

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 March 19, 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

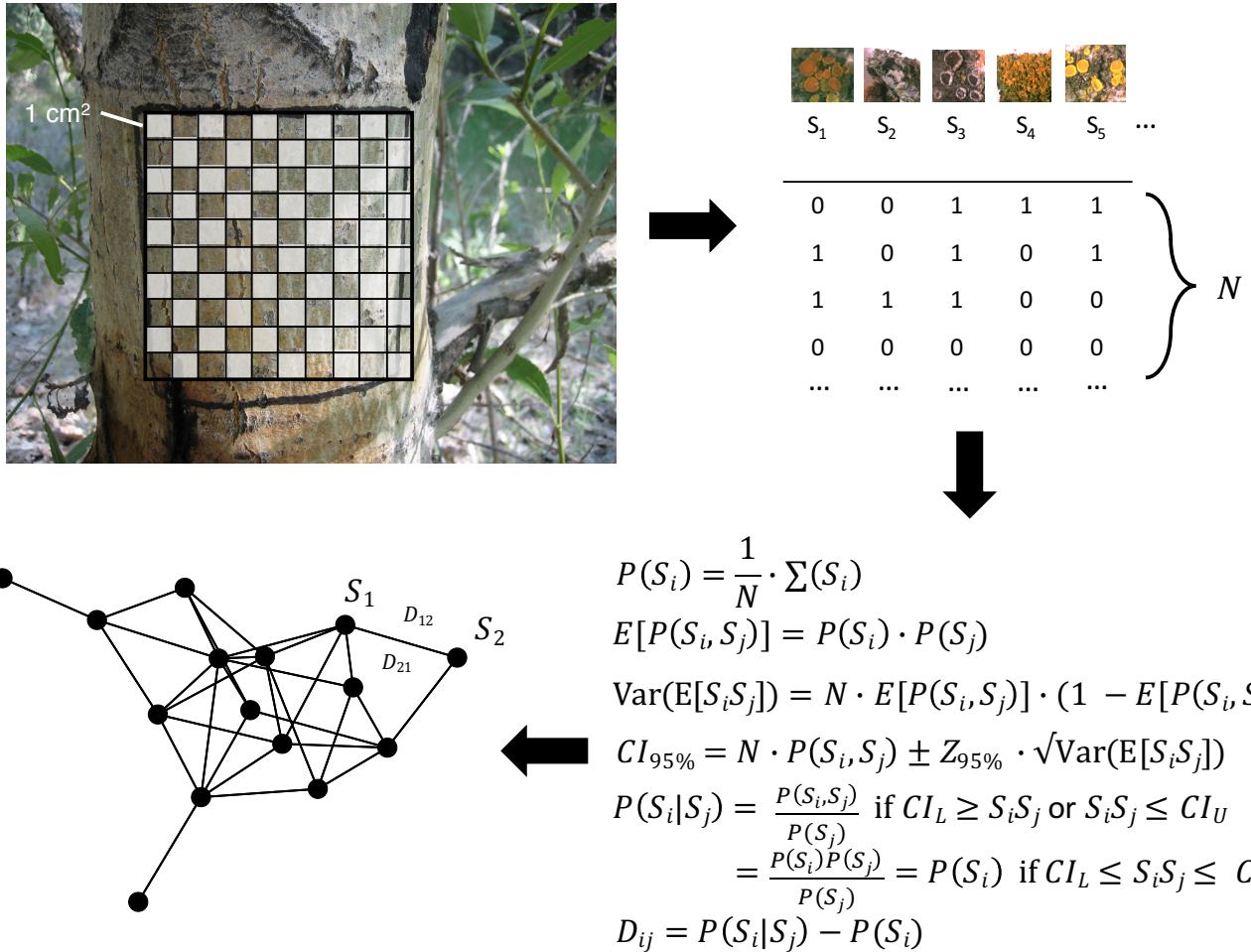


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded 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, $S_i S_j$, to that expected due to chance alone, $E[P(S_i, S_j)] = P(S_i) P(S_j)$, and $P(S_i | S_j)$ reduces to $P(S_i)$, the observed individual probability of species S_i .

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

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. 4); 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 1) 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 $r = 0.084$, $p\text{-value} = 0.556$).

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 2). Bark C:N ratio was also a significant predictor of network similarity, but, as shown previously (see

	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.

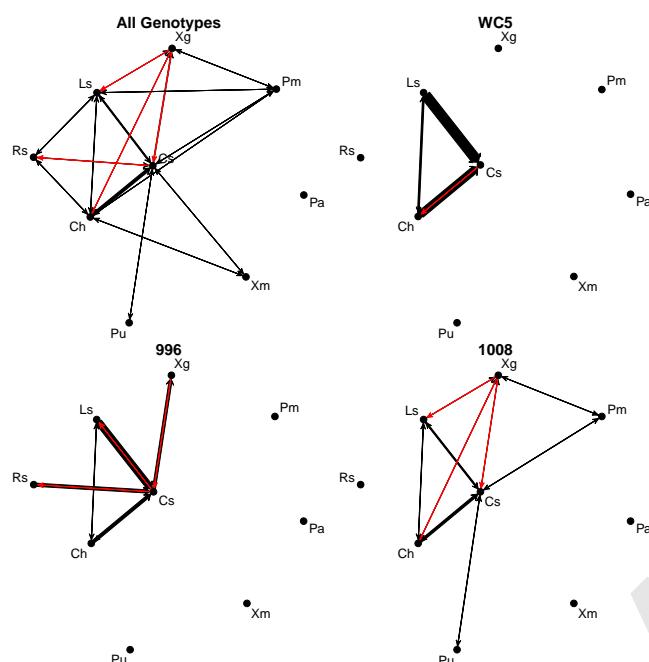


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

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).

Structural model using path analysis based on partial Mantels.

G -> CT -> SR -> Net (0.05, 0.067) (0.24, 0.053) (0.13, 0.041) G -> Net (included as a path within

Response	H2	p-value
Lichen Network Similarity	0.1734	0.0957
Network Centrality	0.3305	0.0196
Number of Network Links	0.3156	0.0267
Lichen Species Diversity	0	0.4558
Lichen Species Richness	0	0.458
Lichen Species Evenness	0	0.4574
Percent Rough Bark	0.3221	0.0128
Condensed Tannins (CT)	0.0041	0.4513

Table 1. Genotypic effects on the associated lichen community.

this model) (0.001, ?)

G -> Net (0.07, 0.042)

ADONIS SSg = 11.093 SSp = 24.428 SST = 35.521

P = 0.053 R2 = 0.31 nperm = 100000

Discussion

- We found:

– Lichen networks genetically based
331

– Several genetically based traits also explained variation in lichen network structure
332
333

– Although correlated with lichen network structure, the network metrics explored were not genetically based
334
335
336
337

• 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 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.
338
339
340
341
342
343
344
345
346
347

• Habitat filtering of communities (richness, composition) vs environmental influence on interactions. Likely a combination of both of these factors.
348
349
350
351

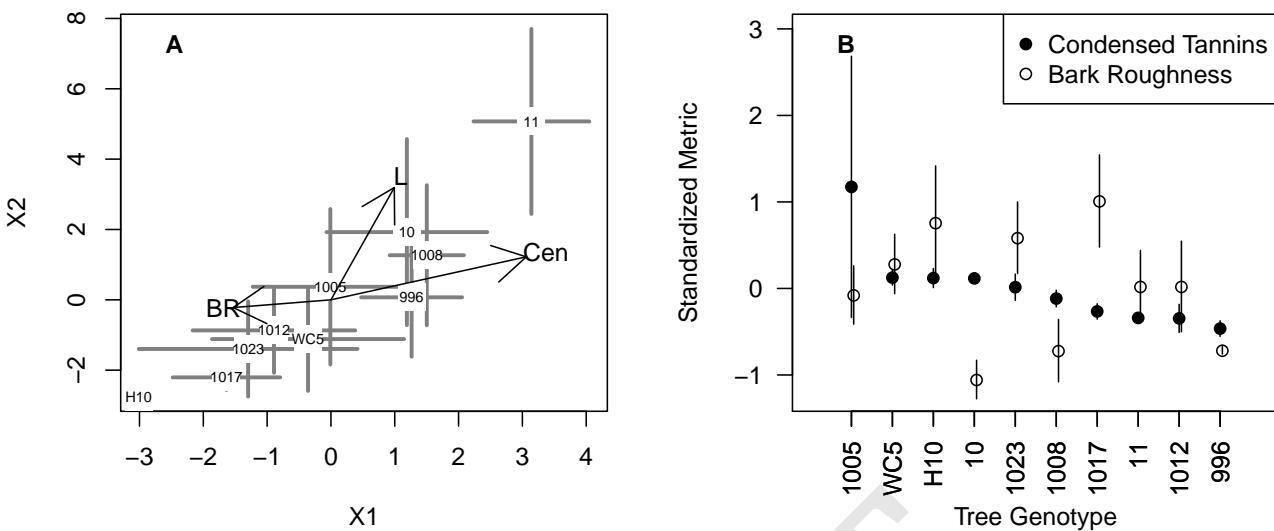


Fig. 4. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized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

- 352 – Lichen network structure correlated with
353 species richness, evenness and diversity
- 354 – Lichen community composition not corre-
355 lated with network structure
- 356 – None of these were genetically based
- 357 • An important consequence for diversity is that
358 genotypes could be supporting unique communi-
359 ties, even if the composition of the communities
360 is the same among individuals and genotypes.
- 361 • Genetic diversity could be influencing the stabili-
362 ty of communities through the effects on inter-
363 actions. Some network structures are likely to
364 be more stable, either in response to disturbance
365 or via self-organized dynamics. Although, none
366 of the metrics examined, such as the number of
367 links, modularity or centrality, showed a genetic
368 signature.
- 369 • Important factors to consider in studies of other
370 ecological networks:
 - 371 – Relative body size
 - 372 – Mobility
 - 373 – Reproductive isolation

- 374 • Future work should consider the potential influ-
375 ence on evolutionary dynamics of communities
- 376 – Network structure influences network sta-
377 bility
- 378 – 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.

- 391 • Genetic assembly rule = similar genetics will
392 have more similar communities
- 393 • What we don't know is whether or not these
394 interactions will also lead to similar interactions
395 among other species.
- 396 • Thus, it would be possible for genetics to not
397 only influence other species directly, but also

398 indirectly by influencing the interactions among
399 other species.

400 We observed significant lichen interaction struc-
401 ture that varied among genotypes of a foundation
402 tree species, narrowleaf cottonwood (*P. angustifo-*
403 *lia*). We found that a genetically based trait, bark
404 roughness, partially explained the variation in lichen
405 interaction networks. Some of this variation in lichen
406 networks was related to both the overall abundance
407 and species richness of lichen; though, statistically
408 controlling for the effect of genotype on these vari-
409 ables indicates that a significant portion of the vari-
410 ance in lichen species richness is due to a factor
411 other than tree genotype. By using network metrics,
412 we were also able to probe for specific characteris-
413 tics of how these networks were responding to tree
414 genotype. We found that both number of links and
415 the centralization of the networks were highly corre-
416 lated with network similarity and that tree genotype
417 significantly predicted network centrality but only
418 marginally predicted the number of network links.
419 This latter result could be due to the relationship
420 between species richness and the number of links
421 in the network, which were significantly correlated
422 with each other. We also found that bark roughness
423 did not significantly predict either the number of
424 links or the centrality of lichen networks, suggesting
425 that bark roughness has some other effect on the
426 structure of the lichen networks. Taken together,
427 these findings support the hypothesis that genotypic
428 variation in a foundation species contributes to the
429 structure of a network of interacting species.

430 **LJL:** I wonder if you need to have so much
431 on richness here. Overall, I think you want to
432 focus on the network responses and patterns
433 among genotype first, and then go into mech-
434 anism later. I think we don't quite have a
435 good mechanism yet so I don't think it needs
436 to come up in the first paragrphah of the dis-
437 cussion.

438 These findings point to the importance of under-
439 standing the community level effects of genetic varia-
440 tion in plant functional traits and highlights the
441 potential for indirect effects of genetic variation to
442 propagate through networks of interacting species
443 and trophic levels.

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

447 Altering the structure of interaction networks
448 presents a means for genetic effects to be magni-
449 fied within the system of interacting species. For
450 example, (8) showed that the genetics based interac-
451 tions of aphid resistant and aphid susceptible trees
452 resulted in different interaction networks of their
453 associated arthropod communities composed of 139
454 species. At the scale of ecosystems, trophic net-
455 works or food webs direct and control the rates of
456 energy and nutrient flux (16). Furthermore, in a
457 predator-prey-plant study, Smith (17), showed that
458 the interactions among species across trophic levels
459 depended on plant genotype.

460 Also, work by (18–20) observed consistent pat-
461 terns of centralized interactions of species modules
462 focused around hubs of plant-fungal interactions. In
463 other words, a small number of plant and fungal
464 symbionts tended to have disproportionate numbers
465 of interactions with other species and likely are the
466 drivers in determining community assembly, struc-
467 ture and dynamics.

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

469 From Thompson2014

- 470 • Pairwise interactions are usually influenced by
471 other species
- 472 • Selection favors the development of small webs
- 473 • Evolution of new lifestyles leads to changes in
474 selection on large and small webs
- 475

476 Specific hypothesis from Thompson2014

477 **LJL:** If I recall, the Elamo paper just looks
478 at genetic correlations between pairwise individ-
479 ual abundances. I would suggest maybe it
480 doesn't deserve to be in this 1st paragraph.
481 Perhaps it actually should be in the 2nd or
482 3rd paragraph, just as a reference that points
483 to the potential for genotype to influence net-
484 works. Definately our 2015 JOE paper goes
485 much further, too, since it has whole com-
486 munities being correalated. But, again, I
487 woudl put both of these as citation in the com-
488 munity genetics paragraphs (2 of 3) instead
489 of the first paragrphah, which focuses on the
490 general network lit.

491 **LJL:** It could be useful to point out that
492 our findings are not related to trophic inter-
493 actions, which is pretty cool. Also,we talk

494 about interaction networks but it is not clear
495 to me if the interactions tend to be positive
496 or negative. Can we get at that with the ap-
497 proach used?

498 TGW: Is there any adaptive component to
499 the tree in having certain lichen communi-
500 ties? e.g., can they feed back to affect tree
501 performance in some way or is this a pas-
502 sive outcome of a trait that affects bark for
503 other adaptive reasons and lichens are pas-
504 sive players that tag along for the ride? I
505 could envision that lichens covering the bark
506 of a tree act as a barrier between insects and
507 pathogens, much like ectomycorrhizae cover
508 fine roots as a first line of defense by invad-
509 ing microorganisms. Uptake of N that gets
510 passed to the tree??

511 LJL: I agree that there is a general over-
512 arching theme that evolution occurs in a com-
513 munity network context, but I'm not sure
514 that we should state that as our main hypoth-
515 esis. It seems more that this is a fundamen-
516 tal foundation for our work. The hypoth-
517 esis is more what we are testing directly, but
518 we don't test this directly. I guess I don't
519 want to give the impression that our com-
520 munities are necessarily the result of each
521 species evolving into its place in the commu-
522 nity on these tree genotypes (although I do
523 understand this as Shuster et al 2006's funda-
524 mental explanation for why we see different
525 communities on different genotypes; I don't
526 necessarily agree that this is the only rea-
527 son we would see different communities on
528 dif genotypes). Most of these are pretty gen-
529 eralist lichens, which could be found on other
530 deciduous trees in the surrounding city or nat-
531 ural areas. I would look at it more like an
532 assembling of lichen species into unique con-
533 figurations on genetically different substrates.
534 There may be some selection for different
535 genotype of lichen during the community as-
536 sembly process but we can't really tell that
537 just by differences in species abundances or
538 coocurneces. I guess to me the evolutionary
539 context that is more direclty related to this
540 work is that the tree genotype is a central
541 controller (indeed a sort of hub species in the
542 network) of network structure. By anchor-

543 ing the lichen network to tree genotype (and
544 variation among networks to variation among
545 tree genotypes), our study highlights the pos-
546 sibility that natural selection acting on the
547 trees may have an extended consequence for
548 the network structure of organisms living on
549 the trees...the extra thing we add to the field
550 is that we show interaction networks are sen-
551 sitive to genotype. I doubt the lichens have
552 a direct effect on tree fitness, but favorabil-
553 ity of some tree genotypes over others during
554 natural selection will then go on to favor and
555 disfavor certain lichen communities of differ-
556 ent network structures. By being sensitive
557 to tree genotype, the lichen community net-
558 works are passive riders on the waves of evolu-
559 tionary dynamics that occur within the tree
560 species they inhabit.

561 MKL: In response to Lamit's comment
562 above, I agree that it is not required that
563 there is co-evolution. Another, perhaps sim-
564 pler, explanation is that there is variation in
565 environmental filtering of lichen individuals
566 created in part by genetic variation in tree
567 individuals.

568 TGW: might be good to cite papers on
569 competition in lichens or other organizing fac-
570 tors to back up the least expected statement.
571 as epiphytes we might not expect them to
572 care.

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

578 MKL: Environmental filtering is evidenced
579 by species richness, but also possibly species
580 interaction varying based on environment as
581 networks varied in terms of sign and magni-
582 tude as well.

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

587 Discussion of network implications for sta-
588 bility with genetics.

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

Although our study was conducted with a community of lichens, these results should be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level (24, 25). In the face of the high degree of complexity and potential context dependency of ecological processes, the current study points to the utility of considering the spatial and temporal scales of interactions, as discussed to some in previous studies (26–28). In the present study, we found that community assembly processes, such as environmental filtering and species interactions, are genetically based. This is likely due, in part, to the large difference in the differences in size and longevity of the lichen and cottonwood individuals with the trees determining the environment in which the lichen occur. We suggest that future work would be aided by determining these modules within the biotic community that include species with similar differences in body-size and time-scales. As heritable variation is the raw material for natural selection to act upon, a genetic basis for interaction network structure indicates evolutionary dynamics should be considered at the community level and that conserving genetic variation is important to consider in efforts to restore or preserve complex species interactions and their associated ecosystem functions (29). With such findings, it appears that we are closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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

1. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming decade. *New Phytol.*
2. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co-extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
3. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly across multiple environments. *Ecology* 96(4):960–971.
4. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486.
5. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia* 170:695–707.
6. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364(1523):1607–16.
7. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foundation species generates network structure that may drive community dynamics and evolution. *Ecology* 97(3):15–0600.1.
8. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation

- | | |
|---|-----|
| species affect community diversity, stability and network structure. <i>Proc. R. Soc. B Biol. Sci.</i> 284(1854):20162703. | 648 |
| 9. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. <i>Ecol. Econ.</i> 71:80–88. | 649 |
| 10. Zytnyska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. <i>Philos. Trans. R. Soc. Lond. B. Biol. Sci.</i> 366:1329–1336. | 650 |
| 11. Thompson JN (2013) <i>Relentless Evolution.</i> (University of Chicago Press), p. 499. | 651 |
| 12. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance. <i>Science</i> 1123412. <i>Science</i> (80-). 312:431–433. | 652 |
| 13. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influences a dominant bark lichen. <i>Fungal Ecol.</i> 4(1):103–109. | 653 |
| 14. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence networks to assess the impacts of climate change. <i>Ecography (Cop.)</i> . 34:897–908. | 654 |
| 15. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics: opportunities for synthesis. <i>Ecosphere</i> 8(8):e01900. | 655 |
| 16. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. <i>Soc. Networks</i> 28:466–484. | 656 |
| 17. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic interactions and selection: trees, aphids and birds. <i>J. Evol. Biol.</i> 24(2):422–9. | 657 |
| 18. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommunity level. | 658 |
| 19. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs in plant-root fungal biomes. <i>J. R. Soc. Interface</i> . | 659 |
| 20. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus networks. <i>Nat. Commun.</i> | 660 |
| 21. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. <i>Ecol. Lett.</i> 14(9):877–85. | 661 |
| 22. Thompson JN, Schwind C, Guimaraes PR, Friberg M (2013) Diversification through multiltrait evolution in a coevolving interaction. <i>Proc. Natl. Acad. Sci.</i> | 662 |
| 23. Bdeir R, et al. (2017) Quantitative trait locus mapping of <i>Populus</i> bark features and stem diameter. <i>BMC Plant Biol.</i> | 663 |
| 24. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and evolution. <i>Philos. Trans. R. Soc. Lond. B. Biol. Sci.</i> 366(1569):1322–8. | 664 |
| 25. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes. | 665 |
| 26. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure. <i>Mol. Ecol.</i> 15:1379–1391. | 666 |
| 27. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to body size yields high degree of intervality. <i>J. Theor. Biol.</i> 271(1):106–113. | 667 |
| 28. Zytnyska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. <i>Oecologia</i> 170(2). | 668 |
| 29. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological networks to habitat loss. <i>Ecol. Lett.</i> 16:844–52. | 669 |

690 Assessment and Results

- 691 Network similarity not genetically based
- 692 Genetically based number of links and centrality but
693 not modularity
- 694 Lichen cover, richness, evenness, diversity and com-
695 position not genetically based
- 696 Roughness genetically based but not bark condensed
697 tannins, CN or pH
- 698 Bark roughness correlation with number of links
699 (yes) and centrality (yes)? <- TODO add figure A
700 = mdc.plot(L, Cen), B = (ch.plot(L,Cen,geno), BR
701 vector))
- 702 Centrality values for species <- censpp.pdf
- 703 Redo haritability calculations
- 704 Jamie double check genotype network permanova in
705 PRIMER
- 706 Jamie double check reml's in R

707 Tables.

Response	H2	p-value
Lichen Network Similarity	0.1734	0.0957
Network Centrality	0.3305	0.0196
Number of Network Links	0.3156	0.0267
Lichen Species Diversity	0	0.4558
Lichen Species Richness	0	0.458
Lichen Species Evenness	0	0.4574
Percent Rough Bark	0.3221	0.0128
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

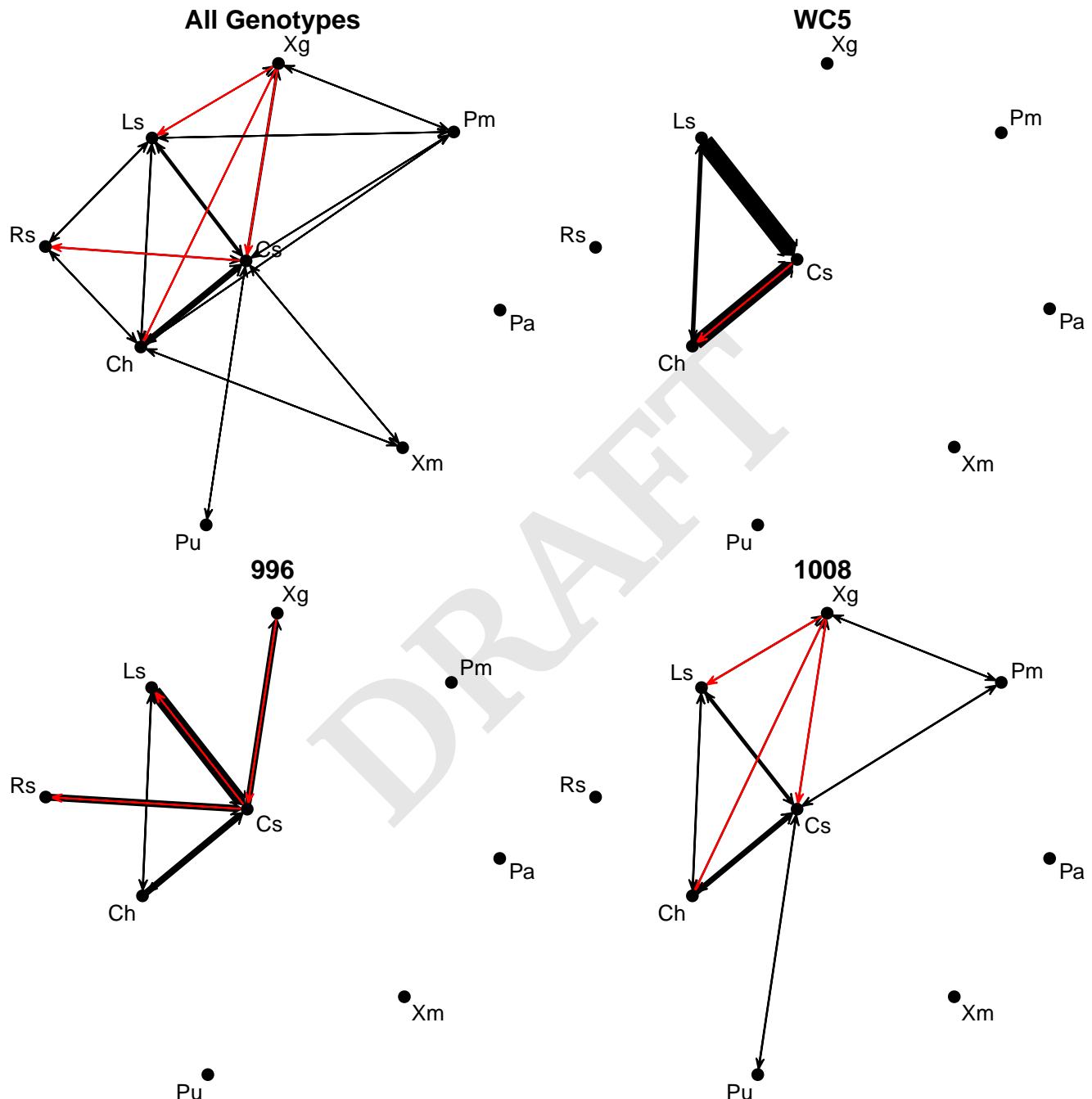


Fig. 1

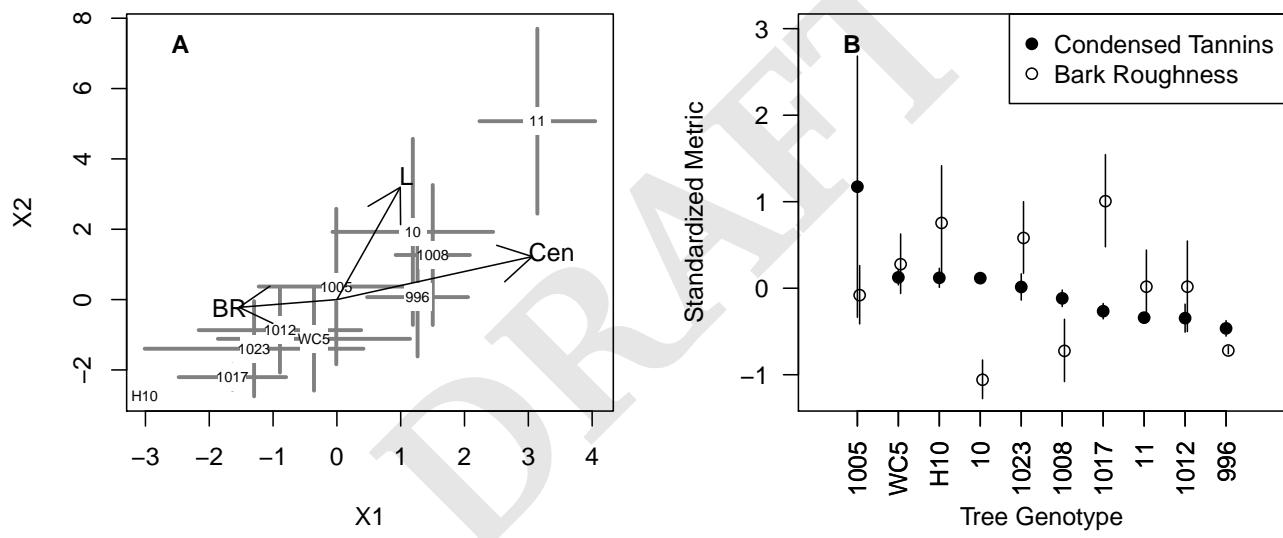


Fig. 2

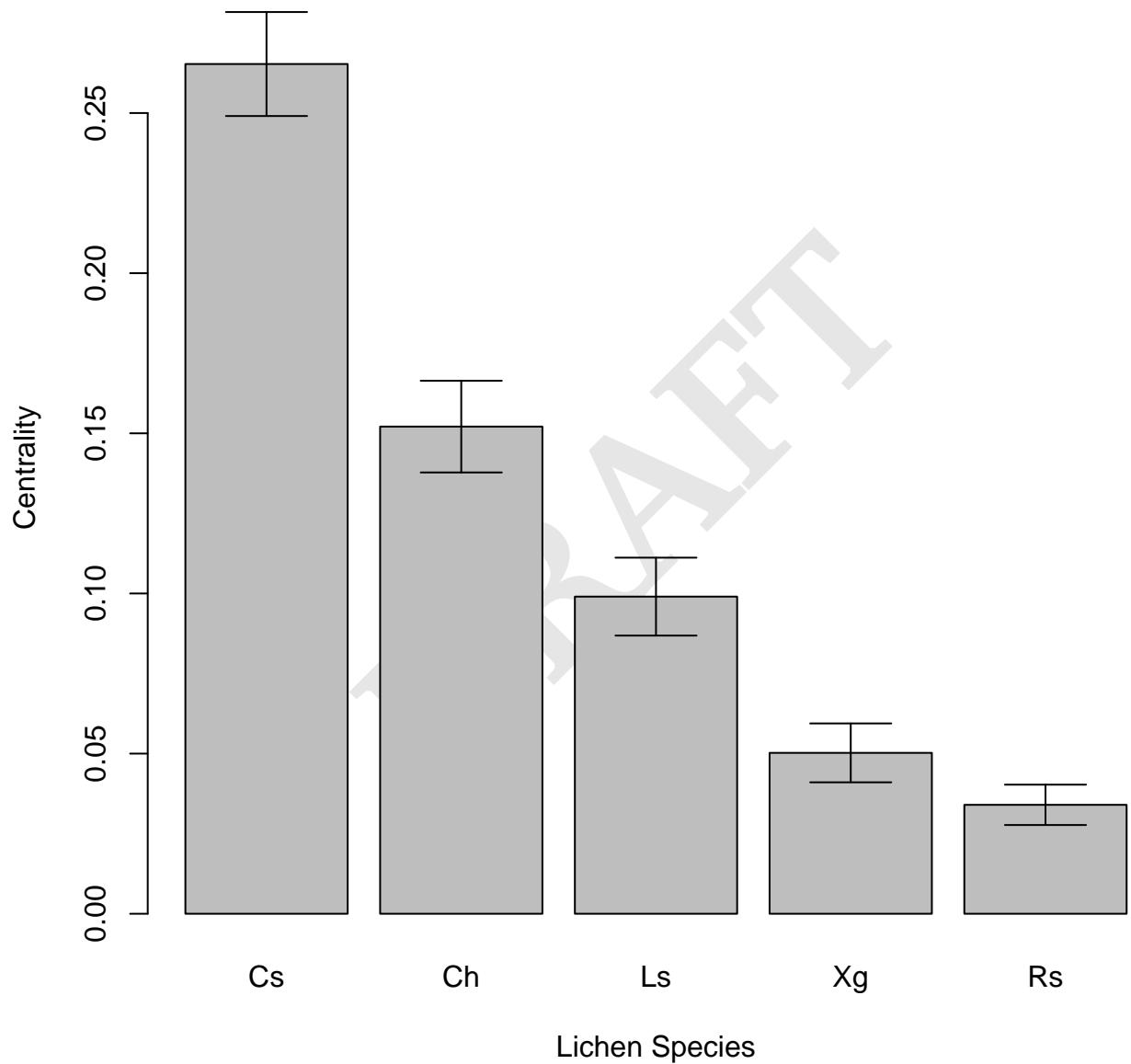


Fig. 3

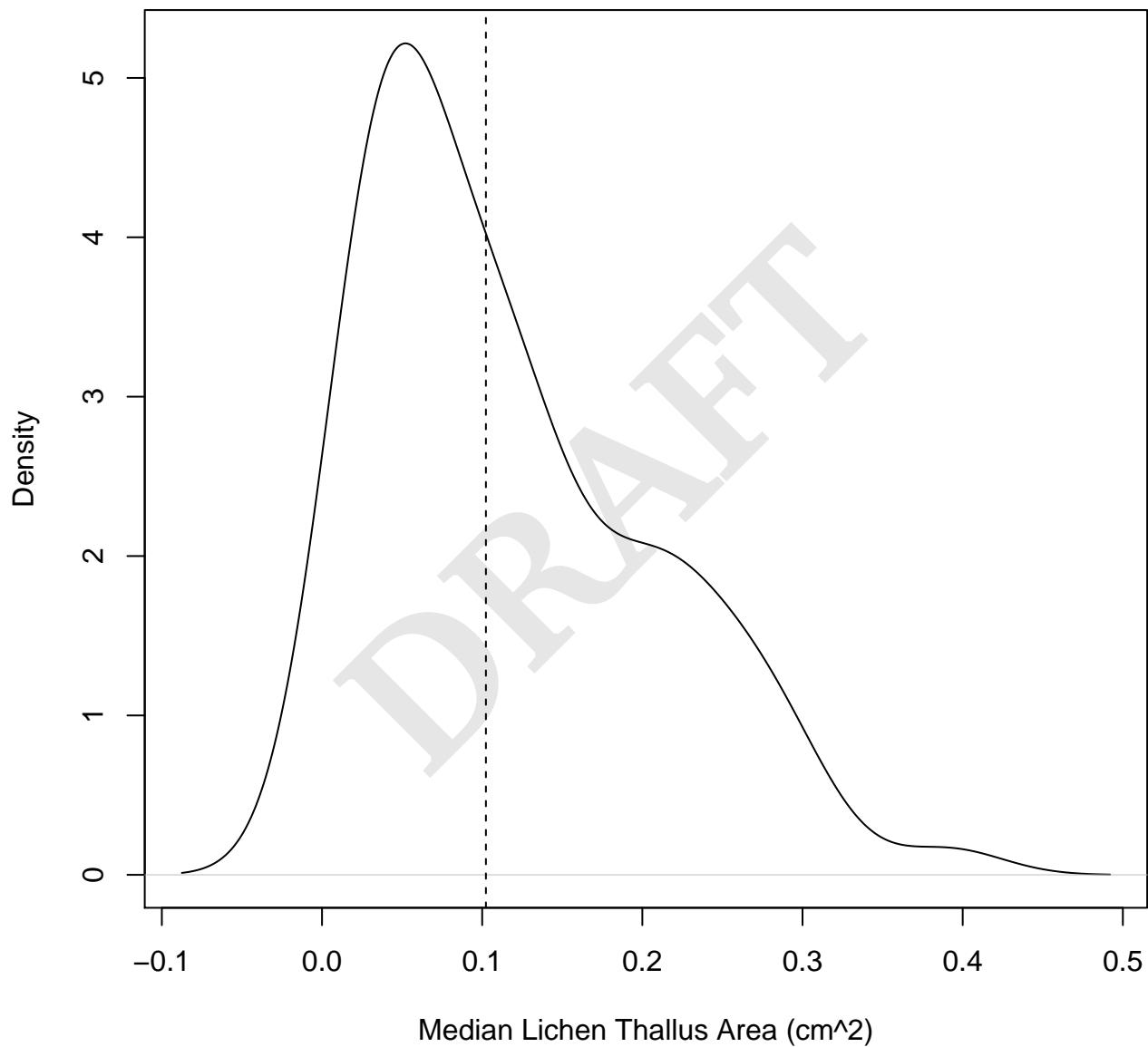


Fig. 4