

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

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1 Biological evolution occurs in the context of complex networks of
2 interacting species in which natural selection defines the structure
3 of ecological networks. Fundamental to this evolutionary process
4 is the discovery of a genetic basis to ecological network structure.
5 Although previous work has demonstrated that tree genotype con-
6 tributes to interaction network structure at the scale of forest stands,
7 the contribution of tree genetics to localized interaction networks
8 at the scale of individual trees has not yet been explored. To test
9 the degree to which tree genetics can contribute to network struc-
10 ture we conducted quantitative modeling of interaction networks. We
11 constructed networks of epiphytic lichen associated with individual
12 trees that were a part of a long-term experimental common garden
13 of genotypes of (*Populus angustifolia*), a foundation species. We
14 found three main results. First, tree genotype significantly predicted
15 lichen network similarity, i.e. trees of the same genotype had more
16 similar lichen networks. Second, bark roughness and condensed
17 tannin concentration were both predicted by tree genotype and cor-
18 related with lichen network similarity. Third, the network metrics, the
19 number of links and centrality, were both correlated with several tree
20 traits, including bark roughness and condensed tannin concentra-
21 tion. We conclude that tree genotype influences lichen interaction
22 network structure with two potential pathways through bark rough-
23 ness and condensed tannin concentration. These results provide
24 evidence that variation in ecological networks can result from genet-
25 ically based variation in foundation species.

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

- 1 Evolution occurs in complex ecosystems
- 2 Community genetics studies have demonstrated
3 the importance of genetics and evolutionary 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.

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

31 **E**volution occurs in the context of complex eco-
 32 logical networks. Initially, evolution in a com-
 33 munity context was focused on examples of highly
 34 co-evolved pairs of species (e.g. Darwin's famous
 35 prediction of the Sphinx Moth and Christmas
 36 Orchid) citeWade2007. However, studies of dif-
 37 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-
 38 combe2002, Strauss2007, Dicke2010, geographic mo-
 39 saics of co-evolution citeThompson2005 and com-
 40 munity genetics citeWhitham2006 have provided an
 41 in-road for ecological network approaches citeFor-
 42 tuna2009, Keith2017, Lau2016 to illuminate a more
 43 complex perspective of the interface between eco-
 44 logical and evolutionary dynamics. There is now
 45 evidence to support that selection tends to occur
 46 among groups of species citeWade2007, Pieterse2007,
 47 Dicke2010 favoring the development of small webs
 48 citeNuismer2006, Guimaraes2007, Gomez2009 and
 49 that genetic variation and phylogenetic relatedness
 50 contributes to variation in community assembly (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 and spatial autocorrelation. Established in 1992,
125 asexually propagated clones of genotyped *P. angustifolia* individuals were obtained from wild collections and
126 planted in fully randomized design at the Ogden Nature
127 Center, Ogden, UT. From the population of established
128 individuals in the common garden, we chose a total of
129 thirteen genotypes, replicated between 3 and 8 times each,
130 for sampling.
131

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

133 The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of 0.12 ± 0.001 cm² (1 S.E.) (see Supporting Information). Based on the median thallus size, we expected thalli observed in each cell to generally be spatially independent of thalli present in other cells but exposed to similar micro-environmental conditions created by the bark and the location of the sampling area on an individual tree. Therefore, we were confident in treating the cell-wise observations in quadrats as independent with respect to lichen-lichen interactions.

134 We also measured several bark traits for each tree:
135 including, bark roughness and chemistry. **INSERT
136 FROM LAMIT.**

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

140 of species occurrences $P(S_i)$ and the joint probability
141 of co-occurrences $P(S_i, S_j)$ using the frequencies of each species and their co-occurrences. We were then able to
142 calculate the conditional probabilities of each species pair
143 as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
144 This yielded a matrix that could possibly be asymmetric, i.e.
145 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another important property of this matrix is that the diagonal
146 (S_{ii}) was equal to one for all species present and zero for
147 species that were not observed in any cell.
148

149 We then applied an analytical procedure to remove non-significant links between species. This procedure determines if the joint probability of a species pair (i.e. $P(S_i, S_j)$) is different from zero (Fig. 2). Here, a confidence interval $CI_{95\%}$ is calculated as $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected frequency of co-occurrences $E(S_i S_j)$ is the total number of cells surveyed (N) times the independent probabilities of each species $P(S_i) * P(S_j)$, $Z_{95\%}$ is the Z-score for 95% from a Z-distribution and the expected variance of $E(S_i S_j)$ is the total number of cells times the expected probability of $S_i S_j$ and its compliment (i.e. $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the observed number of co-occurrence falls outside of the confidence interval, the joint probability $P(S_i, S_j)$ is determined to be equal to the product of the individual probabilities (i.e. $P(S_i)P(S_j)$), and the conditional probability reduces to the individual probability of that species $P(S_i)$. Therefore, unless the co-occurrence of a species pair falls outside the confidence interval, the probability that the observation of one species given the other is no different than simply observing that species alone. This enables us to remove links from a given network by re-scaling the resulting conditional probabilities by subtracting the individual probabilities from the conditional probabilities (i.e. how different the conditional probability is from the independent probability), which makes any species with a non-significant conditional probability zero. The resulting matrix ($\mathbf{D} = D_{ij}$) can be interpreted as how one species impacts another with zero being no effect and values less than or greater than zero interpreted as negative and positive effects, respectively. Here, we will refer to this matrix (\mathbf{D}) as an interaction matrix with the properties that it can be asymmetric (i.e. P_{ij} does not necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e. a species does not influence its own probability of being observed).

150 **Statistical Analyses, Software and Data.** We used a combination of parametric and non-parametric, permutation based frequentist statistical analyses to test for the effects of genetic variation on lichen communities and their interaction networks. To assess the effect of genotype on univariate responses, we used additive, random effects models with Restricted Maximum Likelihood (REML). We used a combination of Least Squares Regression, Analysis of



Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground (A and B, respectively). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

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
255 10^{-12} . Final configurations has the lowest stress with at
256 most a stress level of 0.10.
257

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

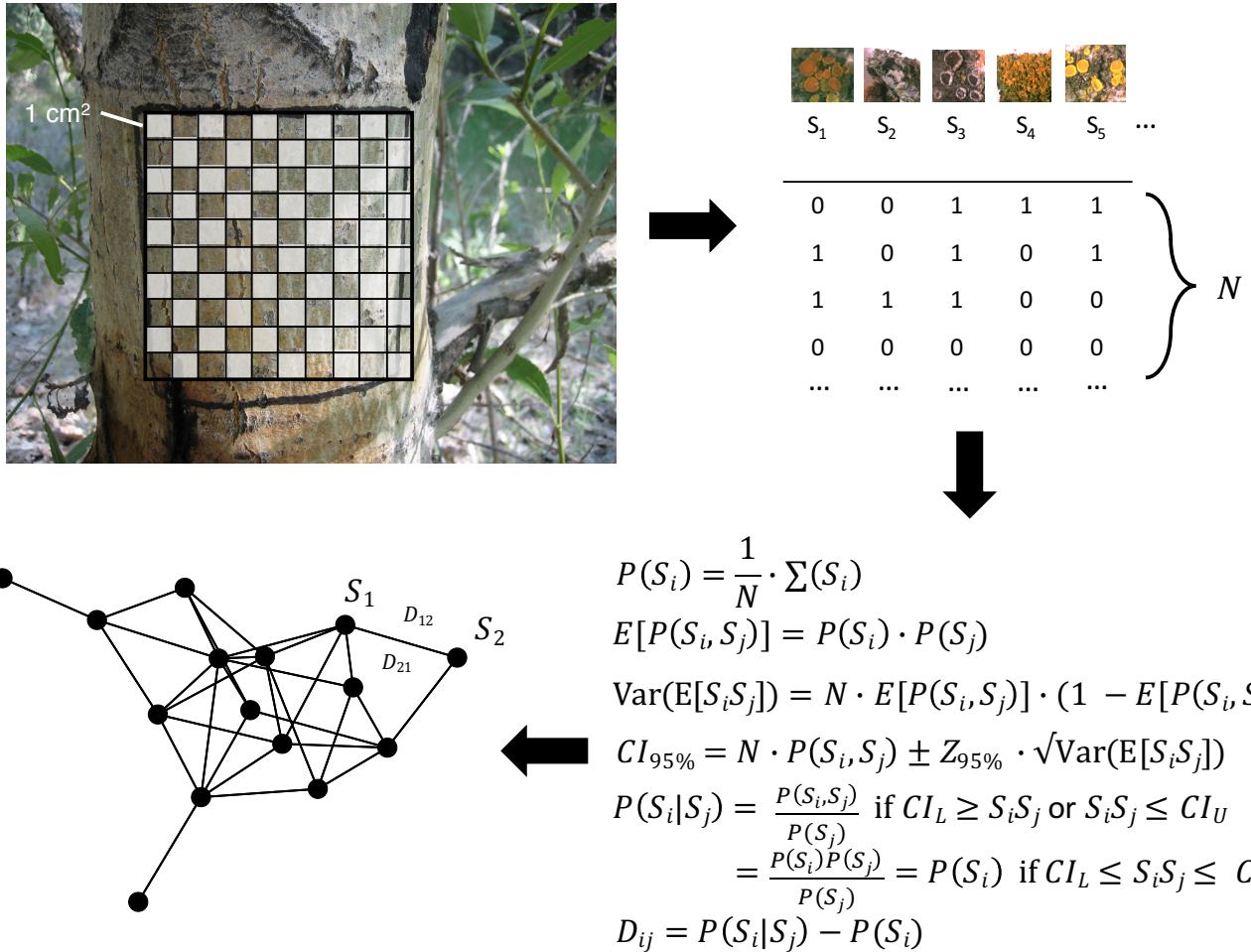


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded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

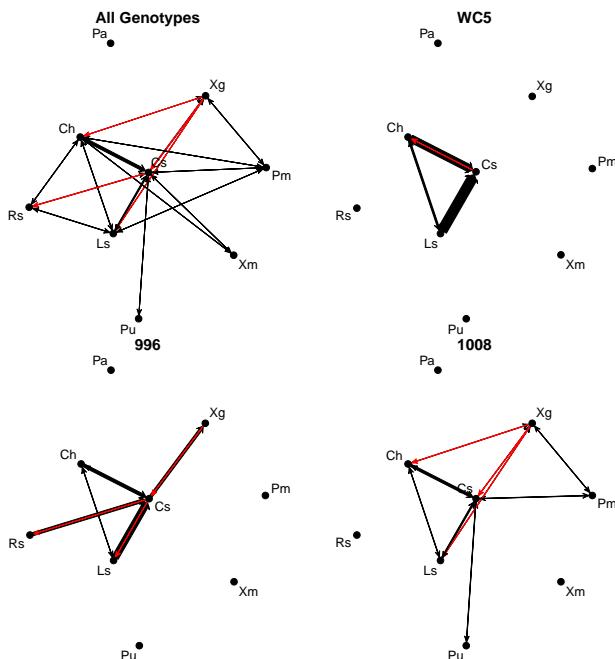


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

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

Response	H2	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.

	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.

- 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. 330
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- Habitat filtering of communities (richness, composition) vs environmental influence on interactions. Likely a combination of both of these factors. 340
341
342
343
- Lichen network structure correlated with species richness, evenness and diversity 344
345
- Lichen community composition not correlated with network structure 346
347
- None of these were genetically based 348
- 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. 349
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- Genetic diversity could be influencing the stability of communities through the effects on interactions. Some network structures are likely to 353
354
355

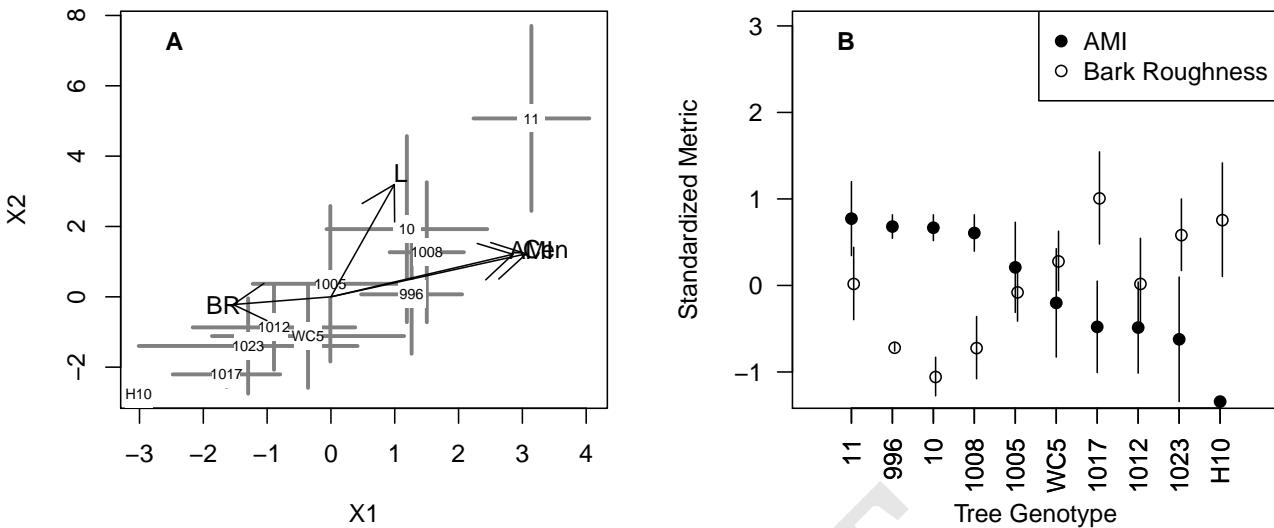


Fig. 4. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized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

be more stable, either in response to disturbance or via self-organized dynamics. Although, none of the metrics examined, such as the number of links, modularity or centrality, showed a genetic signature.

- Important factors to consider in studies of other ecological networks:
 - Relative body size
 - Mobility
 - Reproductive isolation
- Future work should consider the potential influence on evolutionary dynamics of communities
 - Network structure influences network stability
 - Are the communities nested subsets?

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

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

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

We observed significant lichen interaction structure that varied among genotypes of a foundation tree species, narrowleaf cottonwood (*P. angustifolia*). We found that a genetically based trait, bark roughness, partially explained the variation in lichen interaction networks. Some of this variation in lichen networks was related to both the overall abundance and species richness of lichen; though, statistically controlling for the effect of genotype on these variables indicates that a significant portion of the variance in lichen species richness is due to a factor

403 other than tree genotype. By using network metrics,
404 we were also able to probe for specific characteristics
405 of how these networks were responding to tree
406 genotype. We found that both number of links and
407 the centralization of the networks were highly correlated
408 with network similarity and that tree genotype
409 significantly predicted network centrality but only
410 marginally predicted the number of network links.
411 This latter result could be due to the relationship
412 between species richness and the number of links
413 in the network, which were significantly correlated
414 with each other. We also found that bark roughness
415 did not significantly predict either the number of
416 links or the centrality of lichen networks, suggesting
417 that bark roughness has some other effect on the
418 structure of the lichen networks. Taken together,
419 these findings support the hypothesis that genotypic
420 variation in a foundation species contributes to the
421 structure of a network of interacting species.

422 **LJL: I wonder if you need to have so much**
423 **on richness here. Overall, I think you want to**
424 **focus on the network responses and patterns**
425 **among genotype first, and then go into mech-**
426 **anism later. I think we don't quite have a**
427 **good mechanism yet so I don't think it needs**
428 **to come up in the first paragrpah of the dis-**
429 **cussion.**

430 These findings point to the importance of under-
431 standing the community level effects of genetic varia-
432 tion in plant functional traits and highlights the
433 potential for indirect effects of genetic variation to
434 propagate through networks of interacting species
435 and trophic levels.

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

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

452 Also, work by (18–20) observed consistent pat-
453 terns of centralized interactions of species modules
454 focused around hubs of plant-fungal interactions. In
455 other words, a small number of plant and fungal
456 symbionts tended to have disproportionate numbers
457 of interactions with other species and likely are the
458 drivers in determining community assembly, struc-
459 ture and dynamics.

460 More on the importance of ecological networks
461 (21, 22).

462 From Thompson2014

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

468 Specific hypothesis from Thompson2014

469 **LJL: If I recall, the Elamo paper just looks**
470 **at genetic correlations between pairwise indi-**
471 **vidual abundances. I would suggest maybe it**
472 **doesn't deserve to be in this 1st paragraph.**
473 **Perhaps it actually should be in the 2nd or**
474 **3rd paragraph, just as a reference that points**
475 **to the potential for genotype to influence net-**
476 **works. Definately our 2015 JOE paper goes**
477 **much further, too, since it has whole com-**
478 **munities being correalated. But, again, I**
479 **woudl put both of these as citation in the com-**
480 **munity genetics paragraphs (2 of 3) instead**
481 **of the first paragrpah, which focuses on the**
482 **general network lit.**

483 **LJL: It could be useful to point out that**
484 **our findings are not related to trophic inter-**
485 **actions, which is pretty cool. Also,we talk**
486 **about interaction networks but it is not clear**
487 **to me if the interactions tend to be positive**
488 **or negative. Can we get at that with the ap-**
489 **proach used?**

490 **TGW: Is there any adaptive component to**
491 **the tree in having certain lichen communi-**
492 **ties? e.g., can they feed back to affect tree**
493 **performance in some way or is this a pas-**
494 **sive outcome of a trait that affects bark for**
495 **other adaptive reasons and lichens are pas-**
496 **sive players that tag along for the ride? I**
497 **could envision that lichens covering the bark**
498 **of a tree act as a barrier between insects and**

499 pathogens, much like ectomycorrhizae cover
500 fine roots as a first line of defense by invading
501 microorganisms. Uptake of N that gets
502 passed to the tree??

503 **LJL:** I agree that there is a general overar-
504 ching theme that evolution occurs in a com-
505 munity network context, but I'm not sure
506 that we should state that as our main hypoth-
507 esis. It seems more that this is a fundamen-
508 tal foundation for our work. The hypoth-
509 esis is more what we are testing directly, but
510 we don't test this directly. I guess I don't
511 want to give the impression that our com-
512 munities are necessarily the result of each
513 species evolving into its place in the commu-
514 nity on these tree genotypes (although I do
515 understand this as Shuster et al 2006's funda-
516 mental explanation for why we see different
517 communities on different genotypes; I don't
518 necessarily agree that this is the only rea-
519 son we would see different communities on
520 dif genotypes). Most of these are pretty gen-
521 eralist lichens, which could be found on other
522 deciduous trees in the surrounding city or nat-
523 ual areas. I would look at it more like an
524 assembling of lichen species into unique con-
525 figurations on genetically different substrates.
526 There may be some selection for different
527 genotype of lichen during the community as-
528 sembly process but we can't really tell that
529 just by differences in species abundances or
530 coocurneces. I guess to me the evolutionary
531 context that is more directly related to this
532 work is that the tree genotype is a central
533 controller (indeed a sort of hub species in the
534 network) of network structure. By anchor-
535 ing the lichen network to tree genotype (and
536 variation among networks to variation among
537 tree genotypes), our study highlights the pos-
538 sibility that natural selection acting on the
539 trees may have an extended consequence for
540 the network structure of organisms living on
541 the trees... the extra thing we add to the field
542 is that we show interaction networks are sen-
543 sitive to genotype. I doubt the lichens have
544 a direct effect on tree fitness, but favorabil-
545 ity of some tree genotypes over others during
546 natural selection will then go on to favor and
547 disfavor certain lichen communities of differ-

548 ent network structures. By being sensitive
549 to tree genotype, the lichen community net-
550 works are passive riders on the waves of evolu-
551 tionary dynamics that occur within the tree
552 species they inhabit.

553 **MKL:** In response to Lamit's comment
554 above, I agree that it is not required that
555 there is co-evolution. Another, perhaps sim-
556 pler, explanation is that there is variation in
557 environmental filtering of lichen individuals
558 created in part by genetic variation in tree
559 individuals.

560 **TGW:** might be good to cite papers on
561 competition in lichens or other organizing fac-
562 tors to back up the least expected statement.
563 as epiphytes we might not expect them to
564 care.

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

570 **MKL:** Environmental filtering is evidenced
571 by species richness, but also possibly species
572 interaction varying based on environment as
573 networks varied in terms of sign and magni-
574 tude as well.

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

579 **Discussion of network implications for sta-**
580 **bility with genetics.**

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

584 Although our study was conducted with a commu-
585 nity of lichens, these results should be generalized
586 to other groups of diverse organisms around the
587 world that also exhibit significant genetic signals at
588 the community level (24, 25). In the face of the
589 high degree of complexity and potential context de-
590 pendence of ecological processes, the current study
591 points to the utility of considering the spatial and
592 temporal scales of interactions, as discussed to some
593 in previous studies (26–28). In the present study,
594 we found that community assembly processes, such
595 as environmental filtering and species interactions,
596 are genetically based. This is likely due, in part,

to the large difference in the differences in size and longevity of the lichen and cottonwood individuals with the trees determining the environment in which the lichen occur. We suggest that future work would be aided by determining these modules within the biotic community that include species with similar differences in body-size and time-scales. As heritable variation is the raw material for natural selection to act upon, a genetic basis for interaction network structure indicates evolutionary dynamics should be considered at the community level and that conserving genetic variation is important to consider in efforts to restore or preserve complex species interactions and their associated ecosystem functions (29). With such findings, it appears that we are closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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19. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs in plant-root fungal biomes. *J. R. Soc. Interface*. 662
20. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus networks. *Nat. Commun.* 663
21. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. *Ecol. Lett.* 14(9):877–85. 664
22. Thompson JN, Schwind C, Guimarães PR, Friberg M (2013) Diversification through multiltrait evolution in a coevolving interaction. *Proc. Natl. Acad. Sci.* 668
23. Bdeir R, et al. (2017) Quantitative trait locus mapping of *Populus* bark features and stem diameter. *BMC Plant Biol.* 670
24. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1322–8. 672
25. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes. 673
26. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure. *Mol. Ecol.* 15:1379–1391. 674
27. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to body size yields high degree of intervality. *J. Theor. Biol.* 271(1):106–113. 677
28. Zytnyska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2). 679
29. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological networks to habitat loss. *Ecol. Lett.* 16:844–52. 680

- 622 1. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming 623 decade. *New Phytol.*
- 624 2. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co- 625 extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
- 626 3. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly 627 across multiple environments. *Ecology* 96(4):960–971.
- 628 4. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and 629 dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486.
- 630 5. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, 631 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype 632 in a foundation tree species. *Oecologia* 170:695–707.
- 633 6. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant 634 genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 635 364(1523):1607–16.
- 636 7. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in found- 637 nation species generates network structure that may drive community dynamics and evolu- 638 tion. *Ecology* 97(3):15–0600.1.
- 639 8. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation 640 species affect community diversity, stability and network structure. *Proc. R. Soc. B Biol. Sci.* 641 284(1854):20162703.
- 642 9. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecol. Econ.* 643 71:80–88.
- 644 10. Zytnyska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree 645 species influences the associated epiphytic plant and invertebrate communities in a complex 646 forest ecosystem. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366:1329–1336.
- 647 11. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499.
- 648 12. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate 649 Biodiversity Maintenance10.1126/science.1123412. *Science* (80-.). 312:431–433.
- 650 13. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influ- 651 ences a dominant bark lichen. *Fungal Ecol.* 4(1):103–109.
- 652 14. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net- 653 works to assess the impacts of climate change. *Ecography (Cop.)*. 34:897–908.
- 654 15. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics: 655 opportunities for synthesis. *Ecosphere* 8(8):e01900.
- 656 16. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Soc. Networks* 657 28:466–484.
- 658 17. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter- 659 actions and selection: trees, aphids and birds. *J. Evol. Biol.* 24(2):422–9.
- 660 18. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommunity 661 level.

682 **Assessment and Results**

- Network similarity not genetically based
- Genetically based number of links and centrality but not modularity
- Lichen cover, richness, evenness, diversity and composition not genetically based
- Roughness genetically based but not bark condensed tannins, CN or pH
- Bark roughness correlation with number of links (yes) and centrality (yes)? <- TODO add figure A = mdc.plot(L, Cen), B = (ch.plot(L,Cen,geno), BR vector))
- Centrality values for species <- censpp.pdf
- Redo haritability calculations
- Jamie double check genotype network permanova in PRIMER
- Jamie double check reml's in R

699 **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

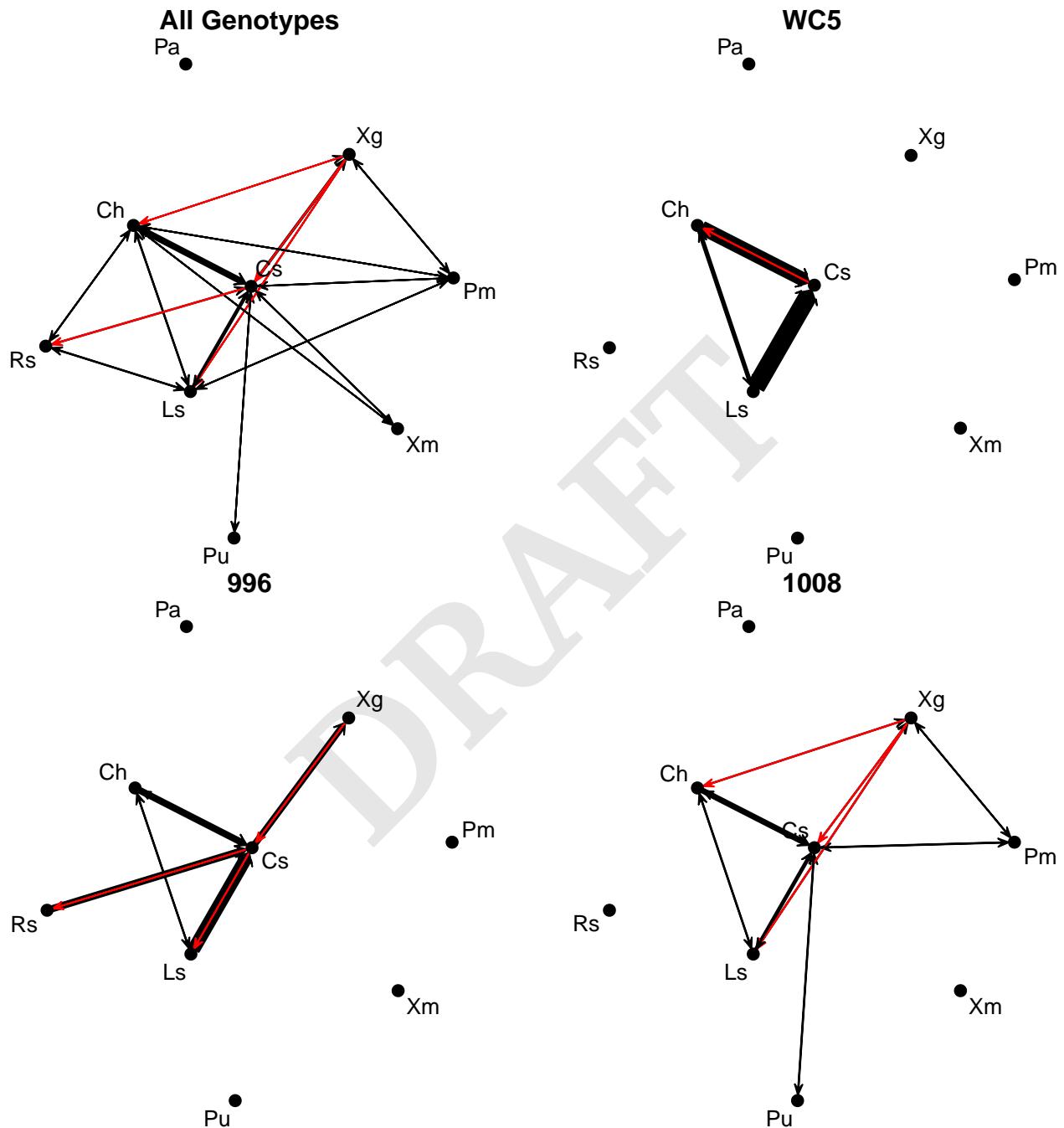


Fig. 1

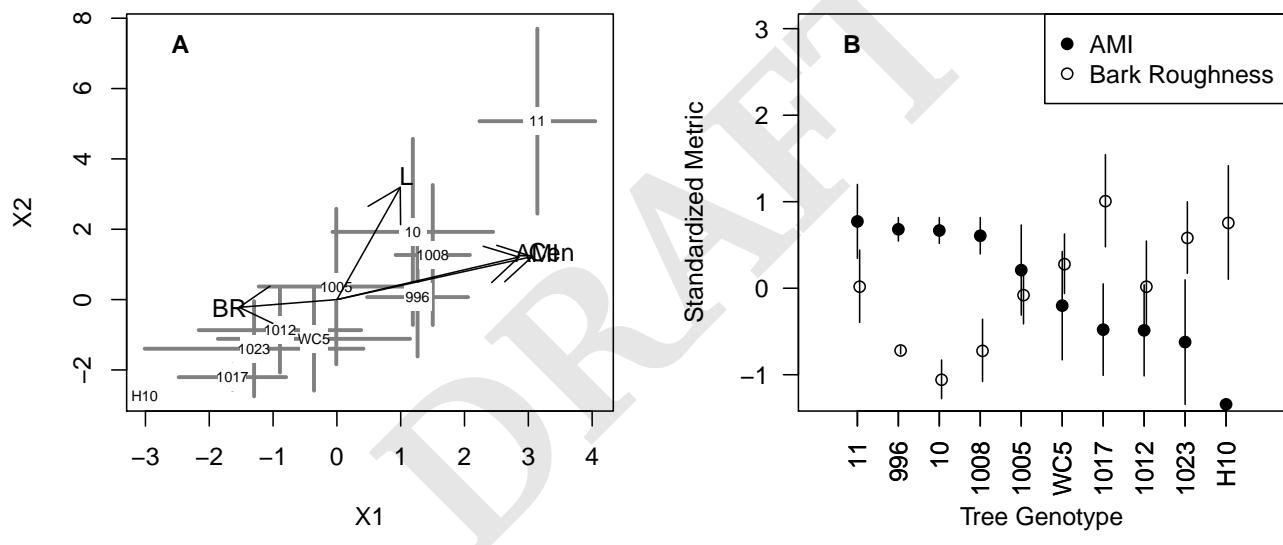


Fig. 2

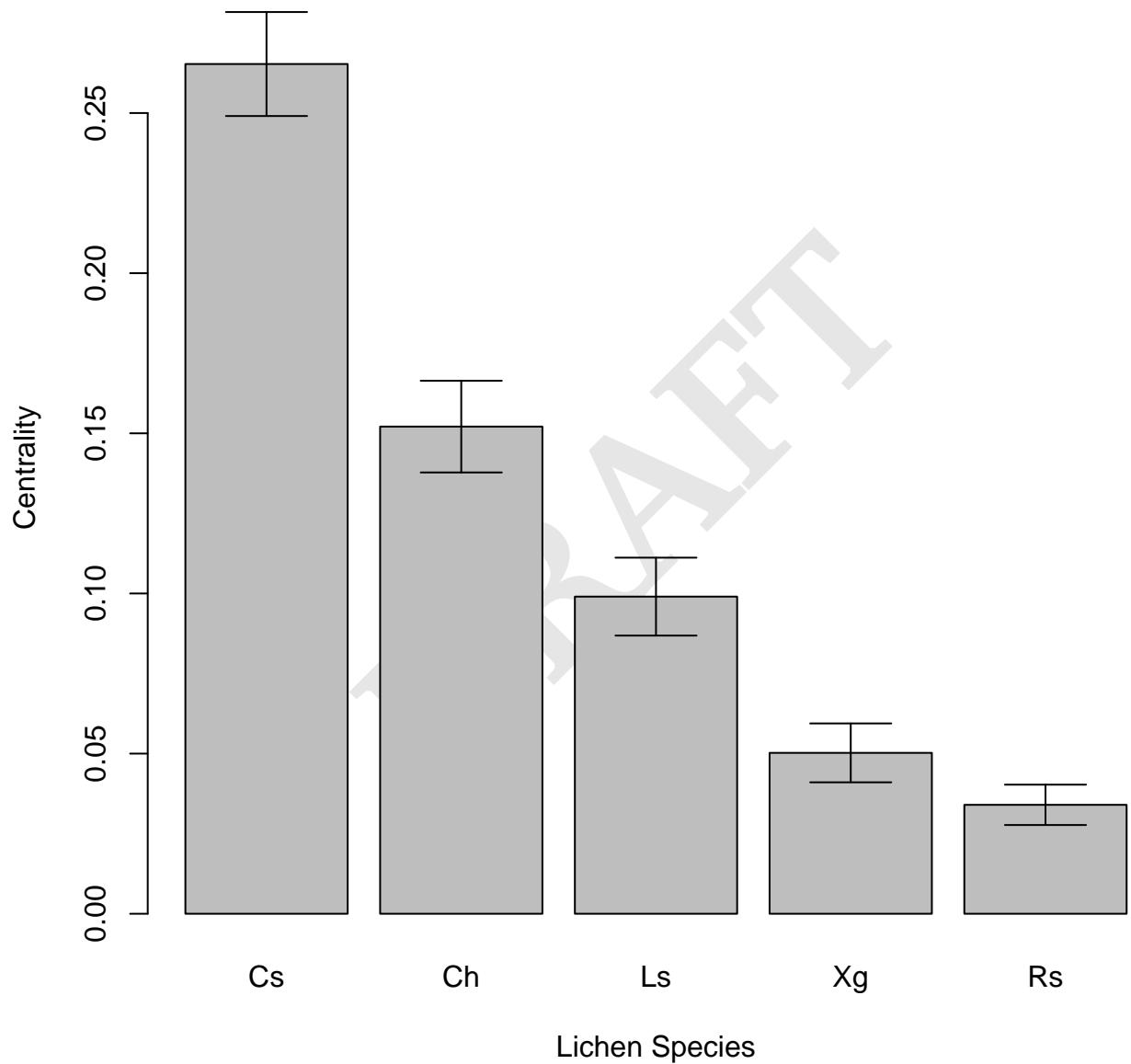


Fig. 3

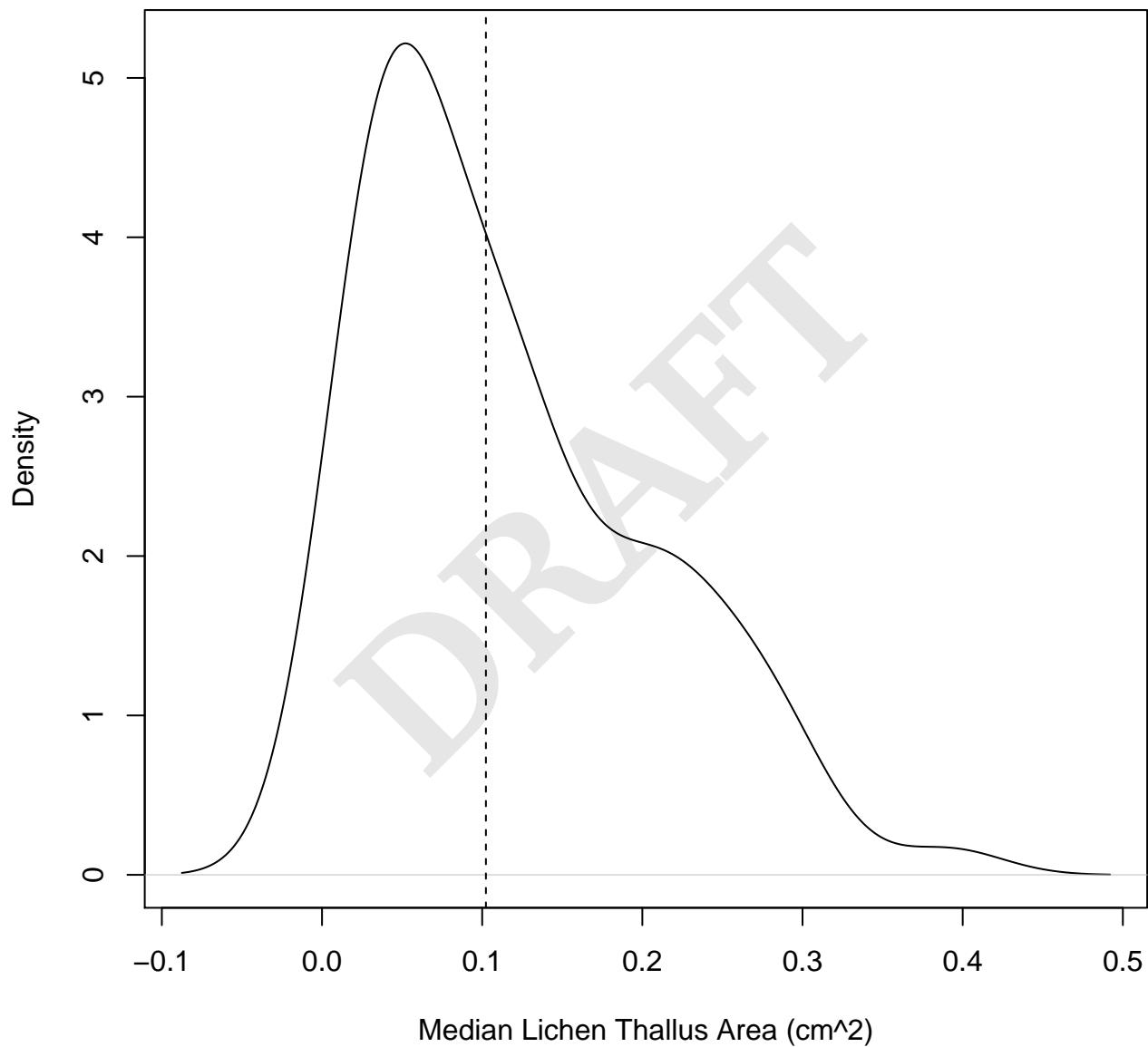


Fig. 4