

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

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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 eco-
2 logical networks. Initially, evolution in a com-
3 munity context was focused on examples of highly
4 co-evolved pairs of species (e.g. Darwin's famous
5 prediction of the Sphinx Moth and Christmas
6 Orchid) citeWade2007. However, studies of dif-
7 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-
8 combe2002, Strauss2007, Dicke2010, geographic mo-
9 saics of co-evolution citeThompson2005 and com-
10 munity genetics citeWhitham2006 have provided an
11 in-road for ecological network approaches citeFor-
12 tuna2009, Keith2017, Lau2016 to illuminate a more
13 complex perspective of the interface between eco-
14 logical and evolutionary dynamics. There is now

15 evidence to support that selection tends to occur
16 among groups of species citeWade2007, Pieterse2007,
17 Dicke2010 favoring the development of small webs
18 citeNuismer2006, Guimaraes2007, Gomez2009 and
19 that genetic variation and phylogenetic relatedness
20 contributes to variation in community assembly (1)
21 and species interactions citeWhitham2006a, Bai-
22 ley2009, Moya-Larano2011, which shapes the eco-
23 logical interaction networks (2).

Community genetics studies (3) have shown that
genetic variation in foundation species (4) plays a
significant role in defining distinct communities of in-

Significance Statement

Evolution occurs in the context of ecosystems com-
prised of complex ecological networks. Research
at the interface of ecology and evolution has prim-
arily focused on pairwise interactions among species
and have rarely included a genetic component to
analyses. Here, we use a long-term common gar-
den 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 genet-
ically based and is correlated with heritable bark
traits, including roughness and condensed tannins.
These findings demonstrate the importance of ge-
netic variation and evolutionary dynamics in shaping
ecological networks as evolved traits.

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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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 (5) that contributes to variation in associated communities (6). The importance of genetic variation in structuring ecological systems was recently reviewed by Des Roches et al. (2018).

Additional work has provided support for the hypothesis that not only does composition vary among genetically distinct genotypes of foundation species, it also impacts the structure of the network of species interactions in these communities. Two studies have demonstrated conceptually (7) and empirically (8). However, studies in the network ecology literature generally do not include a genetic component (see review by Borrett et al. 2014) and community genetics papers do not have data on network structure of associated communities. Currently, we are aware of only one paper that empirically examines the genetic basis of network structure of a community citeBarbour2019.

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 but to date, no studies have examined the genetic basis of networks of interacting species (?). 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 (9, 10). 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 (7, 8, 11, 12). 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, such as Bangert et al. (2005) 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

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 (13).

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 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 = *Candeliariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora*

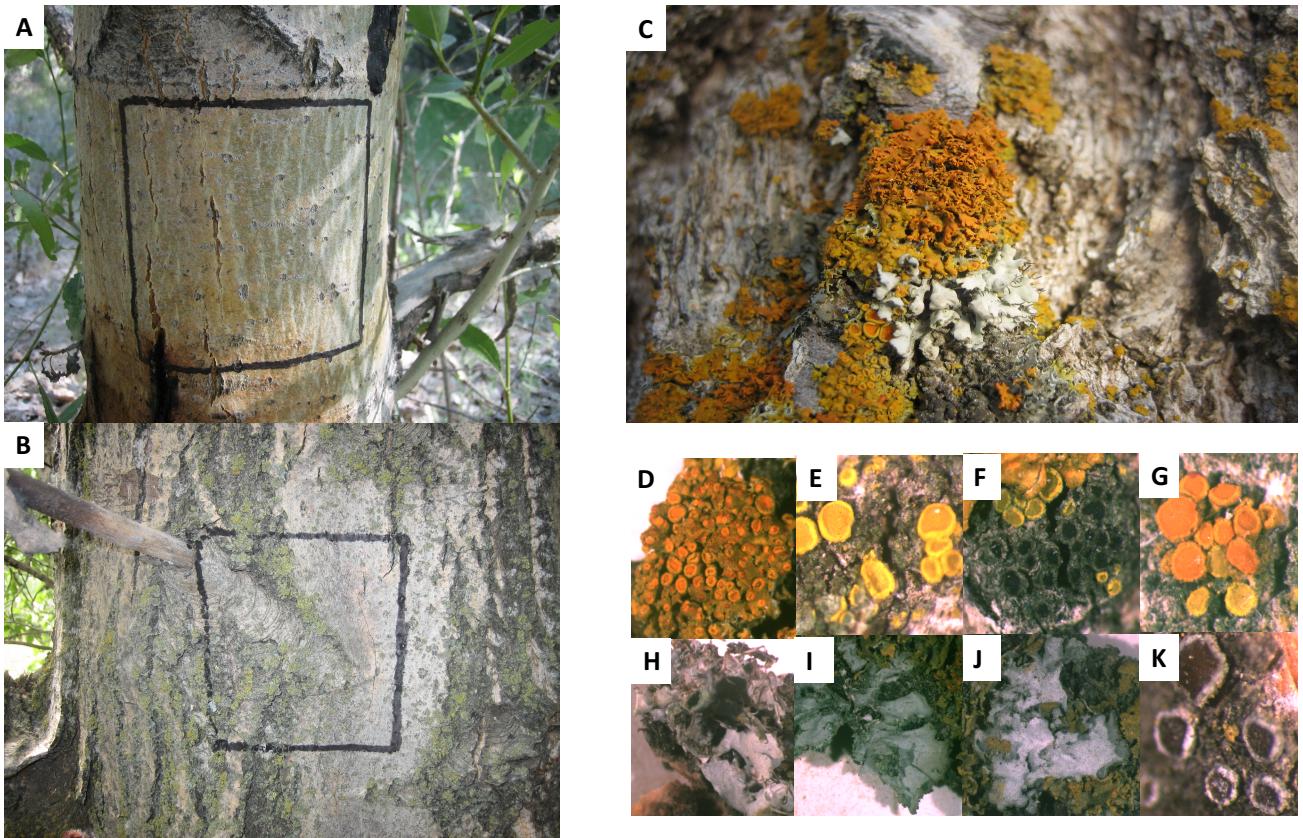


Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground (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).

128 *hagenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*,
129 Pu = *Physcia undulata*. Several other species were
130 not observed in the present study but are known to occur
131 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
132 *ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

133 The cell size and checkerboard sampling pattern was
134 chosen to isolate the individuals in each cell. In a previous
135 survey of lichen thallus size in this common garden, we
136 had observed a median thallus size of $0.12 \pm 0.001 \text{ cm}^2$ (1
137 S.E.) (see Supporting Information). Based on the median
138 thallus size, we expected thalli observed in each cell to
139 generally be spatially independent of thalli present in
140 other cells but exposed to similar micro-environmental
141 conditions created by the bark and the location of the
142 sampling area on an individual tree. Therefore, we were
143 confident in treating the cell-wise observations in quadrats
144 as independent with respect to lichen-lichen interactions.

145 We also measured several bark traits for each tree:
146 including, bark roughness and chemistry. **INSERT**
147 **FROM LAMIT.**

148 **Lichen Network Modeling and Analysis.** For each tree,
149 repeated observations of lichen were made in order to con-

struct replicated interaction networks for each genotype. 150
We quantified the presence of lichen in the 1 cm^2 cells on 151
individual trees of *P. angustifolia*. Unipartite networks 152
were generated using the conditional probabilities of each 153
species pair, i.e. the probability of observing one species 154
given an observation of another species $P(S_i|S_j)$, based 155
on the method developed by (14). To calculate conditional 156
probabilities, we quantified the individual probabilities 157
of species occurrences $P(S_i)$ and the joint probability 158
of co-occurrences $P(S_i, S_j)$ using the frequencies of each 159
species and their co-occurrences. We were then able to 160
calculate the conditional probabilities of each species pair 161
as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability. 162
This yielded a matrix that could possibly be asymmetric, i.e. 163
 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another 164
important property of this matrix is that the diagonal 165
(S_{ii}) was equal to one for all species present and zero for 166
species that were not observed in any cell. 167

168 We then applied an analytical procedure to remove 169
non-significant links between species. This procedure 170
determines if the joint probability of a species pair 171
(i.e. $P(S_i, S_j)$) is different from zero (Fig. 2). Here, a 172
confidence interval $CI_{95\%}$ is calculated as as $CI_{95\%} =$

173 $E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, 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 its own probability of being observed).

205 **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

$$222 \quad \sum x_i \quad [1]$$

- 223 • Node degree
- 224 • Node centrality
- 225 • Network degree

- Centralization
- In vs out
- Pos vs neg

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

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 distance based multivariate statistical approaches, including Permutational Analysis of Variance (PERMANOVA) and Mantel tests. For some analyses, community composition was relativized by species maxima to reduce the effect of the highly abundant *X. galericulata*. For community composition we used Bray-Curtis dissimilarity, which has optimal performance with count data citepMinchen1998. 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) citeecodist to produce dimensionally reduced ordinations of these multi-variate responses and fitted vectors for continuous predictor variables to the ordinated values citevegan. 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 (15)). We calculated the number of interactions or “links” in each network, which provides a measure of the size of the network citepLau2015, Borrett2014. We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network citeButts2005. In a network with a low level of centralization species have similar amount of interaction in the network, while a network



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 (14), 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.

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 citeBeckett2016, 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.

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 at zenodo.com/doi/XXXXXX. All analyses were conducted using the programming language R version 3.6.1 (R De-

velopment Core Team 2019).

Results

Tree genotype influenced lichen network structure. Tree genotype significantly predicted the structural similarity of lichen networks (PERMANOVA: $\text{Pseudo-}F_{9,27} = 3.58$, $H^2 = 0.41$, $p\text{-value} = 0.0537$) (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 ($RLRT = 3.9852$, $H^2 = 0.3309$, $p\text{-value} = 0.0184$) or when separated into out-going only ($RLRT = 3.52$, $H^2 = 0.32$, $p\text{-value} = 0.0255$).

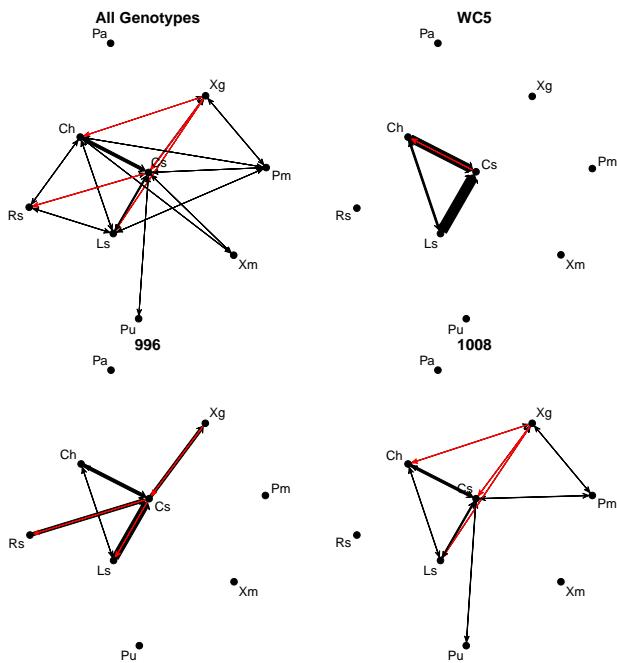


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

	response	statistic	H2	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 network structure.

not for either negative centrality metrics in-coming (RLRT = 0, H² = 0, p-value = 1) or out-going (RLRT = 0, H² = 0, p-value = 0.4543). None of the other species' centralities showed a genotypic response (Supplementary Table 6) with the exception of *X. montana* (RLRT = 2.92, H² = 0.32, p-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).

Genotype indirectly influenced lichen network centralization via the genetically based variation in bark roughness. The percent of rough bark was the only tree trait that displayed a significant response to genotype (RLRT = 4.8526, H² = 0.3221, p-value = 0.0113). None of the other bark traits, condensed tannins (RLRT = 0.0007, H² = 0.0041, p-value = 0.4439), pH (RLRT = 0.00, H² = 0.00, p-value = 1.0000) or carbon-nitrogen Ratio (RLRT = 0.0000, H² = 0.0000, p-value = 1.0000), showed a significant 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, R² = 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, H²

0.0190) or out-going only (RLRT = 3.8615, H² = 0.3193, p-value = 0.0205). Metrics including only positive links also showed a significant effect of tree genotype, including positive degree (RLRT = 3.6925, H² = 0.3242, p-value = 0.0229), positive in-going centralization (RLRT = 4.4812, H² = 0.3487, p-value = 0.0142). Metrics calculated with negative links were not significant, including degree (negative) (RLRT = 0.0327, H² = 0.0318, p-value = 0.3859) and both in-coming (negative) (RLRT = 0.3304, H² = 0.1057, p-value = 0.2508) and out-going centralization (negative) (RLRT = 0.0862, H² = 0.0513, p-value = 0.3446).

The genetic response of network centralization was driven by variation in *Caloplaca holocarpa*. Centralization varied significantly among species (*F*_{8,324} = 7.99, R² = 0.16, p-value << 0.0001). *Caloplaca holocarpa* centralization was the main species to exhibit a significant response to tree genotype in terms of positive centralization for both the in-coming (RLRT = 3.61, H² = 0.32, p-value = 0.0240) and out-going (RLRT = 3.13, H² = 0.30, p-value = 0.0327) perspectives, but

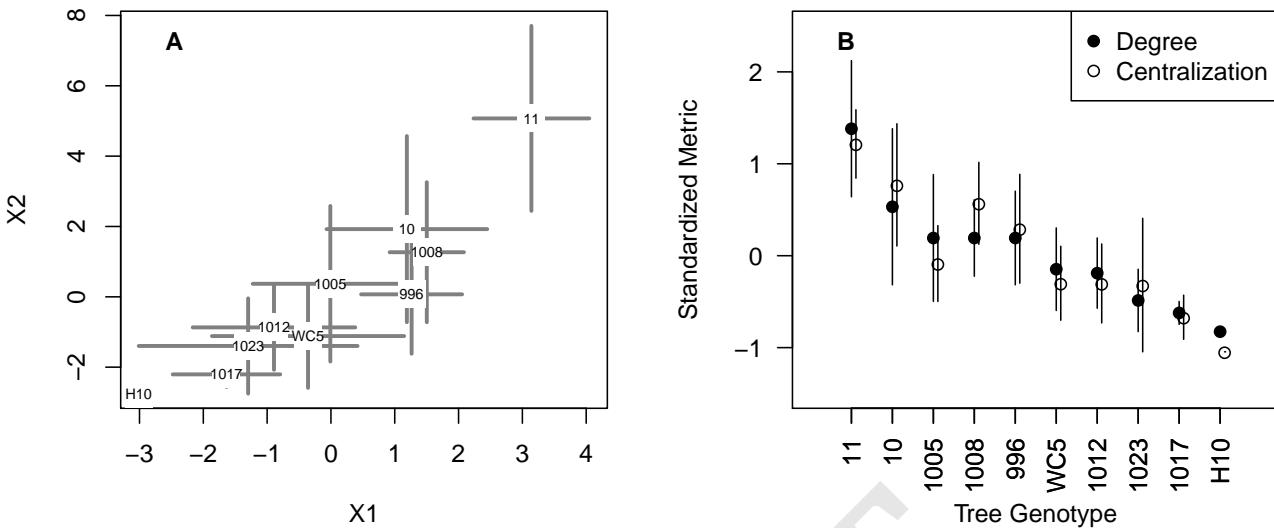


Fig. 4. The similarity of lichen networks varied among tree genotypes. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks ($\pm 1 \text{ S.E.}$). Genotype centroids that are closer together tend to have more similar lichen network structure. B. Plot showing the standardized ($\frac{x - \bar{x}}{\sigma}$) means ($\pm 1 \text{ S.E.}$) 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.

= 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 being positive, including *C. holocarpa*'s centrality being positive both in and out. We don't know specific micro-scopic dynamics, such as photobionts, mycobionts, endolichenic fungi and bacteria, but variation in these underlying interactions could also be playing a role.

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

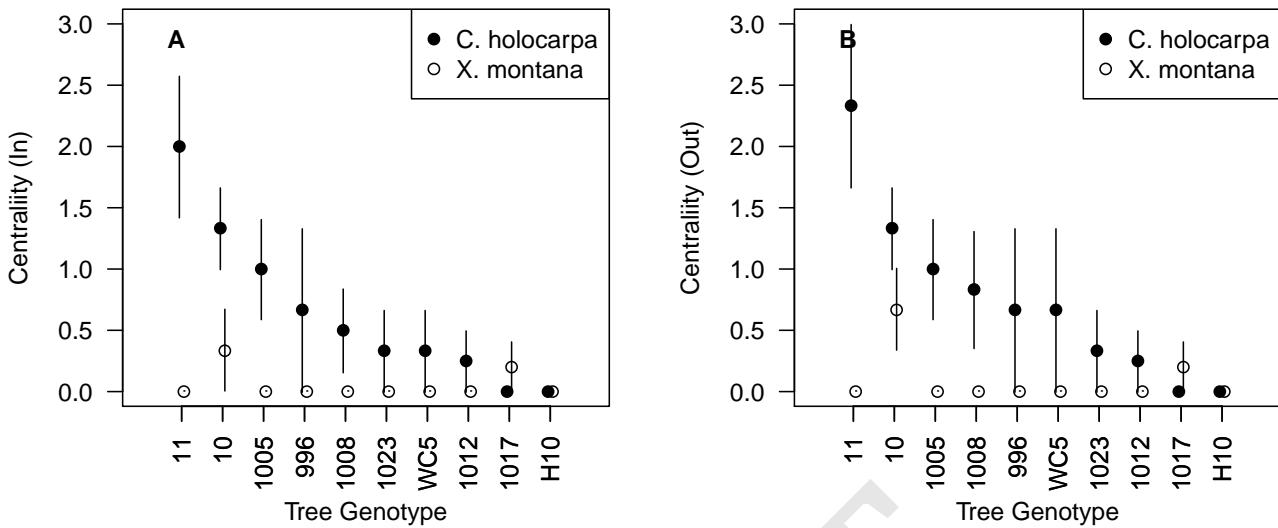


Fig. 5. Dot-plots showing the mean (dot) and ± 1 SE of in-degree (A) and out-degree (B) centrality for two species, *C. holocarpa* and *X. montana*. *Caloplaca holocarpa* centrality was highly variable among genotypes. *Xanthomendoza montana* centrality, both in- and out-degree, was only non-zero for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

among lichen species does vary among genotypes. Community composition of lichen has been observed to be different among these trees, 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 a few species at the center and radiating interactions out from the central core (Leiberman). This is structurally 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.

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

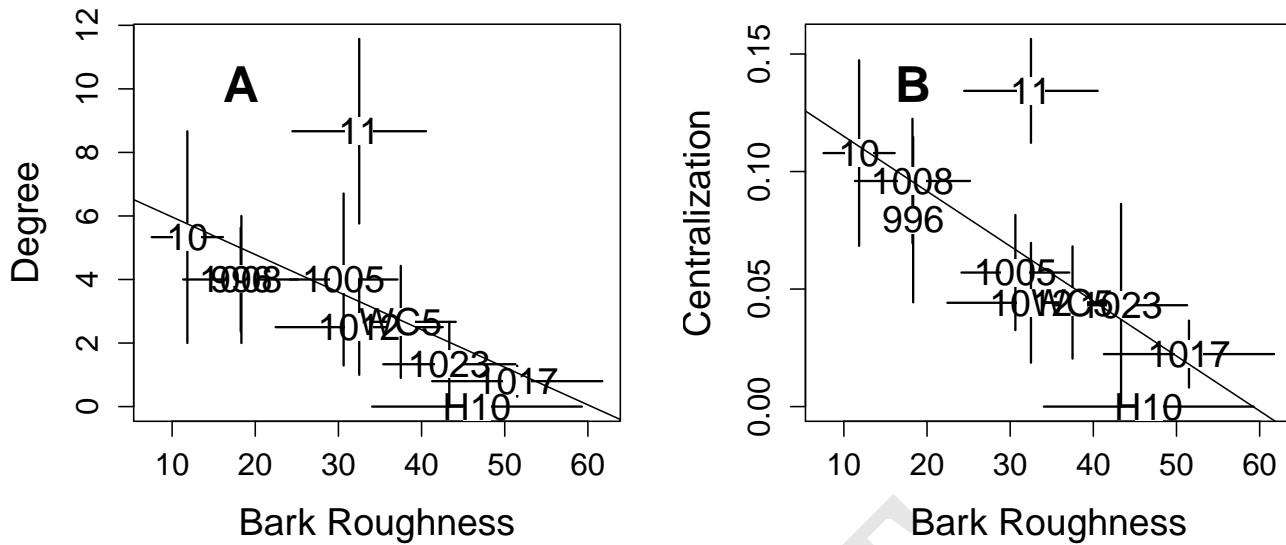


Fig. 6. Bivariate plots of the relationship between bark roughness and three network metrics: A) degree and B) centralization. Each plot displays the genotype mean \pm 1 S.E. for both variables and a least-squares regression calculated using the genotype means.

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). 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.

Future work should consider the potential influence on evolutionary dynamics of the associated communities. The network of interactions of species that are strongly influenced by a foundation species, could amplify the effects of genotype, this serves as a means for genetic effects to increase rather than diffuse through an ecosystem either through space or over time, as has been proposed in the construction of the genetic diffusion hypothesis. Altered abundances can lead to differences in interactions. Genotype effects on abundances of individual abundances may cancel out. Specifically for asexually reproducing species, such as many lichen are, shifting interaction frequencies could lead to evolutionary outcomes, given the potential to take-up symbionts and genetic material from thalli that they come into contact with. Alter-

ing interaction frequencies could differences in the frequencies the exchange of genetic materials among lichen that could then be passed on to vegetative and possibly sexually produced reproductive propagules. The larger scale (stand or region) effects of these "evolutionary units" on each tree would depend on the connectivity and rate of movement of propagules among trees per the geographic mosaic of co-evolution hypothesis developed by Thompson.

- Genetic assembly rule = similar genetics will have more similar communities
- What we don't know is whether or not these interactions will also lead to similar interactions among other species.
- Thus, it would be possible for genetics to not only influence other species directly, but also indirectly by influencing the interactions among other species.

We observed significant lichen interaction structure that varied among genotypes of a foundation tree species, narrowleaf cottonwood (*P. angustifolia*). We found that a genetically based trait, bark roughness, partially explained the variation in lichen interaction networks. Some of this variation in lichen networks was related to both the overall abundance

and species richness of lichen; though, statistically controlling for the effect of genotype on these variables indicates that a significant portion of the variance in lichen species richness is due to a factor other than tree genotype. By using network metrics, we were also able to probe for specific characteristics of how these networks were responding to tree genotype. We found that both number of links and the centralization of the networks were highly correlated with network similarity and that tree genotype significantly predicted network centrality but only marginally predicted the number of network links. This latter result could be due to the relationship between species richness and the number of links in the network, which were significantly correlated with each other. We also found that bark roughness did not significantly predict either the number of links or the centrality of lichen networks, suggesting that bark roughness has some other effect on the structure of the lichen networks. Taken together, these findings support the hypothesis that genotypic variation in a foundation species contributes to the structure of a network of interacting species.

These findings point to the importance of understanding the community level effects of genetic variation in plant functional traits and highlights the potential for indirect effects of genetic variation to propagate through networks of interacting species and trophic levels.

This work corroborates previous findings of the importance of plant genetics in shaping community structure and ecosystem processes. citepBangert2008

Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. For example, (8) 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 (16). Furthermore, in a predator-prey-plant study, Smith (17), showed that the interactions among species across trophic levels depended on plant genotype.

Also, work by (18–20) 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.

More on the importance of ecological networks (21, 22).

From Thompson2014

- Pairwise interactions are usually influenced by other species
- Selection favors the development of small webs
- Evolution of new lifestyles leads to changes in selection on large and small webs

Specific hypothesis from Thompson2014

Bark roughness had previously been shown to be an important tree trait influencing bark lichens (13) that is under strong genetic control (23).

Although our study was conducted with a community of lichens, these results should be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level (24, 25). 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 (26–28). 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. We suggest 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. 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 (29).

With such findings, it appears that we are closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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Supplementary Materials

Tables.

688

689

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

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

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

DRAFT

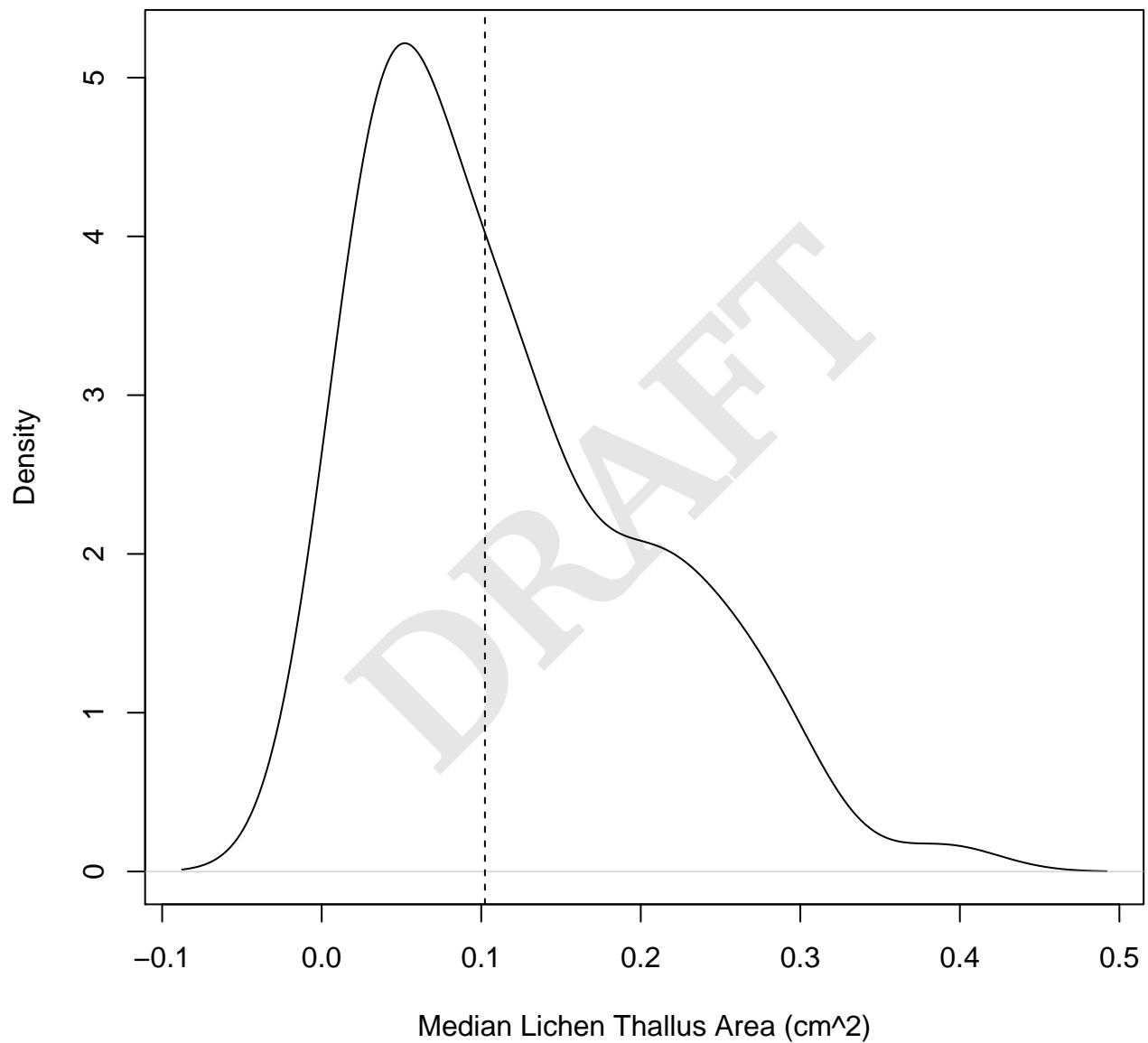


Fig. 1

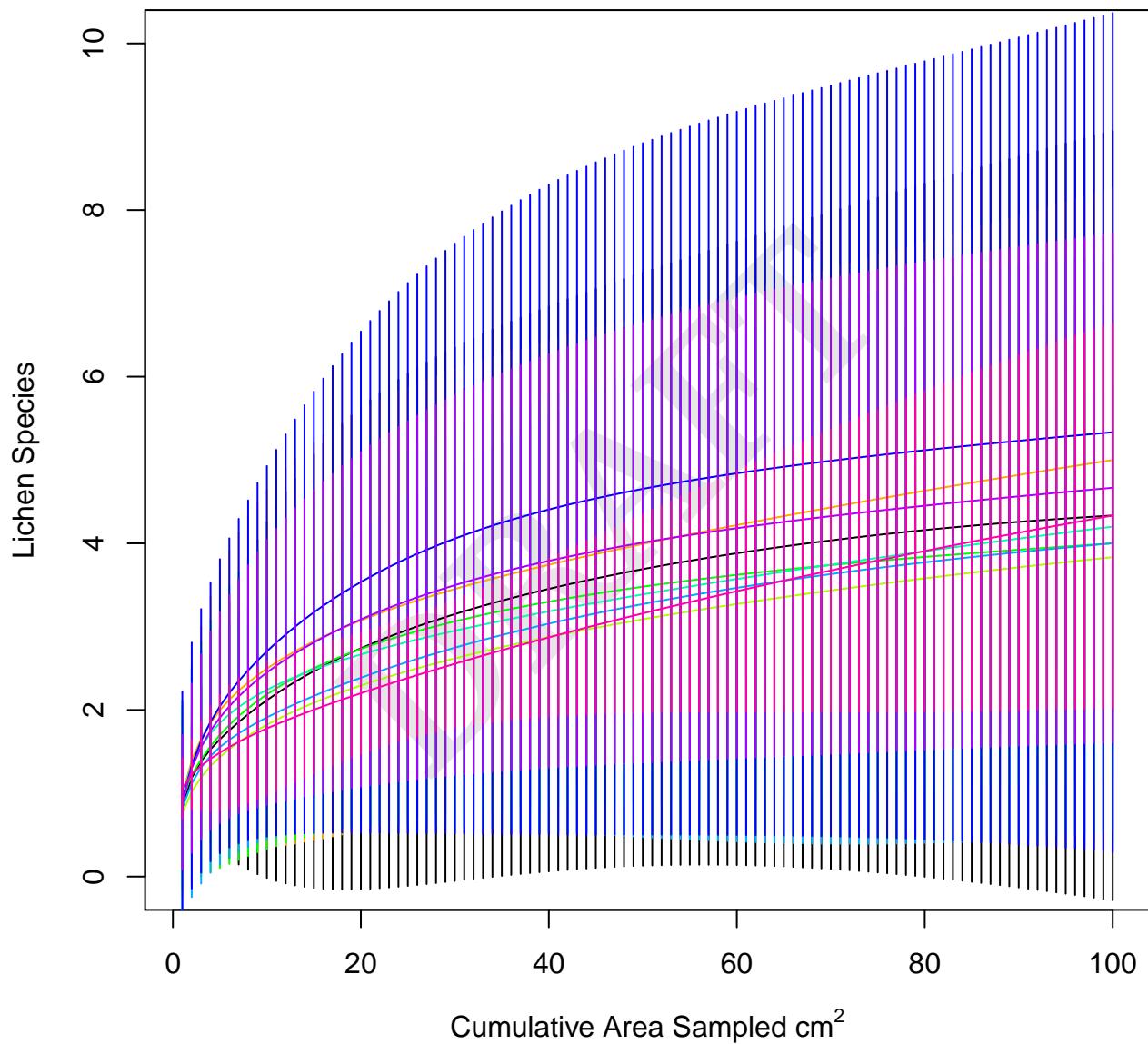


Fig. 2. Species area curve by genotype.

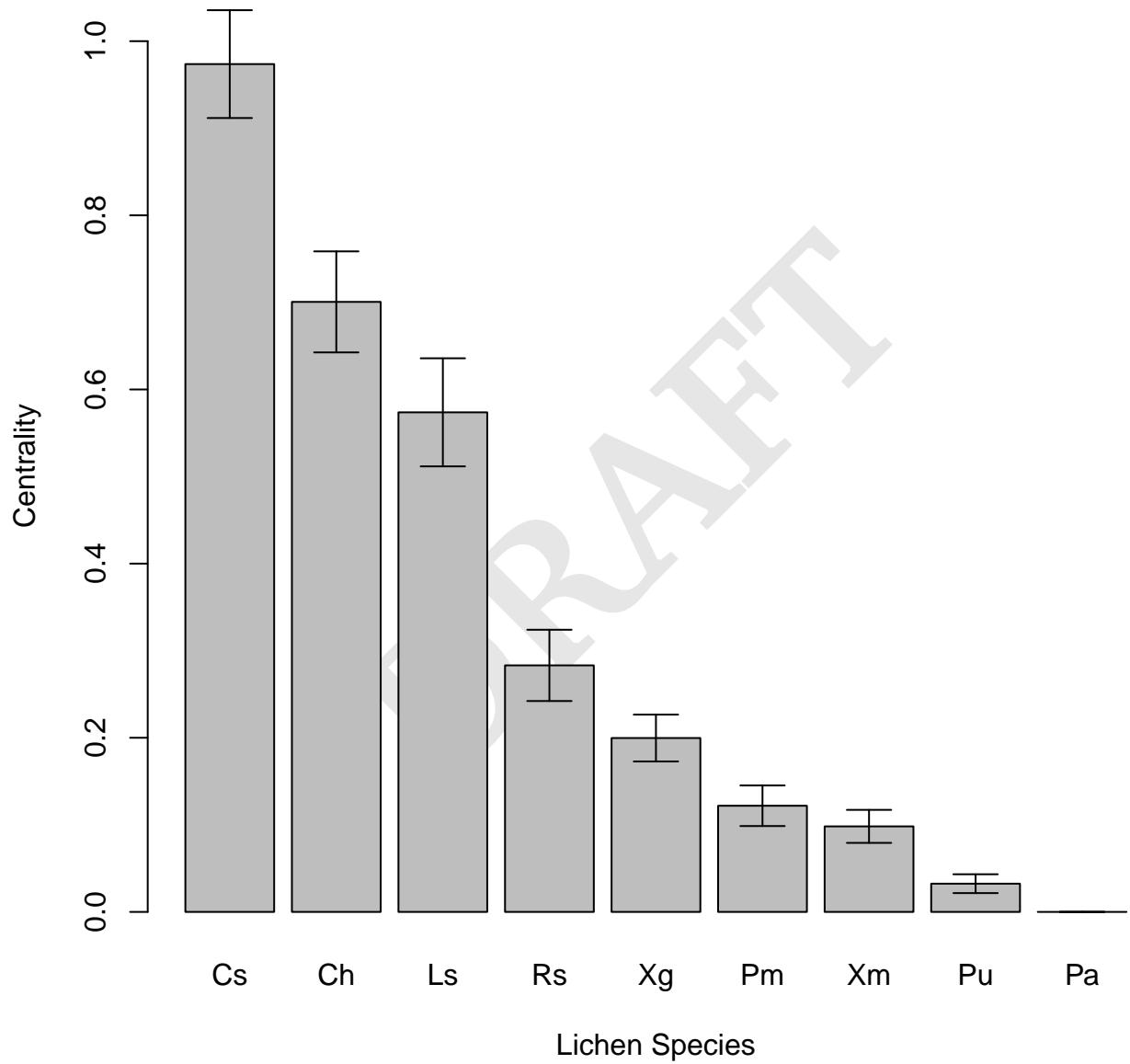


Fig. 3