

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

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

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

This manuscript was compiled on May 1, 2020

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 dynamics in structuring communities and ecosystems
- 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 system is ideal for examining for this kind of study because it:

- 1 Lichen do not move among trees, although
2 asexual propagules could move to other
3 trees in the garden
- 2 Is not unapproachably complex
- 3 Is comprised of distinct individuals that
4 are small enough for an entire community
5 to occur on a single tree but not too small
6 to be quantified easily

Significance Statement

Evolution occurs in the context of ecosystems comprised of complex ecological networks. Research at the interface of ecology and evolution has primarily focused on pairwise interactions among species and have rarely included a genetic component to analyses. Here, we use a long-term common garden experiment to reveal the effect that genotypic variation can have on networks of lichens that occur on the bark of a foundation tree species. We found that lichen interaction network structure is genetically based and is correlated with heritable bark traits, including roughness and condensed tannins. These findings demonstrate the importance of genetic 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.

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

- 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 (1)
 51 and species interactions citeWhitham2006a, Bai-
 52 ley2009, Moya-Larano2011, which shapes the eco-
 53 logical interaction networks (2).

54 Community genetics studies (3) have shown that
 55 genetic variation in foundation species (4) plays a
 56 significant role in defining distinct communities of in-
 57 teracting organisms: such as, endophytes, pathogens,
 58 lichens, arthropods, and soil microbes. Multiple stud-
 59 ies have now demonstrated that genetic variation
 60 influences numerous functional traits (e.g., phyto-
 61 chemical, phenological, morphological) produces a
 62 multivariate phenotype (5) that contributes to varia-
 63 tion in associated communities (6). The importance
 64 of genetic variation in structuring ecological systems
 65 was recently reviewed by Des Roches et al. (2018).

66 Additional work has provided support for the hy-
 67 pothesis that not only does composition vary among
 68 genetically distinct genotypes of foundation species,
 69 it also impacts the structure of the network of species
 70 interactions in these communities. Two studies have
 71 demonstrated conceptually (7) and empirically (8).
 72 However, studies in the network ecology literature
 73 generally do not include a genetic component (see

74 review by Borrett et al. 2014) and community genet-
 75 ics papers do not have data on network structure of
 76 associated communities. Currently, we are aware of
 77 only one paper that empirically examines the genetic
 78 basis of network structure of a community citeBar-
 79 bour2019.

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 (9, 10). 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 (7, 8, 11, 12). 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 biodiveristy.
 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

109 The study was conducted along the Weber River, UT
 110 (USA), which is a cottonwood (*Populus* spp.) dominated
 111 riparian ecosystem. Although two native species, *Populus*
 112 *angustifolia* (James) and *Populus fremontii* (S. Watson),
 113 occur here and are known to hybridize, only pure or
 114 advanced generation backcrosses of *P. angustifolia* were
 115 sampled. Bark lichens have been extensively studied
 116 in this system and provide an ideal system in which to
 117 observe and model lichen interaction networks, as their
 118 sessile nature permits accurate identification of individuals
 119 (13).

120 A long-term, common garden experiment was used
 121

123 to isolate the effect of tree genotype from the effect of
124 the localized microenvironment associated with each individual
125 and spatial autocorrelation. Established in 1992,
126 asexually propagated clones of genotyped *P. angustifolia*
127 individuals were obtained from wild collections and
128 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 **Bark Lichen Observations.** On each tree, presence or absence
134 of each lichen species was assessed in 50 total 1 cm²
135 cells arrayed in a checkerboard pattern. Given the small
136 size and sessile nature of lichens, we were able to rapidly
137 assess lichen interactions by quantifying thalli in close
138 contact. Sampling was restricted to the northern aspect
139 of the trunk to maximize the abundance of lichen and
140 control for the effect of trunk aspect. Two adjacent 10
141 cm² quadrats centered at 50 cm and 85 cm from ground
142 level were sampled (Fig 1 A and B). The observed lichen
143 community included (abbreviations are given for species
144 present in study): Xg = *Xanthomendoza galericulata*, Xm
145 = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Candeliella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were
149 not observed in the present study but are known to occur
150 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

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 cm² (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 **Lichen Network Modeling and Analysis.** For each tree,
168 repeated observations of lichen were made in order to construct
169 replicated interaction networks for each genotype.
170 We quantified the presence of lichen in the 1 cm² cells on
171 individual trees of *P. angustifolia*. Unipartite networks
172 were generated using the conditional probabilities of each
173 species pair, i.e. the probability of observing one species
174 given an observation of another species $P(S_i|S_j)$, based on
175 the method developed by (14). To calculate conditional
176 probabilities, we quantified the individual probabilities

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

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

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



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. Naesbour (D-K).

232 Variance (ANOVA) and correlation tests to quantify and
 233 test for the relationship among other variables. Bark
 234 roughness, lichen cover and species richness were square-
 235 root transformed to meet the assumptions of homogeneity
 236 of variance and normality for these tests.

237 For multivariate response variables, such as lichen com-
 238 munity composition and network structure, we used dis-
 239 tance based multivariate statistical approaches, including
 240 Permutational Analysis of Variance (PERMANOVA) and
 241 Mantel tests. For some analyses, community composition
 242 was relativized by species maxima to reduce the effect
 243 of the highly abundant *X. galericulata*. For community
 244 composition we used Bray-Curtis dissimilarity, which has
 245 optimal performance with count data citepMinchen1998.
 246 To quantify the similarity of lichen networks among indi-
 247 vidual trees, we calculated the pairwise Euclidean distance
 248 of the **D** interaction matrices among all pairs of trees.

249 For visualization of multivariate patterns, we used Non-
 250 metric Multi-Dimensional Scaling (NMDS) citeecodist
 251 to produce dimensionally reduced ordinations of these
 252 multi-variate responses and fitted vectors for continuous
 253 predictor variables to the ordinated values citevegan. Us-
 254 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.

255 For each network, we also calculated metrics that mea-
 256 sure different structural aspects. Although there are many
 257 other metrics, for the sake of simplicity we focus on a sub-
 258 set that represent several interesting features of network
 259 structure (see (15)). We calculated the number of interac-
 260 tions or “links” in each network, which provides a measure
 261 of the size of the network citepLau2015, Borrett2014. We
 262 also calculated the centralization of each network, which
 263 measures the evenness of the distribution of interactions
 264 among the species in the network citeButts2005. In a net-
 265 work with a low level of centralization species have similar
 266 amount of interaction in the network, while a network
 267 with a high level of centralization tends to have one or
 268 small number of species that interact with other species.
 269 We used a related function to calculate the centrality of
 270 each species (i.e. node level centrality) in each network
 271 as well. The modularity of each network was also quanti-
 272 fied using a weighted algorithm citeBeckett2016, which
 273 measures the degree to which a given network is divided
 274 into groups of species more connected to each other than
 275

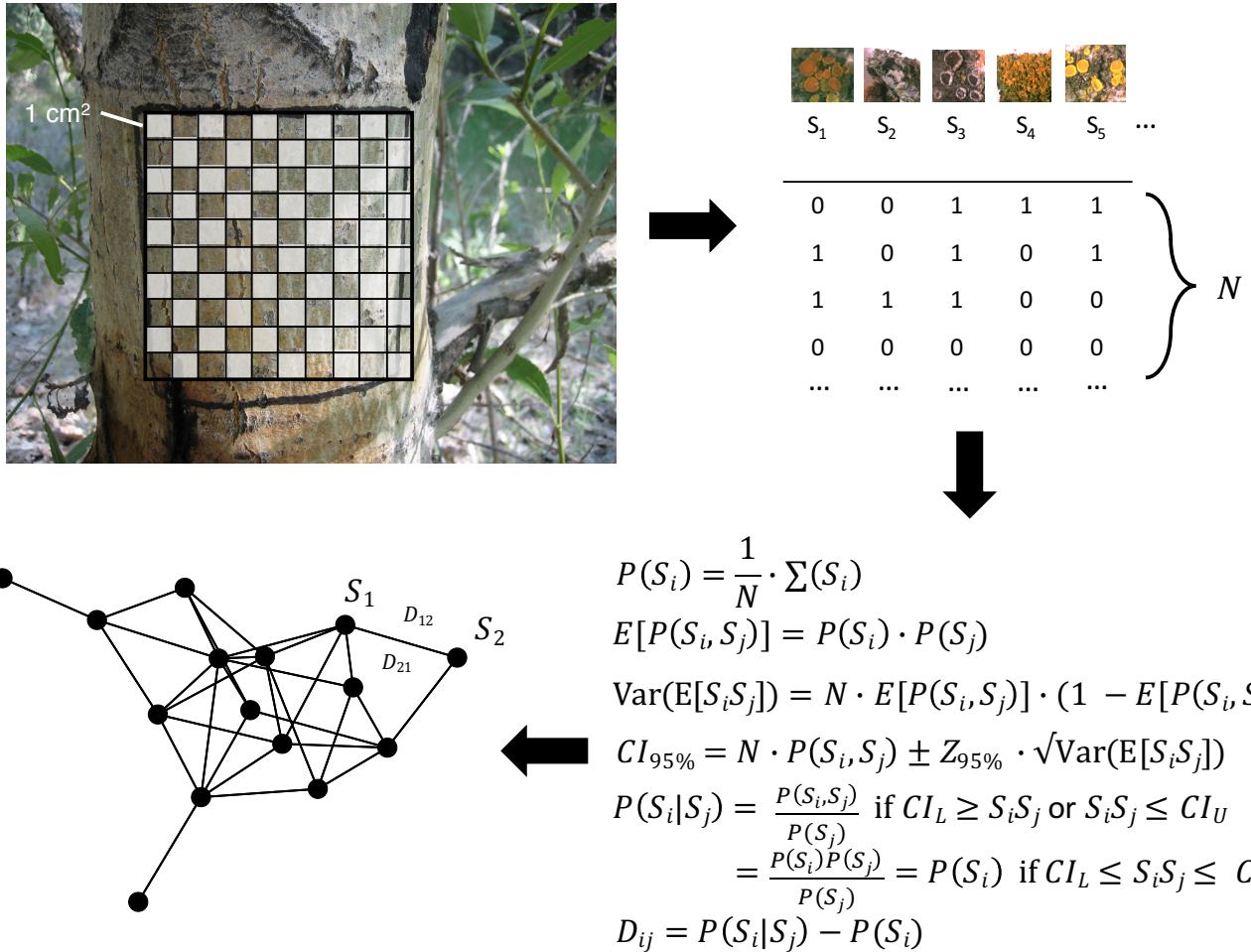


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded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

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

1. Network structure responded to genotype
 - number of links responded
 - centrality responded
 - average mutual information responded

- Figure: chplot network metrics 293
- 2. Genotype indirectly influenced networks through bark roughness 294
 - genotype predicted bark roughness (reml) 296
 - bark roughness predicted network metrics (L, Cen, AMI) 297
 - residuals of BR - geno reml did not predict net metrics 299
- 3. Centrality was a collective response 301
 - centrality varied among species 302
 - individual species centrality did not respond to genotype 303
 - mantel? 305

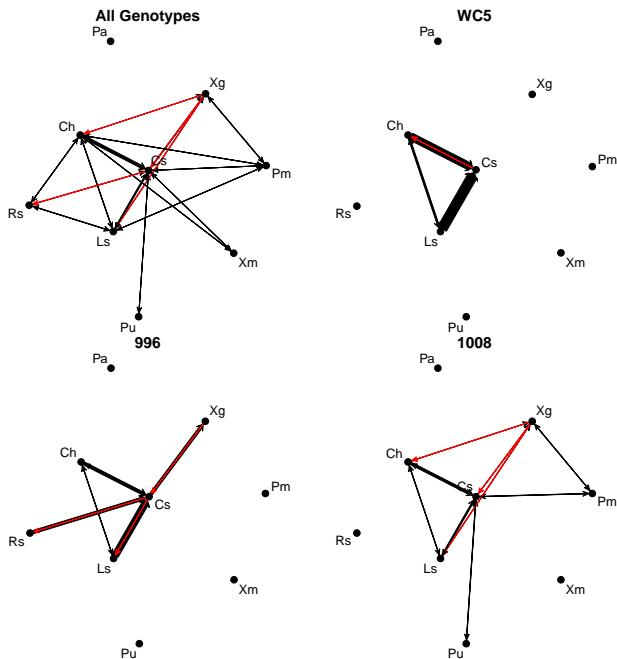


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive) 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	H ²	p-value
Lichen Network Similarity	0.1734	0.0957
Network Ascendancy	0.3308	0.0262
Average Mutual Information	0.3101	0.0274
Network Centrality	0.3305	0.0196
Number of Network Links	0.3156	0.0269
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

Table 1. Genotypic effects on the associated lichen community.

Table 1), there is not sufficient evidence support a genetic basis for it. Bark pH was not a significant predictor of lichen network similarity (Table 2). The abundance, richness, evenness and diversity of the bark lichen community, although also not predicted by tree genotype, were all significantly correlated with lichen network structure (Table 2). 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	257.2922	0.3710	1.7697	0.0957
Residual	27.0000	436.1515	0.6290		
Total	36.0000	693.4437	1.0000		

Table 2. PERMANOVA Pseudo-F Table of lichen network similarity.

Discussion

- We found:
 - Lichen networks genetically based
 - Several genetically based traits also explained variation in lichen network structure
 - Although correlated with lichen network structure, the network metrics explored were not genetically based
- Genetic response of lichen networks is not a function of the abundance, richness, diversity or composition of lichen communities and is not reducible to simple network metrics. This is likely the result of having similar interactions

- 306 • Figure: barplot + post-hoc

307 Network similarity and several tree traits were
308 genetically based. Tree genotype was a significant
309 predictor of network similarity ($H^2 = 0.16$, *p-value*
310 ≤ 0.001). Bark roughness ($H^2 = 0.38$, *p-value*
311 ≤ 0.001) and condensed tannin concentration ($H^2 =$
312 0.28 , *p-value* = 0.014) also showed a signature of tree
313 genotype (Fig. 4); however, this was not the case for
314 other tree traits, bark pH and carbon to nitrogen
315 ratio. Also, none of the lichen network metrics were
316 significantly predicted by tree genotype, either at the
317 scale of the entire network (Table 1) or for individual
318 species (see Supporting Information). Although both
319 showed a response to tree genotype, bark roughness
320 and condensed tannins were not correlated (Pearson's
321 $r = 0.084$, *p-value* = 0.556).

322 Tree traits and lichen community metrics were cor-
323 related with lichen networks. The genetically based
324 traits, bark roughness and condensed tannins were
325 both significant predictors of network similarity (Ta-
326 ble 2). Bark C:N ratio was also a significant predictor
327 of network similarity, but, as shown previously (see

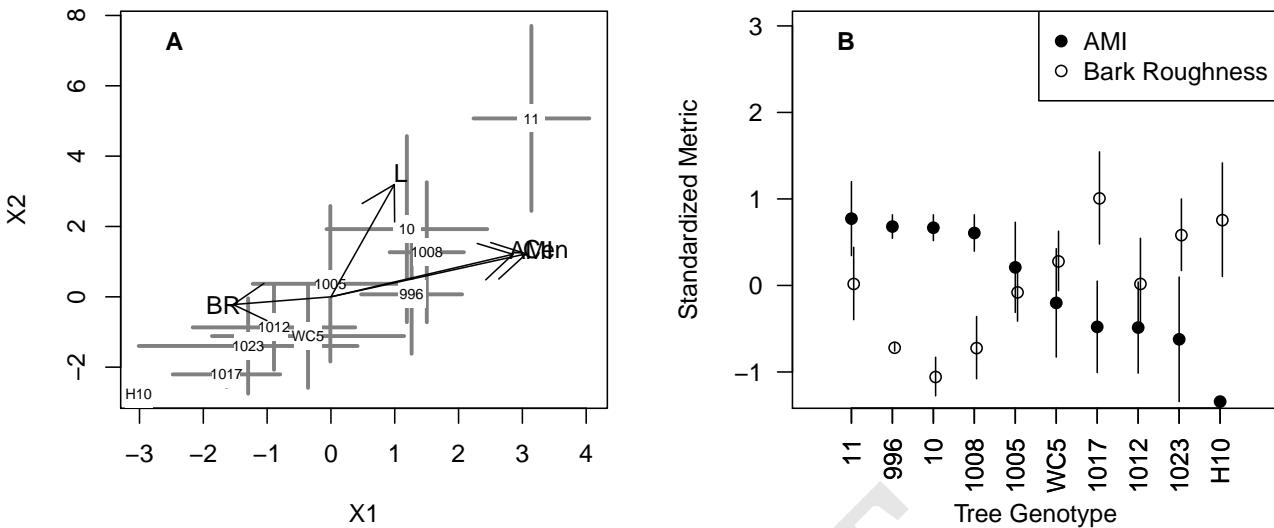


Fig. 4. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized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

among similar species in networks on trees of the same genotype. This is difficult to disentangle with the current set of analyses. Future work should apply a structural statistical approach, such as SEM or path analysis.

- 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

399	in the past and it has worked, e.g., Randy's	446
400	genetic similarity rule.	447
401	• Genetic assembly rule = similar genetics will	448
402	have more similar communities	449
403	• What we don't know is whether or not these	450
404	interactions will also lead to similar interactions	451
405	among other species.	452
406	• Thus, it would be possible for genetics to not	453
407	only influence other species directly, but also	454
408	indirectly by influencing the interactions among	455
409	other species.	456
410	We observed significant lichen interaction struc-	457
411	ture that varied among genotypes of a foundation	458
412	tree species, narrowleaf cottonwood (<i>P. angustifo-</i>	459
413	<i>lia</i>). We found that a genetically based trait, bark	460
414	roughness, partially explained the variation in lichen	461
415	interaction networks. Some of this variation in lichen	462
416	networks was related to both the overall abundance	463
417	and species richness of lichen; though, statistically	464
418	controlling for the effect of genotype on these vari-	465
419	ables indicates that a significant portion of the vari-	466
420	ance in lichen species richness is due to a factor	467
421	other than tree genotype. By using network metrics,	468
422	we were also able to probe for specific characteris-	469
423	tics of how these networks were responding to tree	470
424	genotype. We found that both number of links and	471
425	the centralization of the networks were highly corre-	472
426	lated with network similarity and that tree genotype	473
427	significantly predicted network centrality but only	474
428	marginally predicted the number of network links.	475
429	This latter result could be due to the relationship	476
430	between species richness and the number of links	477
431	in the network, which were significantly correlated	478
432	with each other. We also found that bark roughness	479
433	did not significantly predict either the number of	480
434	links or the centrality of lichen networks, suggesting	481
435	that bark roughness has some other effect on the	482
436	structure of the lichen networks. Taken together,	483
437	these findings support the hypothesis that genotypic	484
438	variation in a foundation species contributes to the	485
439	structure of a network of interacting species.	486
440	LJL: I wonder if you need to have so much	487
441	on richness here. Overall, I think you want to	488
442	focus on the network responses and patterns	489
443	among genotype first, and then go into mech-	490
444	anism later. I think we don't quite have a	491
445	good mechanism yet so I don't think it needs	492
446	to come up in the first paragrpah of the dis-	493
447	cussion.	494
448	These findings point to the importance of under-	495
449	standing the community level effects of genetic vari-	496
450	ation in plant functional traits and highlights the	497
451	potential for indirect effects of genetic variation to	498
452	propagate through networks of interacting species	499
453	and trophic levels.	500
454	This work corroborates previous findings of the	501
455	importance of plant genetics in shaping community	502
456	structure and ecosystem processes. citepBangert2008	503
457	Altering the structure of interaction networks	504
458	presents a means for genetic effects to be magni-	505
459	fied within the system of interacting species. For	506
460	example, (8) showed that the genetics based interac-	507
461	tions of aphid resistant and aphid susceptible trees	508
462	resulted in different interaction networks of their	509
463	associated arthropod communities composed of 139	510
464	species. At the scale of ecosystems, trophic net-	511
465	works or food webs direct and control the rates of	512
466	energy and nutrient flux (16). Furthermore, in a	513
467	predator-prey-plant study, Smith (17), showed that	514
468	the interactions among species across trophic levels	515
469	depended on plant genotype.	516
470	Also, work by (18–20) observed consistent pat-	517
471	terns of centralized interactions of species modules	518
472	focused around hubs of plant-fungal interactions. In	519
473	other words, a small number of plant and fungal	520
474	symbionts tended to have disproportionate numbers	521
475	of interactions with other species and likely are the	522
476	drivers in determining community assembly, struc-	523
477	ture and dynamics.	524
478	More on the importance of ecological networks	525
479	(21, 22).	526
480	From Thompson2014	527
481	• Pairwise interactions are usually influenced by	528
482	other species	529
483	• Selection favors the development of small webs	530
484	• Evolution of new lifestyles leads to changes in	531
485	selection on large and small webs	532
486	Specific hypothesis from Thompson2014	533
487	LJL: If I recall, the Elamo paper just looks	534
488	at genetic correlations between pairwise indi-	535
489	vidual abundances. I would suggest maybe it	536
490	doesn't deserve to be in this 1st paragraph.	537
491	Perhaps it actually should be in the 2nd or	538
492	3rd paragraph, just as a reference that points	539

493 to the potential for genotype to influence net-
494 works. Definately our 2015 JOE paper goes
495 much further, too, since it has whole com-
496 munities being correlationed. But, again, I
497 woudl put both of these as citation in the com-
498 munity genetics paragraphs (2 of 3) instead
499 of the first paragrphah, which focuses on the
500 general network lit.

501 **LJL:** It could be useful to point out that
502 our findings are not related to trophic inter-
503 actions, which is pretty cool. Also,we talk
504 about interaction networks but it is not clear
505 to me if the interactions tend to be positive
506 or negative. Can we get at that with the ap-
507 proach used?

508 **TGW:** Is there any adaptive component to
509 the tree in having certain lichen communi-
510 ties? e.g., can they feed back to affect tree
511 performance in some way or is this a pas-
512 sive outcome of a trait that affects bark for
513 other adaptive reasons and lichens are pas-
514 sive players that tag along for the ride? I
515 could envision that lichens covering the bark
516 of a tree act as a barrier between insects and
517 pathogens, much like ectomycorrhizae cover
518 fine roots as a first line of defense by invad-
519 ing microorganisms. Uptake of N that gets
520 passed to the tree??

521 **LJL:** I agree that there is a general over-
522 arching theme that evolution occurs in a com-
523 munity network context, but I'm not sure
524 that we should state that as our main hypoth-
525 esis. It seems more that this is a fundamen-
526 tal foundation for our work. The hypoth-
527 esis is more what we are testing directly, but
528 we don't test this directly. I guess I don't
529 want to give the impresison that our com-
530 munities are necessarily the result of each
531 species evolving into its place in the commu-
532 nity on these tree genotypes (although I do
533 understand this as Shuster et al 2006's funda-
534 mental explanation for why we see different
535 communities on different genotypes; I don't
536 necessarily agree that this is the only rea-
537 son we woudl see different communities on
538 dif genotypes). Most of these are pretty gen-
539 eralist lichens, which could be found on other
540 deciduous trees in the surounding city or nat-
541 ural areas. I would look at it more like an

542 assembling of lichen species into unique con-
543 figurations on genetically different substrates.
544 There may be some selection for different
545 genotype of lichen during the community as-
546 sembly process but we can't really tell that
547 just by differences in species abundances or
548 cocurneces. I guess to me the evolutionary
549 context that is more direclty related to this
550 work is that the tree genotype is a central
551 controller (indeed a sort of hub species in the
552 network) of network structure. By anchor-
553 ing the lichen network to tree genotype (and
554 variation among networks to variation among
555 tree genotypes) , our study highlights the pos-
556 sibility that natural selection acting on the
557 trees may have an extended consequence for
558 the network structure of organisms living on
559 the trees...the extra thing we add to the field
560 is that we show interaction networks are sen-
561 sitive to genotype. I doubt the lichens have
562 a direct effect on tree fitness, but favorabil-
563 ity of some tree genotypes over others during
564 natural selection will then go on to favor and
565 disfavor certain lichen communities of differ-
566 ent network structures. By being sensitive
567 to tree genotype, the lichen community net-
568 works are passive riders on the waves of evolu-
569 tionary dynamics that occur within the tree
570 species they inhabit.

571 **MKL:** In response to Lamit's comment
572 above, I agree that it is not reuired that
573 there is co-evolution. Another, perhaps sim-
574 pler, explanation is that there is variation in
575 environmental filtering of lichen individuals
576 created in part by genetic variation in tree
577 individuals.

578 **TGW:** might be good to cite papers on
579 competition in lichens or other organizing fac-
580 tors to back up the least expected statement.
581 as epiphytes we might not expect them to
582 care.

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

588 **MKL:** Environmental filtering is evidenced
589 by species richness, but also possibly species
590 interaction varying based on environment as

591 networks varied in terms of sign and magnitude as well.

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

597 Discussion of network implications for stability with genetics.

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

602 Although our study was conducted with a community
603 of lichens, these results should be generalized
604 to other groups of diverse organisms around the
605 world that also exhibit significant genetic signals at
606 the community level (24, 25). In the face of the
607 high degree of complexity and potential context de-
608 pendence of ecological processes, the current study
609 points to the utility of considering the spatial and
610 temporal scales of interactions, as discussed to some
611 in previous studies (26–28). In the present study,
612 we found that community assembly processes, such
613 as environmental filtering and species interactions,
614 are genetically based. This is likely due, in part,
615 to the large difference in the differences in size and
616 longevity of the lichen and cottonwood individuals
617 with the trees determining the environment in which
618 the lichen occur. We suggest that future work would
619 be aided by determining these modules within the
620 biotic community that include species with similar
621 differences in body-size and time-scales. As heritable
622 variation is the raw material for natural selection
623 to act upon, a genetic basis for interaction network
624 structure indicates evolutionary dynamics should be
625 considered at the community level and that con-
626 serving genetic variation is important to consider in
627 efforts to restore or preserve complex species interac-
628 tions and their associated ecosystem functions (29).
629 With such findings, it appears that we are closer to
630 understanding the evolutionary drivers of Darwin’s
631 entangled bank and the interconnectedness of species
632 in complex communities.

633 **ACKNOWLEDGMENTS.** This work was supported by
634 the National Science Foundation grant (DEB-0425908)
635 and Integrative Graduate Research Traineeship (IGERT)
636 fellowships for M.L. and L.L. The Ogden Nature Center
637 staff helped to maintain the common gardens. Lichen
638 sampling was supported by Todd Wojtowicz, Luke Evans
639 and David Solance Smith.

1. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming 640 decade. *New Phytol.* 641
2. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co- 642 extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8. 643
3. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly 644 across multiple environments. *Ecology* 96(4):960–971. 645
4. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and 646 dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486. 647
5. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, 648 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype 649 in a foundation tree species. *Oecologia* 170:695–707. 650
6. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant 651 genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 652 364(1523):1607–16. 653
7. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foun- 654 dation species generates network structure that may drive community dynamics and evolu- 655 tion. *Ecology* 97(3):15–0600.1. 656
8. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation 657 species affect community diversity, stability and network structure. *Proc. R. Soc. B Biol. Sci.* 658 284(1854):20162703. 659
9. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecol. Econ.* 660 71:80–88. 661
10. Zytnyska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree 662 species influences the associated epiphytic plant and invertebrate communities in a complex 663 forest ecosystem. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366:1329–1336. 664
11. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499. 665
12. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate 666 Biodiversity Maintenance10.1126/science.1123412. *Science (80-.).* 312:431–433. 667
13. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influ- 668 ences a dominant bark lichen. *Fungal Ecol.* 4(1):103–109. 669
14. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net- 670 works to assess the impacts of climate change. *EcoGraphy (Cop.)*. 34:897–908. 671
15. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics: 672 opportunities for synthesis. *Ecosphere* 8(8):e01900. 673
16. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Soc. Networks* 674 28:466–484. 675
17. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter- 676 actions and selection: trees, aphids and birds. *J. Evol. Biol.* 24(2):422–9. 677
18. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacom- 678 munity level. 679
19. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs 680 in plant-root fungal biomes. *J. R. Soc. Interface.* 681
20. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus 682 networks. *Nat. Commun.* 683
21. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic 684 networks. *Ecol. Lett.* 14(9):877–85. 685
22. Thompson JN, Schwind C, Guimaraes PR, Friberg M (2013) Diversification through multiltrait 686 evolution in a coevolving interaction. *Proc. Natl. Acad. Sci.* 687
23. Bdeir R, et al. (2017) Quantitative trait locus mapping of Populus bark features and stem 688 diameter. *BMC Plant Biol.* 689
24. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and 690 evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1322–8. 691
25. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes. 692
26. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure. 693 *Mol. Ecol.* 15:1379–1391. 694
27. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to 695 body size yields high degree of intervalty. *J. Theor. Biol.* 271(1):106–113. 696
28. Zytnyska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2). 697
29. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net- 698 works to habitat loss. *Ecol. Lett.* 16:844–52. 699

700 **Assessment and Results**

- 701 • Network similarity not genetically based
- 702 • Genetically based number of links and centrality but
703 not modularity
- 704 • Lichen cover, richness, evenness, diversity and com-
705 position not genetically based
- 706 • Roughness genetically based but not bark condensed
707 tannins, CN or pH
- 708 • Bark roughness correlation with number of links
709 (yes) and centrality (yes)? <- TODO add figure A
710 = mdc.plot(L, Cen), B = (ch.plot(L,Cen,geno), BR
711 vector))
- 712 • Centrality values for species <- censpp.pdf
- 713 • Redo haritability calculations
- 714 • Jamie double check genotype network permanova in
715 PRIMER
- 716 • Jamie double check reml's in R

717 **Tables.**

Response	H2	p-value
Lichen Network Similarity	0.1734	0.0957
Network Ascendency	0.3308	0.0262
Average Mutual Information	0.3101	0.0274
Network Centrality	0.3305	0.0196
Number of Network Links	0.3156	0.0269
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

Table 1. Genotypic effects on the associated lichen community.

	df	SS	R2	F	p-value
geno	9.0000	257.2922	0.3710	1.7697	0.0957
Residual	27.0000	436.1515	0.6290		
Total	36.0000	693.4437	1.0000		

Table 2. PERMANOVA Pseudo-F Table of lichen network similarity.

	Df	SumOfSqs	R2	F	Pr(>F)
geno	9.0000	0.4963	0.1960	0.7315	0.8469
Residual	27.0000	2.0353	0.8040		
Total	36.0000	2.5316	1.0000		

Table 3. Pseudo-F Table of lichen community similarity PERMANOVA.

DRAFT

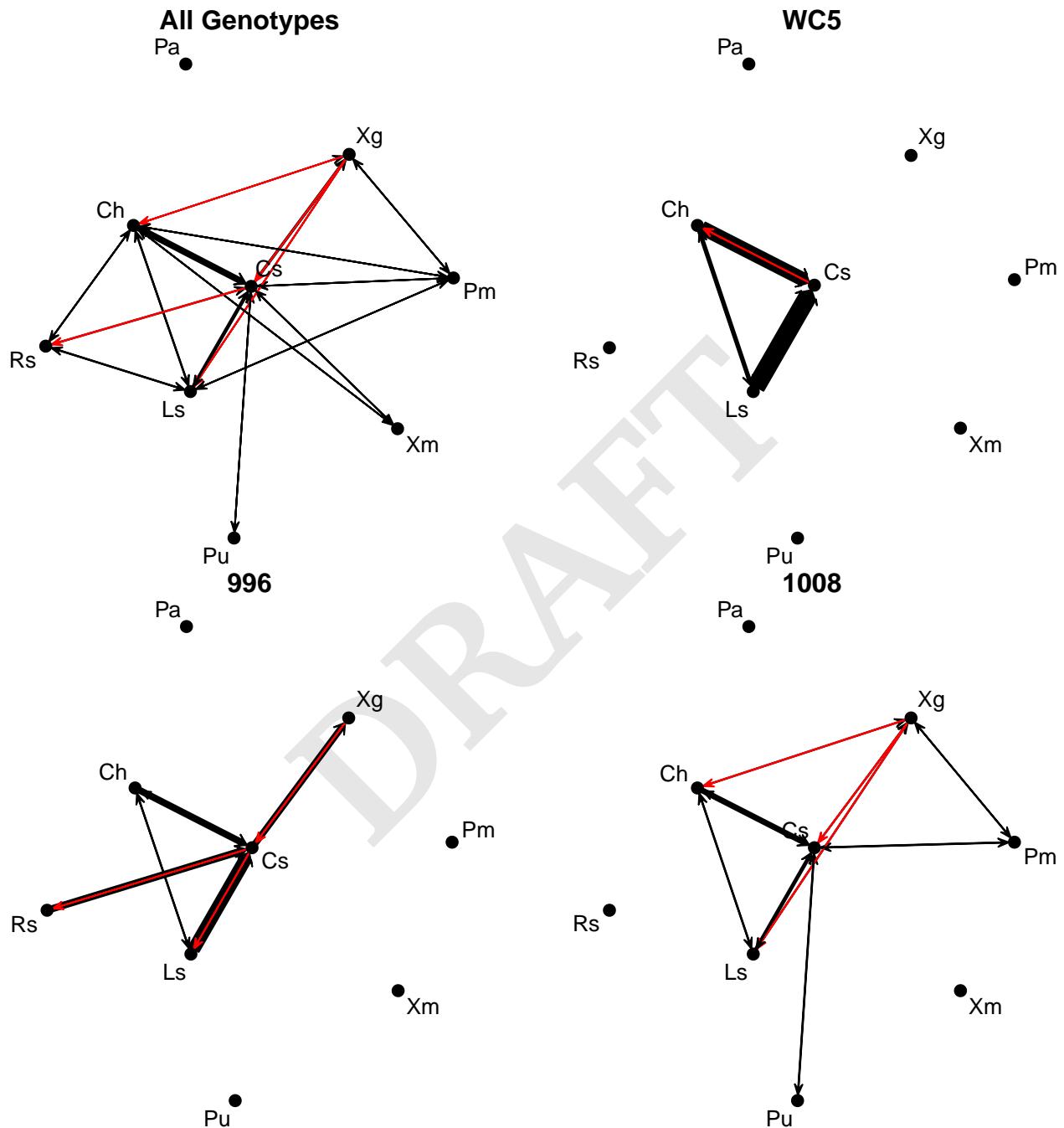


Fig. 1

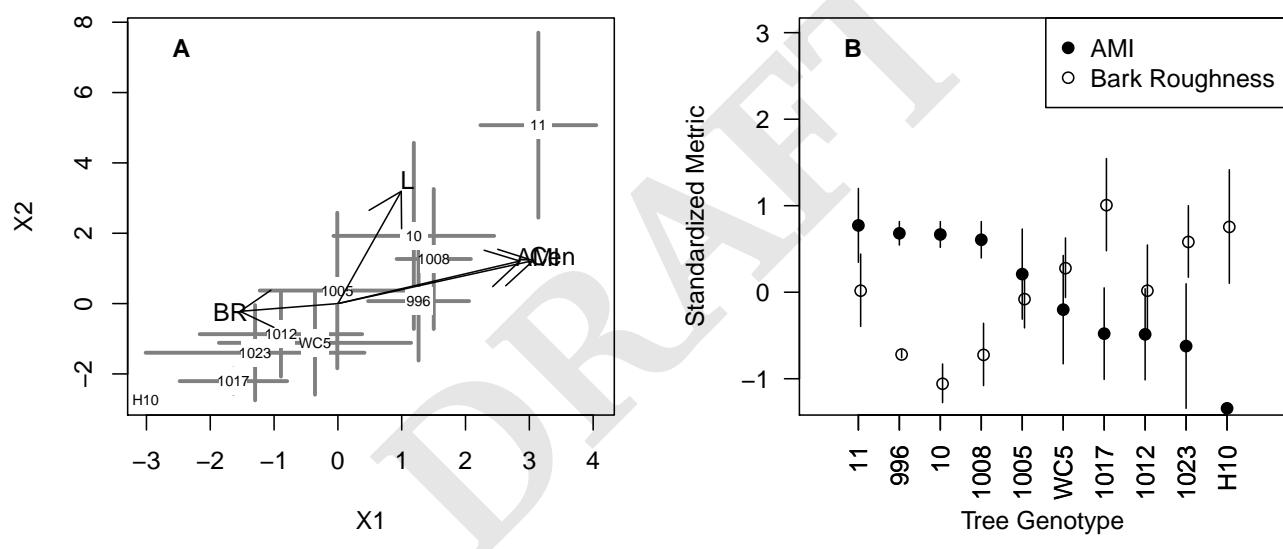


Fig. 2

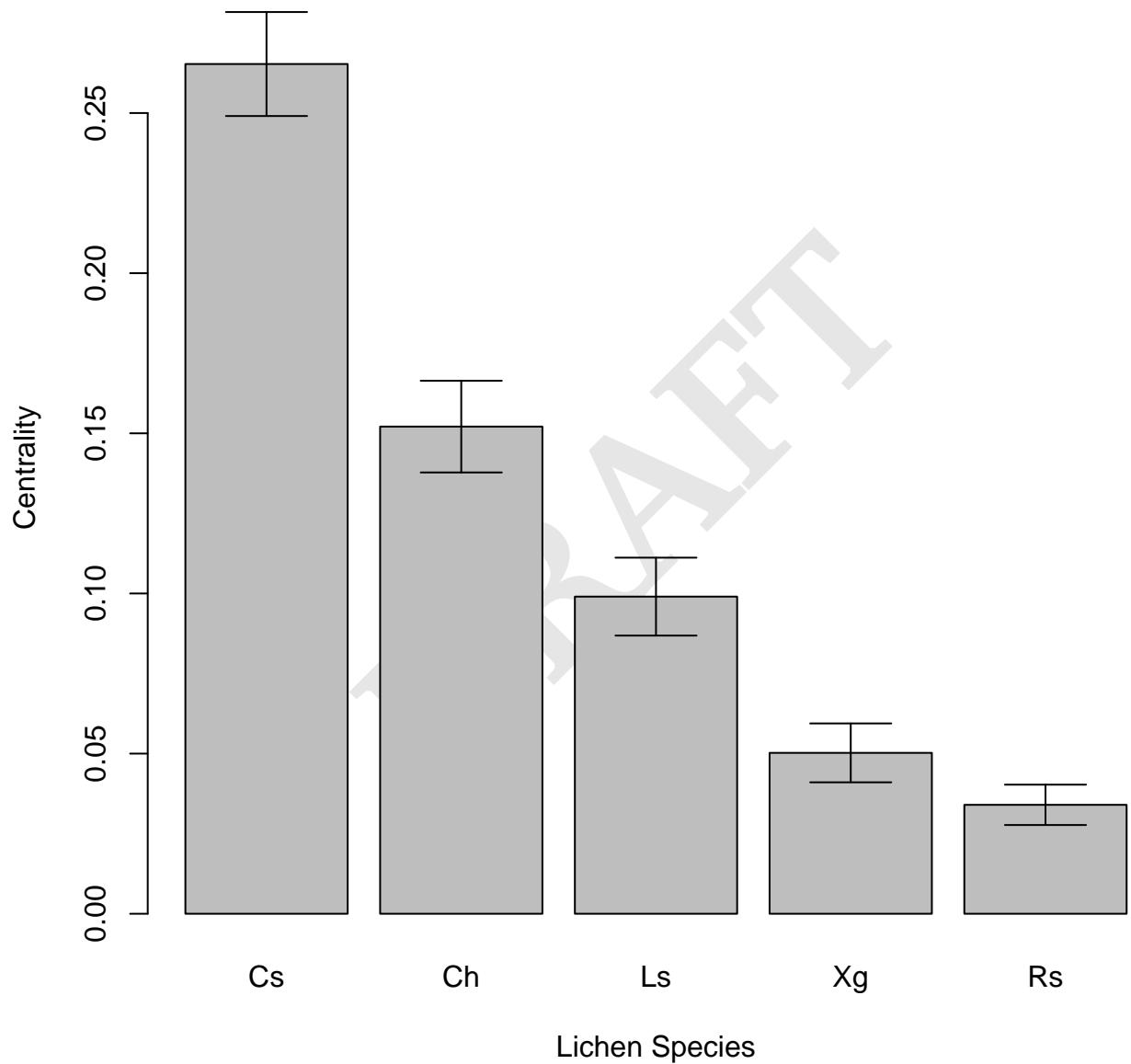


Fig. 3

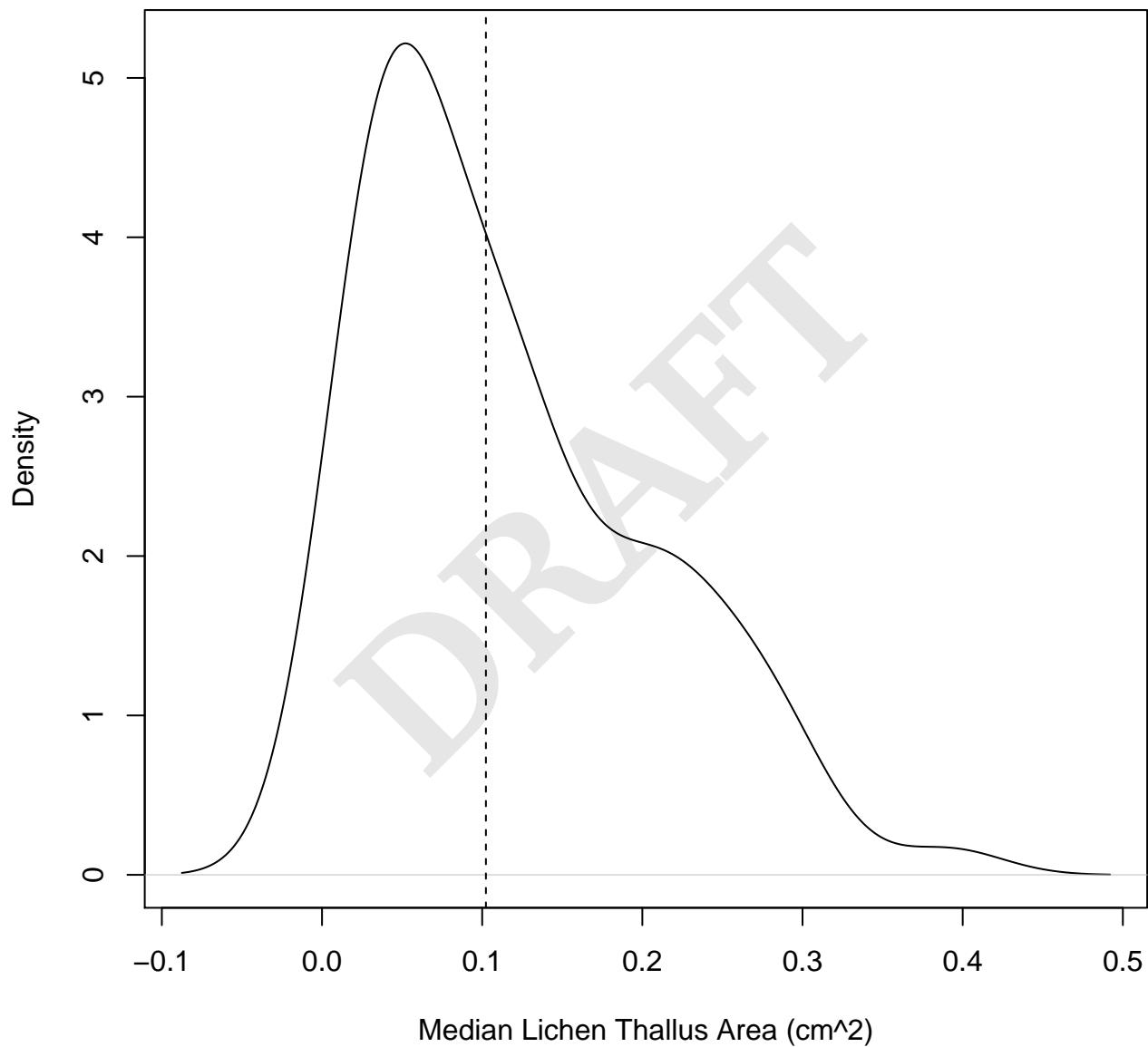


Fig. 4