

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

1 **E**volution occurs in the context of complex ecologi-
2 cal 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 (? ? ?) favoring
14 the development of small webs (? ? ?) and that
15 genetic variation and phylogenetic relatedness
16 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 microevolutionary processes (e.g., the genotype or population level).

LJL: likewise, studies of networks do not

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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31 have a genetic component

32 Community genetics studies (6) have shown that
33 genetic variation in foundation species (7) plays a
34 significant role in defining distinct communities of in-
35 teracting organisms: such as, endophytes, pathogens,
36 lichens, arthropods, and soil microbes. Multiple stud-
37 ies have now demonstrated that genetic variation
38 influences numerous functional traits (e.g., phyto-
39 chemical, phenological, morphological) produces a
40 multivariate phenotype (8) that contributes to varia-
41 tion in associated communities (9).

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

48 Additional work has provided support for the hy-
49 pothesis that not only does composition vary among
50 genetically distinct genotypes of foundation species,
51 it also impacts the structure of the network of species
52 interactions in these communities (1, 10). Also, work
53 by (11–13) observed consistent patterns of central-
54 ized interactions of species modules focused around
55 hubs of plant-fungal interactions. In other words, a
56 small number of plant and fungal symbionts tended
57 to have have disproportionate numbers of interac-
58 tions with other species and likely are the drivers
59 in determining community assembly, structure and
60 dynamics.

61 More on the importance of ecological networks
62 (14, 15).

63 Add a discussion of DesRoches.

64 From Thompson2014

- 65 • Pairwise interactions are usually influenced by
66 other species
- 67 • Selection favors the development of small webs
- 68 • Evolution of new lifestyles leads to changes in
69 selection on large and small webs

70 Specific hypothesis from Thomspson2014

71 Selection in small webs

- 72 •

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

75 in the manuscript. I do like the idea of in-
76 cluding the field data. We might need to
77 cross reference IDs of the lichens in my com-
78 munity composition data on the same trees
79 since there was considerably more diversity
80 than we initially saw out there. We would
81 just want to make sure the same names are
82 applied to taxa in the same quadrats on each
83 tree. It probably wouldn't take long.

84 **LJL:** If I recall, the Elamo paper just looks
85 at genetic correlations between pairwise indi-
86 vidual abundances. I would suggest maybe it
87 doesn't deserve to be in this 1st paragraph.
88 Perhaps it actually should be in the 2nd or
89 3rd paragraph, just as a reference that points
90 to the potential for genotype to influence net-
91 works. Definately our 2015 JOE paper goes
92 much further, too, since it has whole com-
93 munities being correlated. But, again, I
94 woudl put both of these as citation in the com-
95 munity genetics paragraphs (2 of 3) instead
96 of the first paragrpah, which focuses on the
97 general network lit.

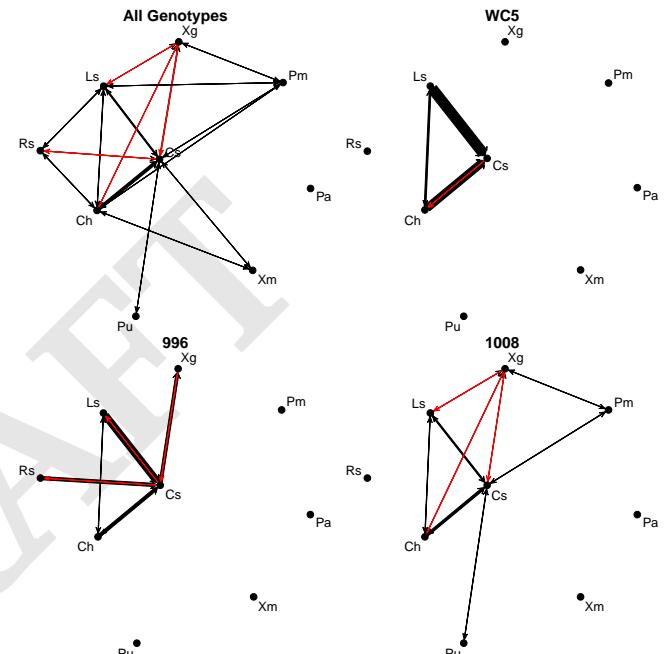
98 Here, we investigate how genetic variation in a
99 foundation tree species determines the structure of a
100 network of interactions among a community of tree
101 associated lichen species. Using a long-term (20+
102 years), common garden experiment with replicated
103 individuals of known genetic identity and a naturally
104 established stand of *Populus angustifolia*. We focused
105 on a model community of 9 epiphytic lichen species,
106 as previous research has demonstrated significant
107 compositional responses of epiphytes to genotypic
108 variation (16, 17). In addition, the life-history char-
109 acteristics of lichens, having highly localized, direct
110 contact interactions and slow population turnover
111 rates, allowed us to assess interactions among lichen
112 species on individual trees. We hypothesize that in
113 natural systems evolution occurs in a community
114 context involving interactions of complex networks
115 of interacting species (1, 10, 18, 19). If correct, we
116 should expect to find that network structure is ge-
117 netically based in which different plant genotypes
118 support different interaction networks and that these
119 interactions networks can function as indicators of
120 ecological dynamics important for conserving biodi-
121 versity. Applying a probability-theory based network
122 modeling approach, we constructed a set of interac-
123 tion network models for the lichens associated with

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

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

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

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



184
Fig. 1. Lichen networks varied in structure among tree genotypes. Network dia-
185 grams of the mean lichen interaction matrices averaged for all trees and for several
186 individual genotypes showing a range of interaction network structure. Directionality
187 (arrowheads) and sign (red = negative, black = positive) of interactions are shown
188 as edges between species (abbreviated by the first letter of the genus and specific
189 epithet), which are scaled by their magnitude. The sign of the interaction is indicative
190 of greater (positive) or lesser (negative) paired occurrences than expected relative
191 to the overall frequency of occurrence of each species. Ecologically, the links in
192 the network are likely the product of multiple types of interactions (e.g. mutualism,
193 parasitism, competition, facilitation) that could vary over both space and time.

Results

184 Network similarity and several tree traits were geneti-
185 cally based. Tree genotype was a significant predictor
186 of network similarity ($H^2 = 0.16$, $p\text{-value} \leq 0.001$).
187 Bark roughness ($H^2 = 0.38$, $p\text{-value} \leq 0.001$) and
188 condensed tannin concentration ($H^2 = 0.28$, $p\text{-value}$
189 = 0.014) also showed a signature of tree genotype
190 (Fig. ??); however, this was not the case for other
191 tree traits, bark pH and carbon to nitrogen ratio.
192 Also, none of the lichen community nor any of the
193

Response	H2	p-value
Lichen Network Similarity	0.16	1e-04
Number of Network Links	0.0695	0.2376
Network Centrality	0.0851	0.1985
Network Modularity	0	1
Percent Lichen Cover	0.0793	0.172
Lichen Species Richness	0	1
Lichen Species Evenness	0.0151	0.3882
Lichen Species Diversity	0.0095	0.4167
Community Composition	0.052	0.1019
Percent Rough Bark	0.3849	1e-04
Condensed Tannins (CT)	0.2803	0.0139
Carbon-Nitrogen (CN) Ratio	0	0.4479
pH	0.0539	0.2939

Table 1. Genotypic effects on the associated lichen community.

	df	SS	R2	F	p-value
Genotype	10.0000	304.9280	0.2335	19.2955	0.0001
Bark Roughness	1.0000	16.2594	0.0124	10.2888	0.0025
pH	1.0000	5.0371	0.0039	3.1874	0.0831
C:N Ratio	1.0000	39.6664	0.0304	25.1005	0.0001
Condensed Tannins	1.0000	70.7702	0.0542	44.7826	0.0001
Percent Cover	1.0000	56.3523	0.0431	35.6592	0.0001
Species Richness	1.0000	332.4174	0.2545	210.3504	0.0001
Species Evenness	1.0000	55.1077	0.0422	34.8716	0.0001
Number of Links	1.0000	326.5265	0.2500	206.6226	0.0001
Network Modularity	1.0000	7.7683	0.0059	4.9157	0.0305
Network Centrality	1.0000	43.7646	0.0335	27.6938	0.0001
Residual	30.0000	47.4091	0.0363		
Total	50.0000	1306.0069	1.0000		

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

lichen network metrics were significantly predicted by tree genotype (Table 1).

Tree traits and lichen community metrics were correlated with lichen networks. The genetically based traits bark roughness ($R^2 = 0.01$, $p\text{-value} = 0.003$) and condensed tannins ($R^2 = 0.05$, $p\text{-value} \leq 0.001$) were both significant predictors of network similarity (Table 2). Bark C:N ratio ($R^2 = 0.03$, $p\text{-value} \leq 0.001$) was correlated with lichen network similarity, as stated above, it was did not show evidence of a genetic response, and bark pH ($R^2 = 0.004$, $p\text{-value} = 0.083$) did not significantly predict network similarity. The abundance, richness, evenness and diversity of the bark lichen communities were all correlated with lichen network similarity (Table 2). Interestingly, lichen network similarity was not correlated with lichen community composition either when species abundances were relativized (Mantel R = 0.09, $p\text{-value} = 0.139$) or not (Mantel R = -0.03, $p\text{-value} = 0.573$). Also, unlike lichen network similarity, lichen community composition was not predicted by tree genotype (Supplementary Table ??).

MKL Wild stands don't have chemistry data so those data were not used.

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

a slightly different sampling method as used in the 2015 paper, and for this specific set of genotypes. But, then does this test of composition just become something necessary just in a methodological variation that justifies the next step of examining network structure. Something to think about. It might be that the NMDS should just go in a supplement, although I do like it here in some ways. It might also be another approach to put the composition and other analyses after the network analysis results are presented. In this way, you could use the composition and results with vectors to help provide resolution on what is driving networks to differ among genotypes.

MKL: Adapt into a table.

TGW: clarify positive vs negative interactions.

MKL: lichen networks in wild stands displayed similar structural patterns. Is it worth adding the wild stand? This will require adding methods, results and more discussion.

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

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

LJL: It seems to me that the first two sentences here are the most important of the results. How can you make them stand out more? Maybe also they should go at the beginning of the previous paragraph, and then move that paragraph to being the first in the Results section.

264 TGW: Here and in earlier paragraphs, a
265 lot of stats are presented some of which are
266 significant and some not. For your topic sen-
267 tence to be accepted, it seems readers need
268 to know how many of the stats need to con-
269 firm the pattern and how many would it take
270 to reject. This paragraph has about 8 stats
271 so need some overarching statement(s). E.g.,
272 7 of 8 analyses support our overarching hy-
273 pothesis that ... Same goes for other such
274 paragraphs such as the 1st and last paras of
275 the Results.

276 Materials and Methods

277 MKL: Need to add more citations of Lamits or
278 other lichen studies.

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

286 The study was conducted along the Weber River, UT
287 (USA), which is a cottonwood (*Populus* spp.) dominated
288 riparian ecosystem. Although two native species, *Populus*
289 *angustifolia* (James) and *Populus fremontii* (S. Watson),
290 occur here and are known to hybridize, only pure or
291 advanced generation backcrosses of *P. angustifolia* were
292 sampled. Bark lichens have been extensively studied
293 in this system and provide an ideal system in which to
294 observe and model lichen interaction networks, as their
295 sessile nature permits accurate identification of individuals
296 (20).

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

308 **Bark Lichen Observations.** LJL: A total pain in
309 the butt but it might be good just to double
310 check that our exact species that occurred in each
311 quadrat are the same as the species that I found
312 in the composition data. No doubt that I woudl
313 have foudn more species but it might be a good
314 double check of things just to see if they roughly
315

correspond. I can do this if you send me your
matrix.

316 LJL: I think this sentence is good and these
317 lichens tend to be ones that were rarer or really
318 easy to miss. So, the list of species you picked
319 up sounds legit to me. One thing... above you
320 say 14 species but only 9 were found. That is a
321 bit redundant with this next sentence that high-
322 lights which species were found and which were
323 not obsered. I would just stick with the second
324 sentence and delete the first sentence.

325 On each tree, presence or absence of each lichen species
326 was assessed in 50 total 1 cm² cells arrayed in a checker-
327 board pattern. Given the small size and sessile nature
328 of lichens, we were able to rapidly assess lichen interac-
329 tions by quantifying thalli in close contact. Sampling
330 was restricted to the northern aspect of the trunk to
331 maximize the abundance of lichen and control for the
332 effect of trunk aspect. Two adjacent 10 cm² quadrats
333 centered at 50 cm and 85 cm from ground level were
334 sampled (Fig 3 A and B). The observed lichen commu-
335 nity included (abbreviations are given for species present
336 in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
337 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella*
338 *subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*,
339 Pm = *Phyciella melanura*, Pa = *Physcia adscendens*,
340 Pu = *Physcia undulata*. Several other species were not
341 obesrvd in the present study but are known to occur
342 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
343 *ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

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

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

363 We also measured the roughness of the bark on indi-
364 vidual trees within each sampling area. Bark roughness
365 had previously been shown to be an important tree trait
366 influencing bark lichens (20) that is under strong genetic
367 control (21). For each tree, the number of cells contain-
368 ing disrupted, fissured bark were counted within each
369 quadrat. The number of rough bark containing cells were
370 371

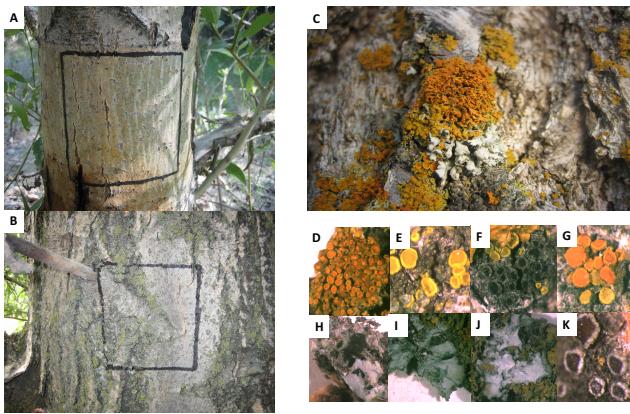


Fig. 3. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community 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*, *Physciella melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbørg (D-K).

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.

Lichen Network Modeling and Analysis. L.J.L: 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. 4). 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 ($D = D_{ij}$) can be interpreted as how one species impacts another with zero being no effect

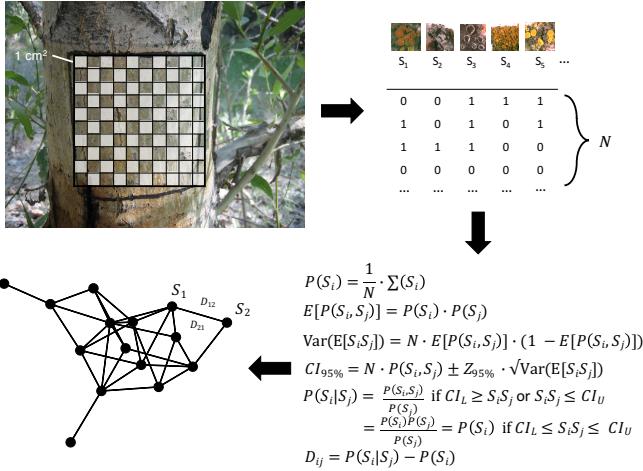


Fig. 4. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (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 .

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 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 multivariate responses and fitted vectors for continuous predictor variables to the ordinated values (?). Using random initial configurations with a maximum of 500 iterations

501 and a change in stress threshold of less than 10^{-12} . Final
502 configurations has the lowest stress with at most a stress
503 level of 0.10.

504 For each network, we also calculated two network met-
505 rics that measure different structural aspects. We cal-
506 culated the number of interactions or “links” in each
507 network, which provides a measure of the size of the net-
508 work (23?). We also calculated the centralization of each
509 network, which measures the evenness of the distribution
510 of interactions among the species in the network (?).
511 In a network with a low level of centralization species
512 have similar amount of interaction in the network, while
513 a network with a high level of centralization tends to
514 one or small subset of species that interact with other
515 species. We used a related function to calculate the cen-
516 trality of each species in each network as well. Although
517 there are many other metrics, see (24), we focus on a
518 subset for the sake of simplicity and because some metrics
519 are not appropriate for our relatively small communities.
520 **In particular, we do not present analysis of the**
521 **modularity (i.e. the degree of sub-grouping) be-**
522 **cause our community has relatively few species to**
523 **form modules.** As with the other response variables,
524 the number of links was log-transformed and centralization
525 scores were square-root transformed to meet variance
526 and normality assumptions.

527 **LJL: I suggest deleting the highlighted part.**
528 **And, just changing the sentence above it to “...be-**
529 **cause some metric (e.g., modularity) are not ap-**
530 **propriate...” Too much emphasis on caviots will**
531 **make some readers be uncertain. But, also, you**
532 **can save some space that way.**

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

540 Discussion

- 541 - Genotypic variation can lead to network variation
- 542 - Traits could both share or be a causal mechanism
- 543 with lichen networks. - Network structure is linked
- 544 to function and dynamics. E.g. stability
 - 545 - Community level selection may be possible, but
 - 546 this is not a necessary factor for evolutionary dynamics
 - 547 to be relevant to ecological networks
 - 548 - What are the conditions in which genetically
 - 549 based ecological network structure could have an
 - 550 effect?
 - 551 - Network structure serves to amplify the signal
 - 552 of genetics

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

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

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

574 We observed significant lichen interaction struc-
575 ture that varied among genotypes of a foundation
576 tree species, narrowleaf cottonwood (*P. angustifo-*
577 *lia*). We found that a genetically based trait, bark
578 roughness, partially explained the variation in lichen
579 interaction networks. Some of this variation in lichen
580 networks was related to both the overall abundance
581 and species richness of lichen; though, statistically
582 controlling for the effect of genotype on these vari-
583 ables indicates that a significant portion of the varia-
584 nce in lichen species richness is due to a factor
585 other than tree genotype. By using network metrics,
586 we were also able to probe for specific characteris-
587 tics of how these networks were responding to tree
588 genotype. We found that both number of links and
589 the centralization of the networks were highly corre-
590 lated with network similarity and that tree genotype
591 significantly predicted network centrality but only
592 marginally predicted the number of network links.
593 This latter result could be due to the relationship
594 between species richness and the number of links
595 in the network, which were significantly correlated
596 with each other. We also found that bark roughness
597 did not significantly predict either the number of
598 links or the centrality of lichen networks, suggesting
599 that bark roughness has some other effect on the

600 structure of the lichen networks. Taken together,
601 these findings support the hypothesis that genotypic
602 variation in a foundation species contributes to the
603 structure of a network of interacting species.

604 **LJL: I wonder if you need to have so much**
605 **on richness here. Overall, I think you want to**
606 **focus on the network responses and patterns**
607 **among genotype first, and then go into mech-**
608 **anism later. I think we don't quite have a**
609 **good mechanism yet so I don't think it needs**
610 **to come up in the first paragrpah of the dis-**
611 **cussion.**

612 These findings point to the importance of under-
613 standing the community level effects of genetic varia-
614 tion in plant functional traits and highlights the
615 potential for indirect effects of genetic variation to
616 propagate through networks of interacting species
617 and trophic levels.

618 This work corroborates previous findings of the
619 importance of plant genetics in shaping community
620 structure and ecosystem processes. (?)

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

634 **LJL: It could be useful to point out that**
635 **our findings are not related to trophic inter-**
636 **actions, which is pretty cool. Also,we talk**
637 **about interaction networks but it is not clear**
638 **to me if the interactions tend to be positive**
639 **or negative. Can we get at that with the ap-**
640 **proach used?**

641 **TGW: Is there any adaptive component to**
642 **the tree in having certain lichen communi-**
643 **ties? e.g., can they feed back to affect tree**
644 **performance in some way or is this a pas-**
645 **sive outcome of a trait that affects bark for**
646 **other adaptive reasons and lichens are pas-**
647 **sive players that tag along for the ride? I**
648 **could envision that lichens covering the bark**

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

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

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

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

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

673 **Discussion of network implications for sta-** 674 **bility with genetics.**

675 Although our study was conducted with a commu-
676 nity of lichens, these results should be generalized
677 to other groups of diverse organisms around the
678 world that also exhibit significant genetic signals at
679 the community level (27, 28). In the face of the
680 high degree of complexity and potential context de-
681 pendence of ecological processes, the current study
682 points to the utility of considering the spatial and
683 temporal scales of interactions, as discussed to some
684 in previous studies (29–31). In the present study,
685 we found that community assembly processes, such
686 as environmental filtering and species interactions,
687 are genetically based. This is likely due, in part,
688 to the large difference in the differences in size and
689 longevity of the lichen and cottonwood individuals
690 with the trees determining the environment in which
691 the lichen occur. We suggest that future work would
692 be aided by determining these modules within the
693 biotic community that include species with similar
694 differences in body-size and time-scales. As heritable
695 variation is the raw material for natural selection
696 to act upon, a genetic basis for interaction network
697 structure indicates evolutionary dynamics should be
698

698 considered at the community level and that con-
 699 serving genetic variation is important to consider in
 700 efforts to restore or preserve complex species interac-
 701 tions and their associated ecosystem functions (32).
 702 With such findings, it appears that we are closer to
 703 understanding the evolutionary drivers of Darwin's
 704 entangled bank and the interconnectedness of species
 705 in complex communities.

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- 713 1. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation
 714 species affect community diversity, stability and network structure. *Proc. R. Soc. B Biol. Sci.*
 715 284(1854):20162703.
- 716 2. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming
 717 decade. *New Phytol.*
- 718 3. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes
 719 to ecosystems. *Nat. Rev. Genet.* 7:510–523.
- 720 4. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure.
Philos. Trans. R. Soc. Lond. B. Biol. Sci. 366(1569):1425–37.
- 721 5. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random co-
 722 extinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
- 723 6. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly
 724 across multiple environments. *Ecology* 96(4):960–971.
- 725 7. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and
 726 dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486.
- 727 8. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic,
 728 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype
 729 in a foundation tree species. *Oecologia* 170:695–707.
- 730 9. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant
 731 genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*
 732 364(1523):1607–16.
- 733 10. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foun-
 734 dation species generates network structure that may drive community dynamics and evolu-
 735 tion. *Ecology* 97(3):15–0600.1.
- 736 11. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacom-
 737 munity level.
- 738 12. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs
 739 in plant-root fungal biomes. *J. R. Soc. Interface.*
- 740 13. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus
 741 networks. *Nat. Commun.*
- 742 14. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic
 743 networks. *Ecol. Lett.* 14(9):877–85.
- 744 15. Thompson JN, Schwind C, Guimaraes PR, Friberg M (2013) Diversification through multitrait
 745 evolution in a coevolving interaction. *Proc. Natl. Acad. Sci.*
- 746 16. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecol. Econ.*
 747 71:80–88.
- 748 17. Zytynska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree
 749 species influences the associated epiphytic plant and invertebrate communities in a complex
 750 forest ecosystem. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366:1329–1336.
- 751 18. Thompson JN (2013) *Relentless Evolution.* (University of Chicago Press), p. 499.
- 752 19. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate
 753 Biodiversity Maintenance 10.1126/science.1123412. *Science* (80-.). 312:431–433.
- 754 20. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influ-
 755 ences a dominant bark lichen. *Fungal Ecol.* 4(1):103–109.
- 756 21. Bdeir R, et al. (2017) Quantitative trait locus mapping of Populus bark features and stem
 757 diameter. *BMC Plant Biol.*
- 758 22. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net-
 759 works to assess the impacts of climate change. *Ecography (Cop.)*. 34:897–908.
- 760 23. Lau MK, Borrett SR, Hines DE, Singh P (2015) enaR: Tools for Ecological Network Analysis.
- 761 24. Lau MK, Borrett SR, Baisier B, Gotelli NJ, Ellison AM (2017) Ecological network metrics:
 762 opportunities for synthesis. *Ecosphere* 8(8):e01900.
- 763 25. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Soc. Networks*
 764 28:466–484.
- 765 26. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter-
 766 actions and selection: trees, aphids and birds. *J. Evol. Biol.* 24(2):422–9.
- 767 27. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and
 768 evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1322–8.

28. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes. 770
29. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure. 771
30. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to 772 body size yields high degree of intervality. *J. Theor. Biol.* 271(1):106–113.
31. Zytynska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2). 773
32. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net- 774 works to habitat loss. *Ecol. Lett.* 16:844–52. 775

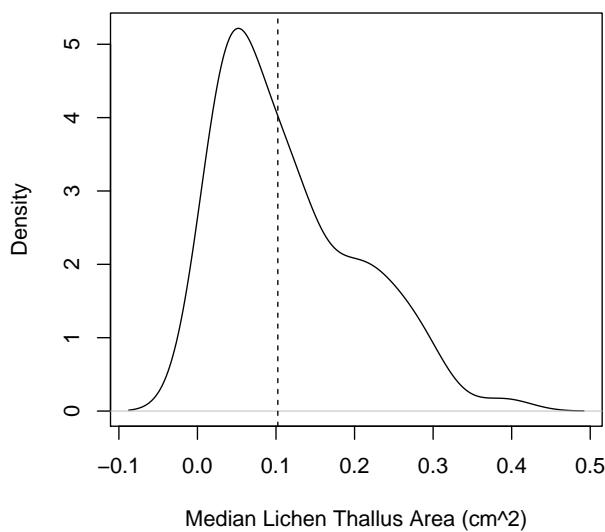


Fig. 1. Density plot of the median lichen thallus area (cm²).

778 **Supplementary Materials**

779 TGW: I know you commented about not talking
 780 about H2 in the text, but since you have the data,
 781 why not? All heritability findings only apply for
 782 the environment or common garden they were
 783 measured in as does the rest of the findings pre-
 784 sented in this paper. MKL: Moved to main text.

	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		