

# 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, bark roughness and condensed  
17 tannin concentration were both predicted by tree genotype and cor-  
18 related with lichen network similarity. Third, the network metrics, the  
19 number of links and centrality, were both correlated with several tree  
20 traits, including bark roughness and condensed tannin concentra-  
21 tion. We conclude that tree genotype influences lichen interaction  
22 network structure with two potential pathways through bark rough-  
23 ness and condensed tannin concentration. These results provide  
24 evidence that variation in ecological networks can result from genet-  
25 ically based variation in foundation species.

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

- 1 • Evolution occurs in complex ecosystems
- 2 • Community genetics studies have demonstrated  
3 the importance of genetics and evolutionary dy-  
4 namics in structuring communities and ecosys-  
5 tems
- 6 • Community composition, ecosystem processes  
7 have been shown to vary in response to the  
8 genetic variation of foundation species
- 9 • Previous studies have examined aspects of net-  
10 works but to date, no studies have examined the  
11 genetic basis of networks of interacting species
- 12 • Here we examine the genetic basis of network  
13 structure on a community of sessile lignicolous

(i.e. bark) lichen on cottonwood trees. This  
14 system is ideal for examining for this kind of  
15 study because it:

- 16 1. Lichen do not move among trees, although  
17 asexual propagules could move to other  
18 trees in the garden
- 19 2. Is not unapproachably complex
- 20 3. Is comprised of distinct individuals that  
21 are small enough for an entire community  
22 to occur on a single tree but not too small  
23 to be quantified easily

## 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.

The authors have no conflicts of interest.

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- 25 • We hypothesize that trees will vary in some  
26 phenotypic traits, such that trees of the same  
27 genotype will tend to have similar traits produc-  
28 ing similarities in lichen network structure. To  
29 test this hypothesis, we use a common garden  
30 of cottonwood trees with lichen communities.

31 **E**volution occurs in the context of complex eco-  
32 logical networks. Initially, evolution in a com-  
33 munity context was focused on examples of highly  
34 co-evolved pairs of species (e.g. Darwin's famous  
35 prediction of the Sphinx Moth and Christmas  
36 Orchid) citeWade2007. However, studies of dif-  
37 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-  
38 combe2002, Strauss2007, Dicke2010, geographic mo-  
39 saics of co-evolution citeThompson2005 and com-  
40 munity genetics citeWhitham2006 have provided an  
41 in-road for ecological network approaches citeFor-  
42 tuna2009, Keith2017, Lau2016 to illuminate a more  
43 complex perspective of the interface between eco-  
44 logical and evolutionary dynamics. There is now  
45 evidence to support that selection tends to occur  
46 among groups of species citeWade2007, Pieterse2007,  
47 Dicke2010 favoring the development of small webs  
48 citeNuismer2006, Guimaraes2007, Gomez2009 and  
49 that genetic variation and phylogenetic relatedness  
50 contributes to variation in community assembly (?  
51 ) and species interactions citeWhitham2006a, Bai-  
52 ley2009, Moya-Larano2011, which shapes the ecolog-  
53 ical interaction networks (? ).

54 Community genetics studies (?) have shown  
55 that genetic variation in foundation species (?)  
56 plays a significant role in defining distinct communi-  
57 ties of interacting organisms: such as, endophytes,  
58 pathogens, lichens, arthropods, and soil microbes.  
59 Multiple studies have now demonstrated that ge-  
60 netic variation influences numerous functional traits  
61 (e.g., phytochemical, phenological, morphological)  
62 produces a multivariate phenotype (?) that con-  
63 tributes to variation in associated communities (?  
64 ). The importance of genetic variation in structur-  
65 ing ecological systems was recently reviewed by Des  
66 Roches et al. (2018).

67 Additional work has provided support for the hy-  
68 pothesis that not only does composition vary among  
69 genetically distinct genotypes of foundation species,  
70 it also impacts the structure of the network of species  
71 interactions in these communities. Two studies have  
72 demonstrated conceptually (?) and empirically (?  
73 ). However, studies in the network ecology litera-

ture generally do not include a genetic component  
74 (see review by Borrett et al. 2014) and community  
75 genetics papers do not have data on network struc-  
76 ture of associated communities. Currently, we are  
77 aware of only one paper that empirically examines  
78 the genetic basis of network structure of a community  
79 citeBarbour2019.

80 Here, we investigate how genetic variation in a  
81 foundation tree species determines the structure of a  
82 network of interactions among a community of tree  
83 associated lichen species. Using a long-term (20+  
84 years), common garden experiment with replicated  
85 individuals of known genetic identity and a naturally  
86 established stand of *Populus angustifolia*. We focused  
87 on a model community of 9 epiphytic lichen species,  
88 as previous research has demonstrated significant  
89 compositional responses of epiphytes to genotypic  
90 variation (? ? ). In addition, the life-history char-  
91 acteristics of lichens, having highly localized, direct  
92 contact interactions and slow population turnover  
93 rates, allowed us to assess interactions among lichen  
94 species on individual trees. We hypothesize that in  
95 natural systems evolution occurs in a community  
96 context involving interactions of complex networks  
97 of interacting species (? ? ? ? ). If correct, we  
98 expect to find that network structure is genetically  
99 based in which different plant genotypes support  
100 different interaction networks and that these interac-  
101 tions networks can function as indicators of ecologi-  
102 cal dynamics important for conserving biodiversity.  
103 Applying a probability-theory based network mod-  
104 eling approach, we constructed a set of interaction  
105 network models for the lichens associated with indi-  
106 vidual trees. Using these models, we then examined  
107 the genetic basis of the structure of these ecological  
108 networks.

## Materials and Methods

110 The study was conducted along the Weber River, UT  
111 (USA), which is a cottonwood (*Populus* spp.) dominated  
112 riparian ecosystem. Although two native species, *Populus*  
113 *angustifolia* (James) and *Populus fremontii* (S. Watson),  
114 occur here and are known to hybridize, only pure or  
115 advanced generation backcrosses of *P. angustifolia* were  
116 sampled. Bark lichens have been extensively studied  
117 in this system and provide an ideal system in which to  
118 observe and model lichen interaction networks, as their  
119 sessile nature permits accurate identification of individuals  
120 (? ).

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

133  
134 **Bark Lichen Observations.** On each tree, presence or absence  
135 of each lichen species was assessed in 50 total 1 cm<sup>2</sup>  
136 cells arrayed in a checkerboard pattern. Given the small  
137 size and sessile nature of lichens, we were able to rapidly  
138 assess lichen interactions by quantifying thalli in close  
139 contact. Sampling was restricted to the northern aspect  
140 of the trunk to maximize the abundance of lichen and  
141 control for the effect of trunk aspect. Two adjacent 10  
142 cm<sup>2</sup> quadrats centered at 50 cm and 85 cm from ground  
143 level were sampled (Fig 1 A and B). The observed lichen  
144 community included (abbreviations are given for species  
145 present in study): Xg = *Xanthomendoza galericulata*, Xm  
146 = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Pm = *Phyciella melanachra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were  
150 not observed in the present study but are known to occur  
151 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia subolivacea*, *Meanelia elegans*.

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

164 We also measured several bark traits for each tree:  
165 including, bark roughness and chemistry. **INSERT  
166 FROM LAMIT.**

167  
168 **Lichen Network Modeling and Analysis.** For each tree,  
169 repeated observations of lichen were made in order to construct  
170 replicated interaction networks for each genotype.  
171 We quantified the presence of lichen in the 1 cm<sup>2</sup> cells on  
172 individual trees of *P. angustifolia*. Unipartite networks  
173 were generated using the conditional probabilities of each  
174 species pair, i.e. the probability of observing one species  
175 given an observation of another species  $P(S_i|S_j)$ , based on  
176 the method developed by (?). To calculate conditional

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

188 We then applied an analytical procedure to remove  
189 non-significant links between species. This procedure  
190 determines if the joint probability of a species pair  
191 (i.e.  $P(S_i, S_j)$ ) is different from zero (Fig. 2). Here, a  
192 confidence interval  $CI_{95\%}$  is calculated as  $CI_{95\%} =$   
193  $E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$ , where the expected  
194 frequency of co-occurrences  $E(S_i S_j)$  is the total number  
195 of cells surveyed ( $N$ ) times the independent probabilities  
196 of each species  $P(S_i) * P(S_j)$ ,  $Z_{95\%}$  is the Z-score  
197 for 95% from a Z-distribution and the expected variance  
198 of  $E(S_i S_j)$  is the total number of cells times the  
199 expected probability of  $S_i S_j$  and its compliment (i.e.  
200  $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$ ). If the  
201 observed number of co-occurrence falls outside of the  
202 confidence interval, the joint probability  $P(S_i, S_j)$  is de-  
203 termined to be equal to the product of the individual  
204 probabilities (i.e.  $P(S_i) * P(S_j)$ ), and the conditional prob-  
205 ability reduces to the individual probability of that species  
206  $P(S_i)$ . Therefore, unless the co-occurrence of a species  
207 pair falls outside the confidence interval, the probabil-  
208 ity that the observation of one species given the other  
209 is no different than simply observing that species alone.  
210 This enables us to remove links from a given network by  
211 re-scaling the resulting conditional probabilities by sub-  
212 tracting the individual probabilities from the conditional  
213 probabilities (i.e. how different the conditional probability  
214 is from the independent probability), which makes any  
215 species with a non-significant conditional probability zero.  
216 The resulting matrix ( $D = D_{ij}$ ) can be interpreted as  
217 how one species impacts another with zero being no effect  
218 and values less than or greater than zero interpreted as  
219 negative and positive effects, respectively. Here, we will  
220 refer to this matrix ( $D$ ) as an interaction matrix with  
221 the properties that it can be asymmetric (i.e.  $P_{ij}$  does not  
222 necessarily equal  $P_{ji}$ ), and the diagonal ( $P_{ii}$ ) is zero (i.e.  
223 a species does not influence its own probability of being  
224 observed).

225  
226 **Statistical Analyses, Software and Data.** We used a com-  
227 bination of parametric and non-parametric, permutation  
228 based frequentist statistical analyses to test for the effects  
229 of genetic variation on lichen communities and their inter-  
230 action networks. To assess the effect of genotype on uni-  
231 variate responses, we used additive, random effects models  
232 with Restricted Maximum Likelihood (REML). We used



**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 ( $10 \text{ cm}^2$ ) 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. Naesbour (D-K).

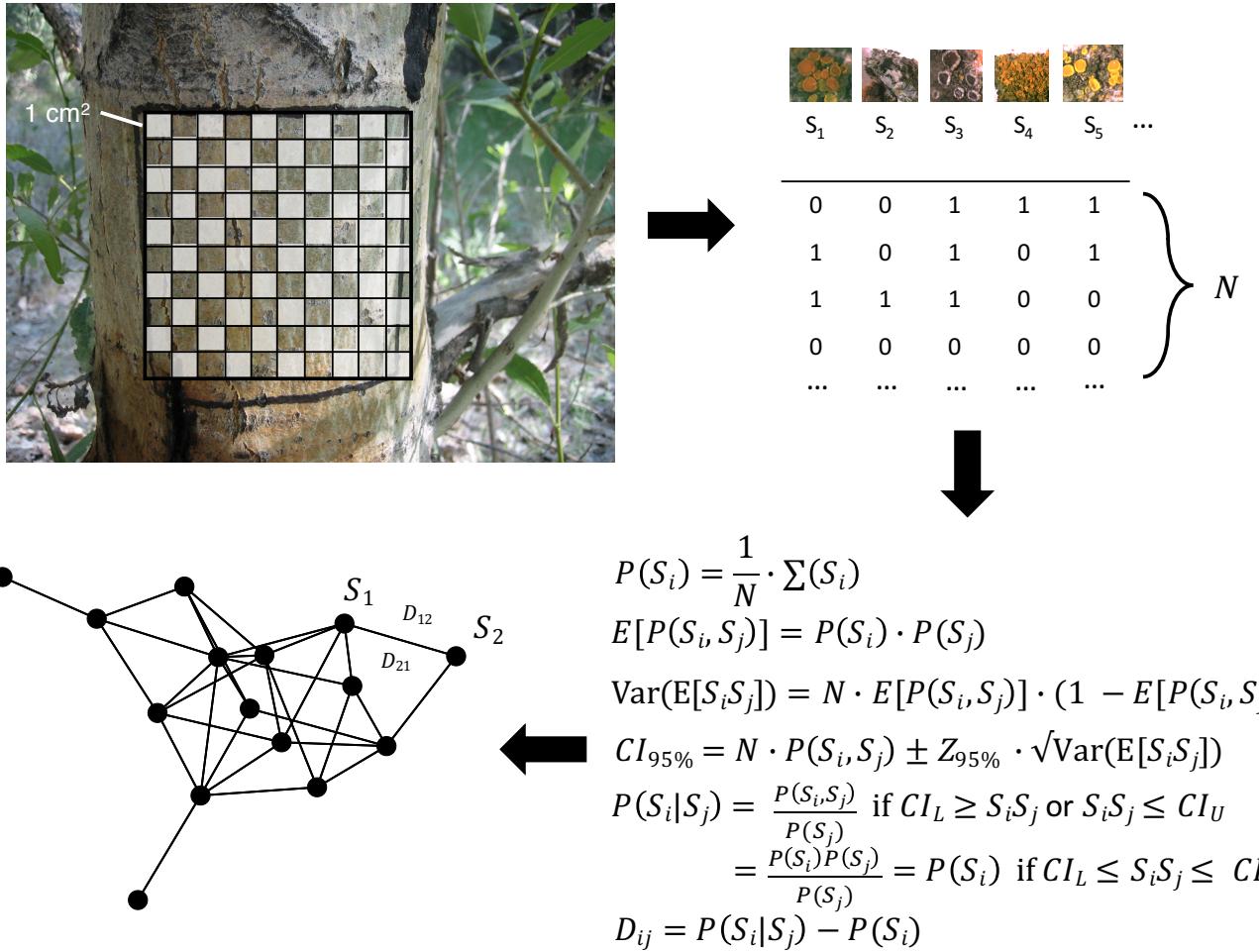
a combination of Least Squares Regression, Analysis of Variance (ANOVA) and correlation tests to quantify and test for the relationship among other variables. Bark roughness, lichen cover and species richness were square-root transformed to meet the assumptions of homogeneity of variance and normality 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 **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. Us-

ing 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 (?)). 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 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



**Fig. 2.** Lichen interaction networks were constructed by conducting field observations in  $1 \text{ cm}^2$  cells within a  $10 \text{ cm}^2$  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 (?), 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,  $S_iS_j$ , 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  $S_i$ .

278 into groups of species more connected to each other than  
 279 other species. As with the other response variables, the  
 280 number of links was log-transformed and both modularity  
 281 and centralization scores were fourth-root transformed to  
 282 meet variance and normality assumptions.

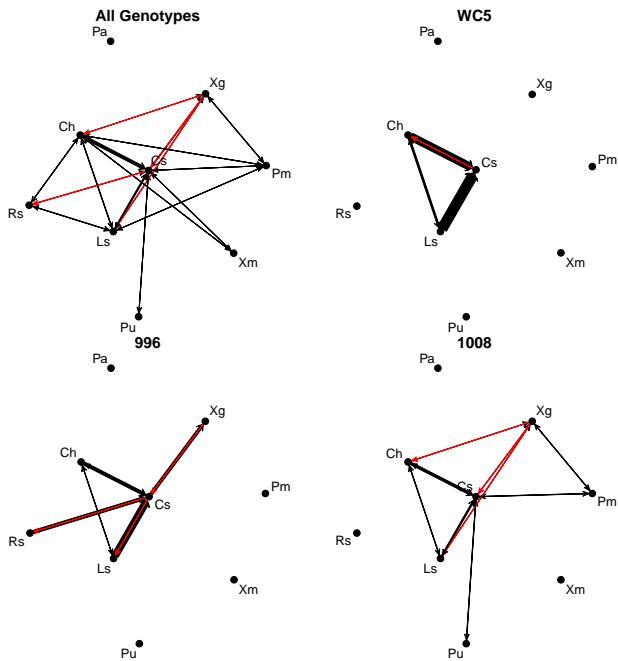
283 All code and data for the project are openly available  
 284 online. Code and data are available at [github.com/  
 285 ecgen/comgen](https://github.com/ecgen/comgen). The project is also archived via Zenodo  
 286 at [zenodo.com/doiXXXXXX](https://zenodo.com/doiXXXXXX). All analyses were conducted  
 287 using the programming language R version 3.6.1 (R De-  
 288 velopment Core Team 2019).

## 289 Results

- 290 1. Genotype influenced lichen network structure
- 291 • Tree genotype significantly predicted the  
 292 similarity of lichen networks (Pseudo- $F_{9,27}$

- = 3.58,  $H^2 = 0.41$ ,  $p\text{-value} = 0.0537$ ). 293
- All network metrics examined responded 294 significantly to tree genotype: including 295 the number of links ( $RLRT = ?$ ,  $H^2 = 296 0.32$ ,  $p\text{-value} = 0.0269$ ), AMI ( $RLRT = ?$ , 297  $H^2 = 0.31$ ,  $p\text{-value} = 0.0268$ ) and degree 298 centralization ( $RLRT = ?$ ,  $H^2 = 0.33$ ,  $p\text{-} 299 value = 0.0196$ ). 300
  - Fig 1. NMDS crosshair with vectors 301
  - Supplementary Table. Vectors 302
  - Supplementary Table. h2-reml 303

2. Genotype impacts were on positive interactions 304 mainly driven by Ch 305
- Tree genotype significantly predicted both 306



**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.

in-degree ( $RLRT = ?, H^2 = 0.35, p\text{-value} = 0.0157$ ) and out-degree ( $RLRT = ?, H^2 = 0.33, p\text{-value} = 0.0195$ ) centralization.

- *Caloplaca holocarpa* centrality was the only species to exhibit a significant response to tree genotype ( $RLRT = ?, H^2 = ?, p\text{-value} = ?$ ).
- Fig 2. dot plot centralization in/out pos/neg
- Table 1. REML H2 Centrality (in/out, pos/neg, spp(in/out, pos/neg))

### 3. Genotype indirectly influenced lichen network centralization via bark roughness

- BR Geno (REML), but not other traits ( $RLRT = ?, H^2 = 0.32, p\text{-value} = 0.0128$ )
- Net BR (PERMANOVA) ( $F_{1,32} = 13.029, R^2 = 0.26, p\text{-value} = 0.0096$ )
- Centrality was significantly correlated with bark roughness ( $F_{1,32} = ?, R^2 = ?, p\text{-value} = ?$ )

- However, tree genotype did not significantly predict the variation in the residuals from the regression of centrality and bark roughness ( $RLRT = ?, H^2 = 0.011, p\text{-value} = 0.4219$ )
- Fig. cross-hair plot Cen BR with trend line
- Table: Network metric and residual responses LM and REML results
- Supplementary Table: cn-trait-perm.tex

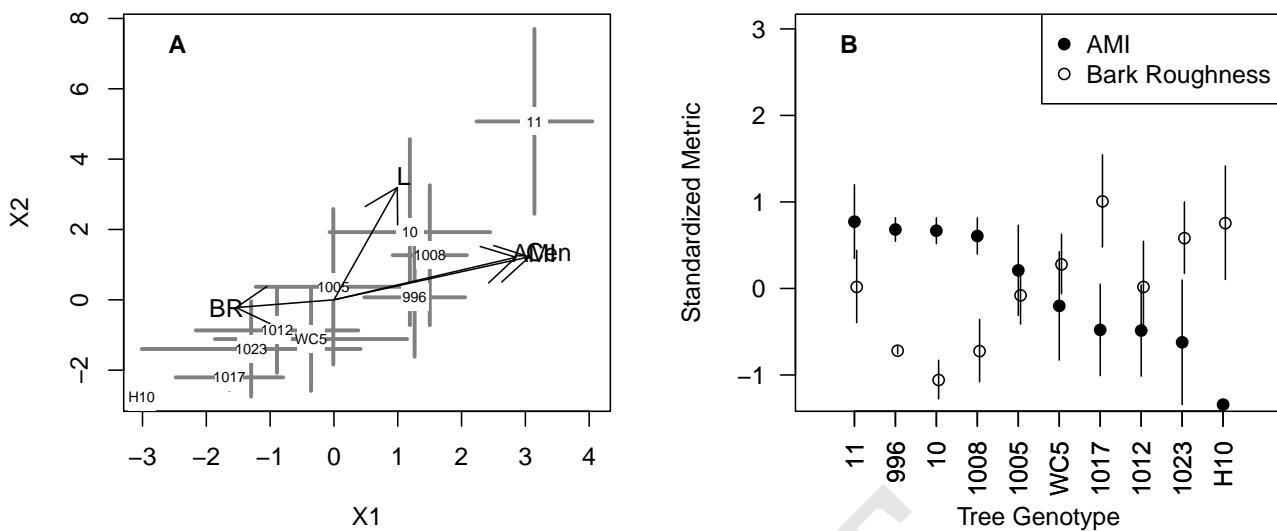
### 4. Net(sim) Other lichen variation (LM) not genetically based (PERMANOVA)

Network similarity and several tree traits were genetically based. Tree genotype was a significant predictor of network similarity ( $H^2 = 0.16, p\text{-value} \leq 0.001$ ). Bark roughness ( $H^2 = 0.38, p\text{-value} \leq 0.001$ ) and condensed tannin concentration ( $H^2 = 0.28, p\text{-value} = 0.014$ ) also showed a signature of tree genotype (Fig. ??); however, this was not the case for other tree traits, bark pH and carbon to nitrogen ratio. Also, none of the lichen network metrics were significantly predicted by tree genotype, either at the scale of the entire network (Table ??) or for individual species (see Supporting Information). Although both showed a response to tree genotype, bark roughness and condensed tannins were not correlated (Pearson's  $r = 0.084, p\text{-value} = 0.556$ ).

	Response	H2	p-value
310	Lichen Network Similarity	0.413	0.0537
311	Average Mutual Information	0.3101	0.0268
312	Degree Centralization	0.3305	0.0196
313	In-degree Centrality	0.3487	0.0157
314	Out-degree Centralization	0.3305	0.0196
315	Number of Network Links	0.3156	0.0269

**Table 1. Genotypic effects on the associated lichen community.**

Tree traits and lichen community metrics were correlated with lichen networks. The genetically based traits, bark roughness and condensed tannins were both significant predictors of network similarity (Table ??). Bark C:N ratio was also a significant predictor of network similarity, but, as shown previously (see Table ??), there is not sufficient evidence to support a genetic basis for it. Bark pH was not significant predictor of lichen network similarity (Table ??). The abundance, richness, evenness and diversity of the bark lichen community, although also



**Fig. 4.** Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ( $R^2 = 0.999$ , stress = 0.008) lichen networks ( $\pm 1$  S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized ( $\frac{x - \bar{x}}{\sigma}$ ) means ( $\pm 1$  S.E.) for the two genetically based tree traits: tree bark roughness and condensed tannin concentration.

not predicted by tree genotype, were all significantly correlated with lichen network structure (Table ??). Lichen community composition was not correlated with lichen network similarity, either when species abundances were relativized (Mantel  $R = -0.09$ ,  $p$ -value = 0.139) or not (Mantel  $R = -0.03$ ,  $p$ -value = 0.573).

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

## Discussion

- We found:
  - Lichen networks genetically based
  - Bark roughness was the primary genetically based trait driving network structure
  - Lichn networks also varied with cover, richness and diversity of the lichen community, which were not correlated with roughness and primarily driven by one dominant species

- What mechanisms could be at play?
- Habitat filtering of communities (richness, composition) vs environmental influence on interactions. Likely a combination of both of these factors.
  - Lichen network structure correlated with species richness, evenness and diversity
  - Lichen community composition not correlated with network structure
  - None of these were genetically based
- An important consequence for diversity is that genotypes could be supporting unique communities, even if the composition of the communities is the same among individuals and genotypes.
- Genetic diversity could be influencing the stability of communities through the effects on interactions. Some network structures are likely to be more stable, either in response to disturbance or via self-organized dynamics. Although, none of the metrics examined, such as the number of links, modularity or centrality, showed a genetic signature.
- Important factors to consider in studies of other ecological networks:

- Relative body size
  - Mobility
  - Reproductive isolation
- Future work should consider the potential influence on evolutionary dynamics of communities
    - Network structure influences network stability
    - Are the communities nested subsets?

**TGW:** I think window is too vague and this topic sentence needs to be much stronger for a journal like PNAS. Might be stronger by saying "Our findings argue there is a genetic component to network structure, which implies that network structure could be subject to selection and networks can evolve."

**TGW:** Could we also make the comparison that genetically more similar trees also have more similar communities? We've done this in the past and it has worked, e.g., Randy's genetic similarity rule.

- 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.

**LJL:** I wonder if you need to have so much on richness here. Overall, I think you want to focus on the network responses and patterns among genotype first, and then go into mechanism later. I think we don't quite have a good mechanism yet so I don't think it needs to come up in the first paragraph of the discussion.

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, (?) 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 (?). Furthermore, in a predator-prey-plant study, Smith (?), showed that the interactions among species across trophic levels depended on plant genotype.

Also, work by (?) 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 (?)

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

**LJL:** If I recall, the Elamo paper just looks at genetic correlations between pairwise individual abundances. I would suggest maybe it doesn't deserve to be in this 1st paragraph. Perhaps it actually should be in the 2nd or 3rd paragraph, just as a reference that points to the potential for genotype to influence networks. Definately our 2015 JOE paper goes much further, too, since it has whole communities being correlated. But, again, I woudl put both of these as citation in the community genetics paragraphs (2 of 3) instead of the first paragrpaah, which focuses on the general network lit.

**LJL:** It could be useful to point out that our findings are not related to trophic interactions, which is pretty cool. Also,we talk about interaction networks but it is not clear to me if the interactions tend to be positive or negative. Can we get at that with the approach used?

**TGW:** Is there any adaptive component to the tree in having certain lichen communities? e.g., can they feed back to affect tree performance in some way or is this a passive outcome of a trait that affects bark for other adaptive reasons and lichens are passive players that tag along for the ride? I could envision that lichens covering the bark of a tree act as a barrier between insects and pathogens, much like ectomycorrhizae cover fine roots as a first line of defense by invading microorganisms. Uptake of N that gets passed to the tree??

499 **LJL:** I agree that there is a general overar- 546  
500 ching theme that evolution occurs in a com- 547  
501 munity network context, but I'm not sure 548  
502 that we should state that as our main hypoth- 549  
503 esis. It seems more that this is a fundamen- 550  
504 tal foundation for our work. The hypoth- 551  
505 esis is more what we are testing directly, but 552  
506 we don't test this directly. I guess I don't 553  
507 want to give the impression that our com- 554  
508 munities are necessarily the result of each 555  
509 species evolving into its place in the commu- 556  
510 nity on these tree genotypes (although I do 557  
511 understand this as Shuster et al 2006's funda- 558  
512 mental explanation for why we see different 559  
513 communities on different genotypes; I don't 560  
514 necessarily agree that this is the only rea- 561  
515 son we woudl see different communities on 562  
516 dif genotypes). Most of these are pretty gen- 563  
517 eralist lichens, which could be found on other 564  
518 decidous trees in the surrounding city or nat- 565  
519 ual areas. I would look at it more like an 566  
520 assembling of lichen species into unique con- 567  
521 figurations on genetically different substrates. 568  
522 There may be some selection for different 569  
523 genotype of lichen during the community as- 570  
524 sembly process but we can't really tell that 571  
525 just by differences in species abundances or 572  
526 coocurneces. I guess to me the evolutionary 573  
527 context that is more direclty related to this 574  
528 work is that the tree genotype is a central 575  
529 controller (indeed a sort of hub species in the 576  
530 network) of network structure. By anchor- 577  
531 ing the lichen network to tree genotype (and 578  
532 variation among networks to variation among 579  
533 tree genotypes) , our study highlights the pos- 580  
534 sibility that natural selection acting on the 581  
535 trees may have an extended consequence for 582  
536 the network structure of organisms living on 583  
537 the trees... the extra thing we add to the field 584  
538 is that we show interaction networks are sen- 585  
539 sitive to genotype. I doubt the lichens have 586  
540 a direct effect on tree fitness, but favorabil- 587  
541 ity of some tree genotypes over others during 588  
542 natural selection will then go on to favor and 589  
543 disfavor certain lichen communities of differ- 590  
544 ent network structures. By being sensitive 591  
545 to tree genotype, the lichen community net- 592  
546 works are passive riders on the waves of evolu- 593  
547 tionary dynamics that occur within the tree 594  
548

species they inhabit.

**MKL:** In response to Lamit's comment above, I agree that it is not required that there is co-evolution. Another, perhaps simpler, explanation is that there is variation in environmental filtering of lichen individuals created in part by genetic variation in tree individuals.

**TGW:** might be good to cite papers on competition in lichens or other organizing factors to back up the least expected statement. as epiphytes we might not expect them to care.

**TGW:** I think we need to emphasize the long-term nature of our common garden study as very few common garden studies of lichens likely exist. Any refs on this? If true might want to mention this up front in intro.

**MKL:** Environmental filtering is evidenced by species richness, but also possibly species interaction varying based on environment as networks varied in terms of sign and magnitude as well.

**MKL:** The effect of bark roughness on network similarity was primarily genetically based, and there are likely other factors at play.

#### Discussion of network implications for stability with genetics.

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

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 (? ?). 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 (? ? ?). 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 (?). 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.

609

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## Assessment and Results

- 725  
 712     • Network similarity not genetically based     726  
 713     • Genetically based number of links and centrality but     727  
 714         not modularity     728  
 715     • Lichen cover, richness, evenness, diversity and com-     729  
 716         position not genetically based     730  
 717     • Roughness genetically based but not bark condensed     731  
 718         tannins, CN or pH     732  
 719     • Bark roughness correlation with number of links     733  
 720         (yes) and centrality (yes)? <- TODO add figure A     734  
 721         = mdc.plot(L, Cen), B = (ch.plot(L,Cen,geno), BR     735  
 722         vector))     736  
 723     • Centrality values for species <- censpp.pdf     737  
 724     • Redo haritability calculations     738  
 725     • Jamie double check genotype network permanova in     739  
 726         PRIMER     740  
 727     • Jamie double check reml's in R     741

## Tables.

742

Response	H2	p-value
Lichen Network Similarity	0.413	0.0537
Average Mutual Information	0.3101	0.0268
Degree Centralization	0.3305	0.0196
In-degree Centrality	0.3487	0.0157
Out-degree Centralization	0.3305	0.0196
Number of Network Links	0.3156	0.0269
Percent Lichen Cover	0	1
Lichen Species Diversity	0	0.4558
Lichen Species Richness	0	0.458
Lichen Species Evenness	0	1
Percent Rough Bark	0.3221	0.0128
pH	0	1
Carbon-Nitrogen (CN) Ratio	0	1
Condensed Tannins (CT)	0.0041	0.4513
BR-L Residuals	0	1
BR-Cen Residuals	0.0113	0.4219
BR-AMI Residuals	0	1

**Table 1. Genotypic effects on the associated lichen community.**

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

	L	Cen	AMI
PC	0.00	-0.46	0.00
SR	0.47	0.00	0.00
SE	0.45	0.00	0.00
SD	0.59	0.33	0.00

## Figures.

743

	R2	p-value
br_L	0.210	0.005
br_Cen	0.190	0.006
br_AMI	0.170	0.012
ct_L	0.020	0.359
ct_Cen	0.010	0.641
ct_AMI	0.000	0.831
ph_L	0.000	0.948
ph_Cen	0.000	0.746
ph_AMI	0.000	0.825
cn_L	0.020	0.432
cn_Cen	0.030	0.345
cn_AMI	0.020	0.435

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

	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		

**Table 4. Pseudo-F Table of lichen community similarity PERMANOVA.**

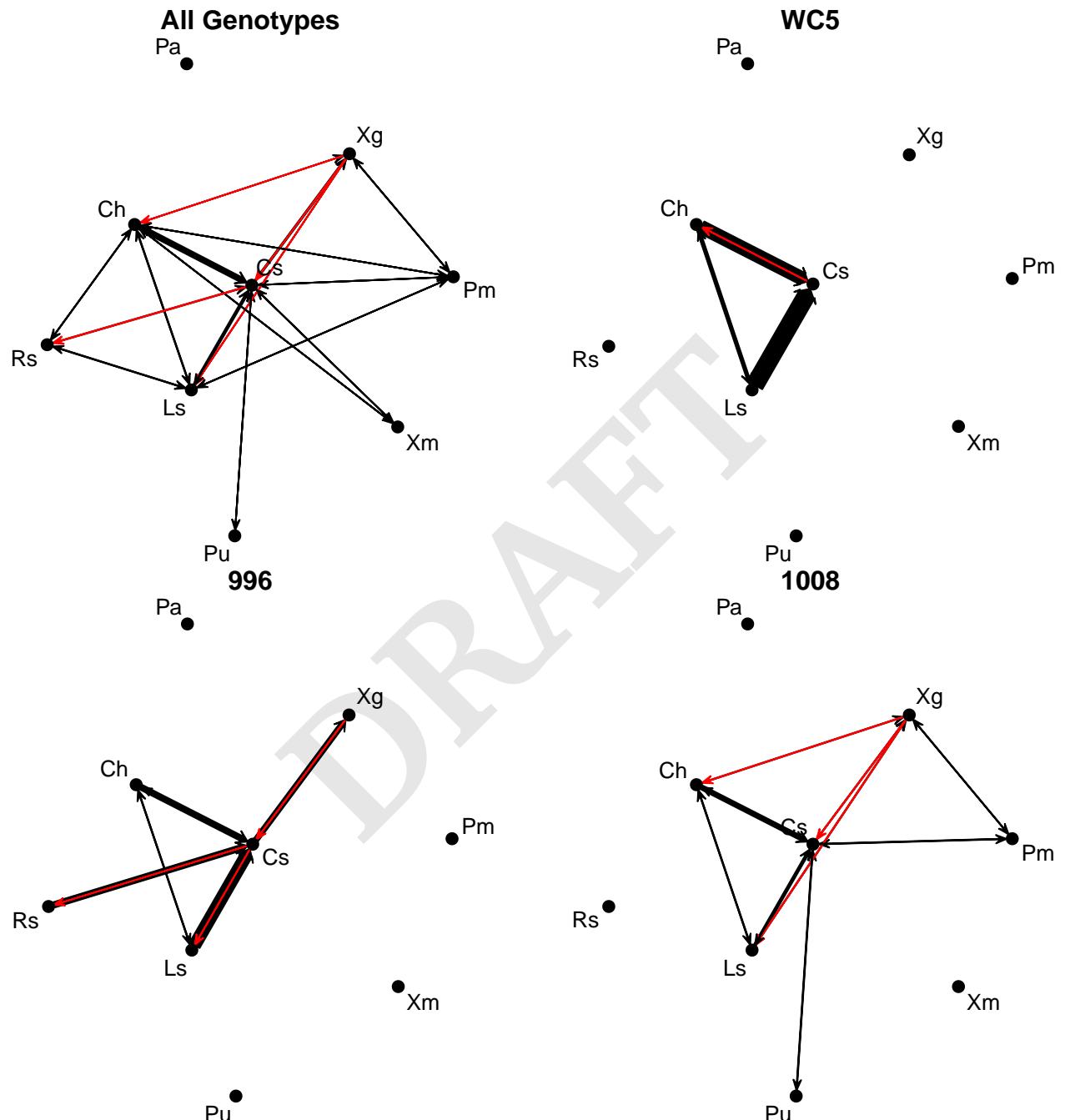


Fig. 1

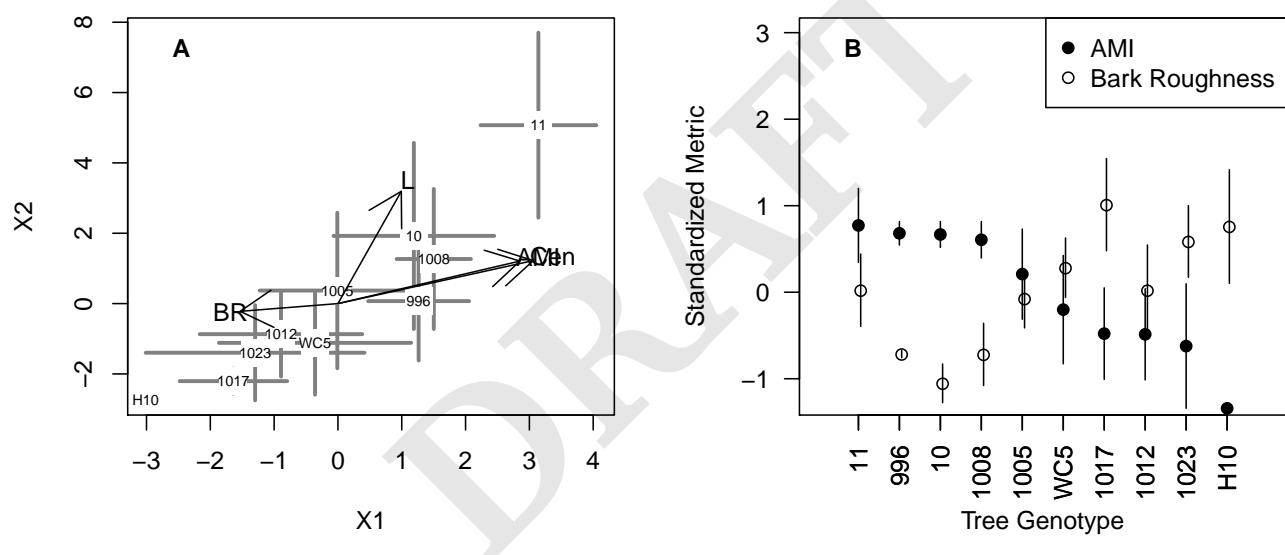


Fig. 2

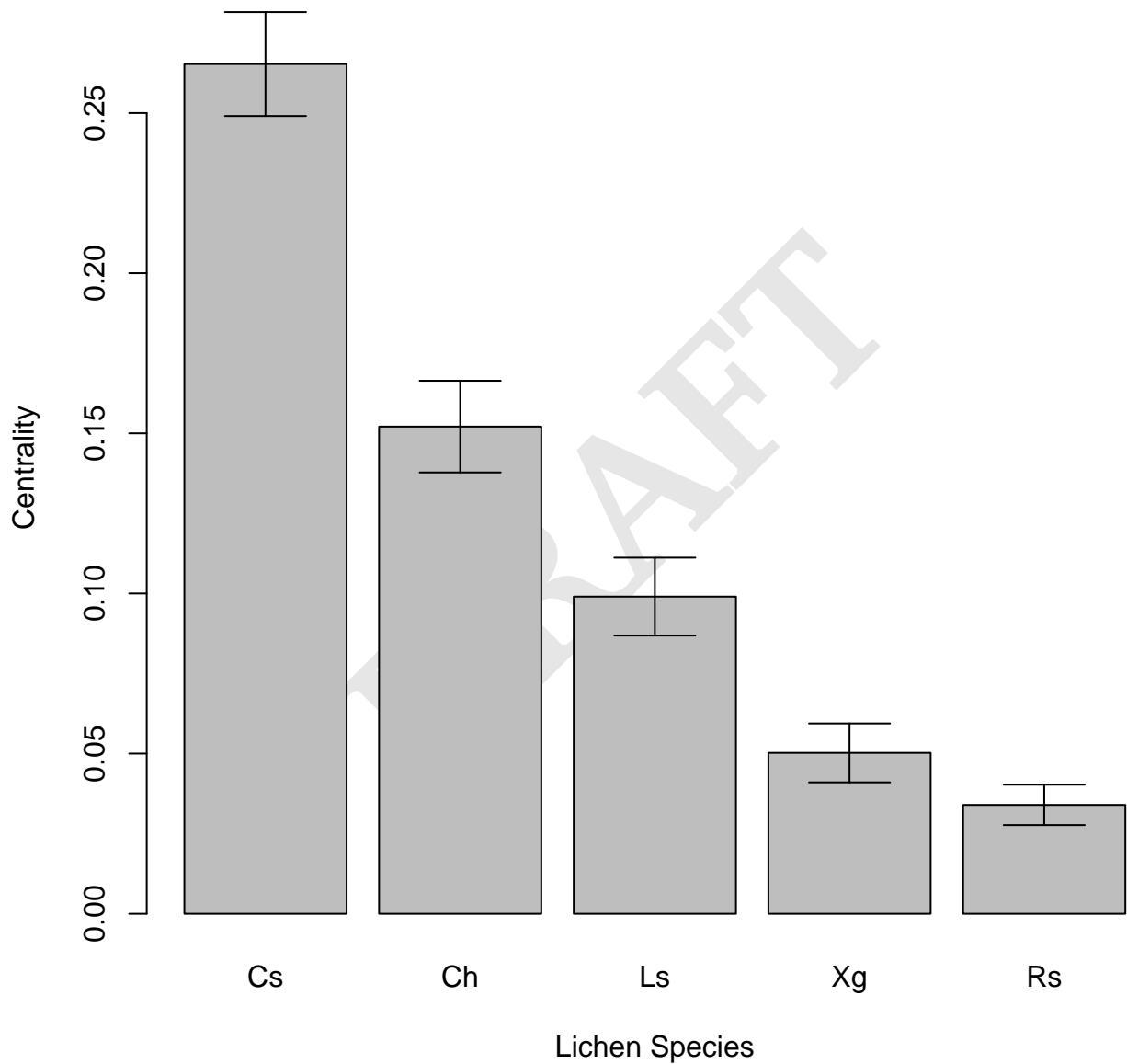


Fig. 3

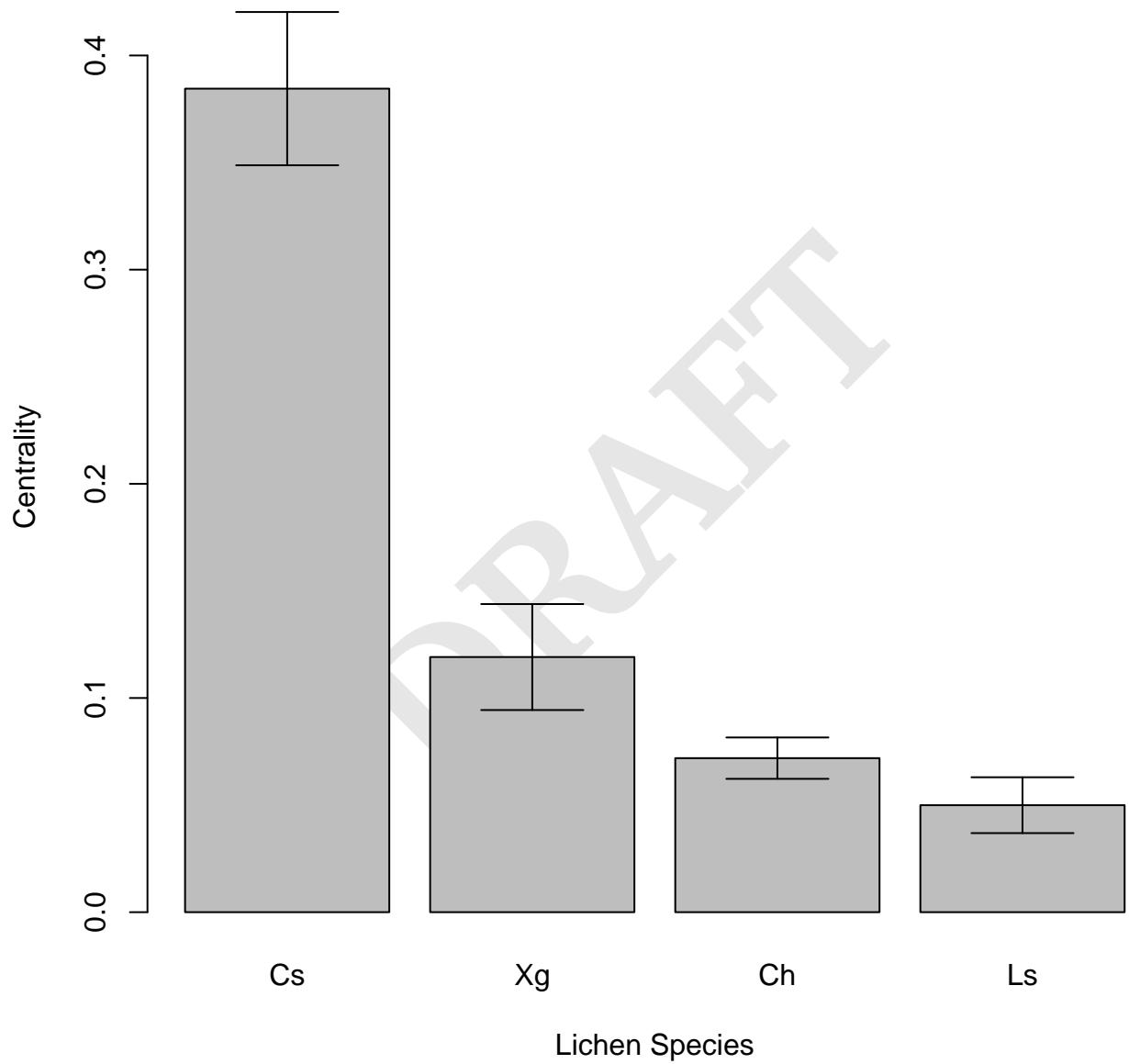


Fig. 4

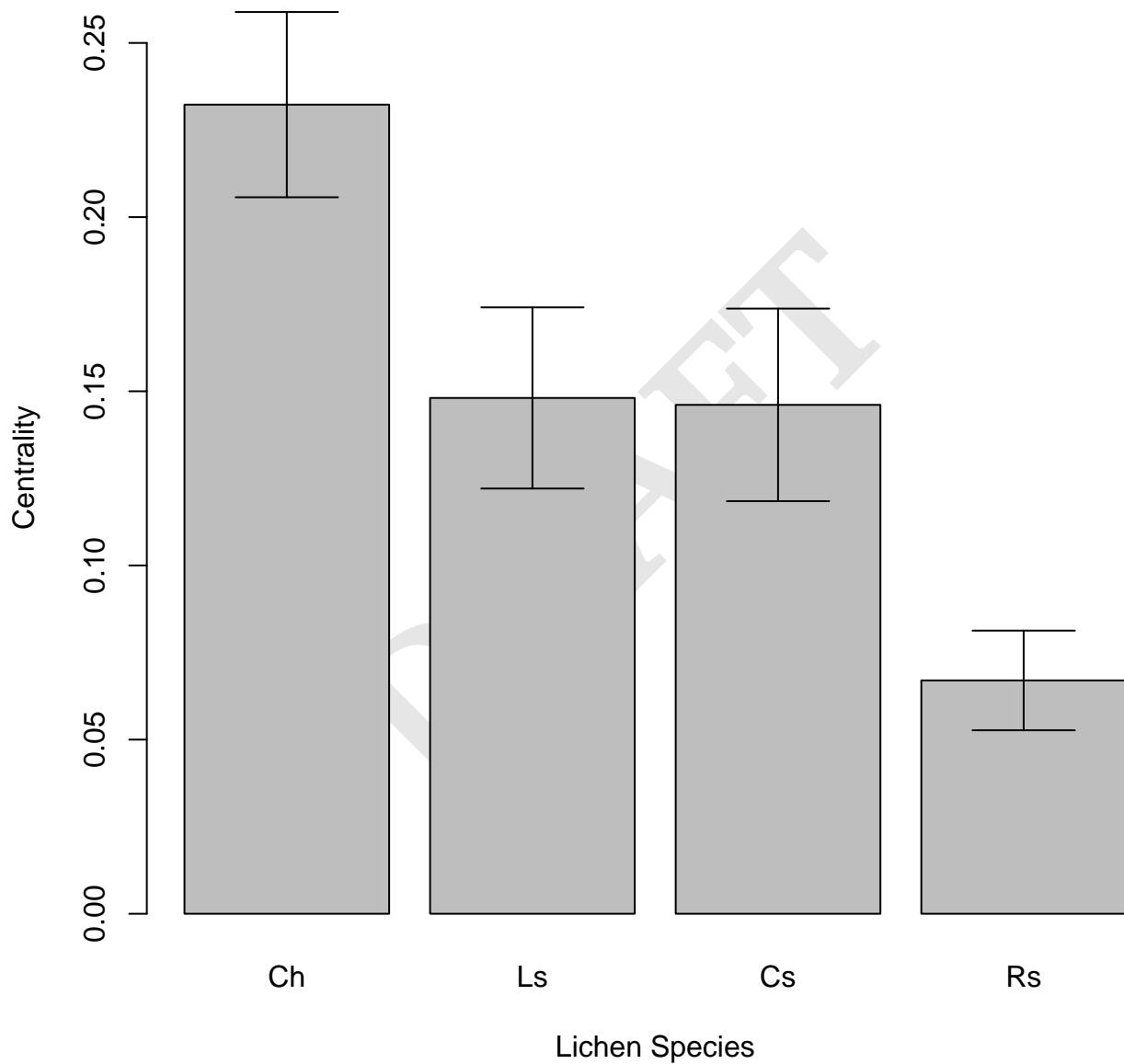
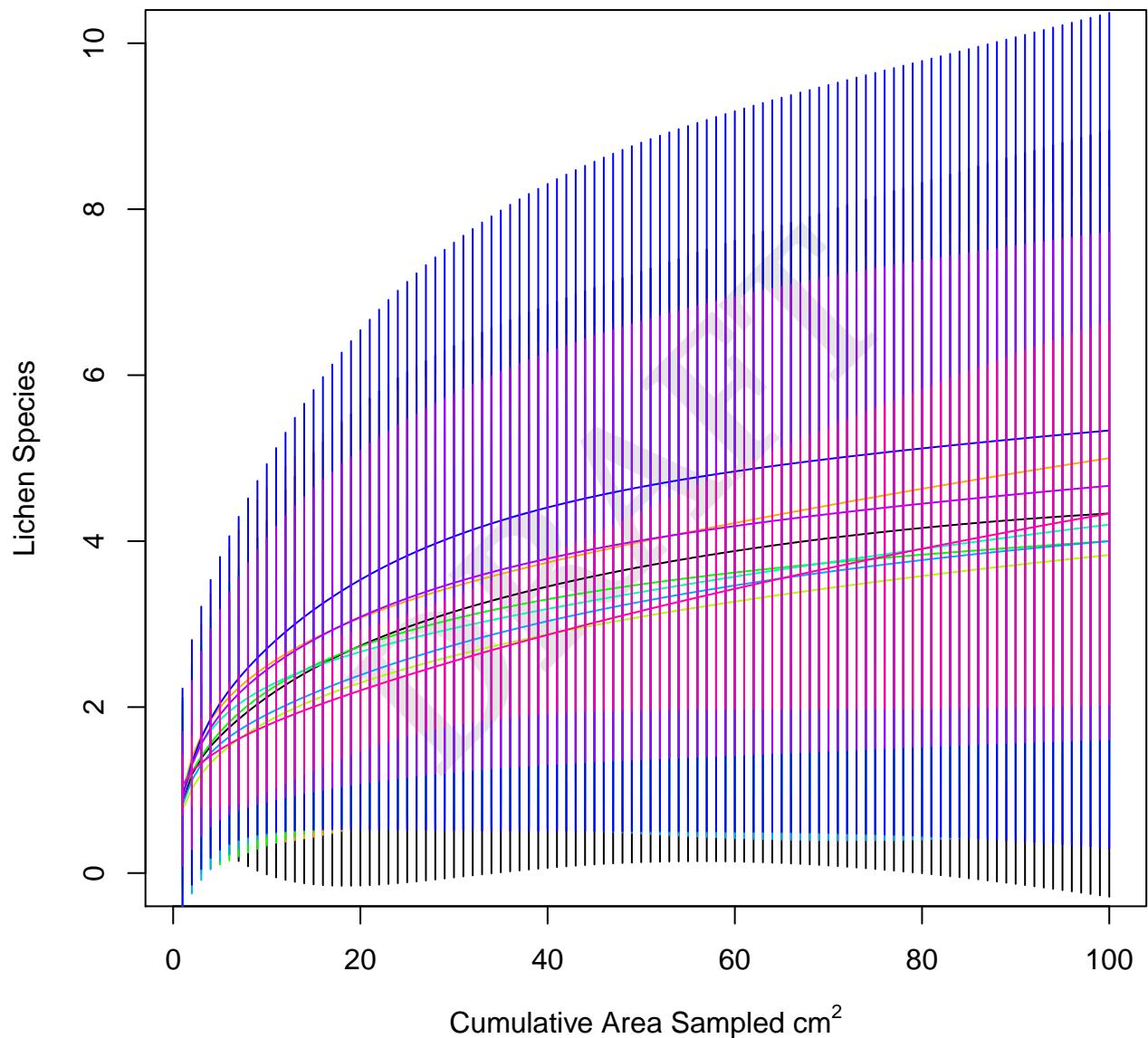


Fig. 5



**Fig. 6.** Species-area curve by genotype.

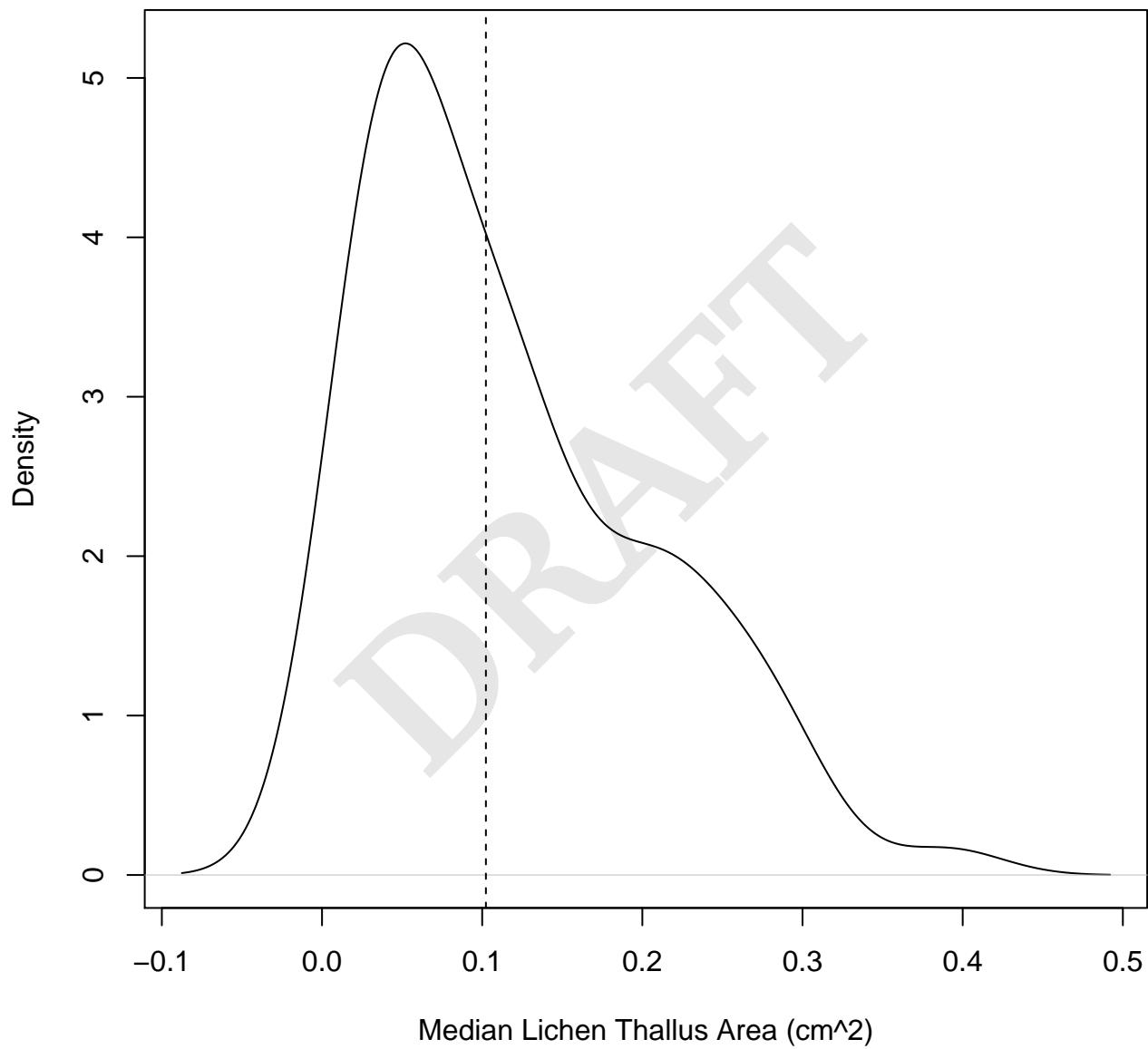


Fig. 7