

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* | com-
mon garden

- 1 • Evolution occurs in complex ecosystems
- 2 • Community genetics studies have demonstrated
3 the importance of genetics and evolutionary dy-
4 namics in structuring communities and ecosys-
5 tems
- 6 • Community composition, ecosystem processes
7 have been shown to vary in response to the
8 genetic variation of foundation species
- 9 • Previous studies have examined aspects of net-
10 works but to date, no studies have examined the
11 genetic basis of networks of interacting species
- 12 • Here we examine the genetic basis of network
13 structure on a community of sessile lignicolous
14 (i.e. bark) lichen on cottonwood trees. This

system is ideal for examining for this kind of
study because it:

- 15 1. Lichen do not move among trees, although
16 asexual propagules could move to other
17 trees in the garden
- 18 2. Is not unapproachably complex
- 19 3. Is comprised of distinct individuals that
20 are small enough for an entire community
21 to occur on a single tree but not too small
22 to be quantified easily

- 23 • We hypothesize that trees will vary in some
24 phenotypic traits, such that trees of the same

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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27 genotype will tend to have similar traits producing
28 similarities in lichen network structure. To
29 test this hypothesis, we use a common garden
30 of cottonwood trees with lichen communities.

31 **E**volution occurs in the context of complex eco-
32 logical networks. Initially, evolution in a com-
33 munity context was focused on examples of highly
34 co-evolved pairs of species (e.g. Darwin's famous
35 prediction of the Sphinx Moth and Christmas
36 Orchid) citeWade2007. However, studies of dif-
37 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-
38 combe2002, Strauss2007, Dicke2010, geographic mo-
39 saics of co-evolution citeThompson2005 and com-
40 munity genetics citeWhitham2006 have provided an
41 in-road for ecological network approaches citeFor-
42 tuna2009, Keith2017, Lau2016 to illuminate a more
43 complex perspective of the interface between eco-
44 logical and evolutionary dynamics. There is now
45 evidence to support that selection tends to occur
46 among groups of species citeWade2007, Pieterse2007,
47 Dicke2010 favoring the development of small webs
48 citeNuismer2006, Guimaraes2007, Gomez2009 and
49 that genetic variation and phylogenetic relatedness
50 contributes to variation in community assembly (1)
51 and species interactions citeWhitham2006a, Bai-
52 ley2009, Moya-Larano2011, which shapes the eco-
53 logical interaction networks (2).

54 **LJL: I think a key point in the first para-**
55 **graph is to point out that networks are be-**
56 **lieve in part to emerge out of complex evo-**
57 **lutionary interactions. But, to-date, the pat-**
58 **terns observed in networks are primarily only**
59 **on the scale of species interactions and phylo-**
60 **genetic signature. There has yet to be much**
61 **work connecting ecological species networks**
62 **at the scale relevant to the actual workings of**
63 **microevolutionary processes (e.g., the geno-**
64 **type or population level).**

65 **LJL: likewise, studies of networks do not**
66 **have a genetic component**

67 Community genetics studies (3) have shown that
68 genetic variation in foundation species (4) plays a
69 significant role in defining distinct communities of in-
70 teracting organisms: such as, endophytes, pathogens,
71 lichens, arthropods, and soil microbes. Multiple stud-
72 ies have now demonstrated that genetic variation
73 influences numerous functional traits (e.g., phyto-
74 chemical, phenological, morphological) produces a
75 multivariate phenotype (5) that contributes to varia-

tion in associated communities (6).

76
77 **LJL: I think the thing that needs to emerge**
78 **in this paragraph is that even though there**
79 **is some incling that genotype is relevant in**
80 **community networks, as based on our few**
81 **past studies, no study has truely examined**
82 **networks on individual trees.**

83 Additional work has provided support for the hy-
84 pothesis that not only does composition vary among
85 genetically distinct genotypes of foundation species,
86 it also impacts the structure of the network of species
87 interactions in these communities (7, 8). Also, work
88 by (9–11) observed consistent patterns of centralized
89 interactions of species modules focused around hubs
90 of plant-fungal interactions. In other words, a small
91 number of plant and fungal symbionts tended to
92 have disproportionate numbers of interactions with
93 other species and likely are the drivers in determining
94 community assembly, structure and dynamics.

95 More on the importance of ecological networks
96 (12, 13).

97 Add a discussion of DesRoches.

98 From Thompson2014

- 99 • Pairwise interactions are usually influenced by
100 other species
- 101 • Selection favors the development of small webs
- 102 • Evolution of new lifestyles leads to changes in
103 selection on large and small webs

104 Specific hypothesis from Thompson2014

105 Selection in small webs

106 **LJL: If I recall, the Elamo paper just looks**
107 **at genetic correlations between pairwise indi-**
108 **vidual abundances. I would suggest maybe it**
109 **doesn't deserve to be in this 1st paragraph.**
110 **Perhaps it actually should be in the 2nd or**
111 **3rd paragraph, just as a reference that points**
112 **to the potential for genotype to influence net-**
113 **works. Definately our 2015 JOE paper goes**
114 **much further, too, since it has whole com-**
115 **munities being correlated. But, again, I**
116 **would put both of these as citation in the com-**
117 **munity genetics paragraphs (2 of 3) instead**
118 **of the first paragrpah, which focuses on the**
119 **general network lit.**

120 Here, we investigate how genetic variation in a
121 foundation tree species determines the structure of a
122 network of interactions among a community of tree

associated lichen species. Using a long-term (20+ years), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *Populus angustifolia*. We focused on a model community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (14, 15). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover rates, allowed us to assess interactions among lichen species on individual trees. We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (7, 8, 16, 17). If correct, we should expect to find that network structure is genetically based in which different plant genotypes support different interaction networks and that these interactions networks can function as indicators of ecological dynamics important for conserving biodiversity. Applying a probability-theory based network modeling approach, we constructed a set of interaction network models for the lichens associated with individual trees. Using these models, we then examined the genetic basis of the structure of these ecological networks.

LJL: I agree that there is a general overarching theme that evolution occurs in a community network context, but I'm not sure that we should state that as our main hypothesis. It seems more that this is a fundamental foundation for our work. The hypothesis 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 communities are necessarily the result of each species evolving into its place in the community on these tree genotypes (although I do understand this as Shuster et al 2006's fundamental explanation for why we see different communities on different genotypes; I don't necessarily agree that this is the only reason we would see different communities on dif genotypes). Most of these are pretty generalist lichens, which could be found on other deciduous trees in the surrounding city or natural areas. I would look at it more like an assembling of lichen species into unique configurations on genetically different substrates.

There may be some selection for different genotype of lichen during the community assembly process but we can't really tell that just by differences in species abundances or coocurneces. I guess to me the evolutionary context that is more direclty 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 anchoring the lichen network to tree genotype (and variation among networks to variation among tree genotypes) , our study highlights the possibility 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 sensitive to genotype. I doubt the lichens have a direct effect on tree fitness, but favorability of some tree genotypes over others during natural selection will then go on to favor and disfavor certain lichen communities of different network structures. By being sensitive to tree genotype, the lichen community networks are passive riders on the waves of evolutionary dynamics that occur within the tree species they inhabit.

MKL: In response to Lamit's comment above, I agree that it is not reuquired that there is co-evolution. Another, perhaps simpler, explanation is that there is variation in environmental filtering of lichen individuals created in part by genetic variation in tree individuals.

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 con-

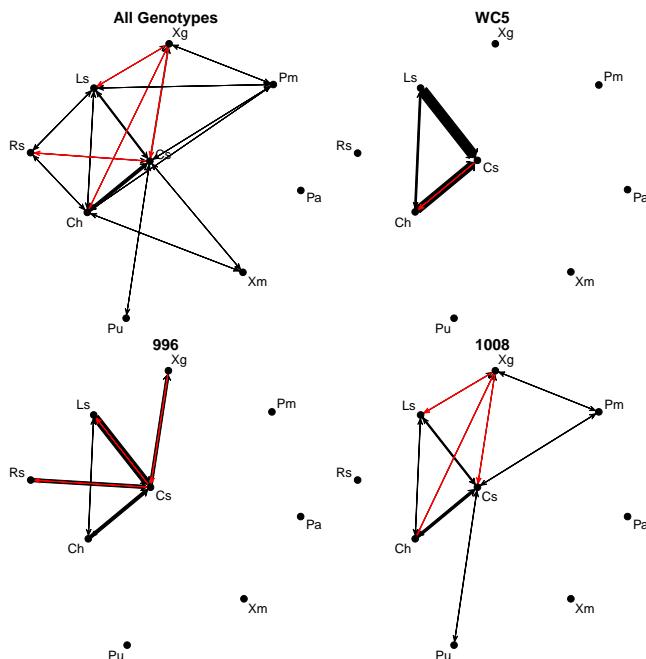


Fig. 1. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

220 densed tannins were not correlated (Pearson's $r =$
221 0.084, $p\text{-value} = 0.556$).

222 Tree traits and lichen community metrics were cor-
223 related with lichen networks. The genetically based
224 traits, bark roughness and condensed tannins were
225 both significant predictors of network similarity (Ta-
226 ble 2). Bark C:N ratio was also a significant predictor
227 of network similarity, but, as shown previously (see
228 Table 1), there is not sufficient evidence support a
229 genetic basis for it. Bark pH was not a significant
230 predictor of lichen network similarity (Table 2). The
231 abundance, richness, evenness and diversity of the
232 bark lichen community, although also not predicted
233 by tree genotype, were all significantly correlated
234 with lichen network structure (Table 2). Lichen com-
235 munity composition was not correlated with lichen
236 network similarity, either when species abundances
237 were relativized (Mantel R = -0.09, $p\text{-value} = 0.139$)
238 or not (Mantel R = -0.03, $p\text{-value} = 0.573$).

239 Materials and Methods

240

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.

	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 net-
work similarity.**

MKL: Need to add more citations of Lamits or
other lichen studies. 241
242

LJL: If you include the field data, I would sug-
243 gest changing the topic sentence for this para-
244 graph and adding something about the field data
245 sampling to this paragrphah. Note, also that we
246 cored and aged each field tree so we can site the
247 age range of the trees here, to make it clear they
248 are within those of the garden. 249

The study was conducted along the Weber River, UT
250 (USA), which is a cottonwood (*Populus* spp.) dominated
251 riparian ecosystem. Although two native species, *Populus*
252 *angustifolia* (James) and *Populus fremontii* (S. Watson),
253 occur here and are known to hybridize, only pure or
254 advanced generation backcrosses of *P. angustifolia* were
255 sampled. Bark lichens have been extensively studied
256 in this system and provide an ideal system in which to
257 observe and model lichen interaction networks, as their
258 sessile nature permits accurate identification of individuals
259 (18). 260

A long-term, common garden experiment was used
261 to isolate the effect of tree genotype from the effect of
262 the localized microenvironment associated with each indi-
263 vidual and spatial autocorrelation. Established in 1992,
264

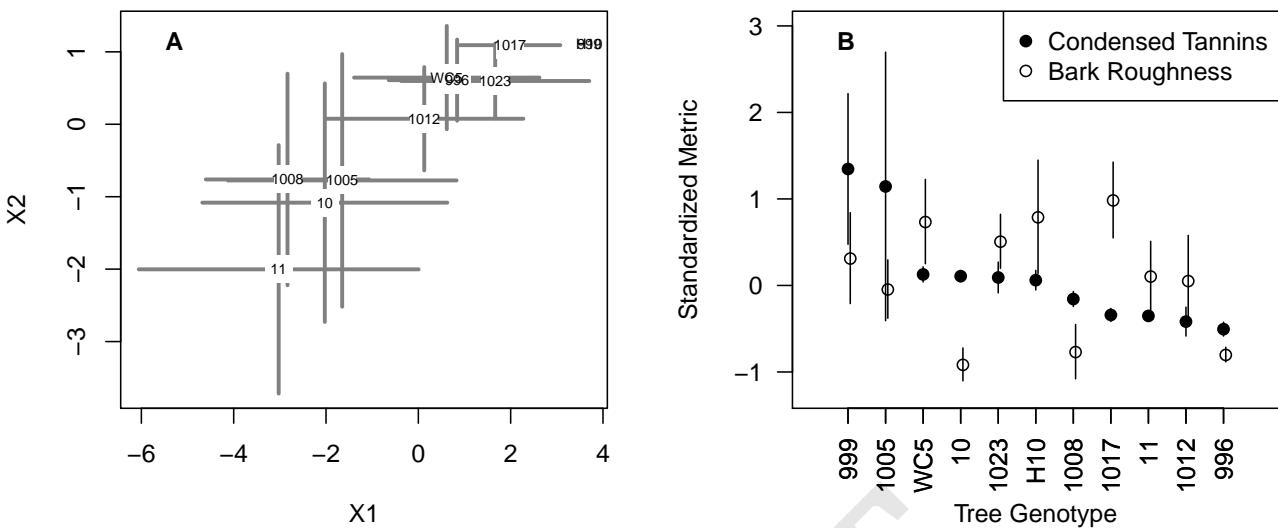


Fig. 2. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized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

265 asexually propagated clones of genotyped *P. angustifolia* individuals were obtained from wild collections and
266 planted in fully randomized design at the Ogden Nature
267 Center, Ogden, UT. From the population of established
268 individuals in the common garden, we chose a total of
269 thirteen genotypes, replicated between 3 and 8 times each,
270 for sampling.
271

272 **Bark Lichen Observations. LJL:** A total pain in
273 the butt but it might be good just to double
274 check that our exact species that occurred in each
275 quadrat are the same as the species that I found
276 in the composition data. No doubt that I would
277 have found more species but it might be a good
278 double check of things just to see if they roughly
279 correspond. I can do this if you send me your
280 matrix.

281 **LJL:** I think this sentence is good and these
282 lichens tend to be ones that were rarer or really
283 easy to miss. So, the list of species you picked
284 up sounds legit to me. One thing... above you
285 say 14 species but only 9 were found. That is a
286 bit redundant with this next sentence that highlights
287 which species were found and which were
288 not observed. I would just stick with the second
289 sentence and delete the first sentence.

290 On each tree, presence or absence of each lichen species
291 was assessed in 50 total 1 cm^2 cells arrayed in a checker-
292 board pattern. Given the small size and sessile nature
293 of lichens, we were able to rapidly assess lichen interac-

tions by quantifying thalli in close contact. Sampling
294 was restricted to the northern aspect of the trunk to
295 maximize the abundance of lichen and control for the
296 effect of trunk aspect. Two adjacent 10 cm^2 quadrats
297 centered at 50 cm and 85 cm from ground level were
298 sampled (Fig 3 A and B). The observed lichen commu-
299 nity included (abbreviations are given for species present
300 in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
301 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella*
302 *subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*,
303 Pm = *Phyciella melanura*, Pa = *Physcia adscendens*,
304 Pu = *Physcia undulata*. Several other species were not
305 obesrvd in the present study but are known to occur
306 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
307 *ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

308
309 The cell size and checkerboard sampling pattern was
310 chosen to isolate the individuals in each cell. In a previous
311 survey of lichen thallus size in this common garden, we
312 had observed a median thallus size of $0.12 \pm 0.001\text{ cm}^2$ (1
313 S.E.) (see Supporting Information). Based on the median
314 thallus size, we expected thalli observed in each cell to
315 generally be spatially independent of thalli present in
316 other cells but exposed to similar micro-environmental
317 conditions created by the bark and the location of the
318 sampling area on an individual tree. Therefore, we were
319 confident in treating the cell-wise observations in quadrats
320 as independent with respect to lichen-lichen interactions.

321 **LJL: My method was overall visual % cover. I**
322 **didn't count P/A of rough bark in each square.**
323 **So, yo might just want to site our Ecology lichen**

324 paper as showing rough bark is important, but
 325 not cite it as the same method. No doubt the
 326 methods should yeild similar results but the ap-
 327 proach is slightly different.

328 We also measured several bark traits for each tree:
 329 including, bark roughness and chemistry.

330 To quantify bark roughness, the number of cells con-
 331 taining disrupted, fissured bark were counted within each
 332 quadrat. The number of rough bark containing cells were
 333 then summed and divided by the total number of cells
 334 surveyed. This was done for all quadrats on all trees in
 335 which lichen communities were also observed. We also
 336 measured several bark chemistry traits: including, pH,
 337 condensed tannins, carbon and nitrogen.

338 **MKL: will need Lamit to add the chemistry**
 339 **methods.**

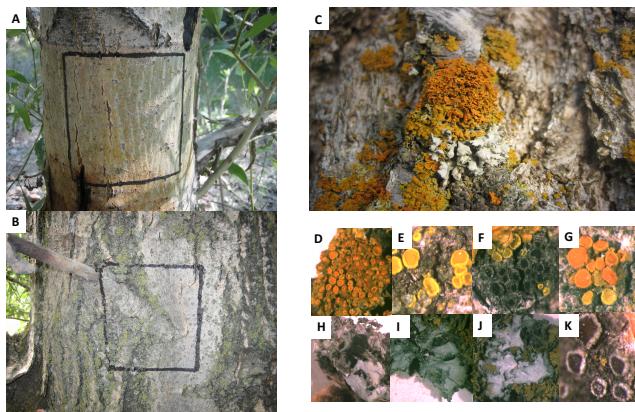


Fig. 3. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community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

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

347 We used the observations of lichens in the 1 cm^2 cells
 348 on individual trees of *P. angustifolia*. Unipartite networks
 349 were generated using the conditional probabilities of each
 350 species pair, i.e. the probability of observing one species
 351 given an observation of another species $P(S_i|S_j)$, based on
 352 the method developed by (20). To calculate conditional
 353 probabilities, we quantified the individual probabilities
 354 of species occurrences $P(S_i)$ and the joint probability
 355 of co-occurrences $P(S_i, S_j)$ using the frequencies of each
 356 species and their co-occurrences. We were then able to

357 calculate the conditional probabilities of each species pair
 358 as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
 359 This yielded a matrix that could possibly be asymmetric, i.e.
 360 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another
 361 important property of this matrix is that the diagonal
 362 (S_{ii}) was equal to one for all species present and zero for
 363 species that were not observed in any cell.

364 **MKL: regarding Lamit's question about the**
 365 **symmetry, the point is that direction of the in-**
 366 **teraction matters. The effect of species A on B**
 367 **can be different from B on A. No the matrix is**
 368 **not necessarily triangular (triangular being that**
 369 **the matrix either above or below the diagonal is**
 370 **completely zero).**

371 We then applied an analytical procedure to remove
 372 non-significant links between species. This procedure
 373 determines if the joint probability of a species pair
 374 (i.e. $P(S_i, S_j)$) is different from zero (Fig. 4). Here, a
 375 confidence interval $CI_{95\%}$ is calculated as $CI_{95\%} =$
 376 $E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected
 377 frequency of co-occurrences $E(S_i S_j)$ is the total number
 378 of cells surveyed (N) times the independent probabili-
 379 ties of each species $P(S_i) * P(S_j)$, $Z_{95\%}$ is the Z-score
 380 for 95% from a Z-distribution and the expected var-
 381 ance of $E(S_i S_j)$ is the total number of cells times the
 382 expected probability of $S_i S_j$ and its compliment (i.e.
 383 $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the
 384 observed number of co-occurrence falls outside of the
 385 confidence interval, the joint probability $P(S_i, S_j)$ is de-
 386 termined to be equal to the product of the individual
 387 probabilities (i.e. $P(S_i)P(S_j)$), and the conditional prob-
 388 ability reduces to the individual probability of that species
 389 $P(S_i)$. Therefore, unless the co-occurrence of a species
 390 pair falls outside the confidence interval, the probabili-
 391 ty that the observation of one species given the other
 392 is no different than simply observing that species alone.
 393 This enables us to remove links from a given network by
 394 re-scaling the resulting conditional probabilities by sub-
 395 tracting the individual probabilities from the conditional
 396 probabilities (i.e. how different the conditional probability
 397 is from the independent probability), which makes any
 398 species with a non-significant conditional probability zero.
 399 The resulting matrix ($D = D_{ij}$) can be interpreted as
 400 how one species impacts another with zero being no effect
 401 and values less than or greater than zero interpreted as
 402 negative and positive effects, respectively. Here, we will
 403 refer to this matrix (D) as an interaction matrix with
 404 the properties that it can be asymmetric (i.e. P_{ij} does not
 405 necessarily equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e.
 406 a species does not influence its own probability of being
 407 observed).

408 **LJL: This approach seems legit and it sound**
 409 **impressive. However, I admit that I think it is**
 410 **a bit above my head and possibly Tom's, too. I**
 411 **have no doubt you did everything correct. But,**
 412 **it might be wise to get a friendly review from a**

413 **mathy person just to be on the safe side. Perhaps**
 414 **Stuart in NC, or Aaron Ellison.**

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

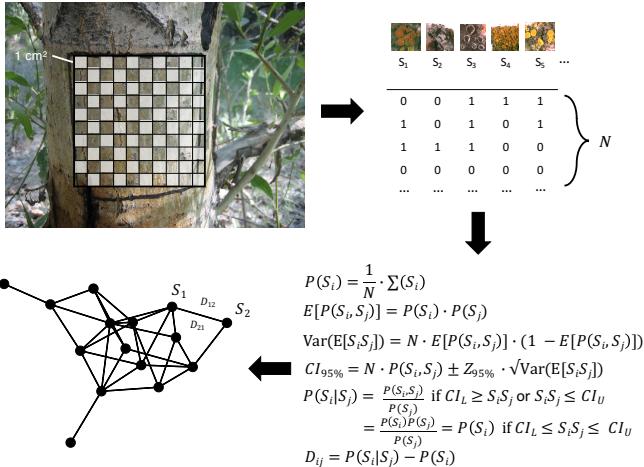


Fig. 4. Lichen interaction networks were constructed by conducting field observations in 1 cm² cells within a 10 cm² grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (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 .

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

429 **MKL: Yeah, it was the latter. I'm using two**
 430 **quadrats lumped together. I'll add more text**
 431 **here to clarify that.**

432 **Statistical Analyses, Software and Data.** We used a com-
 433 bination of parametric and non-parametric, permutation
 434 based frequentist statistical analyses to test for the effects
 435 of genetic variation on lichen communities and their inter-
 436 action networks. To assess the effect of genotype on uni-
 437 variate responses, we used additive, random effects models
 438 with Restricted Maximum Likelihood (REML). We used
 439 a combination of Least Squares Regression, Analysis of
 440 Variance (ANOVA) and correlation tests to quantify and
 441 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 citepMinchen1998. To quantify the similarity of lichen networks among individual trees, we calculated the pairwise Euclidean distance of the **D** interaction matrices among all pairs of trees.

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

For each network, we also calculated two network metrics that measure different structural aspects. We calculated the number of interactions or “links” in each network, which provides a measure of the size of the network citeLau2015, Borrett2014. We also calculated the centralization of each network, which measures the evenness of the distribution of interactions among the species in the network citeButts2005. In a network with a low level of centralization species have similar amount of interaction in the network, while a network with a high level of centralization tends to one or small subset of species that interact with other species. We used a related function to calculate the centrality of each species in each network as well. Although there are many other metrics, see (21), we focus on a subset for the sake of simplicity and because some metrics are not appropriate for our relatively small communities. **In particular, we do not present analysis of the modularity (i.e. the degree of subgrouping) because our community has relatively few species to form modules.** As with the other response variables, the number of links was log-transformed and centralization scores were square-root transformed to meet variance and normality assumptions.

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

We have made all code and data available online. Code is available at github.com/communitygenetics/lcn. Data is available via the Harvard Dataverse (needs project

499 ID). The project is also archived via Zenodo at zenodo.com/doi/XXXXXX. All analyses were conducted using the
500 programming language R version 3.4.2 (R Development
501 Core Team 2018).

502 Discussion

- 503 - Genotypic variation can lead to network variation
- 504 - Traits could both share or be a causal mechanism
505 with lichen networks. - Network structure is linked
506 to function and dynamics. E.g. stability
 - 507 - Community level selection may be possible, but
508 this is not a necessary factor for evolutionary dynamics
509 to be relevant to ecological networks
 - 510 - What are the conditions in which genetically
511 based ecological network structure could have an
512 effect?
 - 513 - Network structure serves to amplify the signal
514 of genetics

515 **TGW: I think window is too vague and this**
516 **topic sentence needs to be much stronger for**
517 **a journal like PNAS. Might be stronger by**
518 **saying "Our findings argue there is a genetic**
519 **component to network structure, which im-**
520 **pplies that network structure could be subject**
521 **to selection and networks can evolve."**

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

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

536 We observed significant lichen interaction struc-
537 ture that varied among genotypes of a foundation
538 tree species, narrowleaf cottonwood (*P. angustifo-*
539 *lia*). We found that a genetically based trait, bark
540 roughness, partially explained the variation in lichen
541 interaction networks. Some of this variation in lichen
542 networks was related to both the overall abundance

498 and species richness of lichen; though, statistically
499 controlling for the effect of genotype on these vari-
500 ables indicates that a significant portion of the varia-
501 nce in lichen species richness is due to a factor
502 other than tree genotype. By using network metrics,
503 we were also able to probe for specific characteris-
504 tics of how these networks were responding to tree
505 genotype. We found that both number of links and
506 the centralization of the networks were highly corre-
507 lated with network similarity and that tree genotype
508 significantly predicted network centrality but only
509 marginally predicted the number of network links.
510 This latter result could be due to the relationship
511 between species richness and the number of links
512 in the network, which were significantly correlated
513 with each other. We also found that bark roughness
514 did not significantly predict either the number of
515 links or the centrality of lichen networks, suggesting
516 that bark roughness has some other effect on the
517 structure of the lichen networks. Taken together,
518 these findings support the hypothesis that genotypic
519 variation in a foundation species contributes to the
520 structure of a network of interacting species.

521 **LJL: I wonder if you need to have so much**
522 **on richness here. Overall, I think you want to**
523 **focus on the network responses and patterns**
524 **among genotype first, and then go into mech-**
525 **anism later. I think we don't quite have a**
526 **good mechanism yet so I don't think it needs**
527 **to come up in the first paragrpah of the dis-**
528 **cussion.**

529 These findings point to the importance of under-
530 standing the community level effects of genetic vari-
531 ation in plant functional traits and highlights the
532 potential for indirect effects of genetic variation to
533 propagate through networks of interacting species
534 and trophic levels.

535 This work corroborates previous findings of the
536 importance of plant genetics in shaping community
537 structure and ecosystem processes. citepBangert2008

538 Altering the structure of interaction networks
539 presents a means for genetic effects to be magni-
540 fied within the system of interacting species. For
541 example, (7) showed that the genetics based interac-
542 tions of aphid resistant and aphid susceptible trees
543 resulted in different interaction networks of their
544 associated arthropod communities composed of 139
545 species. At the scale of ecosystems, trophic net-
546 works or food webs direct and control the rates of
547

energy and nutrient flux (22). Furthermore, in a predator-prey-plant study, Smith (23), showed that the interactions among species across trophic levels depended on plant genotype.

LJL: It could be useful to point out that our findings are not related to trophic interactions, which is pretty cool. Also, we talk about interaction networks but it is not clear to me if the interactions tend to be positive or negative. Can we get at that with the approach used?

TGW: Is there any adaptive component to the tree in having certain lichen communities? e.g., can they feed back to affect tree performance in some way or is this a passive outcome of a trait that affects bark for other adaptive reasons and lichens are passive players that tag along for the ride? I could envision that lichens covering the bark of a tree act as a barrier between insects and pathogens, much like ectomycorrhizae cover fine roots as a first line of defense by invading microorganisms. Uptake of N that gets passed to the tree??

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

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

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

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

Discussion of network implications for stability with genetics.

Bark roughness had previously been shown to be an important tree trait influencing bark lichens (18) that is under strong genetic control (19).

Although our study was conducted with a commu-

nity of lichens, these results should be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level (24, 25). In the face of the high degree of complexity and potential context dependency of ecological processes, the current study points to the utility of considering the spatial and temporal scales of interactions, as discussed to some in previous studies (26–28). In the present study, we found that community assembly processes, such as environmental filtering and species interactions, are genetically based. This is likely due, in part, to the large difference in the differences in size and longevity of the lichen and cottonwood individuals with the trees determining the environment in which the lichen occur. We suggest that future work would be aided by determining these modules within the biotic community that include species with similar differences in body-size and time-scales. As heritable variation is the raw material for natural selection to act upon, a genetic basis for interaction network structure indicates evolutionary dynamics should be considered at the community level and that conserving genetic variation is important to consider in efforts to restore or preserve complex species interactions and their associated ecosystem functions (29). With such findings, it appears that we are closer to understanding the evolutionary drivers of Darwin's entangled bank and the interconnectedness of species in complex communities.

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