

Genotypic variation in a foundation tree directs ecological network structure

Matthew K. Lau^{a,b,1}, Louis J. Lamit^b, Rikke R. Naesbourg^c, Stuart R. Borrett^d, Matthew A. Bowker^e, and Thomas G. Whitham^a

^aDepartment of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011, USA; ^bHarvard Forest, Harvard University, 324 N Main St, Petersham, MA 01366, USA; ^cDepartment of Biology, Syracuse University, 107 College Place Syracuse, NY 13244, USA; ^eUniversity of California Berkeley, Berkeley, CA, USA; ^fDepartment of Biology and Marine Biology, University of North Carolina Wilmington, 601 South College Road, Wilmington, NC, 28403, USA; ^gSchool of Forestry, Northern Arizona University, Flagstaff, AZ 86011, USA

This manuscript was compiled on November 1, 2019

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 of**
13 **genotypes of (*Populus angustifolia*), a foundation species. We found**
14 **three main results: ???.**
15 **We conclude that tree genotype influences lichen interaction network**
16 **structure with one potential pathway being that bark roughness, a ge-**
17 **netically based plant functional trait, alters the presence and overall**
18 **abundance of lichens, which determines the nature and magnitude**
19 **of interactions in the community. These results support the hypoth-**
20 **esis that variation in ecological interaction networks can result from**
21 **genetically based variation in foundation species. This study sug-**
22 **gests a genetic basis to both direct and indirect interactions among**
23 **species that can result in the evolution of 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
33 (9).

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

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

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 substantively to the conceptual development, T.W. established the common garden. All authors contributed to revisions of the manuscript.

The authors have no conflicts of interest.

¹Dr. Matthew K. Lau. E-mail: matthewklau@fas.harvard.edu

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 Thompson2014
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 **JLJ:** 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 correlationated. 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 inter-
99 action networks and that these interactions networks can
100 function as indicators of ecological dynamics important for
101 conserving biodiversity. Applying a probability-theory based
102 network modeling approach, we constructed a set of interac-
103 tion network models for the lichens associated with individual
104 trees. Using these models, we then examined the genetic basis
105 of the structure of these ecological networks.

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

148 **MKL:** In response to Lamit's comment above, I
149 agree that it is not reuquired that there is co-evolution.
150 Another, perhaps simpler, explanation is that there
151 is variation in environmental filtering of lichen indi-
152 viduals created in part by genetic variation in tree
153 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-
159 related with network similarity. Tree genotype significantly
160 predicted network centrality (REML $R^2 = 0.202$, RLRT =
161 2.7801, $p\text{-value} = 0.04012$) but marginally predicted the num-
162 ber of links (REML $R^2 = 0.170$, RLRT = 2.0484, $p\text{-value} =$
163 0.065) (Fig. 3). Total cover was correlated with the number of
164 links (ANOVA $F_1 = 6.867$, $p\text{-value} = 0.0114$) and centrality
165

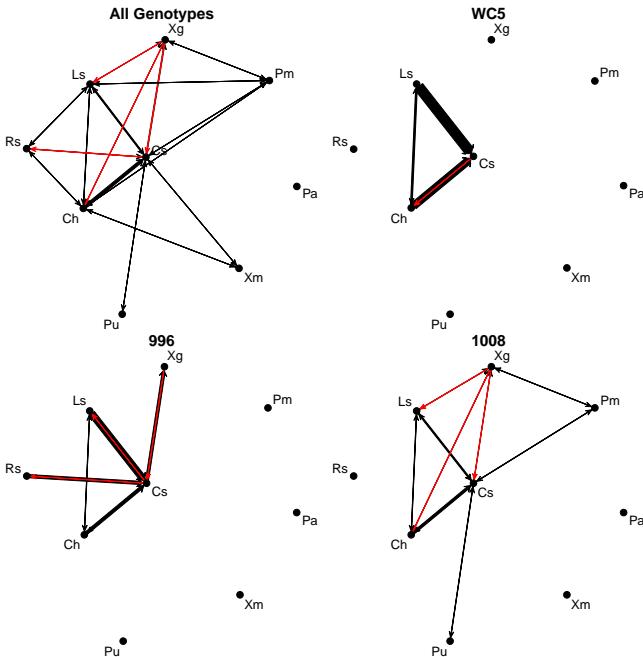


Fig. 1. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

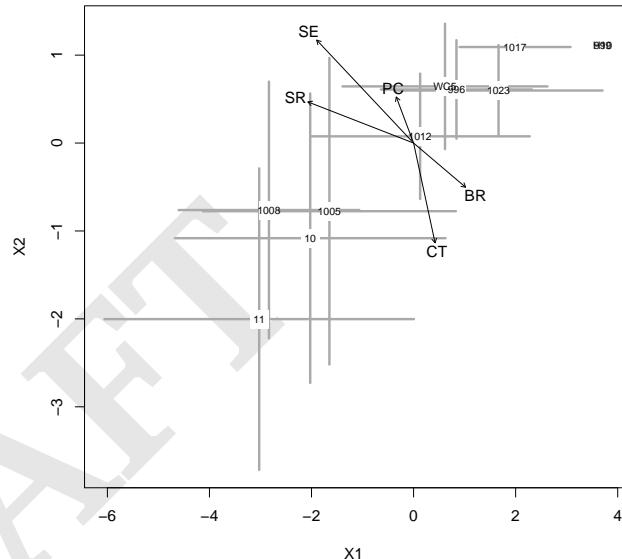


Fig. 2. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.011) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. Arrows show the magnitude and direction of correlation of the ordinated networks with tree bark roughness (BR), percent cover of lichens (PC) and lichen species richness (SR).

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.

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.

(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 ??).

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

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

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

LJL: Since we already published that tree genotypes differ in lichen composition, I wonder if we need to say somewhere in the manuscript why this test was run here. It seems to me it is important to verify this with a slightly different sampling method as used in the 2015 paper, and for this specific set of genotypes. But, then does this test of composition just become something necessary just in a methodological variation that justifies the next step of examining network structure. Something to think about. It might be that the NMDS should just go in a supplement, although I do like it here in some ways. It might also be

193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210

Response	H2	R2	p-value
Percent Rough Bark	0.385	0.385	0
pH	0.054	0.054	0.294
Condensed Tannins (CT)	0.28	0.28	0.014
Carbon-Nitrogen (CN) Ratio	0	0	0.448
Percent Lichen Cover	0.079	0.079	0.172
Lichen Species Richness	0	0	1
Lichen Species Evenness	0.015	0.015	0.388
Lichen Species Diversity	0.01	0.01	0.417
Number of Network Links	0.07	0.07	0.238
Network Modularity	0	0	1
Network Centrality	0.085	0.085	0.199
Lichen Network	0.16	0.233	0.025
Community Composition	0.052	0.173	0.102

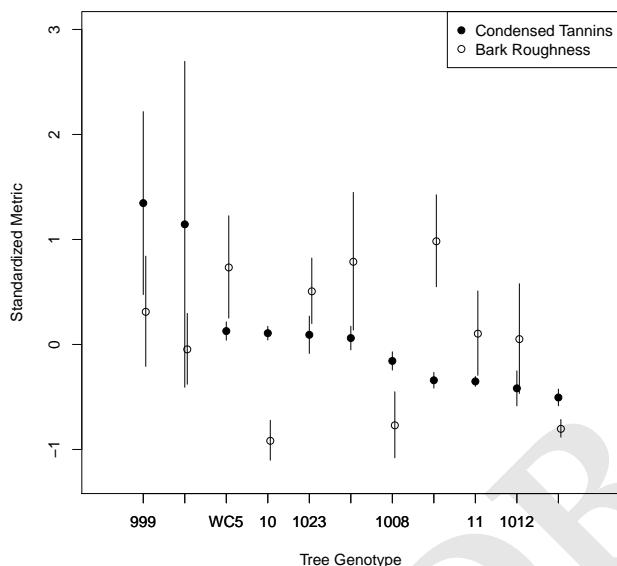


Fig. 3. The impact of tree genotype on lichen network structure was indicative of variation in both the number variation in lichen interactions among species. Plot showing the means (± 1 S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented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

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 support 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 cm^2 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 cm^2 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 hagenii*, Pm = *Phyciella melanochra*, 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 $0.12 \pm 0.001\text{ cm}^2$ (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 individual tree. Therefore, we were confident in treating the cell-wise observations in quadrats as independent with respect to lichen-lichen interactions.

LJL: My method was overall visual % cover. I didn't count P/A of rough bark in each square. So, you might just

want to site our Ecology lichen paper as showing rough bark is important, but not cite it as the same method. No doubt the methods should yeild similar results but the approach is slightly different.

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

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

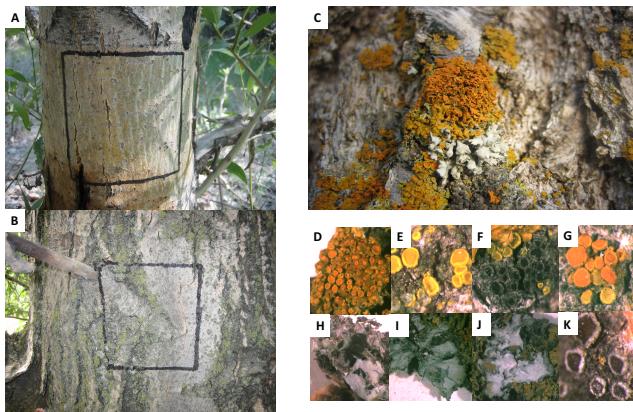


Fig. 4. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community 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).

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

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

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

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

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

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

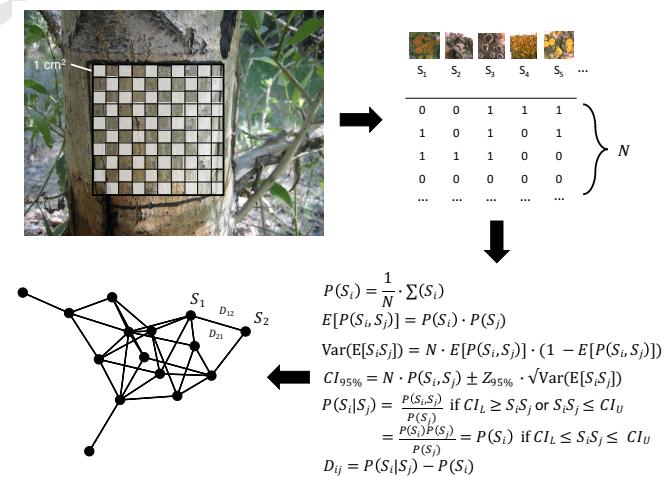


Fig. 5. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (22), we calculated the conditional probabilities, $P(S_i|S_j)$, for all species pairs and removed (i.e. set equal to zero) species pairs whose joint probabilities, $P(S_i, S_j)$, were not significant using a confidence interval based comparison of their observed co-occurrence frequency, $S_i S_j$, to that expected due to chance alone, $E[P(S_i S_j)] = P(S_i) P(S_j)$, and $P(S_i|S_j)$ reduces to $P(S_i)$, the observed individual probability of species S_i .

LJL: I like the details here. THe 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
400 a combination of Least Squares Regression, Analysis of Variance
401 (ANOVA) and correlation tests to quantify and test for the rela-
402 tionship 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
413 lichen networks among individual trees, we calculated the pairwise
414 Euclidean distance of the **D** interaction matrices among all pairs of
415 trees.

416 For visualization of multivariate patterns, we used Non-metric
417 Multi-Dimensional Scaling (NMDS) (?) to produce dimensionally
418 reduced ordinations of these multi-variate responses and fitted
419 vectors for continuous predictor variables to the ordinated values
420 (?). Using random initial configurations with a maximum of 500
421 iterations and a change in stress threshold of less than 10^{-12} . Final
422 configurations has the lowest stress with at most a stress level of
423 0.10.

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

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

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

Discussion

- Genotypic variation can lead to network variation - Network
455 structure is linked to function and dynamics. E.g. stability
456 - Community level selection may be possible, but this is not
457 a necessary factor for evolutionary dynamics to be relevant
458 to ecological networks - What are the conditions in which
459 genetically based ecological network structure could have an
460 effect? - Network structure serves to amplify the signal of
461 genetics
462

TGW: I think window is too vague and this topic
463 sentence needs to be much stronger for a journal like
464 PNAS. Might be stronger by saying "Our findings
465 argue there is a genetic component to network struc-
466 ture, which implies that network structure could be
467 subject to selection and networks can evolve."
468

TGW: Could we also make the comparsion that
469 genetically more similar trees also have more similar
470 communities? We've done this in the past and it has
471 worked, e.g., Randy's genetic similarity rule.
472

- Genetic assembly rule = similar genetics will have more
473 similar communities
474
- What we don't know is whether or not these interactions
475 will also lead to similar interactions among other species.
476
- Thus, it would be possible for genetics to not only influ-
477 ence other species directly, but also indirectly by influen-
478 cing the interactions among other species.
479

We observed significant lichen interaction structure that
480 varied among genotypes of a foundation tree species, narrowleaf
481 cottonwood (*P. angustifolia*). We found that a genetically
482 based trait, bark roughness, partially explained the variation
483 in lichen interaction networks. Some of this variation in lichen
484 networks was related to both the overall abundance and species
485 richness of lichen; though, statistically controlling for the effect
486 of genotype on these variables indicates that a significant
487 portion of the variance in lichen species richness is due to a
488 factor other than tree genotype. By using network metrics, we
489 were also able to probe for specific characteristics of how these
490 networks were responding to tree genotype. We found that
491 both number of links and the centralization of the networks
492 were highly correlated with network similarity and that tree
493 genotype significantly predicted network centrality but only
494 marginally predicted the number of network links. This latter
495 result could be due to the relationship between species richness
496 and the number of links in the network, which were significantly
497 correlated with each other. We also found that bark roughness
498 did not significantly predict either the number of links or the
499 centrality of lichen networks, suggesting that bark roughness
500 has some other effect on the structure of the lichen networks.
501 Taken together, these findings support the hypothesis that
502 genotypic variation in a foundation species contributes to the
503 structure of a network of interacting species.
504

LJL: I wonder if you need to have so much on rich-
505 ness here. Overall, I think you want to focus on the
506 network reponses and patterns among genotype first,
507 and then go into mechanism later. I think we don't
508 quite have a good mechanism yet so I don't think it
509 needs to come up in the first paragrpah of the discus-
510 sion.
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 ecosys-
519 tem 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 associ-
525 ated 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 find-
532 ings 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 play-
542 ers 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 ectomycor-
545 rhizae cover fine roots as a first line of defense by in-
546 vading 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 com-
554 mon 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 sim-
562 ilarity 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 signifi-
569 cant genetic signals at the community level (27, 28). In the
570 face of the high degree of complexity and potential context
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 pro-
575 cesses, 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 con-
586 serving 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.

592 **ACKNOWLEDGMENTS.** This work was supported by the Na-
593 tional Science Foundation grant (DEB-0425908) and Integrative
594 Graduate Research Traineeship (IGERT) fellowships for M.L. and
595 L.L. The Ogden Nature Center staff helped to maintain the common
596 gardens. Lichen sampling was supported by Todd Wojtowicz, Luke
597 Evans and David Solance Smith.

1. Keith AR, Bailey JK, Lau MK, Whitham TG (2017) Genetics-based interactions of foundation species affect community diversity, stability and network structure. *Proc. R. Soc. B Biol. Sci.* 284(1854):20162703. 598
2. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming decade. *New Phytol.* 600
3. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nat. Rev. Genet.* 7:510–523. 603
4. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1425–37. 605
5. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random coextinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8. 607
6. Lamit LJ, et al. (2015) Genotype variation in bark texture drives lichen community assembly across multiple environments. *Ecology* 96(4):960–971. 609
7. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486. 611
8. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic, ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype in a foundation tree species. *Oecologia* 170:695–707. 613
9. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364(1523):1607–16. 615
10. Lau MK, Keith AR, Borrett SR, Shuster SM, Whitham TG (2015) Genotypic variation in foundation species generates network structure that may drive community dynamics and evolution. *Ecology* 97(3):15–0600.1. 617
11. Toju H, et al. (2017) Species-rich networks and eco-evolutionary synthesis at the metacommunity level. 619
12. Toju H, Yamamoto S, Tanabe AS, Hayakawa T, Ishii HS (2016) Network modules and hubs in plant-root fungal biomes. *J. R. Soc. Interface.* 621
13. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) Assembly of complex plant-fungus networks. *Nat. Commun.* 622
14. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic networks. *Ecol. Lett.* 14(9):877–85. 624
15. Thompson JN, Schwind C, Guimaraes PR, Friberg M (2013) Diversification through multitrait evolution in a coevolving interaction. *Proc. Natl. Acad. Sci.* 625
16. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecol. Econ.* 71:80–88. 627
17. Zytnska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree species influences the associated epiphytic plant and invertebrate communities in a complex forest ecosystem. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366:1329–1336. 629
18. Thompson JN (2013) *Relentless Evolution.* (University of Chicago Press), p. 499. 631
19. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate Biodiversity Maintenance10.1126/science.1123412. *Science (80-.).* 312:431–433. 633
20. Lamit L, et al. (2011) Genetically-based trait variation within a foundation tree species influences a dominant bark lichen. *Fungal Ecol.* 4(1):103–109. 640
21. Bdeir R, et al. (2017) Quantitative trait locus mapping of Populus bark features and stem diameter. *BMC Plant Biol.* 642
22. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net-works to assess the impacts of climate change. *Ecography (Cop.)*. 34:897–908. 644
23. Lau MK, Borrett SR, Hines DE, Singh P (2015) enaR: Tools for Ecological Network Analysis. 645

- 647 24. Lau MK, Borrett SR, Baiser B, Gotelli NJ, Ellison AM (2017) Ecological network metrics:
648 opportunities for synthesis. *Ecosphere* 8(8):e01900.
649 25. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Soc. Networks*
650 28:466–484.
651 26. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter-
652 actions and selection: trees, aphids and birds. *J. Evol. Biol.* 24(2):422–9.
653 27. Rountree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and
654 evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1322–8.
655 28. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes.
656 29. Bangert RK, et al. (2006) A genetic similarity rule determines arthropod community structure.
657 *Mol. Ecol.* 15:1379–1391.
658 30. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to body
659 size yields high degree of intervality. *J. Theor. Biol.* 271(1):106–113.
660 31. Zytnyska SE, Khudr MS, Harris E, Preziosi RF (2012) No Title. *Oecologia* 170(2).
661 32. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net-
662 works to habitat loss. *Ecol. Lett.* 16:844–52.

Supplementary Materials

663
664
665
666
667
668
669

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.

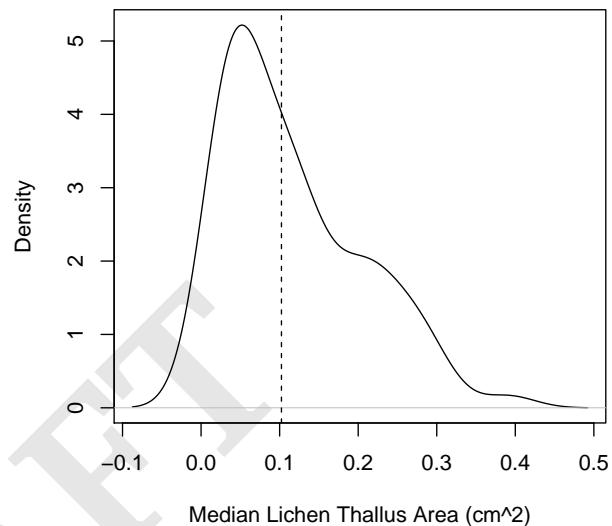


Fig. 1. Density plot of the median lichen thallus area (cm^2).

	Df	SumOfSqs	R2	F	Pr(>F)
geno	10.00	1.85	0.17	1.30	0.10
BR	1.00	0.15	0.01	1.04	0.37
pH	1.00	0.13	0.01	0.92	0.46
CN	1.00	0.17	0.02	1.16	0.31
CT	1.00	0.19	0.02	1.33	0.24
PC	1.00	2.46	0.23	17.33	0.00
SR	1.00	0.55	0.05	3.86	0.00
SE	1.00	0.48	0.05	3.38	0.01
Residual	33.00	4.69	0.44		
Total	50.00	10.65	1.00		