

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 number of network**
19 **links and network centrality were both correlated with bark rough-**
20 **ness; however, none of the network metrics examined were signif-**
21 **icantly correlated with network similarity and this variation is unlikely**
22 **what is underpinning the genetically based similarity of lichen net-**
23 **works. We conclude that tree genotype influences lichen interaction**
24 **network structure with two potential pathways through bark rough-**
25 **ness and condensed tannin concentration. These results provide**
26 **empirical evidence that variation in ecological interaction networks**
27 **can result from genetically based variation in foundation species in**
28 **ecosystems.**

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 para-
graph is to point out that networks are be-
lieve in part to emerge out of complex evo-
lutionary interactions. But, to-date, the pat-
terns observed in networks are primarily only
on the scale of species interactions and phylo-
genetic 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 com-
prised of complex ecological networks. Research
at the interface of ecology and evolution has primar-
ily focused on pairwise interactions among species
and have rarely included a genetic component to
analyses. Here, we use a long-term common gar-
den 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 genet-
ically based tree trait, which alters network structure
both through environmental filtering of species and
altering species interactions. These findings demon-
strate the importance of assessing the impacts of
genetic variation and evolutionary dynamics in shap-
ing 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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28 microevolutionary processes (e.g., the genotype
29 or population level).

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

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

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

59 More on the importance of ecological networks
60 (14, 15).

61 Add a discussion of DesRoches.

62 From Thompson2014

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

68 Specific hypothesis from Thomspon2014
69 Selection in small webs

- 70 •

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

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

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

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

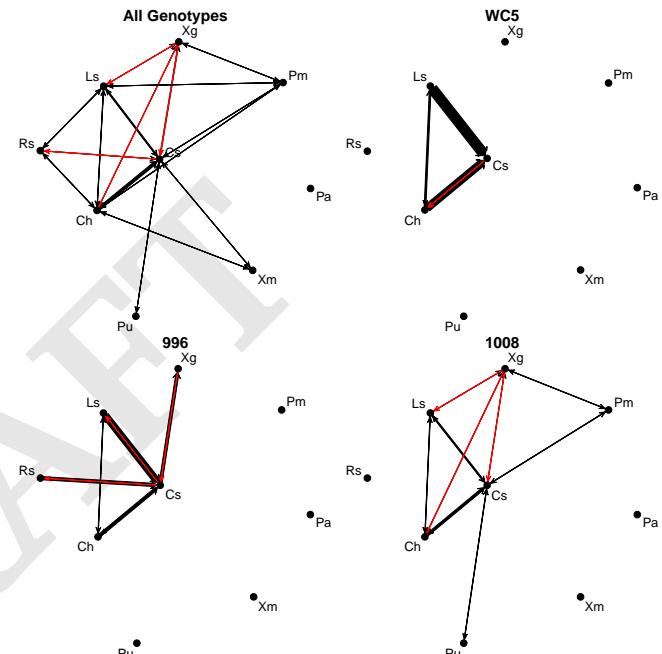


Fig. 1. Lichen networks varied in structure among tree genotypes. Network dia-
grams of the mean lichen interaction matrices averaged for all trees and for several
individual genotypes showing a range of interaction network structure. Directionality
(arrowheads) and sign (red = negative, black = positive)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

Network similarity was genetically based. Tree genotype was a significant predictor of network similarity ($R^2 = 0.23$, $F_{10} = 19.30$, $p\text{-value} \leq 0.001$). The abundance ($R^2 = 0.001$, $F_1 = 3.19$, $p\text{-value} \leq 0.08$), richness ($R^2 = 0.001$, $F_1 = 3.19$, $p\text{-value} \leq 0.08$) and evenness ($R^2 = 0.001$, $F_1 = 3.19$, $p\text{-value} \leq 0.08$) of the bark lichen communities along with the lichen network structural metrics that we examined (number of links, modularity and centrality) were

192 all correlated with lichen network similarity (Table
 193 1). Interestingly, lichen network similarity was not
 194 correlated with lichen community composition either
 195 when species abundances were relativized (Mantel R
 196 = 0.09, *p*-value = 0.139) or not (Mantel R = -0.03, *p*-
 197 value = 0.573). Also, unlike lichen network similarity
 198 , lichen community composition was not predicted
 199 by tree genotype (Supplementary Tables ??).

200 Several tree traits and lichen community and net-
 201 work metrics were significant predictors of lichen
 202 network similarity. Tree bark roughness ($R^2 = 0.01$,
 203 $F_1 = 10.29$, *p*-value ≤ 0.001), carbon to nitrogen
 204 ratio ($R^2 = 0.03$, $F_1 = 25.10$, *p*-value ≤ 0.001), and
 205 condensed tannins ($R^2 = 0.05$, $F_1 = 44.78$, *p*-value
 206 ≤ 0.001) were significantly correlated with lichen
 207 network similarity; however, bark pH ($R^2 = 0.001$,
 208 $F_1 = 3.19$, *p*-value ≤ 0.08) was not (Table 1).

	Df	SumOfSqs	R2	F	Pr(>F)
geno	10	304.93	0.23	19.30	0.0001
BR	1	16.26	0.01	10.29	0.0025
pH	1	5.04	0.00	3.19	0.0831
CN	1	39.67	0.03	25.10	0.0001
CT	1	70.77	0.05	44.78	0.0001
PC	1	56.35	0.04	35.66	0.0001
SR	1	332.42	0.25	210.35	0.0001
SE	1	55.11	0.04	34.87	0.0001
L	1	326.53	0.25	206.62	0.0001
mod.lik	1	7.77	0.01	4.92	0.0305
Cen	1	43.76	0.03	27.69	0.0001
Residual	30	47.41	0.04		
Total	50	1306.01	1.00		

Table 1. Pseudo-F Table of lichen network similarity PERMANOVA.

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

Table 2. Genotypic effects on the associated lichen community.

209 **A. Some genetically based tree traits predicted
 210 lichen network structure.**

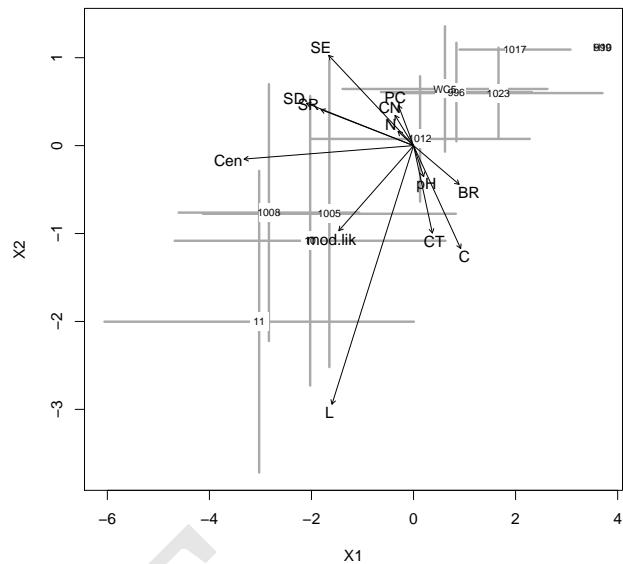


Fig. 2. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.011) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure 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).

B. Wild stand results. MKL: I removed the 211
 212 community similarity figure to simplify the
 213 presentation of the results and improve the
 214 flow.

LJL: Figure looks good. But, maybe making 215
 216 all lines a little thicker would look nicer
 217 and pop more.

LJL: Since we already published that tree 218
 219 genotypes differ in lichen composition, I won-
 220 der if we need to say somewhere in the
 221 manuscript why this test was run here. It
 222 seems to me it is important to verify this with
 223 a slightly different sampling method as used
 224 int eh 2015 paper, and for this specific set of
 225 genotypes. But, then does this test of compo-
 226 sition just become something necessary just
 227 in a methodological variation that justifies
 228 the next step of examing network structure.
 229 Something to think about. It might be that
 230 theNMDS should jsut go in a supplement, al-
 231 though I do like it here in some ways. It
 232 might also be another approach to put the
 233 composition and other analyses after the net-
 234 work analysis results are presented. In this
 235 way, you could use the composition and re-

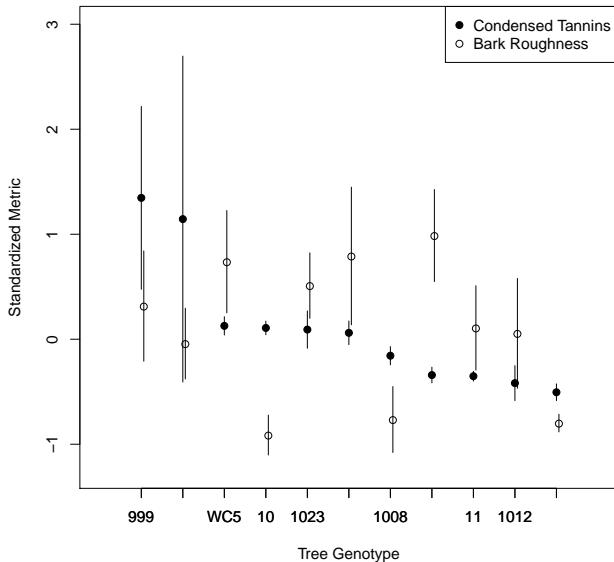


Fig. 3. The impact of tree genotype on lichen network structure was indicative of variation in both the number variation in lichen interactions among species. Plot showing the means (± 1 S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented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

236 sults with vectors to help provide resolution
237 on what is driving networks to differ among
238 genotypes.

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

240 **TGW:** clarify positive vs negative interac-
241 tions.

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

244 **D. Some genetically based tree traits predicted**
245 **lichen network structure.**

246 **E. Wild stand results.** **MKL:** lichen networks in
247 wild stands displayed similar structural pat-
248 terns. Is it worth adding the wild stand?
249 This will require adding methods, results and
250 more discussion.

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

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

256 **LJL:** It seems to me that the first two sen-
257 tences here are the most important of the
258 results. How can you make them stand out
259 more? Maybe also they should go at the be-

gining of the previous paragrph, and then
move that paragraph to being the first in the
REsults section.

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

Materials and Methods

275 **MKL:** Need to add more citations of Lamits or
276 other lichen studies.

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

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

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

306 **Bark Lichen Observations.** **LJL:** A total pain in
307 the butt but it might be good just to double
308 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 would
313 have found 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
316 matrix.

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

326 On each tree, presence or absence of each lichen species
327 was assessed in 50 total 1 cm² cells arrayed in a checker-
328 board pattern. Given the small size and sessile nature
329 of lichens, we were able to rapidly assess lichen interac-
330 tions by quantifying thalli in close contact. Sampling
331 was restricted to the northern aspect of the trunk to
332 maximize the abundance of lichen and control for the
333 effect of trunk aspect. Two adjacent 10 cm² quadrats
334 centered at 50 cm and 85 cm from ground level were
335 sampled (Fig 4 A and B). The observed lichen commu-
336 nity included (abbreviations are given for species present
337 in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
338 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella*
339 *subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*,
340 Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*,
341 Pu = *Physcia undulata*. Several other species were not
342 obesrvd in the present study but are known to occur
343 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
344 *ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

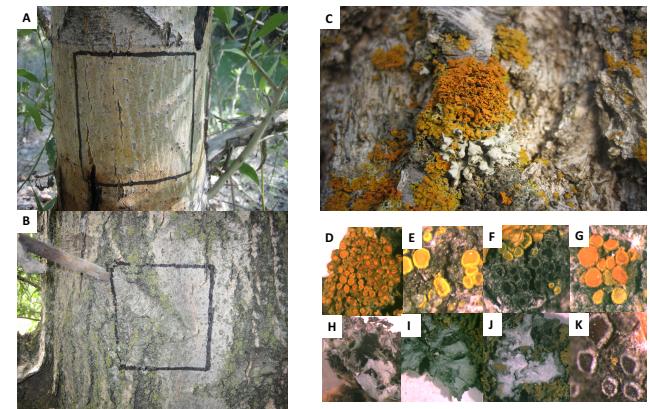
345 The cell size and checkerboard sampling pattern was
346 chosen to isolate the individuals in each cell. In a previous
347 survey of lichen thallus size in this common garden, we
348 had observed a median thallus size of 0.12 ± 0.001 cm²
349 (S.E.) (see Supplementary Fig 1). Based on the median
350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

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

364 We also measured the roughness of the bark on indi-
365 vidual trees within each sampling area. Bark roughness
366 had previously been shown to be an important tree trait

367 influencing bark lichens (20) that is under strong genetic
368 control (21). For each tree, the number of cells contain-
369 ing disrupted, fissured bark were counted within each
370 quadrat. The number of rough bark containing cells were
371 then summed and divided by the total number of cells
372 surveyed. This was done for all quadrats on all trees in
373 which lichen communities were also observed.

374 **MKL: Remove A, then move current B up and
375 C below. You can then present current D-K ver-
376 tically and potentially increase their size.**



377 **Fig. 4.** The communities of bark lichens were observed in a common garden of
378 replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden
379 Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm²) on
380 individual trees (A and B). (C) a photo of a typical community of bark lichen species
381 interacting on the trunk of a cottonwood tree, including one of the more abundant
382 species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen
383 species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp.,
384 *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata*
385 and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

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

393 We used the observations of lichens in the 1 cm² cells
394 on individual trees of *P. angustifolia*. Unipartite networks
395 were generated using the conditional probabilities of each
396 species pair, i.e. the probability of observing one species
397 given an observation of another species $P(S_i|S_j)$, based on
398 the method developed by (22). To calculate conditional
399 probabilities, we quantified the individual probabilities
400 of species occurrences $P(S_i)$ and the joint probability
401 of co-occurrences $P(S_i, S_j)$ using the frequencies of each
402 species and their co-occurrences. We were then able to
403 calculate the conditional probabilities of each species pair
404 as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
405 This yielded a matrix that could possibly be asymmetric, i.e.
406 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another
407 important property of this matrix is that the diagonal
408

399 (S_{ii}) was equal to one for all species present and zero for
400 species that were not observed in any cell.

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

407 We then applied an analytical procedure to remove
408 non-significant links between species. This procedure
409 determines if the joint probability of a species pair
410 (i.e. $P(S_i, S_j)$) is different from zero (Fig. 5). Here, a
411 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
412 of co-occurrences $E(S_i S_j)$ is the total number of cells surveyed (N) times the independent probabilities
413 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
414 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.
415 $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the
416 observed number of co-occurrence falls outside of the
417 confidence interval, the joint probability $P(S_i, S_j)$ is de-
418 termined to be equal to the product of the individual
419 probabilities (i.e. $P(S_i) \dot{P}(S_j)$), and the conditional prob-
420 ability reduces to the individual probability of that species
421 $P(S_i)$. Therefore, unless the co-occurrence of a species
422 pair falls outside the confidence interval, the probability
423 that the observation of one species given the other
424 is no different than simply observing that species alone.
425 This enables us to remove links from a given network by
426 re-scaling the resulting conditional probabilities by sub-
427 tracting the individual probabilities from the conditional
428 probabilities (i.e. how different the conditional probability
429 is from the independent probability), which makes any
430 species with a non-significant conditional probability zero.
431 The resulting matrix ($\mathbf{D} = D_{ij}$) can be interpreted as
432 how one species impacts another with zero being no effect
433 and values less than or greater than zero interpreted as
434 negative and positive effects, respectively. Here, we will
435 refer to this matrix (\mathbf{D}) as an interaction matrix with
436 the properties that it can be asymmetric (i.e. P_{ij} does not
437 necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e.
438 a species does not influence its own probability of being
439 observed).

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

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

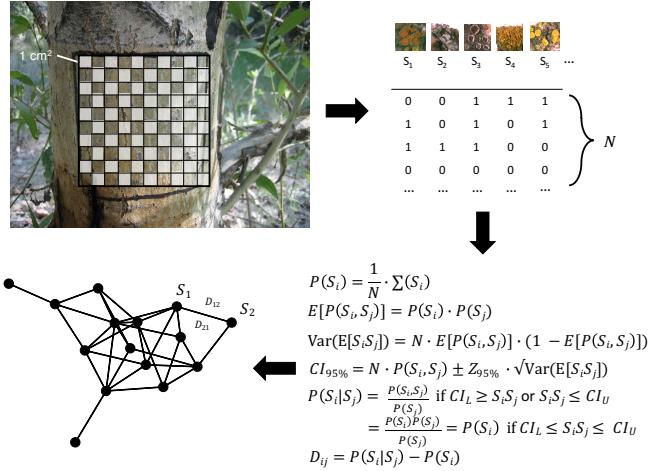


Fig. 5. Lichen interaction networks were constructed by conducting field observations in 1 cm² cells within a 10 cm² grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded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

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

MKL: Yeah, it was the latter. I'm using two
466 quadrats lumped together. I'll add more text
467 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

485 Permutational Analysis of Variance (PerMANOVA) and
486 Mantel tests. For all analyses, community composition
487 was relativized by species maxima to reduce the effect
488 of the highly abundant *X. galericulata*. For community
489 composition we used Bray-Curtis dissimilarity, which has
490 optimal performance with count data (?). To quantify
491 the similarity of lichen networks among individual trees,
492 we calculated the pairwise Euclidean distance of the **D**
493 interaction matrices among all pairs of trees.

494 For visualization of multivariate patterns, we used
495 Non-metric Multi-Dimensional Scaling (NMDS) (?) to
496 produce dimensionally reduced ordinations of these multi-
497 variate responses and fitted vectors for continuous predictor
498 variables to the ordinated values (?). Using random
499 initial configurations with a maximum of 500 iterations
500 and a change in stress threshold of less than 10^{-12} . Final
501 configurations has the lowest stress with at most a stress
502 level of 0.10.

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

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

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

Discussion

- Genotypic variation can lead to network variation -
540 Network structure is linked to function and dynam-
541 ics. E.g. stability - Community level selection may
542 be possible, but this is not a necessary factor for
543 evolutionary dynamics to be relevant to ecological
544 networks - What are the conditions in which geneti-
545 cally based ecological network structure could have
546 an effect? - Network structure serves to amplify the
547 signal of genetics
548

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

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

- Genetic assembly rule = similar genetics will
561 have more similar communities
562
- What we don't know is whether or not these
563 interactions will also lead to similar interactions
564 among other species.
565
- Thus, it would be possible for genetics to not
566 only influence other species directly, but also
567 indirectly by influencing the interactions among
568 other species.
569

We observed significant lichen interaction struc-
570 ture that varied among genotypes of a foundation
571 tree species, narrowleaf cottonwood (*P. angustifo-*
572 *lia*). We found that a genetically based trait, bark
573 roughness, partially explained the variation in lichen
574 interaction networks. Some of this variation in lichen
575 networks was related to both the overall abundance
576 and species richness of lichen; though, statistically
577 controlling for the effect of genotype on these vari-
578 ables indicates that a significant portion of the vari-
579 ance in lichen species richness is due to a factor
580 other than tree genotype. By using network metrics,
581 we were also able to probe for specific characteris-
582 tics of how these networks were responding to tree
583 genotype. We found that both number of links and
584

585	the centralization of the networks were highly corre-	634
586	lated with network similarity and that tree genotype	635
587	significantly predicted network centrality but only	636
588	marginally predicted the number of network links.	
589	This latter result could be due to the relationship	
590	between species richness and the number of links	
591	in the network, which were significantly correlated	
592	with each other. We also found that bark roughness	
593	did not significantly predict either the number of	
594	links or the centrality of lichen networks, suggesting	
595	that bark roughness has some other effect on the	
596	structure of the lichen networks. Taken together,	
597	these findings support the hypothesis that genotypic	
598	variation in a foundation species contributes to the	
599	structure of a network of interacting species.	
600	LJL: I wonder if you need to have so much	
601	on richness here. Overall, I think you want to	
602	focus on the network responses and patterns	
603	among genotype first, and then go into mech-	
604	anism later. I think we don't quite have a	
605	good mechanism yet so I don't think it needs	
606	to come up in the first paragraph of the dis-	
607	cussion.	
608	These findings point to the importance of under-	
609	standing the community level effects of genetic variation	
610	in plant functional traits and highlights the	
611	potential for indirect effects of genetic variation to	
612	propagate through networks of interacting species	
613	and trophic levels.	
614	This work corroborates previous findings of the	
615	importance of plant genetics in shaping community	
616	structure and ecosystem processes. (?)	
617	Altering the structure of interaction networks	
618	presents a means for genetic effects to be magni-	
619	fied within the system of interacting species. For	
620	example, (1) showed that the genetics based interac-	
621	tions of aphid resistant and aphid susceptible trees	
622	resulted in different interaction networks of their	
623	associated arthropod communities composed of 139	
624	species. At the scale of ecosystems, trophic net-	
625	works or food webs direct and control the rates of	
626	energy and nutrient flux (25). Furthermore, in a	
627	predator-prey-plant study, Smith (26), showed that	
628	the interactions among species across trophic levels	
629	depended on plant genotype.	
630	LJL: It could be useful to point out that	
631	our findings are not related to trophic inter-	
632	actions, which is pretty cool. Also, we talk	
633	about interaction networks but it is not clear	
634	to me if the interactions tend to be positive	634
635	or negative. Can we get at that with the ap-	635
636	proach used?	636
637	TGW: Is there any adaptive component to	637
638	the tree in having certain lichen communi-	638
639	ties? e.g., can they feed back to affect tree	639
640	performance in some way or is this a pas-	640
641	sive outcome of a trait that affects bark for	641
642	other adaptive reasons and lichens are pas-	642
643	sive players that tag along for the ride? I	643
644	could envision that lichens covering the bark	644
645	of a tree act as a barrier between insects and	645
646	pathogens, much like ectomycorrhizae cover	646
647	fine roots as a first line of defense by invad-	647
648	ing microorganisms. Uptake of N that gets	648
649	passed to the tree??	649
650	TGW: might be good to cite papers on	650
651	competition in lichens or other organizing fac-	651
652	tors to back up the least expected statement.	652
653	as epiphytes we might not expect them to	653
654	care.	654
655	TGW: I think we need to emphasize the	655
656	long-term nature of our common garden	656
657	study as very few common garden studies of	657
658	lichens likely exist. Any refs on this? If true	658
659	might want to mention this up front in intro.	659
660	MKL: Environmental filtering is evidenced	660
661	by species richness, but also possibly species	661
662	interaction varying based on environment as	662
663	networks varied in terms of sign and magni-	663
664	tude as well.	664
665	MKL: The effect of bark roughness on	665
666	network similarity was primarily genetically	666
667	based, and there are likely other factors at	667
668	play.	668
669	Discussion of network implications for sta-	669
670	stability with genetics.	670
671	Although our study was conducted with a commu-	671
672	nity of lichens, these results should be generalized	672
673	to other groups of diverse organisms around the	673
674	world that also exhibit significant genetic signals at	674
675	the community level (27, 28). In the face of the	675
676	high degree of complexity and potential context de-	676
677	pendency of ecological processes, the current study	677
678	points to the utility of considering the spatial and	678
679	temporal scales of interactions, as discussed to some	679
680	in previous studies (29–31). In the present study,	680
681	we found that community assembly processes, such	681
682	as environmental filtering and species interactions,	682

are genetically based. This is likely due, in part, to the large difference in the differences in size and longevity of the lichen and cottonwood individuals with the trees determining the environment in which the lichen occur. We suggest that future work would be aided by determining these modules within the biotic community that include species with similar differences in body-size and time-scales. As heritable variation is the raw material for natural selection to act upon, a genetic basis for interaction network structure indicates evolutionary dynamics should be considered at the community level and that conserving genetic variation is important to consider in efforts to restore or preserve complex species interactions and their associated ecosystem functions (32). With such findings, it appears that we are closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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774 **Supplementary Materials**

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

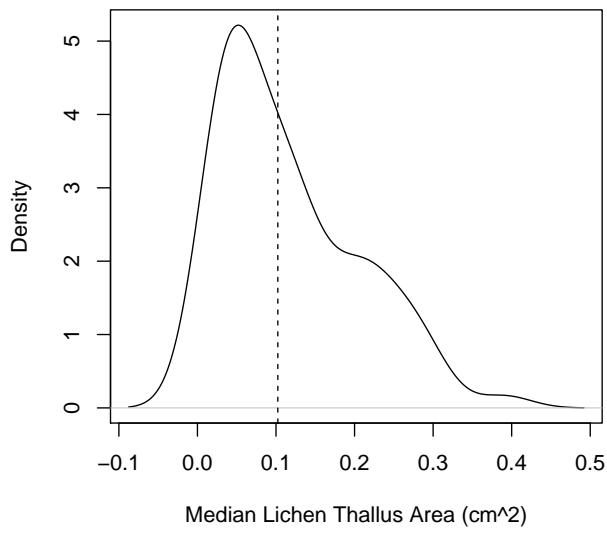


Fig. 1. Density plot of the median lichen thallus area (cm^2).

	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		