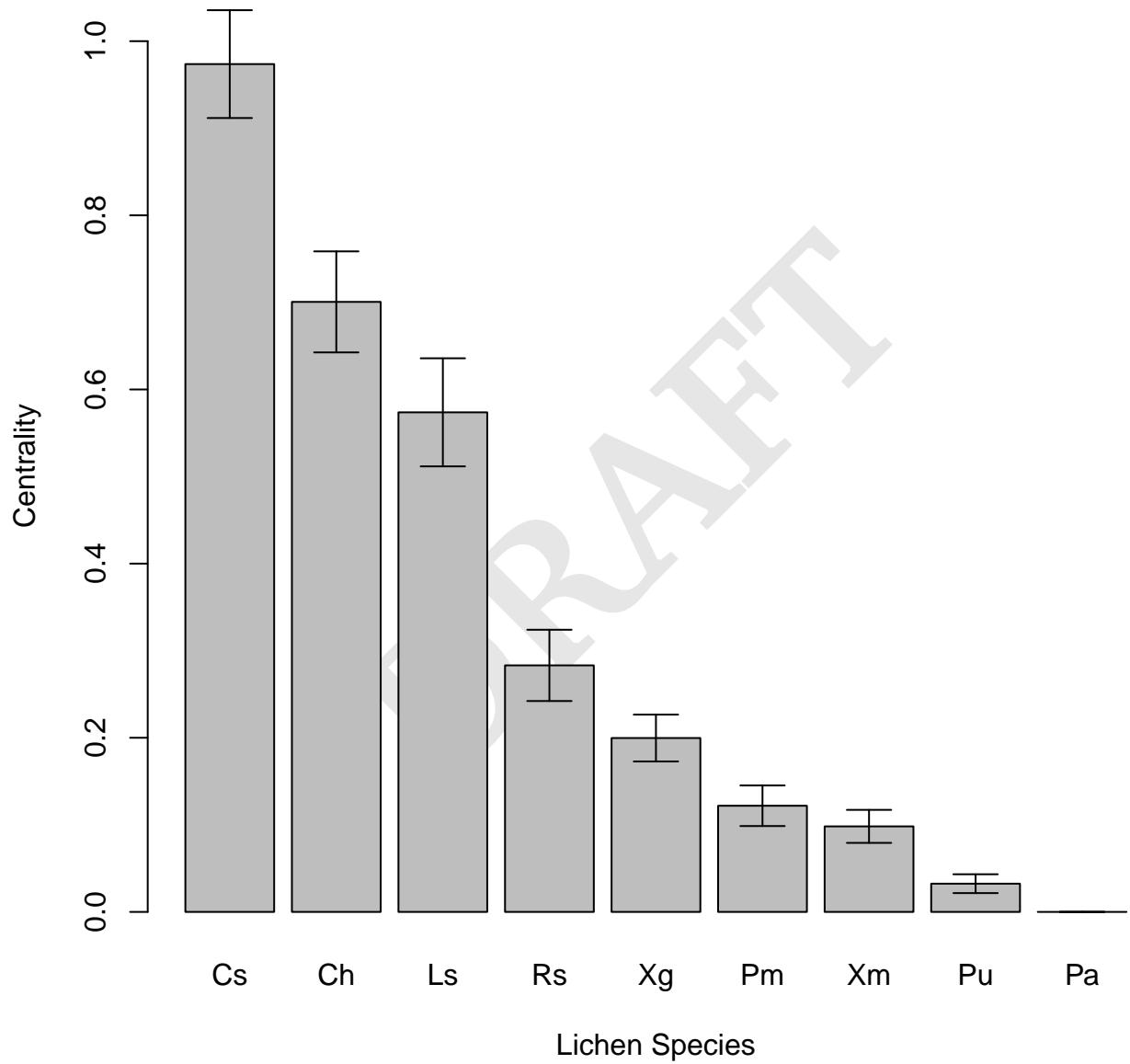


Fig. 3