

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 a foundation species (*Populus angustifolia*).** We
14 **found three main results: 1) bark roughness and lichen communi-**
15 **ties displayed significant responses to tree genotype, 2) tree geno-**
16 **type contributed to lichen network structure, explaining a third of**
17 **the variation in lichen interaction networks, and 3) different aspects**
18 **of lichen network structure, including the number of interactions and**
19 **centralization, responded to tree genotype, primarily as a function**
20 **of the number of species present and to a lesser extent the abun-**
21 **dance of lichens. We conclude that tree genotype influences lichen**
22 **interaction network structure with one potential pathway being that**
23 **bark roughness, a genetically based plant functional trait, alters the**
24 **presence and overall abundance of lichens, which determines the**
25 **nature and magnitude of interactions in the community. These re-**
26 **sults support the hypothesis that variation in ecological interaction**
27 **networks can result from genetically based variation in foundation**
28 **species. This study suggests a genetic basis to both direct and in-**
29 **direct interactions among species that can result in the evolution of**
30 **complex communities.**

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

1 **E**volution occurs in the context of complex ecological net-
2 **works. Initially, evolution in a community context was**
3 **focused on examples of highly co-evolved pairs of species (e.g.**
4 **Darwin's famous prediction of the Sphinx Moth and Christmas**
5 **Orchid) (?). However, studies of diffuse co-evolution (*sensu***
6 **(?)) (? ? ?), geographic mosaics of co-evolution (?)**
7 **and community genetics (?) have provided an in-road for**
8 **ecological network approaches (1 ? ?) to illuminate a more**
9 **complex perspective of the interface between ecological and**
10 **evolutionary dynamics. There is now evidence to support that**
11 **selection tends to occur among groups of species (? ? ?)**
12 **favoring the development of small webs (? ? ?) and that**
13 **genetic variation and phylogenetic relatedness contributes to**
14 **variation in community assembly (2) and species interactions**
15 **(3? , 4), which shapes the ecological interaction networks (5).**

16 **LJL:** I think a key point in the first paragraph is to
17 **point out that networks are believed in part to emerge**
18 **out of complex evolutionary interactions. But, to-**
19 **date, the patterns observed in networks are primarily**
20 **only on the scale of species interactions and phylo-**
21 **genetic signature. There has yet to be much work**
22 **connecting ecological species networks at the scale**
23 **relevant to the actual workings of microevolutionary**
24 **processes (e.g., the genotype or population level).**

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

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

38 Additional work has provided support for the hypothesis
39 that not only does composition vary among genetically distinct
40 genotypes of foundation species, it also impacts the structure

Significance Statement

Evolution occurs in the context of ecosystems comprised of complex ecological networks. Research at the interface of ecology and evolution has primarily focused on pairwise interactions among species and have rarely included a genetic component to analyses. Here, we use a long-term common garden experiment to reveal the effect that genotypic variation can have on networks of lichens that occur on the bark of a foundation tree species. We found that lichen interaction networks respond to a genetically based tree trait, which alters network structure both through environmental filtering of species and altering species interactions. These findings demonstrate the importance of assessing the impacts of genetic variation and evolutionary dynamics in shaping ecological networks as evolved traits.

M.L. and L.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 substantially to the conceptual development, T.W. established the common garden. All authors contributed to revisions of the manuscript.

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42 of the network of species interactions in these communities
43 (1, 10). Also, work by (11–13) observed consistent patterns
44 of centralized interactions of species modules focused around
45 hubs of plant-fungal interactions. In other words, a small
46 number of plant and fungal symbionts tended to have have
47 disproportionate numbers of interactions with other species
48 and likely are the drivers in determining community assembly,
49 structure and dynamics.

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

51 Add a discussion of DesRoches.

52 From Thompson2014

- 53 • Pairwise interactions are usually influenced by other
54 species
- 55 • Selection favors the development of small webs
- 56 • Evolution of new lifestyles leads to changes in selection on
57 large and small webs

58 Specific hypothesis from Thomspn2014

59 Selection in small webs

60 •

61 You mention the naturally established stand but
62 then that data doesn't get included in the manuscript.
63 I do like the idea of including the field data. We
64 might need to cross reference IDs of the lichens in
65 my community composition data on the same trees
66 since there was considerably more diversity than we
67 initially saw out there. We would just want to make
68 sure the same names are applied to taxa in the same
69 quadrats on each tree. It probably wouldn't take long.

70 **LJL:** If I recall, the Elamo paper just looks at ge-
71 netic correlations between pairwise individual abun-
72 dances. I would suggest maybe it doesn't deserve to
73 be in this 1st paragraph. Perhaps it actually should
74 be in the 2nd or 3rd paragraph, just as a reference
75 that points to the potential for genotype to influence
76 networks. Definately our 2015 JOE paper goes much
77 further, too, since it has whole communities being
78 correlationed. But, again, I woudl put both of these
79 as citation in the community genetics paragraphs (2
80 of 3) instead of the first paragrpah, which focuses on
81 the general network lit.

82 Here, we investigate how genetic variation in a foundation
83 tree species determines the structure of a network of inter-
84 actions among a community of tree associated lichen species.
85 Using a long-term (20+ years), common garden experiment
86 with replicated individuals of known genetic identity and a nat-
87 urally established stand of *Populus angustifolia*. We focused on
88 a model community of 9 epiphytic lichen species, as previous
89 research has demonstrated significant compositional responses
90 of epiphytes to genotypic variation (16, 17). In addition, the
91 life-history characteristics of lichens, having highly localized,
92 direct contact interactions and slow population turnover rates,
93 allowed us to assess interactions among lichen species on indi-
94 vidual trees. We hypothesize that in natural systems evolution
95 occurs in a community context involving interactions of com-
96 plex networks of interacting species (1, 10, 18, 19). If correct,
97 we should expect to find that network structure is genetically
98 based in which different plant genotypes support different in-
99 teraction networks and that these interactions networks can

function as indicators of ecological dynamics important for
100 conserving biodiversity. Applying a probability-theory based
101 network modeling approach, we constructed a set of interac-
102 tion network models for the lichens associated with individual
103 trees. Using these models, we then examined the genetic basis
104 of the structure of these ecological networks.

105 **LJL:** I agree that there is a general overarching
106 theme that evolution occurs in a community network
107 context, but I'm not sure that we should state that
108 as our main hypothesis. It seems more that this is a
109 fundamental foundation for our work. The hypoth-
110 esis is more what we are testing directly, but we don't
111 test this directly. I guess I don't want to give the
112 impression that our communities are necessarily the
113 result of each species evolving into its place in the
114 community on these tree genotypes (although I do
115 understand this as Shuster et al 2006's fundamen-
116 tal explanation for why we see different communi-
117 ties on different genotypes; I don't necessarily agree that
118 this is the only reason we woudl see different com-
119 munities on dif genotypes). Most of these are pretty
120 generalist lichens, which could be found on other de-
121 ciduous trees in the surrounding city or natural areas.
122 I would look at it more like an assembling of lichen
123 species into unique configurations on genetically dif-
124 ferent substrates. There may be some selection for
125 different genotype of lichen during the community as-
126 sembly process but we can't really tell that just by
127 differences in species abundances or coocurneces. I
128 guess to me the evolutionary context that is more di-
129 rectly related to this work is that the tree genotype
130 is a central controller (indeed a sort of hub species
131 in the network) of network structure. By anchoring
132 the lichen network to tree genotype (and variation
133 among networks to variation among tree genotypes), our
134 study highlights the possibility that natural selec-
135 tion acting on the trees may have an extended conse-
136 quence for the network structure of organisms living
137 on the trees...the extra thing we add to the field
138 is that we show interaction networks are sensitive to
139 genotype. I doubt the lichens have a direct effect on
140 tree fitness, but favorability of some tree genotypes
141 over others during natural selection will then go on to
142 favor and disfavor certain lichen communities of dif-
143 ferent network structures. By being sensitive to tree
144 genotype, the lichen community networks are passive
145 riders on the waves of evolutionary dynamics that oc-
146 cur within the tree species they inhabit.

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

Results

154 Networks were more similar as a result of having similar num-
155 bers of interactions and distribution of interactions. The
156 number of links (PerMANOVA $R^2 = 0.392$, $F_1 = 72.4348$,
157 $p\text{-value} = 0.001$) and network centrality (PerMANOVA R^2
158 = 0.309, $F_1 = 57.0440$, $p\text{-value} = 0.001$) were highly cor-

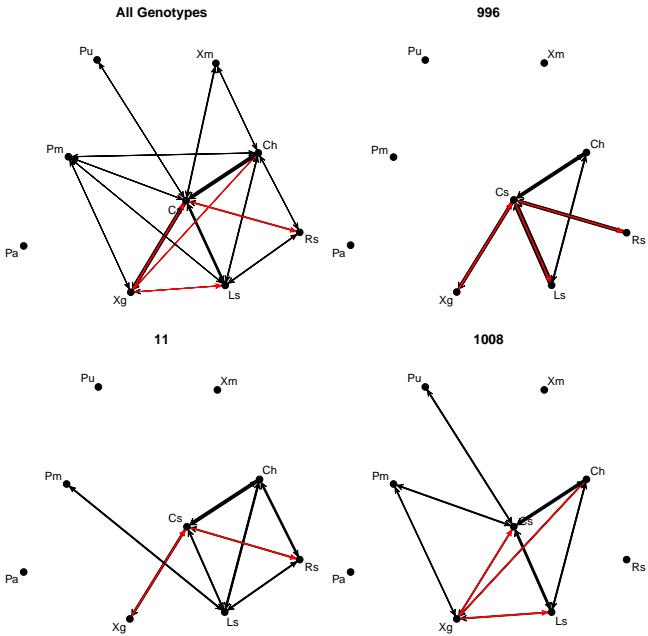


Fig. 1. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

	Df	SumOfSqs	R2	F	Pr(>F)
geno	10.00	304.93	0.23	2.37	0.03
BR	1.00	16.26	0.01	1.26	0.27
pH	1.00	5.04	0.00	0.39	0.57
CN	1.00	39.67	0.03	3.08	0.08
CT	1.00	70.77	0.05	5.49	0.03
PC	1.00	56.35	0.04	4.37	0.04
SR	1.00	332.42	0.25	25.78	0.00
SE	1.00	55.11	0.04	4.27	0.04
Residual	33.00	425.47	0.33		
Total	50.00	1306.01	1.00		

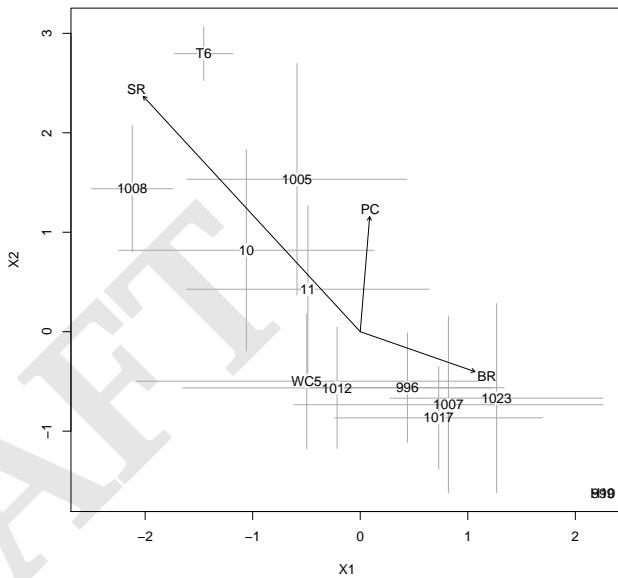


Fig. 2. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.011) lichen networks ($\pm 1\text{ S.E.}$). Centroids that are closer are more similar in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

related with network similarity. Tree genotype significantly predicted network centrality (REML $R^2 = 0.202$, RLRT = 2.7801, p -value = 0.04012) but marginally predicted the number of links (REML $R^2 = 0.170$, RLRT = 2.0484, p -value = 0.065) (Fig. 3). Total cover was correlated with the number of links (ANOVA $F_1 = 6.867$, p -value = 0.0114) and centrality (ANOVA $F_1 = 8.093$, p -value = 0.0063). Lichen species richness was also correlated with the number of links (ANOVA $F_1 = 29.436$, p -value = 0.000015) and centrality (ANOVA $F_1 = 39.488$, p -value < 0.000001). Bark roughness, however, did not significantly predict either the number of links (ANOVA $F_1 = 2.897$, p -value = 0.0946) or the centrality (ANOVA $F_1 = 2.591$, p -value = 0.1134) of lichen networks (Supplementary Tables ?? and ??).

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

MKL: Adapt into a table.

TGW: clarify positive vs negative interactions.

C. Tree genotypes support distinct lichen networks. MKL: Combine 1 and 2

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

Response	H2	R2	p-value
Percent Rough Bark	0.378	0.378	0
pH	0.055	0.055	0.292
Condensed Tannins (CT)	0.28	0.28	0.014
Carbon-Nitrogen (CN) Ratio	0	0	1
Percent Lichen Cover	0.09	0.09	0.157
Lichen Species Richness	0	0	1
Lichen Species Evenness	0.015	0.015	0.394
Lichen Species Diversity	0	0	1
Number of Network Links	0.078	0.078	0.216
Network Modularity	0	0	1
Network Centrality	0.082	0.082	0.2
Lichen Network	0.16	0.233	0.025
Community Composition	0.052	0.173	0.102

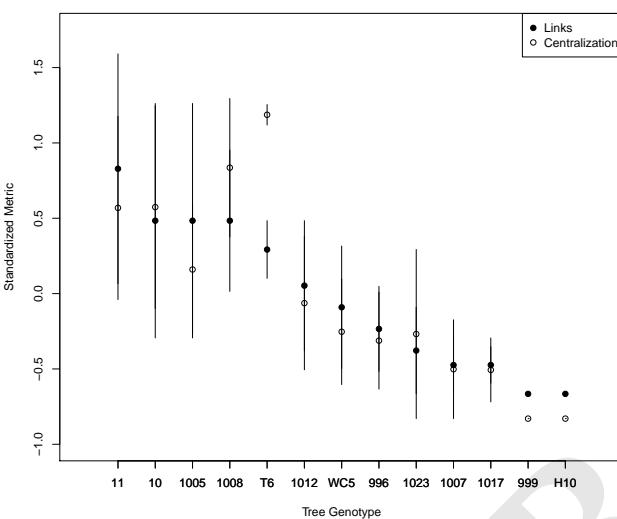


Fig. 3. The impact of tree genotype on lichen network structure was indicative of variation in both the number variation in lichen interactions among species. Plot showing the means (± 1 S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented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

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

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

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

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

TGW: Here and in earlier paragraphs, a lot of stats are presented some of which are significant and some not. For your topic sentence to be accepted, it seems readers need to know how many of the stats need to confirm the pattern and how many would it take to reject. This paragraph has about 8 stats so need some overarching statement(s). E.g., 7 of 8 analyses sup-

port our overarching hypothesis that ... Same goes for other such paragraphs such as the 1st and last paras of the Results.

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

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

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

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

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

On each tree, presence or absence of each lichen species was assessed in 50 total 1 cm² cells arrayed in a checkerboard pattern. Given the small size and sessile nature of lichens, we were able to rapidly assess lichen interactions by quantifying thalli in close contact. Sampling was restricted to the northern aspect of the trunk to maximize the abundance of lichen and control for the effect of trunk aspect. Two adjacent 10 cm² quadrats centered at 50 cm and 85 cm from ground level were sampled (Fig 4 A and B). The observed lichen community included (abbreviations are given for species present in study): Xg = *Xanthomendoza galericulata*, Xm = *X. montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora Augenii*, Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several other species were not observed in the present study but are known to occur in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of 0.12 ± 0.001 cm² (S.E.) (see Supplementary Fig 1). Based on the median thallus size, we expected thalli observed in each cell to generally be spatially independent of thalli present in other cells but exposed to similar micro-environmental conditions created by the bark and the location of the sampling area on an

296 individual tree. Therefore, we were confident in treating the cell-wise
297 observations in quadrats as independent with respect to lichen-lichen
298 interactions.

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

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

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

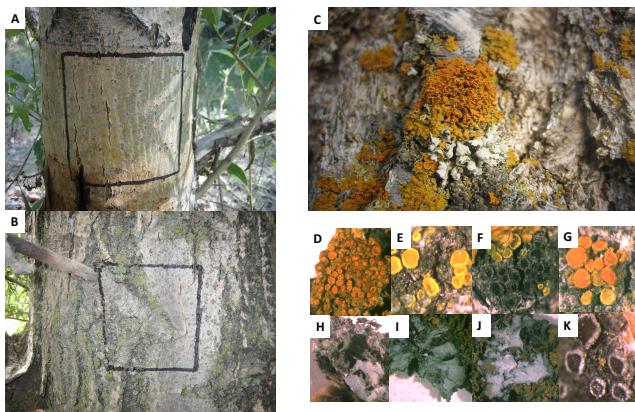


Fig. 4. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourgh (D-K).

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

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

335 **MKL:** regarding Lamit's question about the symmetry,
336 the point is that direction of the interaction matters. The

340 effect of species A on B can be different from B on A.
341 No the matrix is not necessarily triangular (triangular being
342 that the matrix either above or below the diagonal is
343 completely zero).

344 We then applied an analytical procedure to remove non-
345 significant links between species. This procedure determines if
346 the joint probability of a species pair (i.e. $P(S_i, S_j)$) is different
347 from zero (Fig. 5). Here, a confidence interval $CI_{95\%}$ is calculated
348 as as $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected
349 frequency of co-occurrences $E(S_i S_j)$ is the total number of cells
350 surveyed (N) times the independent probabilities of each species
351 $P(S_i) * P(S_j)$, $Z_{95\%}$ is the Z-score for 95% from a Z-distribution
352 and the expected variance of $E(S_i S_j)$ is the total number of cells
353 times the expected probability of $S_i S_j$ and its compliment (i.e.
354 $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$). If the observed
355 number of co-occurrence falls outside of the confidence interval,
356 the joint probability $P(S_i, S_j)$ is determined to be equal to the
357 product of the individual probabilities (i.e. $P(S_i)P(S_j)$), and the
358 conditional probability reduces to the individual probability of that
359 species $P(S_i)$. Therefore, unless the co-occurrence of a species
360 pair falls outside the confidence interval, the probability that the
361 observation of one species given the other is no different than
362 simply observing that species alone. This enables us to remove
363 links from a given network by re-scaling the resulting conditional
364 probabilities by subtracting the individual probabilities from the
365 conditional probabilities (i.e. how different the conditional probabili-
366 ty is from the independent probability), which makes any species
367 with a non-significant conditional probability zero. The resulting
368 matrix ($\mathbf{D} = D_{ij}$) can be interpreted as how one species impacts
369 another with zero being no effect and values less than or greater
370 than zero interpreted as negative and positive effects, respectively.
371 Here, we will refer to this matrix (\mathbf{D}) as an interaction matrix with
372 the properties that it can be asymmetric (i.e. P_{ij} does not necessarily
373 equal P_{ji}), and the diagonal (P_{ii}) is zero (i.e. a species does not
374 influence it's own probability of being observed).

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

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

383 **LJL:** I like the details here. THHe one thing is that it sort
384 of makes the reader think there is only one quadrat on a
385 tree but infact there were two. I think you want to make
386 sure to be explicit about the two. For analytical purposes,
387 was all the data lumped so there was really functionally a
388 20cm by 10cm grid (just split into two pieces). Or, was the
389 network made for each of the two grids and them averaged
390 or combined in some way? My understanding is that it
391 was more the first than the latter.

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

394 **Statistical Analyses, Software and Data.** We used a combination of
395 parametric and non-parametric, permutation based frequentist sta-
396 tistical analyses to test for the effects of genetic variation on lichen
397 communities and their interaction networks. To assess the effect of
398 genotype on univariate responses, we used additive, random effects
399 models with Restricted Maximum Likelihood (REML). We used a
400 combination of Least Squares Regression, Analysis of Variance
401 (ANOVA) and correlation tests to quantify and test for the relation-
402 ship among other variables. Bark roughness, lichen cover and species
403 richness were square-root transformed to meet the assumptions of
404 homogeneity of variance and normality for these tests.

405 For multivariate response variables, such as lichen community
406 composition and network structure, we used distance based multi-
407 variate statistical approaches, including Permutational Analysis of
408 Variance (PerMANOVA) and Mantel tests. For all analyses, com-
409 munity composition was relativized by species maxima to reduce
410 the effect of the highly abundant *X. galericulata*. For community
411 composition we used Bray-Curtis dissimilarity, which has optimal
412 performance with count data (?). To quantify the similarity of

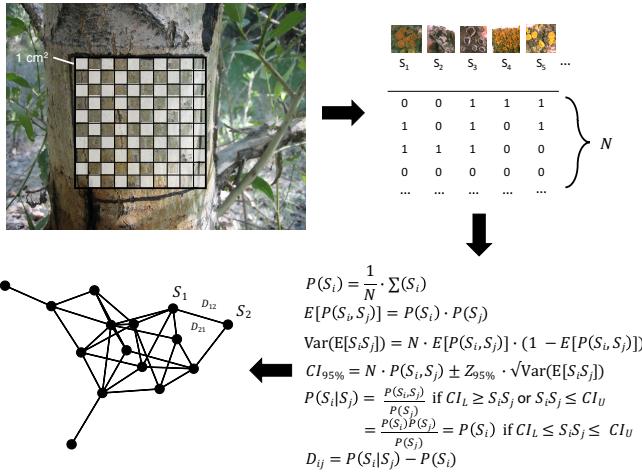


Fig. 5. Lichen interaction networks were constructed by conducting field observations in 1 cm² cells within a 10 cm² grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded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

lichen networks among individual trees, we calculated the pairwise Euclidean distance of the \mathbf{D} interaction matrices among all pairs of trees.

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

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

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 ID). The project is also archived via Zenodo at zenodo.com/doi/XXXXXX. All analyses were conducted using the programming language R version 3.4.2 (R09).

Development Core Team 2018).

453

Discussion

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

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

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

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

We observed significant lichen interaction structure that varied among genotypes of a foundation tree species, narrowleaf cottonwood (*P. angustifolia*). We found that a genetically based trait, bark roughness, partially explained the variation in lichen interaction networks. Some of this variation in lichen networks was related to both the overall abundance and species richness of lichen; though, statistically controlling for the effect of genotype on these variables indicates that a significant portion of the variance in lichen species richness is due to a factor other than tree genotype. By using network metrics, we were also able to probe for specific characteristics of how these networks were responding to tree genotype. We found that both number of links and the centralization of the networks were highly correlated with network similarity and that tree genotype significantly predicted network centrality but only marginally predicted the number of network links. This latter result could be due to the relationship between species richness and the number of links in the network, which were significantly correlated with each other. We also found that bark roughness did not significantly predict either the number of links or the centrality of lichen networks, suggesting that bark roughness has some other effect on the structure of the lichen networks. Taken together, these findings support the hypothesis that genotypic variation in a foundation species contributes to the structure of a network of interacting species.

LJL: I wonder if you need to have so much on richness here. Overall, I think you want to focus on the network reponses and patterns among genotype first, and then go into mechanism later. I think we don't quite have a good mechanism yet so I don't think it

510 needs to come up in the first paragrpah of the discussion.
511

512 These findings point to the importance of understanding the
513 community level effects of genetic variation in plant functional
514 traits and highlights the potential for indirect effects of genetic
515 variation to propagate through networks of interacting species
516 and trophic levels.

517 This work corroborates previous findings of the importance
518 of plant genetics in shaping community structure and ecosystem
519 processes. (?)

520 Altering the structure of interaction networks presents a
521 means for genetic effects to be magnified within the system of
522 interacting species. For example, (1) showed that the genetics
523 based interactions of aphid resistant and aphid susceptible
524 trees resulted in different interaction networks of their associated
525 arthropod communities composed of 139 species. At the
526 scale of ecosystems, trophic networks or food webs direct and
527 control the rates of energy and nutrient flux (25). Furthermore,
528 in a predator-prey-plant study, Smith (26), showed that the
529 interactions among species across trophic levels depended on
530 plant genotype.

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

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

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

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

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

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

564 **Discussion of network implications for stability
565 with genetics.**

566 Although our study was conducted with a community of
567 lichens, these results should be generalized to other groups of
568 diverse organisms around the world that also exhibit significant
569 genetic signals at the community level (27, 28). In the
570 face of the high degree of complexity and potential context⁴²
571

dependency of ecological processes, the current study points
572 to the utility of considering the spatial and temporal scales of
573 interactions, as discussed to some in previous studies (29–31).
574 In the present study, we found that community assembly processes,
575 such as environmental filtering and species interactions,
576 are genetically based. This is likely due, in part, to the large
577 difference in the differences in size and longevity of the lichen
578 and cottonwood individuals with the trees determining the
579 environment in which the lichen occur. We suggest that future
580 work would be aided by determining these modules within the
581 biotic community that include species with similar differences
582 in body-size and time-scales. As heritable variation is the raw
583 material for natural selection to act upon, a genetic basis for
584 interaction network structure indicates evolutionary dynamics
585 should be considered at the community level and that conserving
586 genetic variation is important to consider in efforts
587 to restore or preserve complex species interactions and their
588 associated ecosystem functions (32). With such findings, it
589 appears that we are closer to understanding the evolutionary
590 drivers of Darwin's entangled bank and the interconnectedness
591 of species in complex communities.

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596 gardens. Lichen sampling was supported by Todd Wojtowicz, Luke
597 Evans and David Solance Smith.

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Supplementary Materials

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

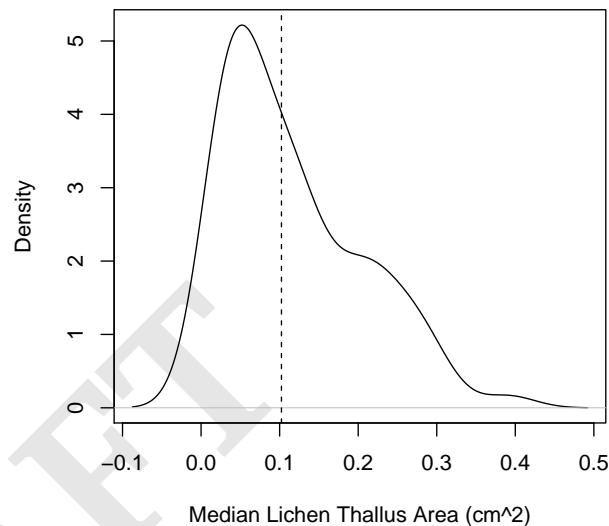


Fig. 1. Density plot of the median lichen thallus area (cm²).

	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		