

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 eco-
2 logical networks. Initially, evolution in a com-
3 munity context was focused on examples of highly
4 co-evolved pairs of species (e.g. Darwin's famous
5 prediction of the Sphinx Moth and Christmas
6 Orchid) citeWade2007. However, studies of dif-
7 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-
8 combe2002, Strauss2007, Dicke2010, geographic mo-
9 saics of co-evolution citeThompson2005 and com-
10 munity genetics citeWhitham2006 have provided an
11 in-road for ecological network approaches citeFor-
12 tuna2009, Keith2017, Lau2016 to illuminate a more
13 complex perspective of the interface between eco-
14 logical and evolutionary dynamics. There is now
15 evidence to support that selection tends to occur
16 among groups of species citeWade2007, Pieterse2007,

Dicke2010 favoring the development of small webs
citeNuismer2006, Guimaraes2007, Gomez2009 and
that genetic variation and phylogenetic relatedness
contributes to variation in community assembly (1)
and species interactions citeWhitham2006a, Bai-
ley2009, Moya-Larano2011, which shapes the eco-
logical interaction networks (2).

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

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 network structure is genet-
ically based and is correlated with heritable bark
traits, including roughness and condensed tannins.
These findings demonstrate the importance of ge-
netic 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 work connecting ecological species networks
32 at the scale relevant to the actual workings of
33 microevolutionary processes (e.g., the genotype
34 or population level).

35 **LJL: likewise, studies of networks do not**
36 **have a genetic component**

37 Community genetics studies (3) have shown that
38 genetic variation in foundation species (4) plays a
39 significant role in defining distinct communities of in-
40 teracting organisms: such as, endophytes, pathogens,
41 lichens, arthropods, and soil microbes. Multiple stud-
42 ies have now demonstrated that genetic variation
43 influences numerous functional traits (e.g., phyto-
44 chemical, phenological, morphological) produces a
45 multivariate phenotype (5) that contributes to varia-
46 tion in associated communities (6).

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

53 Additional work has provided support for the hy-
54 pothesis that not only does composition vary among
55 genetically distinct genotypes of foundation species,
56 it also impacts the structure of the network of species
57 interactions in these communities (7, 8). Also, work
58 by (9–11) observed consistent patterns of centralized
59 interactions of species modules focused around hubs
60 of plant-fungal interactions. In other words, a small
61 number of plant and fungal symbionts tended to
62 have disproportionate numbers of interactions with
63 other species and likely are the drivers in determining
64 community assembly, structure and dynamics.

65 More on the importance of ecological networks
66 (12, 13).

67 Add a discussion of DesRoches.
68 From Thompson2014

- 69 • Pairwise interactions are usually influenced by
70 other species
- 71 • Selection favors the development of small webs
- 72 • Evolution of new lifestyles leads to changes in
73 selection on large and small webs

74 Specific hypothesis from Thompson2014
75 Selection in small webs

76 **LJL: If I recall, the Elamo paper just looks**
77 **at genetic correlations between pairwise indi-**
78 **vidual abundances. I would suggest maybe it**
79 **doesn't deserve to be in this 1st paragraph.**
80 **Perhaps it actually should be in the 2nd or**
81 **3rd paragraph, just as a reference that points**
82 **to the potential for genotype to influence net-**
83 **works. Definately our 2015 JOE paper goes**
84 **much further, too, since it has whole com-**
85 **munities being correlationed. But, again, I**
86 **woudl put both of these as citation in the com-**
87 **munity genetics paragraphs (2 of 3) instead**
88 **of the first paragrpah, which focuses on the**
89 **general network lit.**

90 Here, we investigate how genetic variation in a
91 foundation tree species determines the structure of a
92 network of interactions among a community of tree
93 associated lichen species. Using a long-term (20+
94 years), common garden experiment with replicated
95 individuals of known genetic identity and a naturally
96 established stand of *Populus angustifolia*. We focused
97 on a model community of 9 epiphytic lichen species,
98 as previous research has demonstrated significant
99 compositional responses of epiphytes to genotypic
100 variation (14, 15). In addition, the life-history char-
101 acteristics of lichens, having highly localized, direct
102 contact interactions and slow population turnover
103 rates, allowed us to assess interactions among lichen
104 species on individual trees. We hypothesize that in
105 natural systems evolution occurs in a community
106 context involving interactions of complex networks
107 of interacting species (7, 8, 16, 17). If correct, we
108 should expect to find that network structure is ge-
109 netically based in which different plant genotypes
110 support different interaction networks and that these
111 interactions networks can function as indicators of
112 ecological dynamics important for conserving biodi-
113 versity. Applying a probability-theory based network
114 modeling approach, we constructed a set of interac-
115 tion network models for the lichens associated with
116 individual trees. Using these models, we then ex-
117 amined the genetic basis of the structure of these
118 ecological networks.

119 **LJL: I agree that there is a general overar-**
120 **ching theme that evolution occurs in a com-**
121 **munity network context, but I'm not sure**
122 **that we should state that as our main hypoth-**
123 **esis. It seems more that this is a fundamen-**
124 **tal foundation for our work. The hypoth-**

sis is more what we are testing directly, but
 we don't test this directly. I guess I don't
 want to give the impression that our com-
 munities are necessarily the result of each
 species evolving into its place in the commu-
 nity on these tree genotypes (although I do
 understand this as Shuster et al 2006's funda-
 mental explanation for why we see different
 communities on different genotypes; I don't
 necessarily agree that this is the only rea-
 son we would see different communities on
 dif genotypes). Most of these are pretty gen-
 eralist lichens, which could be found on other
 deciduous trees in the surrounding city or nat-
 ural areas. I would look at it more like an
 assembling of lichen species into unique con-
 figurations on genetically different substrates.
 There may be some selection for different
 genotype of lichen during the community as-
 sembly process but we can't really tell that
 just by differences in species abundances or
 coocurrence. I guess to me the evolutionary
 context that is more directly related to this
 work is that the tree genotype is a central
 controller (indeed a sort of hub species in the
 network) of network structure. By anchor-
 ing the lichen network to tree genotype (and
 variation among networks to variation among
 tree genotypes), our study highlights the pos-
 sibility that natural selection acting on the
 trees may have an extended consequence for
 the network structure of organisms living on
 the trees...the extra thing we add to the field
 is that we show interaction networks are sen-
 sitive to genotype. I doubt the lichens have
 a direct effect on tree fitness, but favorabil-
 ity of some tree genotypes over others during
 natural selection will then go on to favor and
 disfavor certain lichen communities of differ-
 ent network structures. By being sensitive
 to tree genotype, the lichen community net-
 works are passive riders on the waves of evolu-
 tionary dynamics that occur within the tree
 species they inhabit.

MKL: In response to Lamit's comment
 above, I agree that it is not required that
 there is co-evolution. Another, perhaps sim-
 pler, explanation is that there is variation in
 environmental filtering of lichen individuals

created in part by genetic variation in tree
 individuals.

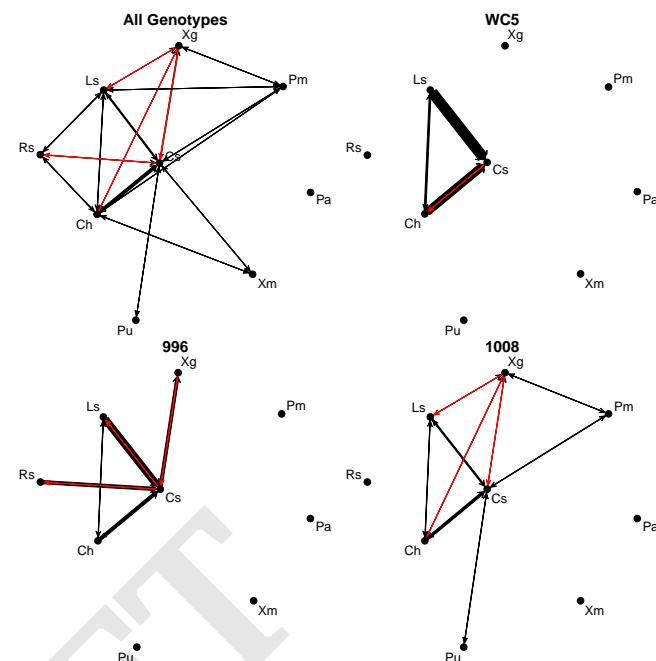


Fig. 1. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

Results

Network similarity and several tree traits were genetically based. Tree genotype was a significant predictor of network similarity ($H^2 = 0.16$, $p\text{-value} \leq 0.001$). Bark roughness ($H^2 = 0.38$, $p\text{-value} \leq 0.001$) and condensed tannin concentration ($H^2 = 0.28$, $p\text{-value} = 0.014$) also showed a signature of tree genotype (Fig. 2); however, this was not the case for other tree traits, bark pH and carbon to nitrogen ratio. Also, none of the lichen network metrics were significantly predicted by tree genotype, either at the scale of the entire network (Table 1) or for individual species (see Supporting Information). Although both showed a response to tree genotype, bark roughness and condensed tannins were not correlated (Pearson's $r = 0.084$, $p\text{-value} = 0.556$).

Tree traits and lichen community metrics were correlated with lichen networks. The genetically based traits, bark roughness and condensed tannins were

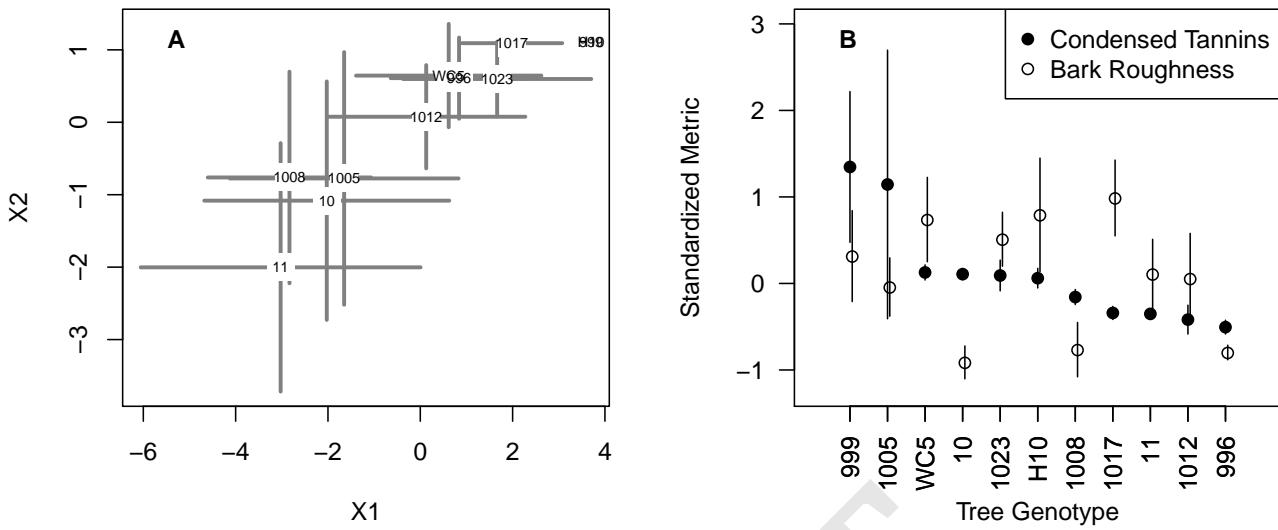


Fig. 2. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized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

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	0.4492
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.

were relativized (Mantel R = -0.09, *p*-value = 0.139) or not (Mantel R = -0.03, *p*-value = 0.573).

	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.

Materials and Methods

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

LJL: If you include the field data, I would suggest changing the topic sentence for this paragraph and adding something about the field data sampling to this paragraph. Note, also that we cored and aged each field tree so we can site the

both significant predictors of network similarity (Table 2). Bark C:N ratio was also a significant predictor of network similarity, but, as shown previously (see Table 1), there is not sufficient evidence support a genetic basis for it. Bark pH was not a significant predictor of lichen network similarity (Table 2). The abundance, richness, evenness and diversity of the bark lichen community, although also not predicted by tree genotype, were all significantly correlated with lichen network structure (Table 2). Lichen community composition was not correlated with lichen network similarity, either when species abundances

218 age range of the trees here, to make it clear they
219 are within those of the garden.

220 The study was conducted along the Weber River, UT
221 (USA), which is a cottonwood (*Populus* spp.) dominated
222 riparian ecosystem. Although two native species, *Populus*
223 *angustifolia* (James) and *Populus fremontii* (S. Watson),
224 occur here and are known to hybridize, only pure or
225 advanced generation backcrosses of *P. angustifolia* were
226 sampled. Bark lichens have been extensively studied
227 in this system and provide an ideal system in which to
228 observe and model lichen interaction networks, as their
229 sessile nature permits accurate identification of individuals
230 (18).

231 A long-term, common garden experiment was used
232 to isolate the effect of tree genotype from the effect of
233 the localized microenvironment associated with each indi-
234 vidual and spatial autocorrelation. Established in 1992,
235 asexually propagated clones of genotyped *P. angustifolia*
236 individuals were obtained from wild collections and
237 planted in fully randomized design at the Ogden Nature
238 Center, Ogden, UT. From the population of established
239 individuals in the common garden, we chose a total of
240 thirteen genotypes, replicated between 3 and 8 times each,
241 for sampling.

242 **Bark Lichen Observations.** LJL: A total pain in
243 the butt but it might be good just to double
244 check that our exact species that occurred in each
245 quadrat are the same as the species that I found
246 in the composition data. No doubt that I would
247 have found more species but it might be a good
248 double check of things just to see if they roughly
249 correspond. I can do this if you send me your
250 matrix.

251 LJL: I think this sentence is good and these
252 lichens tend to be ones that were rarer or really
253 easy to miss. So, the list of species you picked
254 up sounds legit to me. One thing... above you
255 say 14 species but only 9 were found. That is a
256 bit redundant with this next sentence that high-
257 lights which species were found and which were
258 not observed. I would just stick with the second
259 sentence and delete the first sentence.

260 On each tree, presence or absence of each lichen species
261 was assessed in 50 total 1 cm² cells arrayed in a checker-
262 board pattern. Given the small size and sessile nature
263 of lichens, we were able to rapidly assess lichen interac-
264 tions by quantifying thalli in close contact. Sampling
265 was restricted to the northern aspect of the trunk to
266 maximize the abundance of lichen and control for the
267 effect of trunk aspect. Two adjacent 10 cm² quadrats
268 centered at 50 cm and 85 cm from ground level were
269 sampled (Fig 3 A and B). The observed lichen commu-
270 nity included (abbreviations are given for species present
271 in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
272 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella*

273 *subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*,
274 Pm = *Phyciella melanura*, Pa = *Physcia adscendens*,
275 Pu = *Physcia undulata*. Several other species were not
276 observed in the present study but are known to occur
277 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
278 *ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

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

291 **LJL: My method was overall visual % cover. I**
292 **didn't count P/A of rough bark in each square.**
293 **So, yo might just want to site our Ecology lichen**
294 **paper as showing rough bark is important, but**
295 **not cite it as the same method. No doubt the**
296 **methods should yeild similar results but the ap-**
297 **proach is slightly different.**

298 We also measured the roughness of the bark on indi-
299 vidual trees within each sampling area. Bark roughness
300 had previously been shown to be an important tree trait
301 influencing bark lichens (18) that is under strong genetic
302 control (19). For each tree, the number of cells contain-
303 ing disrupted, fissured bark were counted within each
304 quadrat. The number of rough bark containing cells were
305 then summed and divided by the total number of cells
306 surveyed. This was done for all quadrats on all trees in
307 which lichen communities were also observed.

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

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

318 We used the observations of lichens in the 1 cm² cells
319 on individual trees of *P. angustifolia*. Unipartite networks
320 were generated using the conditional probabilities of each
321 species pair, i.e. the probability of observing one species
322 given an observation of another species $P(S_i|S_j)$, based on
323 the method developed by (20). To calculate conditional
324 probabilities, we quantified the individual probabilities
325 of species occurrences $P(S_i)$ and the joint probability
326 of co-occurrences $P(S_i, S_j)$ using the frequencies of each
327 species and their co-occurrences. We were then able to

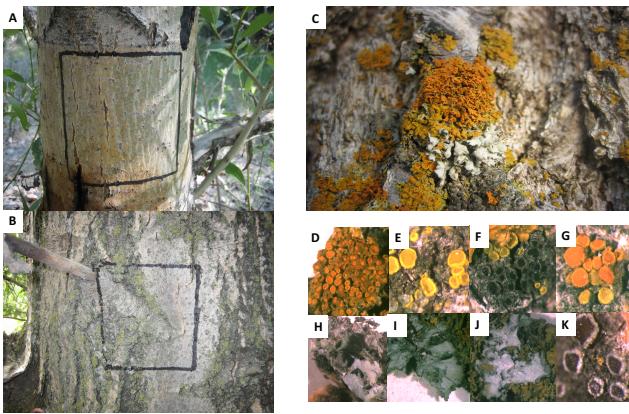


Fig. 3. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community 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. Naesbourn (D-K).

328 calculate the conditional probabilities of each species pair
 329 as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
 330 This yielded a matrix that could possibly be asymmetric, i.e.
 331 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another
 332 important property of this matrix is that the diagonal
 333 (S_{ii}) was equal to one for all species present and zero for
 334 species that were not observed in any cell.

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

341 We then applied an analytical procedure to remove
 342 non-significant links between species. This procedure
 343 determines if the joint probability of a species pair
 344 (i.e. $P(S_i, S_j)$) is different from zero (Fig. 4). Here, a
 345 confidence interval $CI_{95\%}$ is calculated as as $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected fre-
 346 quency of co-occurrences $E(S_i S_j)$ is the total number of cells surveyed (N) times the independent probabili-
 347 ties of each species $P(S_i) * P(S_j)$, $Z_{95\%}$ is the Z-score
 348 for 95% from a Z-distribution and the expected vari-
 349 ance of $E(S_i S_j)$ is the total number of cells times the
 350 expected probability of $S_i S_j$ and its compliment (i.e.
 351 $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the
 352 observed number of co-occurrence falls outside of the
 353 confidence interval, the joint probability $P(S_i, S_j)$ is de-
 354 termined to be equal to the product of the individual
 355 probabilities (i.e. $P(S_i) P(S_j)$), and the conditional prob-
 356 ability reduces to the individual probability of that species
 357 $P(S_i)$. Therefore, unless the co-occurrence of a species
 358 pair falls outside the confidence interval, the probabil-

ity that the observation of one species given the other
 362 is no different than simply observing that species alone.
 363 This enables us to remove links from a given network by
 364 re-scaling the resulting conditional probabilities by sub-
 365 tracting the individual probabilities from the conditional
 366 probabilities (i.e. how different the conditional probability
 367 is from the independent probability), which makes any
 368 species with a non-significant conditional probability zero.
 369 The resulting matrix ($D = D_{ij}$) can be interpreted as
 370 how one species impacts another with zero being no effect
 371 and values less than or greater than zero interpreted as
 372 negative and positive effects, respectively. Here, we will
 373 refer to this matrix (D) as an interaction matrix with
 374 the properties that it can be asymmetric (i.e. P_{ij} does not
 375 necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e.
 376 a species does not influence its own probability of being
 377 observed).

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

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

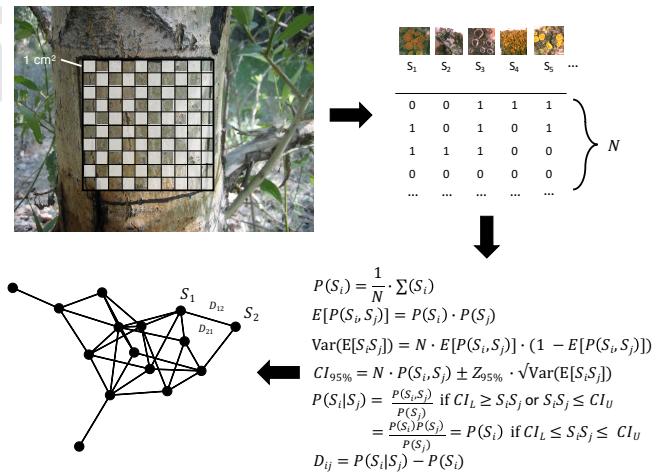


Fig. 4. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (20), 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 .

389 **LJL:** I like the details here. THe one thing is
 390 that it sort of makes the reader think there is only
 391 one quadrat on a tree but infact there were two. I
 392 think you want to make sure to be explicit about

393 the two. For analytical purposes, was all the data
394 lumped so there was really functionally a 20cm
395 by 10cm grid (just split into two pieces). Or, was
396 the network made for each of the two grids and
397 them averaged or combined in some way? My
398 understanding is that it was more the first than
399 the latter.

400 MKL: Yeah, it was the latter. I'm using two
401 quadrats lumped together. I'll add more text
402 here to clarify that.

403 **Statistical Analyses, Software and Data.** We used a com-
404 bination of parametric and non-parametric, permutation
405 based frequentist statistical analyses to test for the effects
406 of genetic variation on lichen communities and their inter-
407 action networks. To assess the effect of genotype on uni-
408 variate responses, we used additive, random effects models
409 with Restricted Maximum Likelihood (REML). We used
410 a combination of Least Squares Regression, Analysis of
411 Variance (ANOVA) and correlation tests to quantify and
412 test for the relationship among other variables. Bark
413 roughness, lichen cover and species richness were square-
414 root transformed to meet the assumptions of homogeneity
415 of variance and normality for these tests.

416 For multivariate response variables, such as lichen com-
417 munity composition and network structure, we used dis-
418 tance based multivariate statistical approaches, including
419 Permutational Analysis of Variance (PerMANOVA) and
420 Mantel tests. For all analyses, community composition
421 was relativized by species maxima to reduce the effect
422 of the highly abundant *X. galericulata*. For community
423 composition we used Bray-Curtis dissimilarity, which has
424 optimal performance with count data citepMinchen1998.
425 To quantify the similarity of lichen networks among indi-
426 vidual trees, we calculated the pairwise Euclidean distance
427 of the **D** interaction matrices among all pairs of trees.

428 For visualization of multivariate patterns, we used Non-
429 metric Multi-Dimensional Scaling (NMDS) citeecodist
430 to produce dimensionally reduced ordinations of these
431 multi-variate responses and fitted vectors for continuous
432 predictor variables to the ordinated values citevegan.
433 Using random initial configurations with a maximum of 500
434 iterations and a change in stress threshold of less than
435 10^{-12} . Final configurations has the lowest stress with at
436 most a stress level of 0.10.

437 For each network, we also calculated two network met-
438 rics that measure different structural aspects. We cal-
439 culated the number of interactions or "links" in each
440 network, which provides a measure of the size of the net-
441 work citepLau2015, Borrett2014. We also calculated the
442 centralization of each network, which measures the even-
443 ness of the distribution of interactions among the species
444 in the network citepButts2005. In a network with a low
445 level of centralization species have similar amount of inter-
446 action in the network, while a network with a high level of
447 centralization tends to one or small subset of species that

448 interact with other species. We used a related function
449 to calculate the centrality of each species in each network
450 as well. Although there are many other metrics, see (21),
451 we focus on a subset for the sake of simplicity and be-
452 cause some metrics are not appropriate for our relatively
453 small communities. **In particular, we do not present**
454 **analysis of the modularity (i.e. the degree of sub-**
455 **grouping) because our community has relatively**
456 **few species to form modules.** As with the other re-
457 sponse variables, the number of links was log-transformed
458 and centralization scores were square-root transformed to
459 meet variance and normality assumptions.

460 **LJL: I suggest deleting the highlighted part.**
461 **And, just changing the sentence above it to "...be-**
462 **cause some metric (e.g., modularity) are not ap-**
463 **propriate..." Too much emphasis on caviots will**
464 **make some readers be uncertain. But, also, you**
465 **can save some space that way.**

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

Discussion

473 - Genotypic variation can lead to network variation
474 - Traits could both share or be a causal mechanism
475 with lichen networks. - Network structure is linked
476 to function and dynamics. E.g. stability

477 - Community level selection may be possible, but
478 this is not a necessary factor for evolutionary dynam-
479 ics to be relevant to ecological networks

480 - What are the conditions in which genetically
481 based ecological network structure could have an
482 effect?

483 - Network structure serves to amplify the signal
484 of genetics

485 **TGW: I think window is too vague and this**
486 **topic sentence needs to be much stronger for**
487 **a journal like PNAS. Might be stronger by**
488 **saying "Our findings argue there is a genetic**
489 **component to network structure, which im-**
490 **pplies that network structure could be subject**
491 **to selection and networks can evolve."**

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

- 498 • Genetic assembly rule = similar genetics will 545
 499 have more similar communities 546
 500 • What we don't know is whether or not these 547
 501 interactions will also lead to similar interactions 548
 502 among other species. 549
 503 • Thus, it would be possible for genetics to not 550
 504 only influence other species directly, but also 551
 505 indirectly by influencing the interactions among 552
 506 other species. 553

507 We observed significant lichen interaction struc- 554
 508 ture that varied among genotypes of a foundation 555 tree species, narrowleaf cottonwood (*P. angustifo-* 556 *lia*). We found that a genetically based trait, bark 557 roughness, partially explained the variation in lichen 558 interaction networks. Some of this variation in lichen 559 networks was related to both the overall abundance 560 and species richness of lichen; though, statistically 561 controlling for the effect of genotype on these vari- 562 ables indicates that a significant portion of the varia- 563 nce in lichen species richness is due to a factor 564 other than tree genotype. By using network metrics, 565 we were also able to probe for specific characteris- 566 tics of how these networks were responding to tree 567 genotype. We found that both number of links and 568 the centralization of the networks were highly corre- 569 lated with network similarity and that tree genotype 570 significantly predicted network centrality but only 571 marginally predicted the number of network links. 572 This latter result could be due to the relationship 573 between species richness and the number of links 574 in the network, which were significantly correlated 575 with each other. We also found that bark roughness 576 did not significantly predict either the number of 577 links or the centrality of lichen networks, suggesting 578 that bark roughness has some other effect on the 579 structure of the lichen networks. Taken together, 580 these findings support the hypothesis that genotypic 581 variation in a foundation species contributes to the 582 structure of a network of interacting species. 583

584 **LJL: I wonder if you need to have so much 585 on richness here. Overall, I think you want to 586 focus on the network responses and patterns 587 among genotype first, and then go into mech- 588 anism later. I think we don't quite have a 589 good mechanism yet so I don't think it needs 590 to come up in the first paragrpah of the dis- 591 cussion.**

592 These findings point to the importance of under- 593 standing the community level effects of genetic vari- 594 ation in plant functional traits and highlights the 595 potential for indirect effects of genetic variation to 596 propagate through networks of interacting species 597 and trophic levels. 598

599 This work corroborates previous findings of the 600 importance of plant genetics in shaping community 601 structure and ecosystem processes. citepBangert2008 602

603 Altering the structure of interaction networks 604 presents a means for genetic effects to be magni- 605 fied within the system of interacting species. For 606 example, (7) showed that the genetics based interac- 607 tions of aphid resistant and aphid susceptible trees 608 resulted in different interaction networks of their 609 associated arthropod communities composed of 139 610 species. At the scale of ecosystems, trophic net- 611 works or food webs direct and control the rates of 612 energy and nutrient flux (22). Furthermore, in a 613 predator-prey-plant study, Smith (23), showed that 614 the interactions among species across trophic levels 615 depended on plant genotype. 616

617 **LJL: It could be useful to point out that 618 our findings are not related to trophic inter- 619 actions, which is pretty cool. Also, we talk 620 about interaction networks but it is not clear 621 to me if the interactions tend to be positive 622 or negative. Can we get at that with the ap- 623 proach used?** 624

625 **TGW: Is there any adaptive component to 626 the tree in having certain lichen communi- 627 ties? e.g., can they feed back to affect tree 628 performance in some way or is this a pas- 629 sive outcome of a trait that affects bark for 630 other adaptive reasons and lichens are pas- 631 sive players that tag along for the ride? I 632 could envision that lichens covering the bark 633 of a tree act as a barrier between insects and 634 pathogens, much like ectomycorrhizae cover 635 fine roots as a first line of defense by invad- 636 ing microorganisms. Uptake of N that gets 637 passed to the tree??** 638

639 **TGW: might be good to cite papers on 640 competition in lichens or other organizing fac- 641 tors to back up the least expected statement. 642 as epiphytes we might not expect them to 643 care.** 644

645 **TGW: I think we need to emphasize the 646 long-term nature of our common garden** 647

594 study as very few common garden studies of
595 lichens likely exist. Any refs on this? If true
596 might want to mention this up front in intro.

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

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

606 Discussion of network implications for sta- 607 bility with genetics.

608 Although our study was conducted with a commu-
609 nity of lichens, these results should be generalized
610 to other groups of diverse organisms around the
611 world that also exhibit significant genetic signals at
612 the community level (24, 25). In the face of the
613 high degree of complexity and potential context de-
614 pendence of ecological processes, the current study
615 points to the utility of considering the spatial and
616 temporal scales of interactions, as discussed to some
617 in previous studies (26–28). In the present study,
618 we found that community assembly processes, such
619 as environmental filtering and species interactions,
620 are genetically based. This is likely due, in part,
621 to the large difference in the differences in size and
622 longevity of the lichen and cottonwood individuals
623 with the trees determining the environment in which
624 the lichen occur. We suggest that future work would
625 be aided by determining these modules within the
626 biotic community that include species with similar
627 differences in body-size and time-scales. As heritable
628 variation is the raw material for natural selection
629 to act upon, a genetic basis for interaction network
630 structure indicates evolutionary dynamics should be
631 considered at the community level and that con-
632 serving genetic variation is important to consider in
633 efforts to restore or preserve complex species inter-
634 actions and their associated ecosystem functions (29).
635 With such findings, it appears that we are closer to
636 understanding the evolutionary drivers of Darwin’s
637 entangled bank and the interconnectedness of species
638 in complex communities.

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645

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