

Genotypic variation in a foundation tree directs ecological network structure

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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 of**
13 **genotypes of (*Populus angustifolia*), a foundation species. We found**
14 **three main results. First, tree genotype significantly predicted lichen**
15 **network similarity, i.e. trees of the same genotype had more simi-**
16 **lar lichen networks. Second, bark roughness and condensed tannin**
17 **concentration were both predicted by tree genotype and correlated**
18 **with lichen network similarity. Third, lichen network similarity was**
19 **most strongly correlated with the number of network links and to a**
20 **lesser degree with network centrality and modularity; however, none**
21 **of these was correlated with any of the genetically based tree traits.**
22 **We conclude that tree genotype influences lichen interaction network**
23 **structure with two potential pathways through bark roughness and**
24 **condensed tannin concentration. These results support the hypoth-**
25 **esis that variation in ecological interaction networks can result from**
26 **genetically based variation in foundation species. This study sug-**
27 **gests a genetic basis to both direct and indirect interactions among**
28 **species that can result in the evolution of complex communities.**

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

1 **E**volution occurs in the context of complex ecolog-
2 **ical networks. Initially, evolution in a community**
3 **context was focused on examples of highly co-evolved**
4 **pairs of species (e.g. Darwin's famous prediction of**
5 **the Sphinx Moth and Christmas Orchid) (?). How-**
6 **ever, studies of diffuse co-evolution (*sensu* (?))**
7 **(? ? ?), geographic mosaics of co-evolution (?)**
8 **and community genetics (?) have provided an**
9 **in-road for ecological network approaches (1? ?) to**
10 **illuminate a more complex perspective of the inter-**
11 **face between ecological and evolutionary dynamics.**
12 **There is now evidence to support that selection tends**
13 **to occur among groups of species (? ? ?) favor-**
14 **ing the development of small webs (? ? ?) and**

that genetic variation and phylogenetic relatedness contributes to variation in community assembly (2) and species interactions (3? , 4), which shapes the ecological interaction networks (5).

LJL: I think a key point in the first paragraph is to point out that networks are believed in part to emerge out of complex evolutionary interactions. But, to-date, the patterns observed in networks are primarily only on the scale of species interactions and phylogenetic signature. There has yet to be much work connecting ecological species networks at the scale relevant to the actual workings of

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 networks respond to a genetically based tree trait, which alters network structure both through environmental filtering of species and altering species interactions. These findings demonstrate the importance of assessing the impacts of genetic variation and evolutionary dynamics in shaping ecological networks as evolved traits.

M.L. and L.J.L. conceived the study, M.L. and L.J.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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microevolutionary processes (e.g., the genotype or population level).

Community genetics studies (6) have shown that genetic variation in foundation species (7) plays a significant role in defining distinct communities of interacting organisms: such as, endophytes, pathogens, lichens, arthropods, and soil microbes. Multiple studies have now demonstrated that genetic variation influences numerous functional traits (e.g., phytochemical, phenological, morphological) produces a multivariate phenotype (8) that contributes to variation in associated communities (9).

LJL: I think the thing that needs to emerge in this paragraph is that even though there is some incling that genotype is relevant in community networks, as based on our few past studies, no study has truely examined networks on individual trees.

Additional work has provided support for the hypothesis that not only does composition vary among genetically distinct genotypes of foundation species, it also impacts the structure of the network of species interactions in these communities (1, 10). Also, work by (11–13) observed consistent patterns of centralized interactions of species modules focused around hubs of plant-fungal interactions. In other words, a small number of plant and fungal symbionts tended to have have disproportionate numbers of interactions with other species and likely are the drivers in determining community assembly, structure and dynamics.

More on the importance of ecological networks (14, 15).

Add a discussion of DesRoches.

From Thompson2014

- Pairwise interactions are usually influenced by other species
- Selection favors the development of small webs
- Evolution of new lifestyles leads to changes in slection on large and small webs

Specific hypothesis from Thomspon2014
Selection in small webs

-

You mention the naturally established stand but then that data doesn't get included

in the manuscript. I do like the idea of including the field data. We might need to cross reference IDs of the lichens in my community composition data on the same trees since there was considerably more diversity than we initially saw out there. We would just want to make sure the same names are applied to taxa in the same quatrats on each tree. It probably wouldn't take long.

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

Here, we investigate how genetic variation in a foundation tree species determines the structure of a network of interactions among a community of tree associated lichen species. Using a long-term (20+ years), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *Populus angustifolia*. We focused on a model community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (16, 17). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover rates, allowed us to assess interactions among lichen species on individual trees. We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (1, 10, 18, 19). If correct, we should expect to find that network structure is genetically based in which different plant genotypes support different interaction networks and that these interactions networks can function as indicators of ecological dynamics important for conserving biodiversity. Applying a probability-theory based network modeling approach, we constructed a set of interaction network models for the lichens associated with

122 individual trees. Using these models, we then ex-
123 amined the genetic basis of the structure of these
124 ecological networks.

125 **LJL:** I agree that there is a general over-
126 ching theme that evolution occurs in a com-
127 munity network context, but I'm not sure
128 that we should state that as our main hypoth-
129 esis. It seems more that this is a fundamen-
130 tal foundation for our work. The hypoth-
131 esis is more what we are testing directly, but
132 we don't test this directly. I guess I don't
133 want to give the impression that our com-
134 munities are necessarily the result of each
135 species evolving into its place in the commu-
136 nity on these tree genotypes (although I do
137 understand this as Shuster et al 2006's funda-
138 mental explanation for why we see different
139 communities on different genotypes; I don't
140 necessarily agree that this is the only rea-
141 son we would see different communities on
142 dif genotypes). Most of these are pretty gen-
143 eralist lichens, which could be found on other
144 deciduous trees in the surrounding city or nat-
145 ual areas. I would look at it more like an
146 assembling of lichen species into unique con-
147 figurations on genetically different substrates.
148 There may be some selection for different
149 genotype of lichen during the community as-
150 sembly process but we can't really tell that
151 just by differences in species abundances or
152 coocurneces. I guess to me the evolutionary
153 context that is more direclty related to this
154 work is that the tree genotype is a central
155 controller (indeed a sort of hub species in the
156 network) of network structure. By anchor-
157 ing the lichen network to tree genotype (and
158 variation among networks to variation among
159 tree genotypes), our study highlights the pos-
160 sibility that natural selection acting on the
161 trees may have an extended consequence for
162 the network structure of organisms living on
163 the trees...the extra thing we add to the field
164 is that we show interaction networks are sen-
165 sitive to genotype. I doubt the lichens have
166 a direct effect on tree fitness, but favorabil-
167 ity of some tree genotypes over others during
168 natural selection will then go on to favor and
169 disfavor certain lichen communities of differ-
170 ent network structures. By being sensitive

171 to tree genotype, the lichen community net-
172 works are passive riders on the waves of evolu-
173 tionary dynamics that occur within the tree
174 species they inhabit.

175 **MKL:** In response to Lamit's comment
176 above, I agree that it is not required that
177 there is co-evolution. Another, perhaps sim-
178 pler, explanation is that there is variation in
179 environmental filtering of lichen individuals
180 created in part by genetic variation in tree
181 individuals.

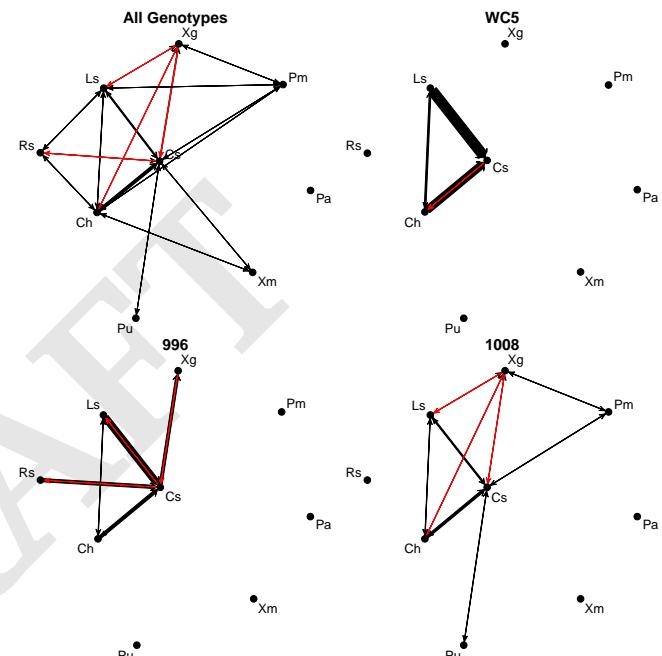


Fig. 1. Lichen networks varied in structure among tree genotypes. Network dia-
grams of the mean lichen interaction matrices averaged for all trees and for several
individual genotypes showing a range of interaction network structure. Directionality
(arrowheads) and sign (red = negative, black = positive) 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.

Results

Networks were more similar as a result of having
similar numbers of interactions and distribution of
interactions. The number of links (PerMANOVA R^2
= 0.392, $F_1 = 72.4348$, p -value = 0.001) and network
centrality (PerMANOVA R^2 = 0.309, $F_1 = 57.0440$,
 p -value = 0.001) were highly correlated with network
similarity. Tree genotype significantly predicted net-
work centrality (REML R^2 = 0.202, RLRT = 2.7801,
 p -value = 0.04012) but marginally predicted the num-

	Df	SumOfSqs	R2	F	Pr(>F)
geno	10.00	304.93	0.23	19.30	0.00
BR	1.00	16.26	0.01	10.29	0.00
pH	1.00	5.04	0.00	3.19	0.08
CN	1.00	39.67	0.03	25.10	0.00
CT	1.00	70.77	0.05	44.78	0.00
PC	1.00	56.35	0.04	35.66	0.00
SR	1.00	332.42	0.25	210.35	0.00
SE	1.00	55.11	0.04	34.87	0.00
L	1.00	326.53	0.25	206.62	0.00
mod.lik	1.00	7.77	0.01	4.92	0.03
Cen	1.00	43.76	0.03	27.69	0.00
Residual	30.00	47.41	0.04		
Total	50.00	1306.01	1.00		

Response	H2	R2	p-value
Percent Rough Bark	0.385	0.385	0
pH	0.054	0.054	0.294
Condensed Tannins (CT)	0.28	0.28	0.014
Carbon-Nitrogen (CN) Ratio	0	0	0.448
Percent Lichen Cover	0.079	0.079	0.172
Lichen Species Richness	0	0	1
Lichen Species Evenness	0.015	0.015	0.388
Lichen Species Diversity	0.01	0.01	0.417
Number of Network Links	0.07	0.07	0.238
Network Modularity	0	0	1
Network Centrality	0.085	0.085	0.199
Lichen Network	0.16	0.233	0
Community Composition	0.052	0.173	0.102

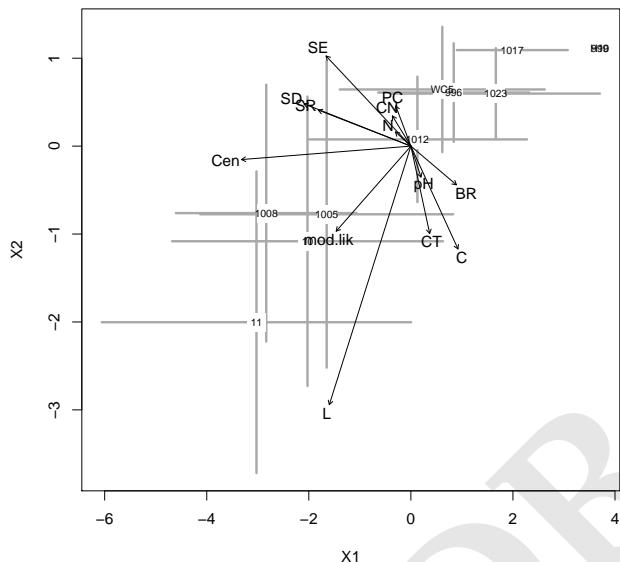


Fig. 2. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.01) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. Arrows show the magnitude and direction of correlation of the ordinated networks with tree bark roughness (BR), percent cover of lichens (PC) and lichen species richness (SR).

ber of links (REML $R^2 = 0.170$, RLRT = 2.0484, p -value = 0.065) (Fig. 3). Total cover was correlated with the number of links (ANOVA $F_1 = 6.867$, p -value = 0.0114) and centrality (ANOVA $F_1 = 8.093$, p -value = 0.0063). Lichen species richness was also correlated with the number of links (ANOVA $F_1 = 29.436$, p -value = 0.000015) and centrality (ANOVA $F_1 = 39.488$, p -value < 0.000001). Bark roughness, however, did not significantly predict either the number of links (ANOVA $F_1 = 2.897$, p -value = 0.0946) or the centrality (ANOVA $F_1 = 2.591$, p -value = 0.1134) of lichen networks (Supplementary Tables ?? and ??).

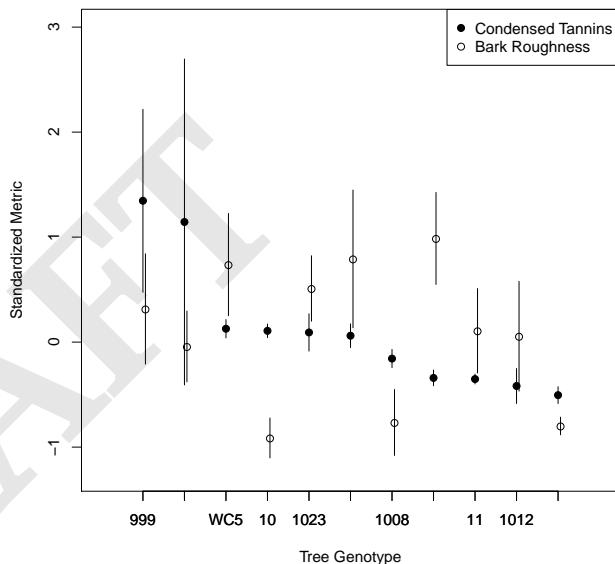


Fig. 3. The impact of tree genotype on lichen network structure was indicative of variation in both the number variation in lichen interactions among species. Plot showing the means (± 1 S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented as standardized scores ($\frac{x - \bar{x}}{s}$).

A. Some genetically based tree traits predicted lichen network structure.

B. Wild stand results. MKL: I removed the community similarity figure to simplify the presentation of the results and improve the flow.

LJL: Figure looks good. But, maybe making all lines a little thicker would look nicer and pop more.

LJL: Since we already published that tree genotypes differ in lichen composition, I wonder if we need to say somewhere in the manuscript why this test was run here. It seems to me it is important to verify this with

219 a slightly different sampling method as used
220 int eh 2015 paper, and for this specific set of
221 genotypes. But, then does this test of compo-
222 sition just become something necessary just
223 in a methodological variation that justifies
224 the next step of examing network structure.
225 Something to think about. It might be that
226 theNMDS should jsut go in a supplement, al-
227 though I do like it here in some ways. It
228 might also be another approach to put the
229 composition and other analyses after the net-
230 work analysis results are presented. In this
231 way, you could use the composition and re-
232 sults with vectors to help provide resolution
233 on what is driving networks to differ among
234 genotypes.

235 **MKL:** Adapt into a table.

236 **TGW:** clarify positive vs negative interac-
237 tions.

238 **C. Tree genotypes support distinct lichen net-
239 works.** **MKL:** Combine 1 and 2

240 **D. Some genetically based tree traits predicted
241 lichen network structure.**

242 **E. Wild stand results.** **MKL:** lichen networks in
243 wild stands displayed similar structural pat-
244 terns. Is it worth adding the wild stand?
245 This will requite adding methods, results and
246 more discussion.

247 **MKL:** Add the network metrics as vectors.
248 Also add the wild stand as a point of reference
249 or add as a supplementary figure.

250 **MKL:** Need to re-organize the flow of the
251 results.

252 **LJL:** It seems to me that the first two sen-
253 tences here are the most important of the
254 results. How can you make them stand out
255 more? Maybe also they should go at the be-
256 gining of the previous paragrphah, and then
257 move that paragraph to being the first in the
258 REsults section.

259 **TGW:** Here and in earlier paragraphs, a
260 lot of stats are presented some of which are
261 significant and some not. For your topic sen-
262 tence to be accepted, it seems readers need
263 to know how many of the stats need to con-
264 firm the pattern and how many would it take
265 to reject. This paragraph has about 8 stats

so need some overarching statement(s). E.g.,
266 7 of 8 analyses support our overarching hy-
267 pothesis that ... Same goes for other such
268 paragraphs such as the 1st and last paras of
269 the Results.

Materials and Methods

271 **MKL:** Need to add more citations of Lamits or
272 other lichen studies.

273 **LJL:** If you include the field data, I would sug-
274 gest changing the topic sentence for this para-
275 graph and adding something about the field data
276 sampling to this paragrpah. Note, also that we
277 cored and aged each field tree so we can site the
278 age range of the trees here, to make it clear they
279 are within those of the garden.

280 The study was conducted along the Weber River, UT
281 (USA), which is a cottonwood (*Populus* spp.) dominated
282 riparian ecosystem. Although two native species, *Populus*
283 *angustifolia* (James) and *Populus fremontii* (S. Watson),
284 occur here and are known to hybridize, only pure or
285 advanced generation backcrosses of *P. angustifolia* were
286 sampled. Bark lichens have been extensively studied
287 in this system and provide an ideal system in which to
288 observe and model lichen interaction networks, as their
289 sessile nature permits accurate identification of individuals
290 (20).

291 A long-term, common garden experiment was used
292 to isolate the effect of tree genotype from the effect of
293 the localized microenvironment associated with each indi-
294 vidual and spatial autocorrelation. Established in 1992,
295 asexually propagated clones of genotyped *P. angustifo-*
296 *lia* individuals were obtained from wild collections and
297 planted in fully randomized design at the Ogden Nature
298 Center, Ogden, UT. From the population of established
299 individuals in the common garden, we chose a total of
300 thirteen genotypes, replicated between 3 and 8 times each,
301 for sampling.

302 **Bark Lichen Observations.** **LJL:** A total pain in
303 the butt but it might be good just to double
304 check that our exact species that occurred in each
305 quadrat are the same as the species that I found
306 in the composition data. No doubt that I would
307 have founrn more species but it might be a good
308 double check of things just to see if they roughly
309 correspond. I can do this if you send me your
310 matrix.

311 **LJL:** I think this sentence is good and these
312 lichens tend to be ones that were rarer or really
313 easy to miss. So, the list of species you picked
314 up sounds legit to me. One thing... above you
315 say 14 species but only 9 were found. That is a
316 317

bit redundant with this next sentence that highlights which species were found and which were not obsered. I would just stick with the second sentence and delete the first sentence.

On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm^2 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 cm^2 quadrats centered at 50 cm and 85 cm from ground level were sampled (Fig 4 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 = *Candelariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Pm = *Phyciella melanachra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were not obesrvd in the present study but are known to occur in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

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 $0.12 \pm 0.001 \text{ cm}^2$ (S.E.) (see Supplementary Fig 1). 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.

LJL: My method was overal visual % cover. I didn't count P/A of rough bark in each square. So, yo might just want to site our Ecology lichen paper as showing rough bark is important, but not cite it as the same method. No doubt the methods should yeild similar results but the approach is slightly different.

We also measured the roughness of the bark on individual trees within each sampling area. Bark roughness had previously been shown to be an important tree trait influencing bark lichens (20) that is under strong genetic control (21). For each tree, the number of cells containing disrupted, fissured bark were counted within each quadrat. The number of rough bark containing cells were then summed and divided by the total number of cells surveyed. This was done for all quadrats on all trees in which lichen communities were also observed.

MKL: Remove A, then move current B up and C below. You can then present current D-K vertically and potentially increase their size.

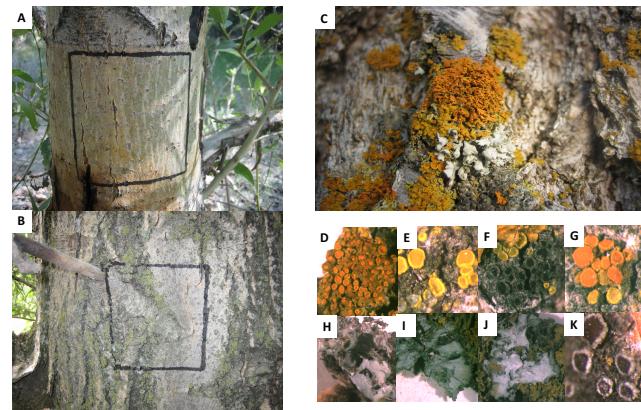


Fig. 4. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community 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 melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

Lichen Network Modeling and Analysis. LJL: This seems like a key point, one that really makes the study above and beyond. I would make it clear with the phrasing that individual networks were created for each individual tree sampled, in this way we had replicated networks for each tree genotypes.

We used the observations of lichens in the 1 cm^2 cells on individual trees of *P. angustifolia*. Unipartite networks were generated using the conditional probabilities of each species pair, i.e. the probability of observing one species given an observation of another species $P(S_i|S_j)$, based on the method developed by (22). To calculate conditional probabilities, we quantified the individual probabilities of species occurrences $P(S_i)$ and the joint probability of co-occurrences $P(S_i, S_j)$ using the frequencies of each species and their co-occurrences. We were then able to calculate the conditional probabilities of each species pair as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability. This yielded a matrix that could possibly be asymmetric, i.e. $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another important property of this matrix is that the diagonal (S_{ii}) was equal to one for all species present and zero for species that were not observed in any cell.

MKL: regarding Lamit's question about the symmetry, the point is that direction of the interaction matters. The effect of species A on B can be different from B on A. No the matrix is not necessarily triangular (triangular being that the matrix either above or below the diagonal is completely zero).

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. 5). 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).

LJL: This approach seems legit and it sound impressive. However, I admit that I think it is a bit above my head and possibly Tom's, too. I have no doubt you did everything correct. But, it might be wise to get a friendly review from a mathy person just to be on the safe side. Perhaps Stuart in NC, or Aaron Ellison.

MKL: agreed. This seems like a job for Bowker or Stuart. They can take a look on the next round of reviews.

LJL: I like the details here. THe one thing is that it sort of makes the reader think there is only one quadrat on a tree but infact there were two. I think you want to make sure to be explicit about the two. For analytical purposes, was all the data lumped so there was really functionally a 20cm by 10cm grid (just split into two pieces). Or, was the network made for each of the two grids and them averaged or combined in some way? My understanding is that it was more the first than the latter.

MKL: Yeah, it was the latter. I'm using two

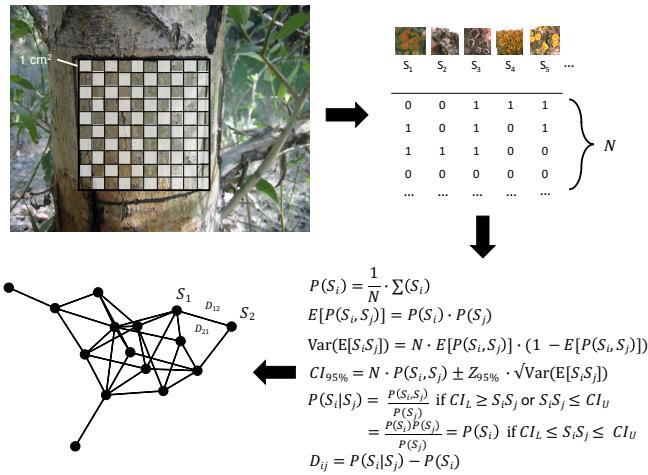


Fig. 5. Lichen interaction networks were constructed by conducting field observations in 1 cm² cells within a 10 cm² grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (22), 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 .

quadrats lumped together. I'll add more text here to clarify that.

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

For multivariate response variables, such as lichen community composition and network structure, we used distance based multivariate statistical approaches, including Permutational Analysis of Variance (PerMANOVA) and Mantel tests. For all analyses, community composition was relativized by species maxima to reduce the effect of the highly abundant *X. galericulata*. For community composition we used Bray-Curtis dissimilarity, which has optimal performance with count data (?). To quantify the similarity of lichen networks among individual trees, we calculated the pairwise Euclidean distance of the \mathbf{D} interaction matrices among all pairs of trees.

For visualization of multivariate patterns, we used Non-metric Multi-Dimensional Scaling (NMDS) (?) to produce dimensionally reduced ordinations of these multi-

493 variate responses and fitted vectors for continuous predictor variables to the ordinated values (?). Using 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.

499 For each network, we also calculated two network metrics that measure different structural aspects. We calculated the number of interactions or “links” in each network, which provides a measure of the size of the network (23?). We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network (?). In a network with a low level of centralization species have similar amount of interaction in the network, while a network with a high level of centralization tends to one or small subset of species that interact with other species. We used a related function to calculate the centrality of each species in each network as well. Although there are many other metrics, see (24), we focus on a subset for the sake of simplicity and because some metrics are not appropriate for our relatively small communities. In particular, we do not present analysis of the modularity (i.e. the degree of sub-grouping) because our community has relatively few species to form modules. As with the other response variables, the number of links was log-transformed and centralization scores were square-root transformed to meet variance and normality assumptions.

522 **LJL: I suggest deleting the highlighted part.**
523 **And, just changing the sentence above it to “...because**
524 **some metric (e.g., modularity) are not appropriate...” Too much emphasis on caviots will**
525 **make some readers be uncertain. But, also, you can save some space that way.**

528 We have made all code and data available online. Code
529 is available at github.com/communitygenetics/lcn. Data
530 is available via the Harvard Dataverse (needs project
531 ID). The project is also archived via Zenodo at zenodo.com/doi/XXXXXX. All analyses were conducted using the
532 programming language R version 3.4.2 (R Development
533 Core Team 2018).

535 Discussion

536 - Genotypic variation can lead to network variation -
537 Network structure is linked to function and dynamics. E.g. stability - Community level selection may
538 be possible, but this is not a necessary factor for
539 evolutionary dynamics to be relevant to ecological
540 networks - What are the conditions in which genetically
541 based ecological network structure could have
542 an effect? - Network structure serves to amplify the
543 signal of genetics

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

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

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

566 We observed significant lichen interaction structure
567 that varied among genotypes of a foundation
568 tree species, narrowleaf cottonwood (*P. angustifolia*).
569 We found that a genetically based trait, bark
570 roughness, partially explained the variation in lichen
571 interaction networks. Some of this variation in lichen
572 networks was related to both the overall abundance
573 and species richness of lichen; though, statistically
574 controlling for the effect of genotype on these variables
575 indicates that a significant portion of the variance
576 in lichen species richness is due to a factor
577 other than tree genotype. By using network metrics,
578 we were also able to probe for specific characteristics
579 of how these networks were responding to tree
580 genotype. We found that both number of links and
581 the centralization of the networks were highly correlated
582 with network similarity and that tree genotype
583 significantly predicted network centrality but only
584 marginally predicted the number of network links.
585 This latter result could be due to the relationship
586 between species richness and the number of links
587 in the network, which were significantly correlated
588 with each other. We also found that bark roughness
589 did not significantly predict either the number of
590 links or the centrality of lichen networks, suggesting
591 that bark roughness has some other effect on the

592 structure of the lichen networks. Taken together,
593 these findings support the hypothesis that genotypic
594 variation in a foundation species contributes to the
595 structure of a network of interacting species.

596 **LJL: I wonder if you need to have so much**
597 **on richness here. Overall, I think you want to**
598 **focus on the network responses and patterns**
599 **among genotype first, and then go into mech-**
600 **anism later. I think we don't quite have a**
601 **good mechanism yet so I don't think it needs**
602 **to come up in the first paragrpah of the dis-**
603 **cussion.**

604 These findings point to the importance of under-
605 standing the community level effects of genetic varia-
606 tion in plant functional traits and highlights the
607 potential for indirect effects of genetic variation to
608 propagate through networks of interacting species
609 and trophic levels.

610 This work corroborates previous findings of the
611 importance of plant genetics in shaping community
612 structure and ecosystem processes. (?)

613 Altering the structure of interaction networks
614 presents a means for genetic effects to be magni-
615 fied within the system of interacting species. For
616 example, (1) showed that the genetics based interac-
617 tions of aphid resistant and aphid susceptible trees
618 resulted in different interaction networks of their
619 associated arthropod communities composed of 139
620 species. At the scale of ecosystems, trophic net-
621 works or food webs direct and control the rates of
622 energy and nutrient flux (25). Furthermore, in a
623 predator-prey-plant study, Smith (26), showed that
624 the interactions among species across trophic levels
625 depended on plant genotype.

626 **LJL: It could be useful to point out that**
627 **our findings are not related to trophic inter-**
628 **actions, which is pretty cool. Also,we talk**
629 **about interaction networks but it is not clear**
630 **to me if the interactions tend to be positive**
631 **or negative. Can we get at that with the ap-**
632 **proach used?**

633 **TGW: Is there any adaptive component to**
634 **the tree in having certain lichen communi-**
635 **ties? e.g., can they feed back to affect tree**
636 **performance in some way or is this a pas-**
637 **sive outcome of a trait that affects bark for**
638 **other adaptive reasons and lichens are pas-**
639 **sive players that tag along for the ride? I**
640 **could envision that lichens covering the bark**

of a tree act as a barrier between insects and
pathogens, much like ectomycorrhizae cover
fine roots as a first line of defense by invad-
ing microorganisms. Uptake of N that gets
passed to the tree??

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

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

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

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

Discussion of network implications for sta- bility with genetics.

Although our study was conducted with a commu-
nity 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 (27, 28). In the face of the
high degree of complexity and potential context de-
pendency 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 (29–31). 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

690 considered at the community level and that con-
 691 serving genetic variation is important to consider in
 692 efforts to restore or preserve complex species interac-
 693 tions and their associated ecosystem functions (32).
 694 With such findings, it appears that we are closer to
 695 understanding the evolutionary drivers of Darwin's
 696 entangled bank and the interconnectedness of species
 697 in complex communities.

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

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- 767
- 768
- 769

770 **Supplementary Materials**

771 TGW: I know you commented about not talking
772 about H2 in the text, but since you have the data,
773 why not? All heritability findings only apply for
774 the environment or common garden they were
775 measured in as does the rest of the findings pre-
776 sented in this paper. MKL: Moved to main text.

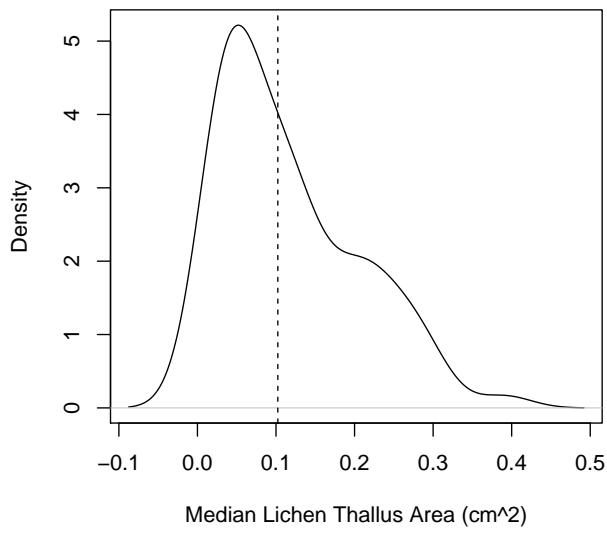


Fig. 1. Density plot of the median lichen thallus area (cm^2).

	Df	SumOfSqs	R2	F	Pr(>F)
geno	10.00	1.85	0.17	1.30	0.10
BR	1.00	0.15	0.01	1.04	0.37
pH	1.00	0.13	0.01	0.92	0.46
CN	1.00	0.17	0.02	1.16	0.31
CT	1.00	0.19	0.02	1.33	0.24
PC	1.00	2.46	0.23	17.33	0.00
SR	1.00	0.55	0.05	3.86	0.00
SE	1.00	0.48	0.05	3.38	0.01
Residual	33.00	4.69	0.44		
Total	50.00	10.65	1.00		