

# Genotypic variation in a foundation tree directs ecological network structure

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1 **Biological evolution occurs in the context of complex networks of**  
2 **interacting species in which natural selection defines the structure**  
3 **of ecological networks. Fundamental to this evolutionary process**  
4 **is the discovery of a genetic basis to ecological network structure.**  
5 **Although previous work has demonstrated that tree genotype con-**  
6 **tributes to interaction network structure at the scale of forest stands,**  
7 **the contribution of tree genetics to localized interaction networks**  
8 **at the scale of individual trees has not yet been explored. To test**  
9 **the degree to which tree genetics can contribute to network struc-**  
10 **ture we conducted quantitative modeling of interaction networks. We**  
11 **constructed networks of epiphytic lichen associated with individual**  
12 **trees that were a part of a long-term experimental common garden**  
13 **of genotypes of (*Populus angustifolia*), a foundation species. We**  
14 **found three main results. First, tree genotype significantly predicted**  
15 **lichen network similarity, i.e. trees of the same genotype had more**  
16 **similar lichen networks. Second, bark roughness and condensed**  
17 **tannin concentration were both predicted by tree genotype and cor-**  
18 **related with lichen network similarity. Third, the network metrics, the**  
19 **number of links and centrality, were both correlated with several tree**  
20 **traits, including bark roughness and condensed tannin concentra-**  
21 **tion. We conclude that tree genotype influences lichen interaction**  
22 **network structure with two potential pathways through bark rough-**  
23 **ness and condensed tannin concentration. These results provide**  
24 **evidence that variation in ecological networks can result from genet-**  
25 **ically based variation in foundation species.**

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

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

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

LJL: I think a key point in the first para-  
graph is to point out that networks are be-  
lieve in part to emerge out of complex evo-  
lutionary interactions. But, to-date, the pat-  
terns observed in networks are primarily only  
on the scale of species interactions and phylo-  
genetic signature. There has yet to be much

## Significance Statement

Evolution occurs in the context of ecosystems com-  
prised of complex ecological networks. Research  
at the interface of ecology and evolution has primar-  
ily focused on pairwise interactions among species  
and have rarely included a genetic component to  
analyses. Here, we use a long-term common gar-  
den experiment to reveal the effect that genotypic  
variation can have on networks of lichens that occur  
on the bark of a foundation tree species. We found  
that lichen interaction network structure is genet-  
ically based and is correlated with heritable bark  
traits, including roughness and condensed tannins.  
These findings demonstrate the importance of ge-  
netic variation and evolutionary dynamics in shaping  
ecological networks as evolved traits.

M.L. and L.L. conceived the study, M.L. and L.L. conducted the field work, R.N. assisted in lichen identifications, M.L. wrote the first draft of the manuscript, S.B. and T.W. contributed substantively to the conceptual development, T.W. established the common garden. All authors contributed to revisions of the manuscript.

The authors have no conflicts of interest.

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31 work connecting ecological species networks  
32 at the scale relevant to the actual workings of  
33 microevolutionary processes (e.g., the genotype  
34 or population level).

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

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

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

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

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

67 Add a discussion of DesRoches.  
68 From Thompson2014

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

74 Specific hypothesis from Thompson2014  
75 Selection in small webs

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

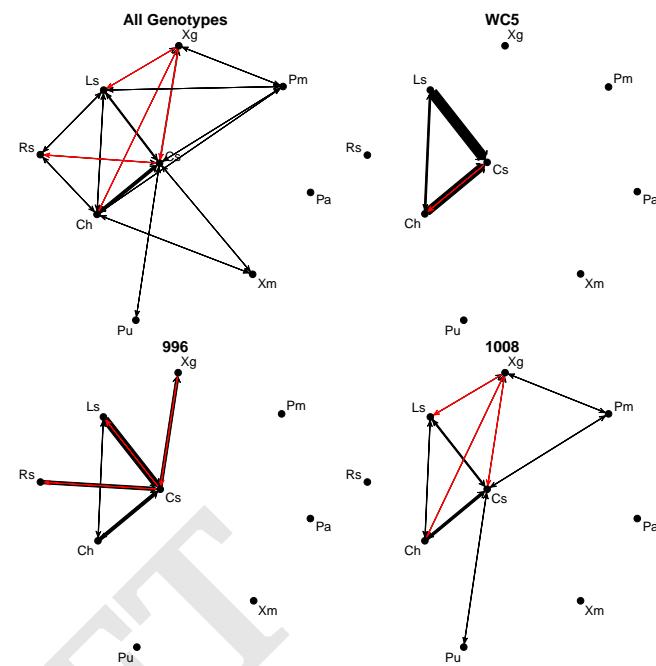
90 Here, we investigate how genetic variation in a  
91 foundation tree species determines the structure of a  
92 network of interactions among a community of tree  
93 associated lichen species. Using a long-term (20+  
94 years), common garden experiment with replicated  
95 individuals of known genetic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

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

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

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

created in part by genetic variation in tree  
 individuals.

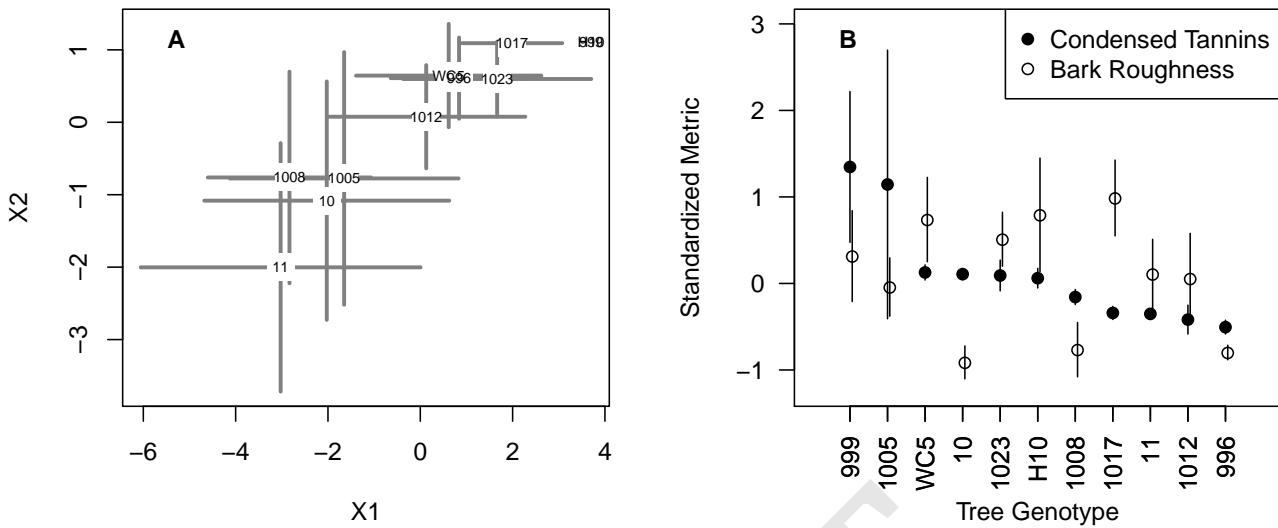


**Fig. 1.** Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

## Results

Network similarity and several tree traits were genetically based. Tree genotype was a significant predictor of network similarity ( $H^2 = 0.16$ ,  $p\text{-value} \leq 0.001$ ). Bark roughness ( $H^2 = 0.38$ ,  $p\text{-value} \leq 0.001$ ) and condensed tannin concentration ( $H^2 = 0.28$ ,  $p\text{-value} = 0.014$ ) also showed a signature of tree genotype (Fig. 2); however, this was not the case for other tree traits, bark pH and carbon to nitrogen ratio. Also, none of the lichen community nor any of the lichen network metrics were significantly predicted by tree genotype (Table 1). Although both showed a response to tree genotype, bark roughness and condensed tannins were not correlated (Pearson's  $r = 0.084$ ,  $p\text{-value} = 0.556$ ).

Tree traits and lichen community metrics were correlated with lichen networks. The genetically based traits, bark roughness and condensed tannins were both significant predictors of network similarity (Ta-



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

Response	H2	p-value
Lichen Network Similarity	0.16	1e-04
Number of Network Links	0.0695	0.2376
Network Centrality	0.0851	0.1985
Network Modularity	0	0.4492
Percent Lichen Cover	0.0793	0.172
Lichen Species Richness	0	1
Lichen Species Evenness	0.0151	0.3882
Lichen Species Diversity	0.0095	0.4167
Community Composition	0.052	0.1019
Percent Rough Bark	0.3849	1e-04
Condensed Tannins (CT)	0.2803	0.0139
Carbon-Nitrogen (CN) Ratio	0	0.4479
pH	0.0539	0.2939

**Table 1. Genotypic effects on the associated lichen community.**

or not (Mantel R = -0.03, p-value = 0.573).

	df	SS	R2	F	p-value
Genotype	10.0000	304.9280	0.2335	19.2955	0.0001
Bark Roughness	1.0000	16.2594	0.0124	10.2888	0.0025
pH	1.0000	5.0371	0.0039	3.1874	0.0831
C:N Ratio	1.0000	39.6664	0.0304	25.1005	0.0001
Condensed Tannins	1.0000	70.7702	0.0542	44.7826	0.0001
Percent Cover	1.0000	56.3523	0.0431	35.6592	0.0001
Species Richness	1.0000	332.4174	0.2545	210.3504	0.0001
Species Evenness	1.0000	55.1077	0.0422	34.8716	0.0001
Number of Links	1.0000	326.5265	0.2500	206.6226	0.0001
Network Modularity	1.0000	7.7683	0.0059	4.9157	0.0305
Network Centrality	1.0000	43.7646	0.0335	27.6938	0.0001
Residual	30.0000	47.4091	0.0363		
Total	50.0000	1306.0069	1.0000		

**Table 2. PERMANOVA Pseudo-F Table of lichen network similarity.**

## Materials and Methods

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

LJL: If you include the field data, I would suggest changing the topic sentence for this paragraph and adding something about the field data sampling to this paragrpah. 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.

ble 2). Bark C:N ratio was also a significant predictor of network similarity, but, as shown previously (see Table 1), there is not sufficient evidence support a genetic basis for it. Bark pH was not a significant predictor of lichen network similarity (Table 2). The abundance, richness, evenness and diversity of the bark lichen community, although also not predicted by tree genotype, were all significantly correlated with lichen network structure (Table 2). Lichen community composition was not correlated with lichen network similarity, either when species abundances were relativized (Mantel R = -0.09, p-value = 0.139)

The study was conducted along the Weber River, UT<sup>24</sup> (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 (18).

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<sup>2</sup> 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<sup>2</sup> quadrats centered at 50 cm and 85 cm from ground level were sampled (Fig 3 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 melanachra*, Pa = *Physcia adscendens*.

<sup>2</sup>Bu = *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 sublivacea*, *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 cm<sup>2</sup> (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 yield 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 (18) that is under strong genetic control (19). 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.

**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<sup>2</sup> 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 (20). 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.



**Fig. 3.** The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area ( $10 \text{ cm}^2$ ) on individual trees (A and B). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Physciella melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesboung (D-K).

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.

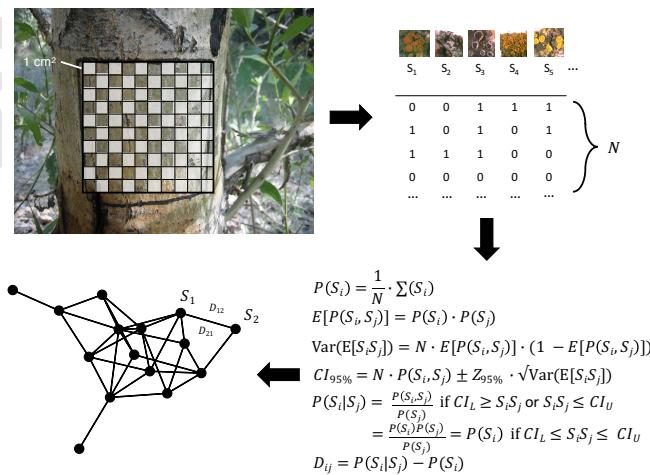
**MKL:** regarding Lamit's question about the symmetry, the point is that direction of the interaction matters. The effect of species A on B can be different from B on A. No the matrix is not necessarily triangular (triangular being that the matrix either above or below the diagonal is completely zero).

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. 4). 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 ( $\mathbf{D} = D_{ij}$ ) 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 ( $\mathbf{D}$ ) 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 ( $P_{ii}$ ) 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. 4.** Lichen interaction networks were constructed by conducting field observations in  $1 \text{ cm}^2$  cells within a  $10 \text{ cm}^2$  grid on each tree using a checkerboard pattern (grey cells). Thus, a set of  $N$  total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (20), we calculated the conditional probabilities,  $P(S_i|S_j)$ , for all species pairs and removed (i.e. set equal to zero) species pairs whose joint probabilities,  $P(S_i S_j)$ , were not significant using a confidence interval based comparison of their observed co-occurrence frequency,  $S_i S_j$ , to that expected due to chance alone,  $E[P(S_i S_j)] = P(S_i)P(S_j)$ , and  $P(S_i|S_j)$  reduces to  $P(S_i)$ , the observed individual probability of species  $S_i$ .

**LJL:** I like the details here. THe one thing is that it sort of makes the reader think there is only one quadrat on a tree but infact there were two. I think you want to make sure to be explicit about the two. For analytical purposes, was all the data lumped so there was really functionally a 20cm

396 by 10cm grid (just split into two pieces). Or, was  
397 the network made for each of the two grids and  
398 them averaged or combined in some way? My  
399 understanding is that it was more the first than  
400 the latter.

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

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

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

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

438 For each network, we also calculated two network met-  
439 rics that measure different structural aspects. We cal-  
440 culated the number of interactions or "links" in each  
441 network, which provides a measure of the size of the net-  
442 work citepLau2015, Borrett2014. We also calculated the  
443 centralization of each network, which measures the even-  
444 ness of the distribution of interactions among the species  
445 in the network citeButts2005. In a network with a low  
446 level of centralization species have similar amount of inter-  
447 action in the network, while a network with a high level of  
448 centralization tends to one or small subset of species that  
449 interact with other species. We used a related function  
450 to calculate the centrality of each species in each network

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

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

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

## 472 Discussion

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

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

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

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

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

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

- 497 • 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.

ation in plant functional traits and highlights the potential for indirect effects of genetic variation to propagate through networks of interacting species and trophic levels.

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

Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. For example, (7) showed that the genetics based interactions of aphid resistant and aphid susceptible trees resulted in different interaction networks of their associated arthropod communities composed of 139 species. At the scale of ecosystems, trophic networks or food webs direct and control the rates of energy and nutrient flux (22). Furthermore, in a predator-prey-plant study, Smith (23), showed that the interactions among species across trophic levels depended on plant genotype.

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

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

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

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

499 have more similar communities 546 500 • What we don't know is whether or not these 547 interactions will also lead to similar interactions 548 among other species. 549

502 • Thus, it would be possible for genetics to not 550 only influence other species directly, but also 551 indirectly by influencing the interactions among 552 other species. 553

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

586 **LJL: It could be useful to point out that 587 our findings are not related to trophic 588 interactions, which is pretty cool. Also, we 589 talk about interaction networks but it is not 590 clear to me if the interactions tend to be positive 591 or negative. Can we get at that with the 592 approach used?**

596 **TGW: Is there any adaptive component to 597 the tree in having certain lichen communities? 598 e.g., can they feed back to affect tree 599 performance in some way or is this a 600 passive outcome of a trait that affects bark for 601 other adaptive reasons and lichens are 602 passive players that tag along for the ride? I 603 could envision that lichens covering the bark 604 of a tree act as a barrier between insects and 605 pathogens, much like ectomycorrhizae cover 606 fine roots as a first line of defense by invading 607 microorganisms. Uptake of N that gets 608 passed to the tree??**

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626 have more similar communities 627 628 • What we don't know is whether or not these 629 interactions will also lead to similar interactions 630 among other species. 631

635 We observed significant lichen interaction 660 structure that varied among genotypes of a foundation 661 tree species, narrowleaf cottonwood (*P. angustifolia*). 662 We found that a genetically based trait, bark 663 roughness, partially explained the variation in lichen 664 interaction networks. Some of this variation in lichen 665 networks was related to both the overall abundance 666 and species richness of lichen; though, statistically 667 controlling for the effect of genotype on these variables 668 indicates that a significant portion of the variance 669 in lichen species richness is due to a factor 670 other than tree genotype. By using network metrics, 671 we were also able to probe for specific characteristics 672 of how these networks were responding to tree 673 genotype. We found that both number of links and 674 the centralization of the networks were highly correlated 675 with network similarity and that tree genotype 676 significantly predicted network centrality but only 677 marginally predicted the number of network links. 678 This latter result could be due to the relationship 679 between species richness and the number of links 680 in the network, which were significantly correlated 681 with each other. We also found that bark roughness 682 did not significantly predict either the number of 683 links or the centrality of lichen networks, suggesting 684 that bark roughness has some other effect on the 685 structure of the lichen networks. Taken together, 686 these findings support the hypothesis that genotypic 687 variation in a foundation species contributes to the 688 structure of a network of interacting species. 689

692 **LJL: It could be useful to point out that 693 our findings are not related to trophic 694 interactions, which is pretty cool. Also, we 695 talk about interaction networks but it is not 696 clear to me if the interactions tend to be positive 697 or negative. Can we get at that with the 698 approach used?**

702 **TGW: Is there any adaptive component to 703 the tree in having certain lichen communities? 704 e.g., can they feed back to affect tree 705 performance in some way or is this a 706 passive outcome of a trait that affects bark for 707 other adaptive reasons and lichens are 708 passive players that tag along for the ride? I 709 could envision that lichens covering the bark 710 of a tree act as a barrier between insects and 711 pathogens, much like ectomycorrhizae cover 712 fine roots as a first line of defense by invading 713 microorganisms. Uptake of N that gets 714 passed to the tree??**

718 **TGW: might be good to cite papers on 719 competition in lichens or other organizing 720 factors to back up the least expected statement. 721 as epiphytes we might not expect them to 722 care.**

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might want to mention this up front in intro<sup>634</sup>  
596 **MKL: Environmental filtering is evidenced<sup>635</sup>**  
597 by species richness, but also possibly species  
598 interaction varying based on environment as  
599 networks varied in terms of sign and magni-  
600 tude as well.

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

#### 605 **Discussion of network implications for sta- 606 bility with genetics.**

607 Although our study was conducted with a commu-  
608 nity of lichens, these results should be generalized  
609 to other groups of diverse organisms around the  
610 world that also exhibit significant genetic signals at  
611 the community level (24, 25). In the face of the  
612 high degree of complexity and potential context de-  
613 pendence of ecological processes, the current study  
614 points to the utility of considering the spatial and  
615 temporal scales of interactions, as discussed to some  
616 in previous studies (26–28). In the present study,  
617 we found that community assembly processes, such  
618 as environmental filtering and species interactions,  
619 are genetically based. This is likely due, in part,  
620 to the large difference in the differences in size and  
621 longevity of the lichen and cottonwood individuals  
622 with the trees determining the environment in which  
623 the lichen occur. We suggest that future work would  
624 be aided by determining these modules within the  
625 biotic community that include species with similar  
626 differences in body-size and time-scales. As heritable  
627 variation is the raw material for natural selection  
628 to act upon, a genetic basis for interaction network  
629 structure indicates evolutionary dynamics should be  
630 considered at the community level and that con-  
631 serving genetic variation is important to consider iff<sup>634</sup>  
632 efforts to restore or preserve complex species interac-  
633 tions and their associated ecosystem functions (29).  
634 With such findings, it appears that we are closer to  
635 understanding the evolutionary drivers of Darwin’s  
636 entangled bank and the interconnectedness of species  
637 in complex communities.<sup>704</sup>

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639 the National Science Foundation grant (DEB-0425908)  
640 and Integrative Graduate Research Traineeship (IGERT)  
641 fellowships for M.L. and L.L. The Ogden Nature Center  
642 staff helped to maintain the common gardens. Lichen  
643 sampling was supported by Todd Wojtowicz, Luke Evans

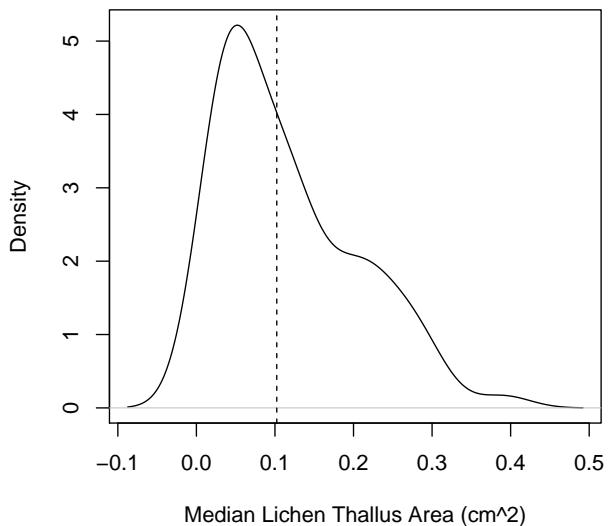
599 and David Solance Smith.

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705 **Supplementary Materials**

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

711



**Fig. 1.** Density plot of the median lichen thallus area ( $\text{cm}^2$ ).

	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		