

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 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 (?) and com-**
8 **munity genetics (?) have provided an in-road for**
9 **ecological network approaches (8? ?) 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 (1) and species
17 interactions (? ? ?), which shapes the ecological
18 interaction networks (2).

19 Community genetics studies (3) have shown that
20 genetic variation in foundation species (4) plays a
21 significant role in defining distinct communities of in-
22 teracting organisms: such as, endophytes, pathogens,
23 lichens, arthropods, and soil microbes. Multiple stud-
24 ies have now demonstrated that genetic variation
25 influences numerous functional traits (e.g., phyto-
26 chemical, phenological, morphological) produces a

Significance Statement

Evolution occurs in the context of ecosystems com-
prised of complex ecological networks. Research
at the interface of ecology and evolution has primar-
ily 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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27 multivariate phenotype (5) that contributes to variation
28 in associated communities (6). The importance
29 of genetic variation in structuring ecological systems
30 was recently reviewed by Des Roches et al. (2018).

31 Additional work has provided support for the hy-
32 pothesis that not only does composition vary among
33 genetically distinct genotypes of foundation species,
34 it also impacts the structure of the network of species
35 interactions in these communities. Two studies have
36 demonstrated conceptually (7) and empirically (8).
37 However, studies in the network ecology literature
38 generally do not include a genetic component (see
39 review by Borrett et al. 2014) and community genet-
40 ics papers do not have data on network structure of
41 associated communities. Currently, we are aware of
42 only one paper that empirically examines the genetic
43 basis of network structure of a community (?).

44 Here, we investigate how genetic variation in a
45 foundation tree species determines the structure of
46 a network of interactions among a community of
47 tree associated lichen species. Previous studies have
48 examined aspects of networks but to date, no studies
49 have examined the genetic basis of networks of inter-
50 acting species (?). Here we examine the genetic
51 basis of network structure on a community of sessile
52 lignicolous (i.e. bark) lichen on cottonwood trees.
53 Using a long-term (20+ years), common garden ex-
54 periment with replicated individuals of known genetic
55 identity and a naturally established stand of *Populus*
56 *angustifolia*. We focused on a model community of
57 9 epiphytic lichen species, as previous research has
58 demonstrated significant compositional responses of
59 epiphytes to genotypic variation (9, 10). In addi-
60 tion, the life-history characteristics of lichens, having
61 highly localized, direct contact interactions and slow
62 population turnover rates, allowed us to assess inter-
63 actions among lichen species on individual trees.
64 We hypothesize that in natural systems evolution oc-
65 curs in a community context involving interactions of
66 complex networks of interacting species (7, 8, 11, 12).
67 If correct, we expect to find that network structure is
68 genetically based in which different plant genotypes
69 support different interaction networks and that these
70 interactions networks can function as indicators of
71 ecological dynamics important for conserving biodi-
72 versity. Applying a probability-theory based network
73 modeling approach, we constructed a set of interac-
74 tion network models for the lichens associated with
75 individual trees. Using these models, we then ex-

76 amined the genetic basis of the structure of these
77 ecological networks. Based on previous community
78 genetics studies, such as Bangert et al. (2005) which
79 proposed the community similarity rule, we hypoth-
80 esize that trees will vary in some phenotypic traits
81 and those trees of the same genotype will tend to
82 have similar traits leading to similarities in lichen
83 network structure.

Materials and Methods

84 The study was conducted along the Weber River, UT
85 (USA), which is a cottonwood (*Populus* spp.) dominated
86 riparian ecosystem. Although two native species, *Populus*
87 *angustifolia* (James) and *Populus fremontii* (S. Watson),
88 occur here and are known to hybridize, only pure or
89 advanced generation backcrosses of *P. angustifolia* were
90 sampled. Bark lichens have been extensively studied
91 in this system and provide an ideal system in which to
92 observe and model lichen interaction networks, as their
93 sessile nature permits accurate identification of individuals
94 (13).

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

106 **Bark Lichen Observations.** On each tree, presence or
107 absence of each lichen species was assessed in 50 total 1 cm²
108 cells arrayed in a checkerboard pattern. Given the small
109 size and sessile nature of lichens, we were able to rapidly
110 assess lichen interactions by quantifying thalli in close
111 contact. Sampling was restricted to the northern aspect
112 of the trunk to maximize the abundance of lichen and
113 control for the effect of trunk aspect. Two adjacent 10
114 cm² quadrats centered at 50 cm and 85 cm from ground
115 level were sampled (Fig 1 A and B). The observed lichen
116 community included (abbreviations are given for species
117 present in study): Xg = *Xanthomendoza galericulata*, Xm
118 = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Cande-
119 lariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora
120 hagenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscen-
121 dens*, Pu = *Physcia undulata*. Several other species were
122 not observed in the present study but are known to occur
123 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia
124 ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

125 The cell size and checkerboard sampling pattern was
126

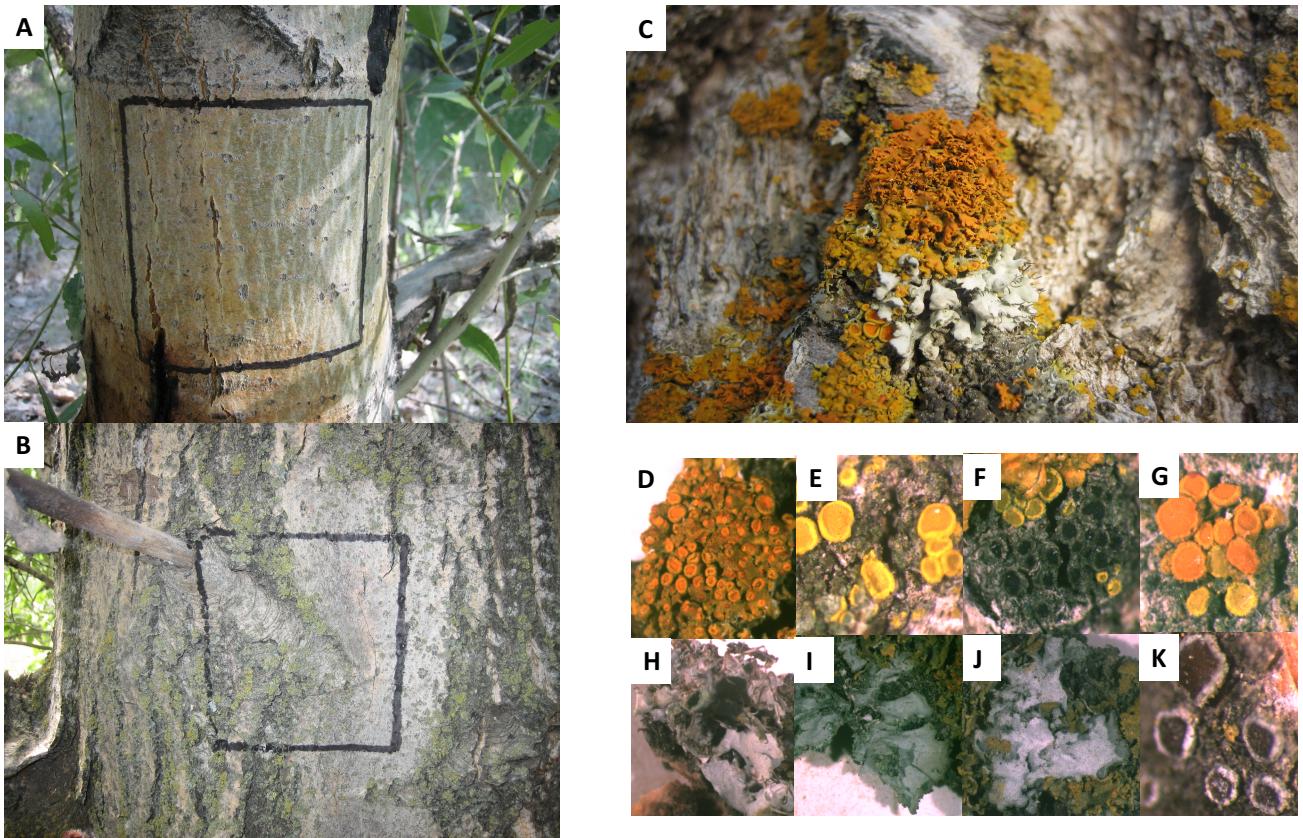


Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground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

chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of $0.12 \pm 0.001 \text{ cm}^2$ (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 and chemistry. **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 cm^2 cells on individual trees of *P. angustifolia*. Unipartite networks were generated using the conditional probabilities of each species pair, i.e. the probability of observing one species given an observation of another species $P(S_i|S_j)$, based on

the method developed by (14). 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 $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, 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

173 expected probability of $S_i S_j$ and its compliment (i.e.
 174 $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the
 175 observed number of co-occurrence falls outside of the
 176 confidence interval, the joint probability $P(S_i, S_j)$ is de-
 177 termined to be equal to the product of the individual
 178 probabilities (i.e. $P(S_i)P(S_j)$), and the conditional prob-
 179 ability reduces to the individual probability of that species
 180 $P(S_i)$. Therefore, unless the co-occurrence of a species
 181 pair falls outside the confidence interval, the probabil-
 182 ity that the observation of one species given the other
 183 is no different than simply observing that species alone.
 184 This enables us to remove links from a given network by
 185 re-scaling the resulting conditional probabilities by sub-
 186 tracting the individual probabilities from the conditional
 187 probabilities (i.e. how different the conditional probability
 188 is from the independent probability), which makes any
 189 species with a non-significant conditional probability zero.
 190 The resulting matrix ($\mathbf{D} = D_{ij}$) can be interpreted as
 191 how one species impacts another with zero being no effect
 192 and values less than or greater than zero interpreted as
 193 negative and positive effects, respectively. Here, we will
 194 refer to this matrix (\mathbf{D}) as an interaction matrix with
 195 the properties that it can be asymmetric (i.e. P_{ij} does not
 196 necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e.
 197 a species does not influence its own probability of being
 198 observed).

199 **Network Metrics.** To quantify the structural variation of
 200 lichen networks we calculated several metrics at both
 201 the node and whole-network level. For individual nodes
 202 (i.e. species) in each network, we calculated both the
 203 degree Eq. (1) and the centrality. We also calculated two
 204 similar global network metrics: degree and centralization.
 205 The first was network degree, which is a count of the
 206 total number of links in a network. As the networks
 207 contained not only positive and negative connections,
 208 as well as directional connections (both in-coming and
 209 out-going), we calculated the same network metrics for
 210 all combinations of these types of connections in each
 211 network. Although there are many more possible network
 212 metrics that could have been examined, we chose to focus
 213 on a restricted set for the sake of clarity. Also, degree and
 214 centrality form the basis of many other network metrics.

ADD EQUATIONS FOR METRICS

216
$$\sum x_i \quad [1]$$

217 • Node degree
 218 • Node centrality
 219 • Network degree
 220 • Centralization
 221 • In vs out
 222 • Pos vs neg

223 To calculate separate metrics for positive and negative
 224 links, we applied methods for calculating the centrality
 225 accounting for the sign differences (? and Borgatti 2014).
 226 We used the `signnet` package version ????, which is avail-
 227 able at ????

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

241 For multivariate response variables, such as lichen com-
 242 munity composition and network structure, we used dis-
 243 tance based multivariate statistical approaches, including
 244 Permutational Analysis of Variance (PERMANOVA) and
 245 Mantel tests. For some analyses, community composition
 246 was relativized by species maxima to reduce the effect
 247 of the highly abundant *X. galericulata*. For community
 248 composition we used Bray-Curtis dissimilarity, which has
 249 optimal performance with count data (?). To quantify
 250 the similarity of lichen networks among individual trees,
 251 we calculated the pairwise Euclidean distance of the \mathbf{D}
 252 interaction matrices among all pairs of trees.

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

262 For each network, we also calculated metrics that mea-
 263 sure different structural aspects. Although there are many
 264 other metrics, for the sake of simplicity we focus on a
 265 subset that represent several interesting features of net-
 266 work structure (see (15)). We calculated the number of
 267 interactions or “links” in each network, which provides a
 268 measure of the size of the network (? ?). We also calcu-
 269 lated the centralization of each network, which measures
 270 the evenness of the distribution of interactions among
 271 the species in the network (?). In a network with a
 272 low level of centralization species have similar amount of
 273 interaction in the network, while a network with a high
 274 level of centralization tends to have one or small number
 275 of species that interact with other species. We used a
 276 related function to calculate the centrality of each species
 277 (i.e. node level centrality) in each network as well. The

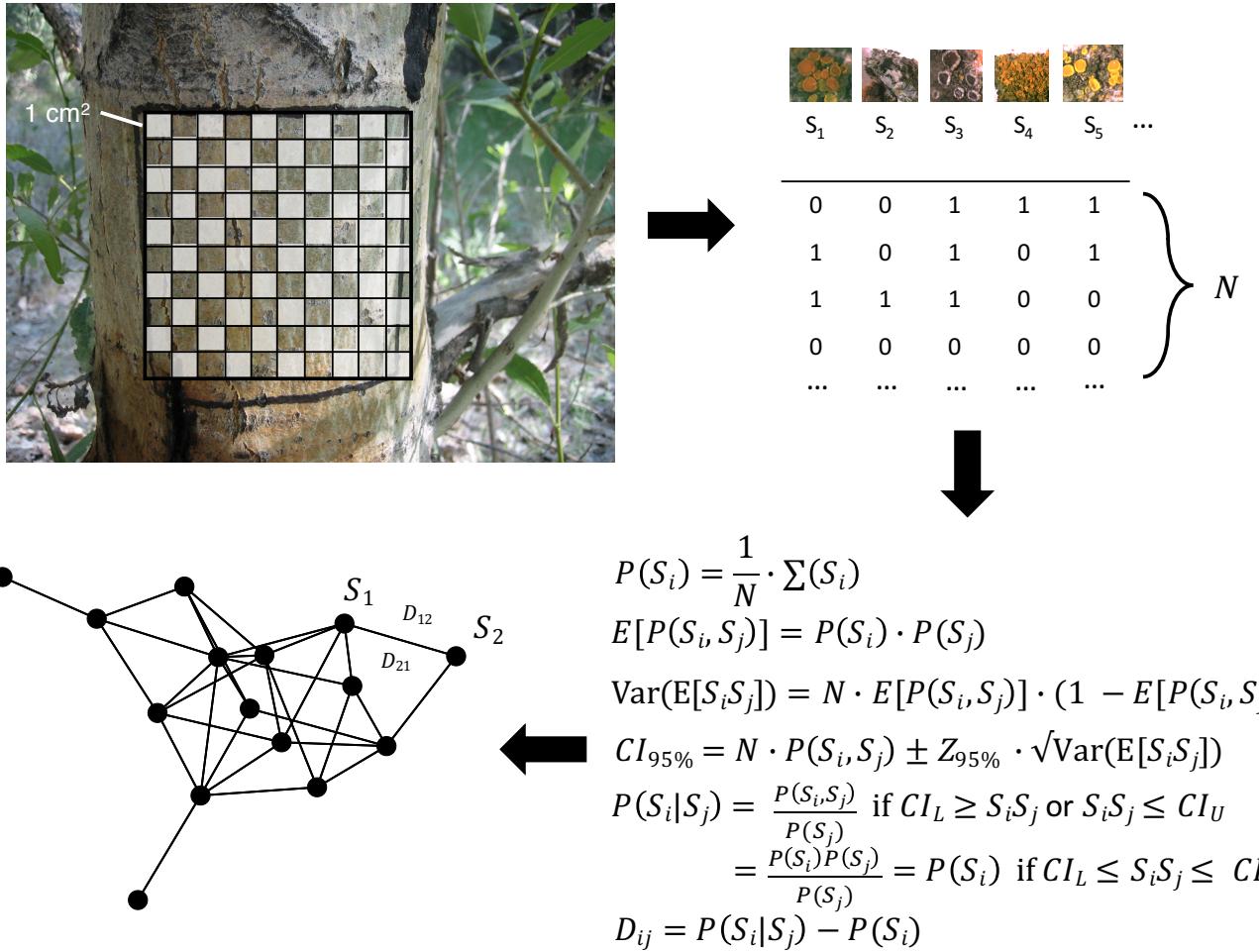


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded 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.

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.

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/doiXXXXXX. All analyses were conducted using the programming language R version 3.6.1 (R Development Core Team 2019).

Results

Tree genotype influenced lichen network structure. Tree genotype significantly predicted the structural similarity of lichen networks (PERMANOVA: Pseudo- $F_{9,27} = 3.58$, $H^2 = 0.41$, $p\text{-value} = 0.0537$) (Fig. 4). Overall network level metrics responded significantly to tree genotype (Table 1), including network degree ($RLRT = 3.52$, $H^2 = 0.32$, $p\text{-value} = 0.0255$) and centralization including both in-coming and out-going links ($RLRT = 4.04$, $H^2 = 0.33$, $p\text{-value} = 0.0184$) or when separated into in-coming only ($RLRT = 3.9852$, $H^2 = 0.3309$, $p\text{-value} = 0.0190$) or out-going only ($RLRT = 3.8615$, $H^2 = 0.3193$, $p\text{-value} = 0.0205$). Metrics including only

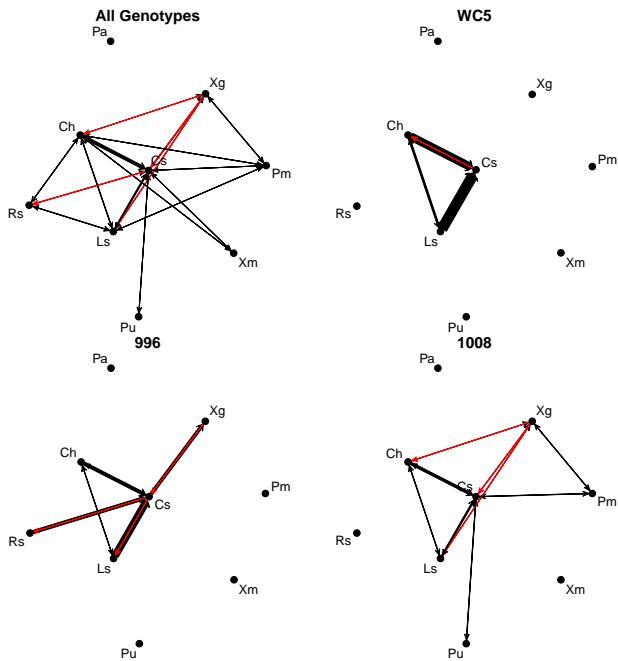


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

positive links also showed a significant effect of tree genotype, including positive degree ($RLRT = 3.6925$, $H^2 = 0.3242$, $p\text{-value} = 0.0229$), positive in-going centralization ($RLRT = 4.4812$, $H^2 = 0.3487$, $p\text{-value} = 0.0142$). Metrics calculated with negative links were not significant, including degree (negative) ($RLRT = 0.0327$, $H^2 = 0.0318$, $p\text{-value} = 0.3859$) and both in-coming (negative) ($RLRT = 0.3304$, $H^2 = 0.1057$, $p\text{-value} = 0.2508$) and out-going centralization (negative) ($RLRT = 0.0862$, $H^2 = 0.0513$, $p\text{-value} = 0.3446$).

The genetic response of network centralization was driven by variation in *Caloplaca holocarpa*. Centrality varied significantly among species ($F_{8,324} = 7.99$, $R^2 = 0.16$, $p\text{-value} << 0.0001$). *Caloplaca holocarpa* centrality was the main species to exhibit a significant response to tree genotype in terms of positive centrality for both the in-coming ($RLRT = 3.61$, $H^2 = 0.32$, $p\text{-value} = 0.0240$) and out-going ($RLRT = 3.13$, $H^2 = 0.30$, $p\text{-value} = 0.0327$) perspectives, but not for either negative centrality metrics in-coming ($RLRT = 0$, $H^2 = 0$, $p\text{-value} = 1$) or out-going

	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.

($RLRT = 0$, $H^2 = 0$, $p\text{-value} = 0.4543$). None of the other species' centralities showed a genotypic response (Supplementary Table 6) with the exception of *X. montana* ($RLRT = 2.92$, $H^2 = 0.32$, $p\text{-value} = 0.0375$); however, the centrality of *X. montana* was much lower overall relative to *C. holocarpa* and the variation in *X. montana* centrality was restricted to two genotypes (Fig. 5).

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 ($RLRT = 4.8526$, $H^2 = 0.3221$, $p\text{-value} = 0.0113$). None of the other bark traits, condensed tannins ($RLRT = 0.0007$, $H^2 = 0.0041$, $p\text{-value} = 0.4439$), pH ($RLRT = 0.00$, $H^2 = 0.00$, $p\text{-value} = 1.0000$) or carbon-nitrogen Ratio ($RLRT = 0.0000$, $H^2 = 0.0000$, $p\text{-value} = 1.0000$), showed a significant response to tree genotype and none other than bark roughness 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- $F_{1,32} = 13.029$, $R^2 = 0.26$, $p\text{-value} = 0.0096$) and other lichen network metrics, including negative correlations with overall network degree ($df = 35$, $t = -2.13$, $r = -0.34$, $p\text{-value} = 0.04$) and centralization ($df = 35$, $t = -2.52$, $r = -0.39$, $p\text{-value} = 0.02$). 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 ($RLRT = 0.00$, $H^2 = 0.00$, $p\text{-value} = 1.0000$) or centralization ($RLRT = 0.00$, $H^2 = 0.00$, $p\text{-value} = 1.0000$), suggesting that

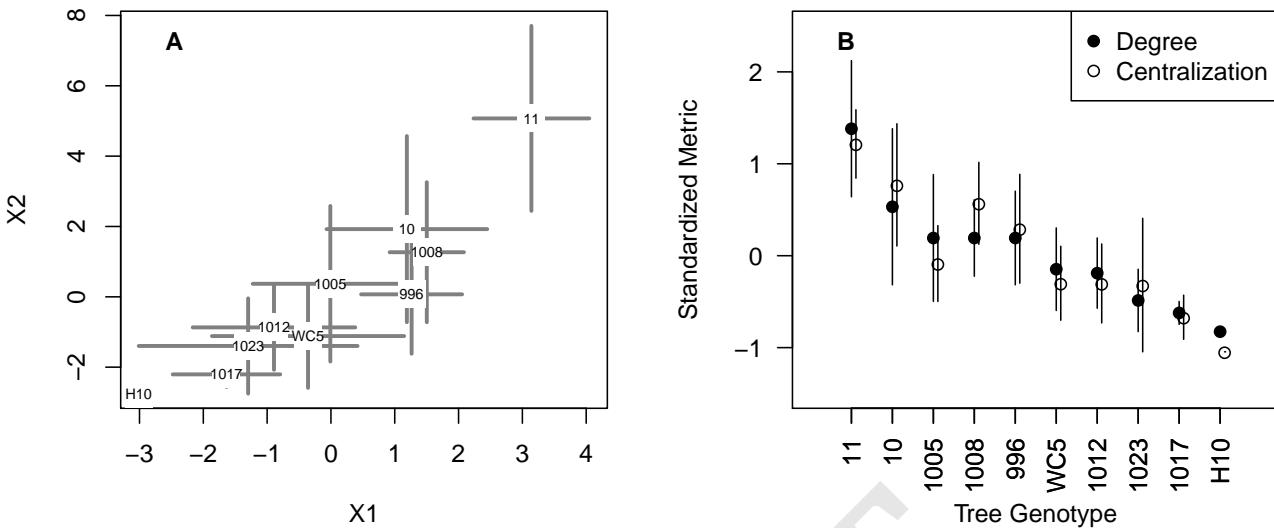


Fig. 4. The similarity of lichen networks varied among tree genotypes. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks ($\pm 1 \text{ S.E.}$). Genotype centroids that are closer together tend to have more similar lichen network structure. B. Plot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

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

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

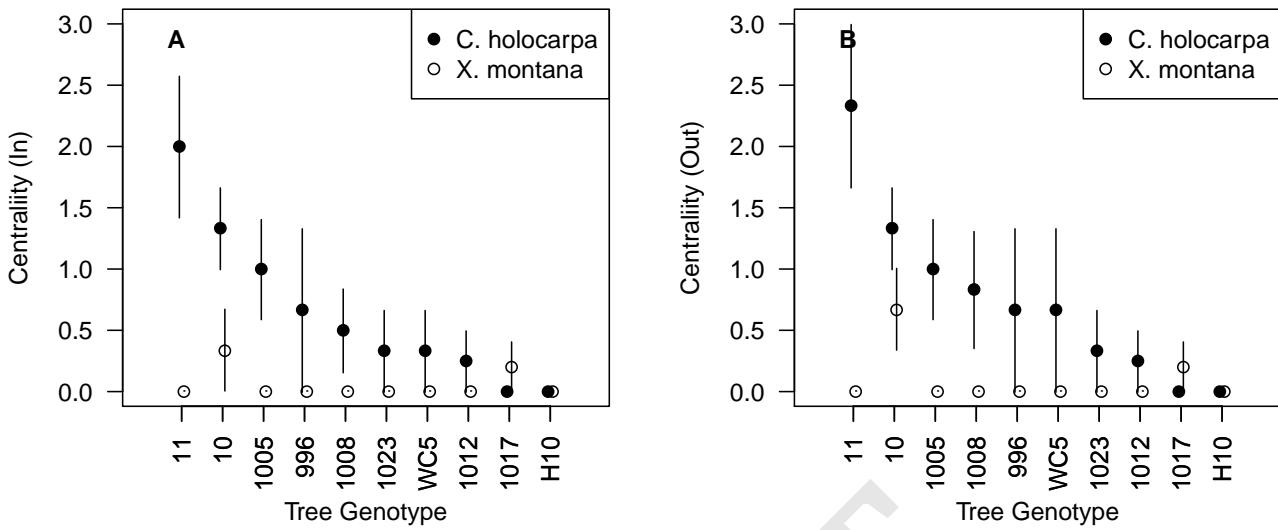


Fig. 5. Dot-plots showing the mean (dot) and ± 1 SE of in-degree (A) and out-degree (B) centrality for two species, *C. holocarpa* and *X. montana*. *Caloplaca holocarpa* centrality was highly variable among genotypes. *Xanthomendoza montana* centrality, both in- and out-degree, was only non-zero for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

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

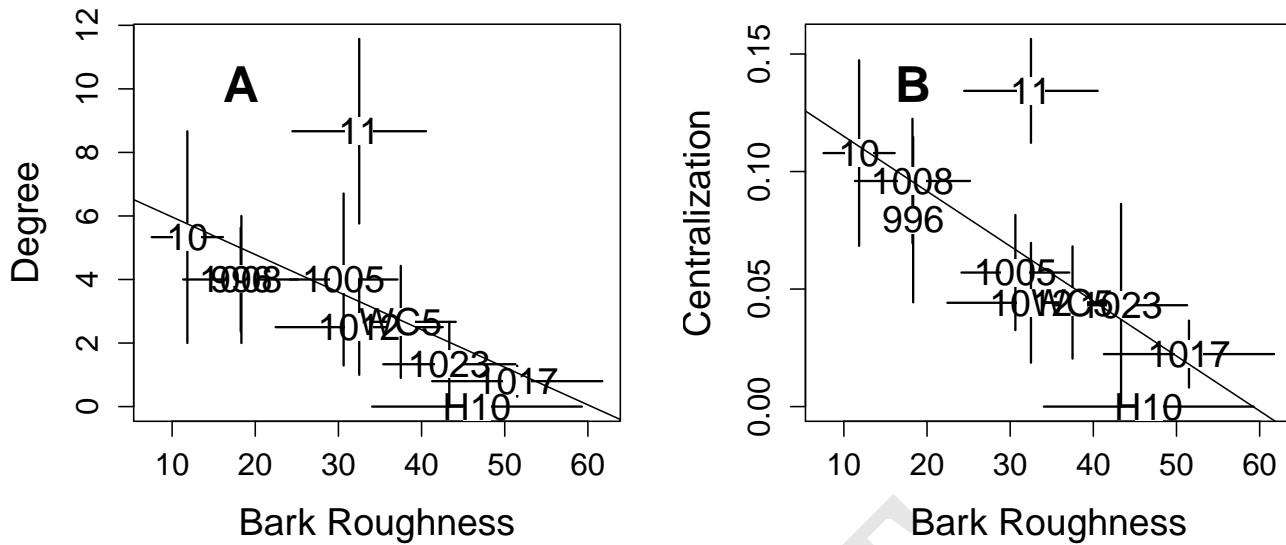


Fig. 6. Bivariate plots of the relationship between bark roughness and three network metrics: A) degree and B) centralization. Each plot displays the genotype mean \pm 1 S.E. for both variables and a least-squares regression calculated using the genotype means.

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.

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 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.

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

523 considered at the community level and that con-
 524 serving genetic variation is important to consider in
 525 efforts to restore or preserve complex species interac-
 526 tions and their associated ecosystem functions (29).
 527 With such findings, it appears that we are closer to
 528 understanding the evolutionary drivers of Darwin's
 529 entangled bank and the interconnectedness of species
 530 in complex communities.

531 Future work should consider the potential influ-
 532 ence on evolutionary dynamics of the associated com-
 533 munities. The network of interactions of species that
 534 are strongly influenced by a foundation species, could
 535 amplify the effects of genotype, this serves as a means
 536 for genetic effects to increase rather than diffuse
 537 through an ecosystem either through space or over
 538 time, as has been proposed in the construction of the
 539 genetic diffusion hypothesis. Altered abundances can
 540 lead to differences in interactions Genotype effects
 541 on abundances of individual abundances may cancel
 542 out. Specifically for asexually reproducing species,
 543 such as many lichen are, shifting interaction frequen-
 544 cies could lead to evolutionary outcomes, given the
 545 potential to take-up symbionts and genetic material
 546 from thalli that they come into contact with. Alter-
 547 ing interaction frequencies could differences in the
 548 frequencies the exchange of genetic materials among
 549 lichen that could then be passed on to vegetative
 550 and possibly sexually produced reproductive propa-
 551 gules. The larger scale (stand or region) effects of
 552 these "evolutionary units" on each tree would de-
 553 pend on the connectivity and rate of movement of
 554 propagules among trees per the geographic mosaic
 555 of co-evolution hypothesis developed by Thompson.

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623 **Supplementary Materials**

624 **Tables.**

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

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

DRAFT

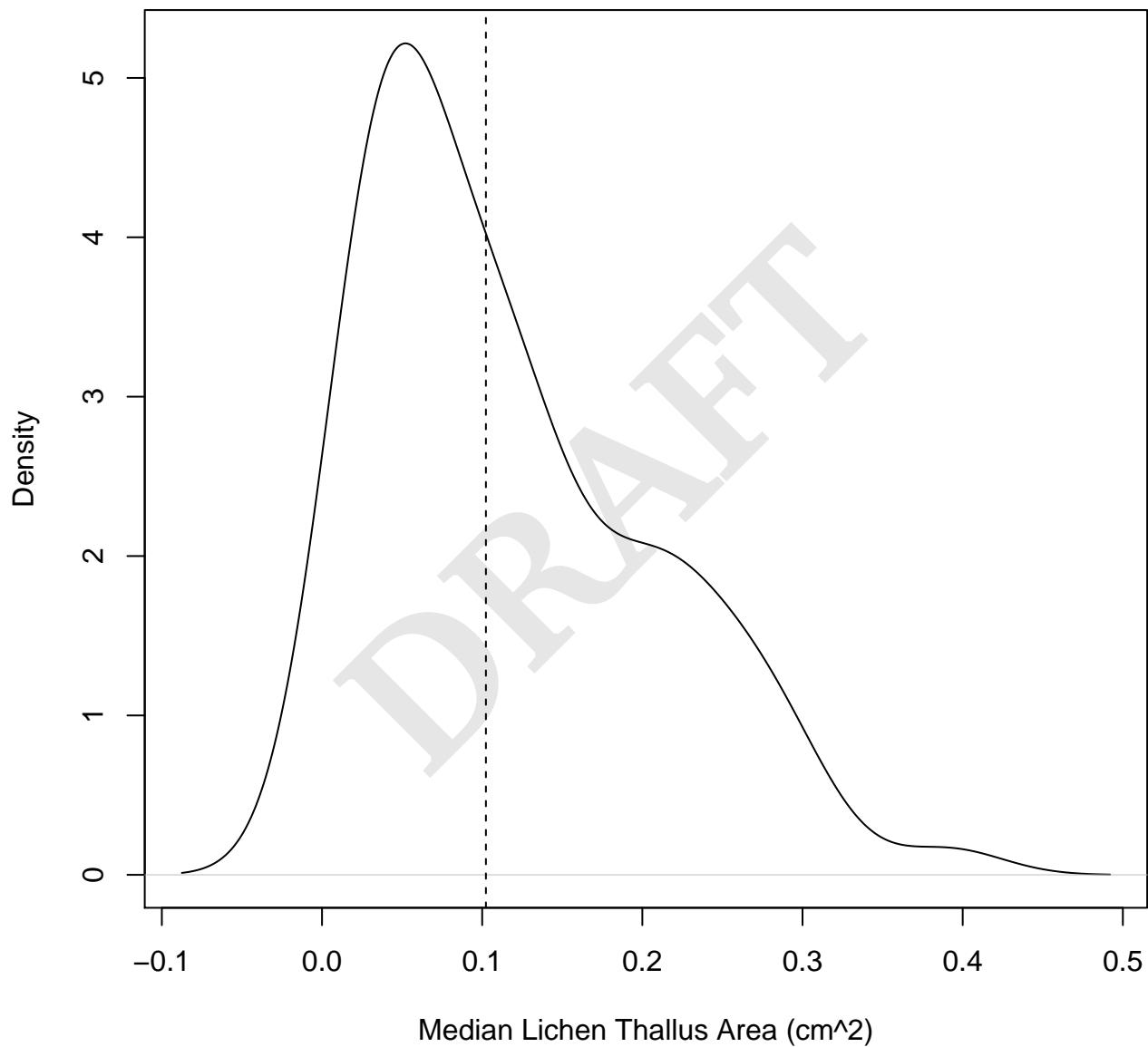


Fig. 1

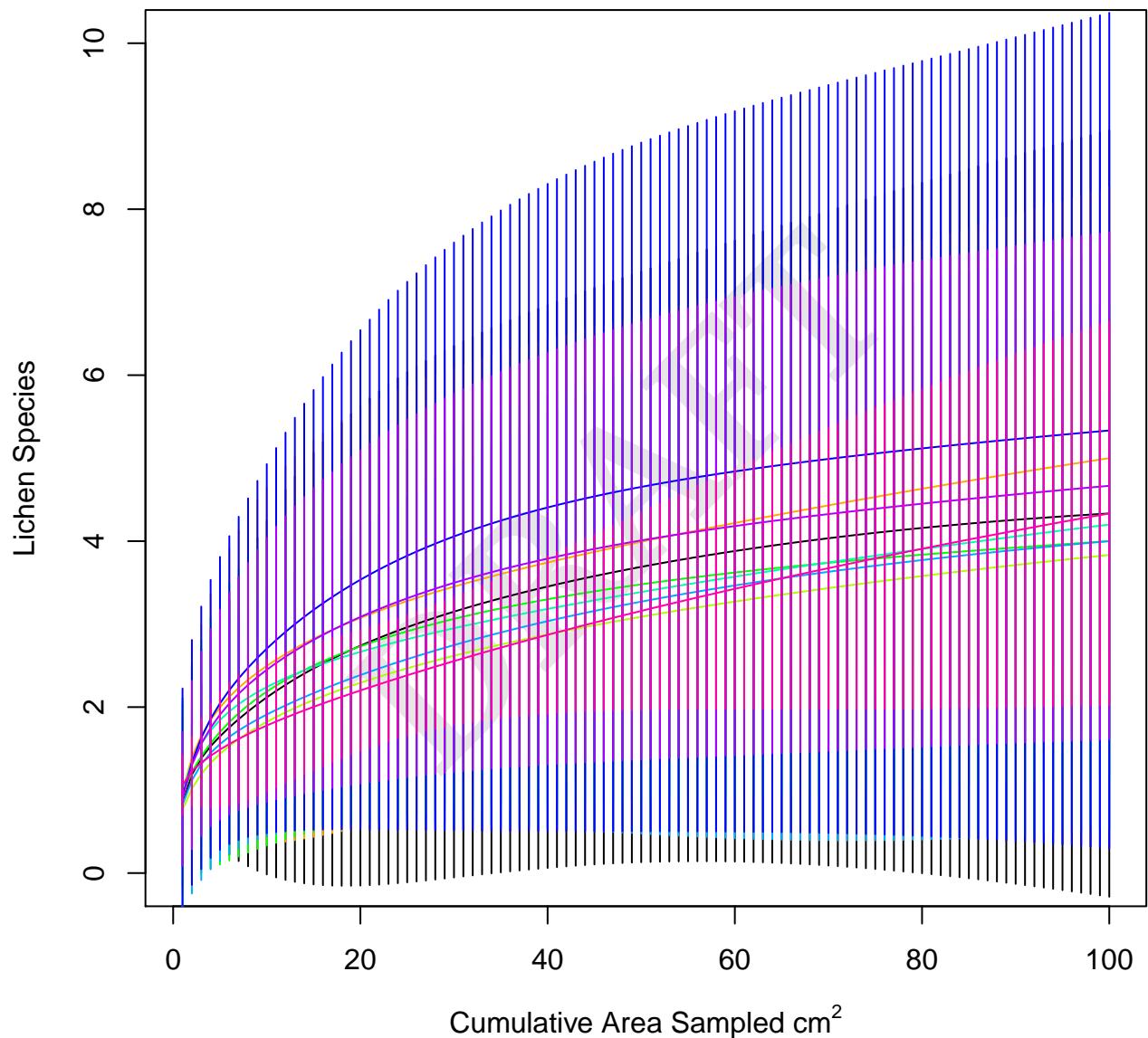


Fig. 2. Species-area curve by genotype.

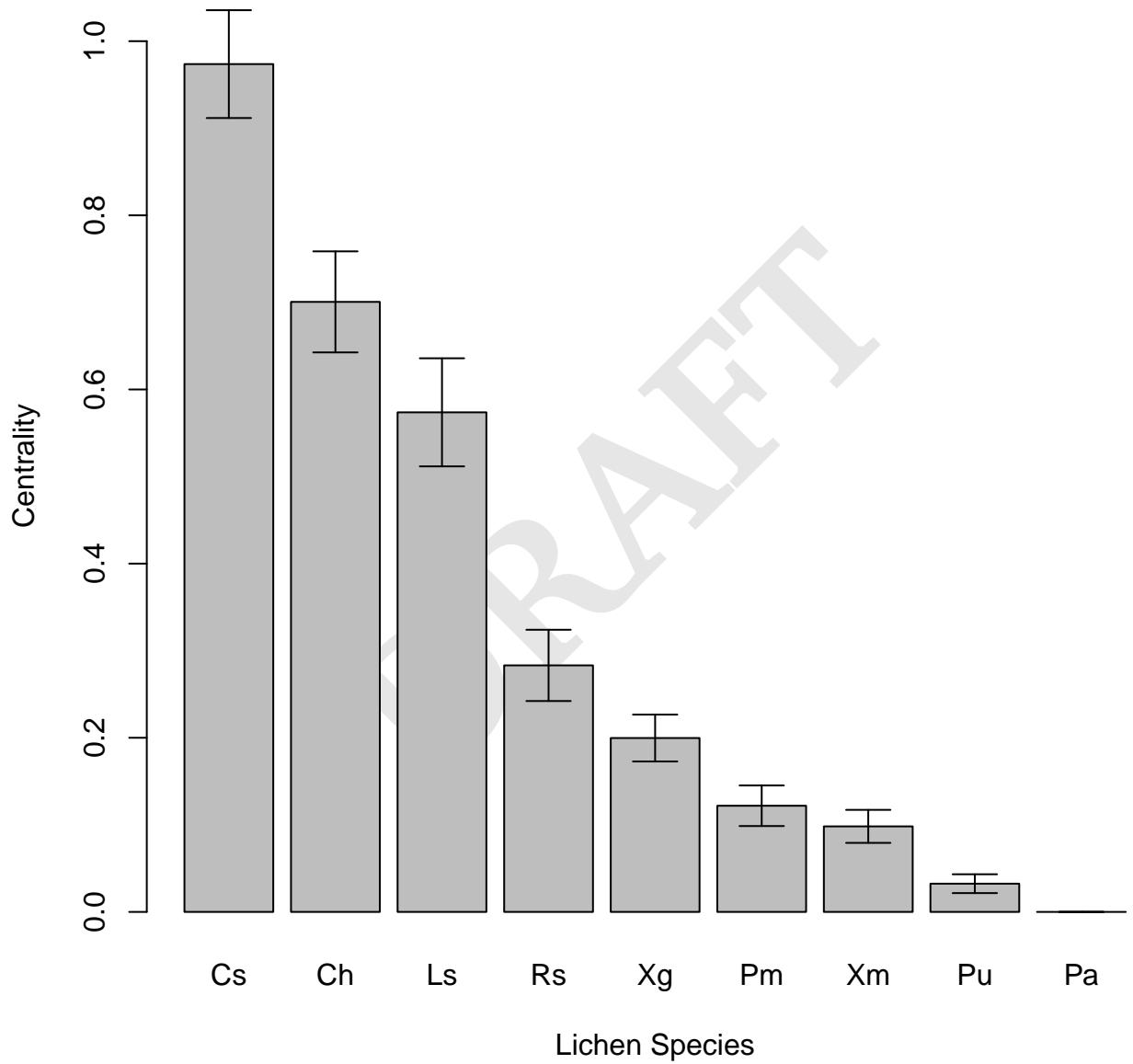


Fig. 3