

# Genotypic variation in a foundation tree directs ecological network structure

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1 **Biological evolution occurs in the context of complex networks of**  
2 **interacting species in which natural selection defines the structure**  
3 **of ecological networks. Fundamental to this evolutionary process**  
4 **is the discovery of a genetic basis to ecological network structure.**  
5 **Although previous work has demonstrated that tree genotype con-**  
6 **tributes to interaction network structure at the scale of forest stands,**  
7 **the contribution of tree genetics to localized interaction networks**  
8 **at the scale of individual trees has not yet been explored. To test**  
9 **the degree to which tree genetics can contribute to network struc-**  
10 **ture we conducted quantitative modeling of interaction networks. We**  
11 **constructed networks of epiphytic lichen associated with individual**  
12 **trees that were a part of a long-term experimental common garden**  
13 **of genotypes of a foundation species (*Populus angustifolia*).** We  
14 **found three main results: 1) bark roughness and lichen communi-**  
15 **ties displayed significant responses to tree genotype, 2) tree geno-**  
16 **type contributed to lichen network structure, explaining a third of**  
17 **the variation in lichen interaction networks, and 3) different aspects**  
18 **of lichen network structure, including the number of interactions and**  
19 **centralization, responded to tree genotype, primarily as a function**  
20 **of the number of species present and to a lesser extent the abun-**  
21 **dance of lichens. We conclude that tree genotype influences lichen**  
22 **interaction network structure with one potential pathway being that**  
23 **bark roughness, a genetically based plant functional trait, alters the**  
24 **presence and overall abundance of lichens, which determines the**  
25 **nature and magnitude of interactions in the community. These re-**  
26 **sults support the hypothesis that variation in ecological interaction**  
27 **networks can result from genetically based variation in foundation**  
28 **species. This study suggests a genetic basis to both direct and in-**  
29 **direct interactions among species that can result in the evolution of**  
30 **complex communities.**

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

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

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

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

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

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

## Significance Statement

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

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

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

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

51 Add a discussion of DesRoches.

52 From Thompson2014

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

58 Specific hypothesis from Thomspn2014

59 Selection in small webs

60 •

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

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

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

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.

## Materials and Methods

154 The study was conducted along the Weber River, UT (USA),  
155 which is a cottonwood (*Populus* spp.) dominated riparian ecosystem.  
156 Although two native species, *Populus angustifolia* (James) and *Pop-*  
157 *ulus fremontii* (S. Watson), occur here and are known to hybridize,  
158 only pure or advanced generation backcrosses of *P. angustifolia*  
159

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).

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 paragrph. 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.

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 founf 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 obsered. 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 1 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*, Pu = *Physcia undulata*. Several other species were not obesrvd in the present study but are known to occur in this region: *Phaeophyscia orbicularis*, *Phaeophyscia ciliata*, *Melanelia subolivacea*, *Meanelia elegantula*.

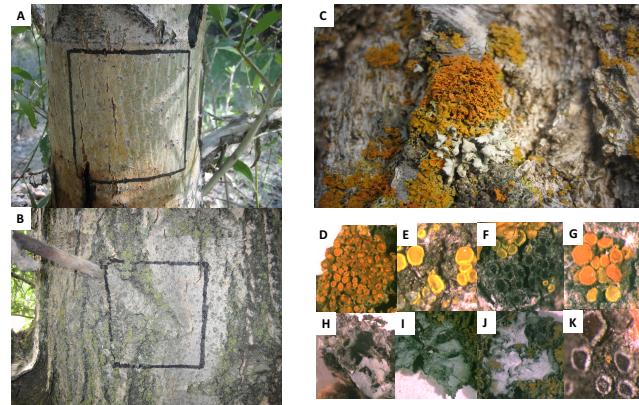
The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous survey of lichen thallus size in this common garden, we had observed a median thallus size of  $0.12 \pm 0.001$  cm<sup>2</sup> (S.E.) (see Supplementary Fig 1). Based on the median thallus 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 overal visual % cover. I didn't count P/A of rough bark in each square. So, yo 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. 1.** 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<sup>2</sup>) 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 melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (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<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 (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.

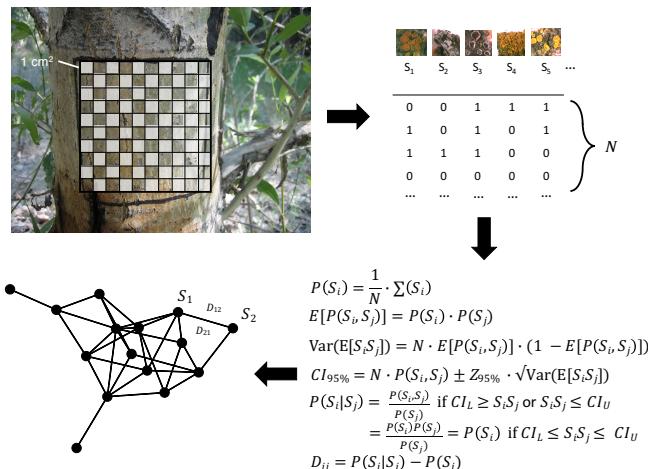
MKL: regarding Lamit's question about the symmetry, the point is that direction of the interaction matters. The effect of species A on B can be different from B on A. No the matrix is not necessarily triangular (triangular being that the matrix either above or below the diagonal is completely zero).

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. 2). Here, a confidence interval  $CI_{95\%}$  is calculated as as  $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$ , where the expected 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.  $P(S_i) \hat{P}(S_j)$ ), 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. 2.** Lichen interaction networks were constructed by conducting field observations in 1 cm<sup>2</sup> cells within a 10 cm<sup>2</sup> 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 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 by 10cm grid (just split into two pieces). Or, was the network made for each of the two grids and them averaged or combined in some way? My understanding is that it was more the first than the latter.

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

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

For multivariate response variables, such as lichen community composition and network structure, we used distance based multivariate statistical approaches, including Permutational Analysis of Variance (PerMANOVA) and Mantel tests. For all analyses, community composition was relativized by species maxima to reduce the effect of the highly abundant *X. galericulata*. For community composition we used Bray-Curtis dissimilarity, which has optimal performance with count data (?). To quantify the similarity of lichen networks among individual trees, we calculated the pairwise Euclidean distance of the  $\mathbf{D}$  interaction matrices among all pairs of trees.

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

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

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

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

## Results

**MKL:** for all figures 1) increase line width, 2) make axis labels bold, 3) increase clarity of vectors and labels.

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

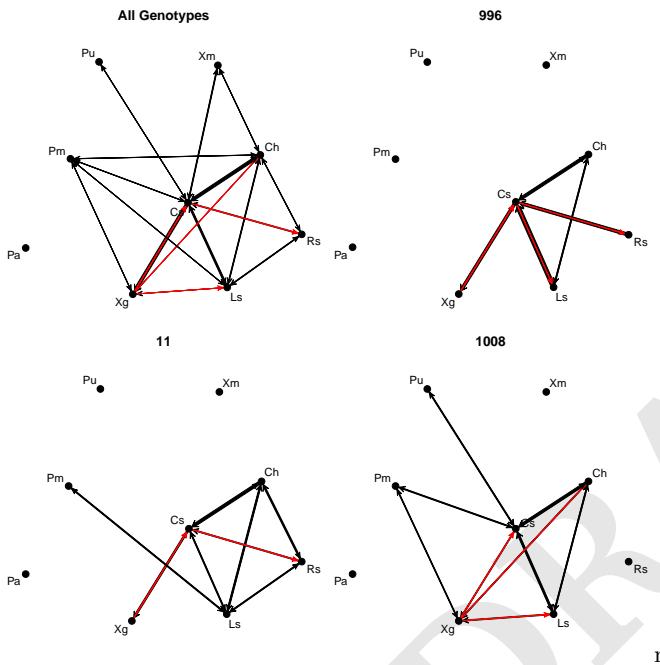
**LJL:** Figure looks good. But, maybe making all lines a little thicker would look nicer and pop more.

**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 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.



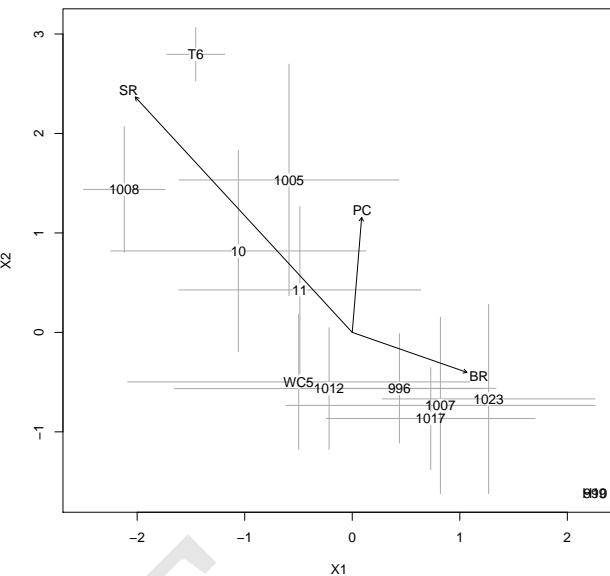
**Fig. 3.** Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive) 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.

We observed significant lichen network structure. This structure varied among genotypes (Fig. 3) and lichen species varied in their importance in the network. *Candaleriella subdeflexa* was generally the most central species (i.e. being the most highly connected) having the highest average centrality (0.73), followed by *Ca. holocarpa* (0.54) and *L. hagenii* (0.40). The centralization of the remaining species were *R. sp.* (0.18), *X. galericulata* (0.14), *P. melanachra* (0.08), *X. montana* (0.06) and *Ph. undulata* (0.02). *Physcia adscendens* was generally not connected to other species in the networks and had a centralization score of zero.

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

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

**LJL:** It seems to me that the first two sentences here are the most important of the results. How can you make them stand out more? Maybe also they should go at the



**Fig. 4.** Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ( $R^2 = 0.999$ , stress = 0.011) lichen networks ( $\pm 1$  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).

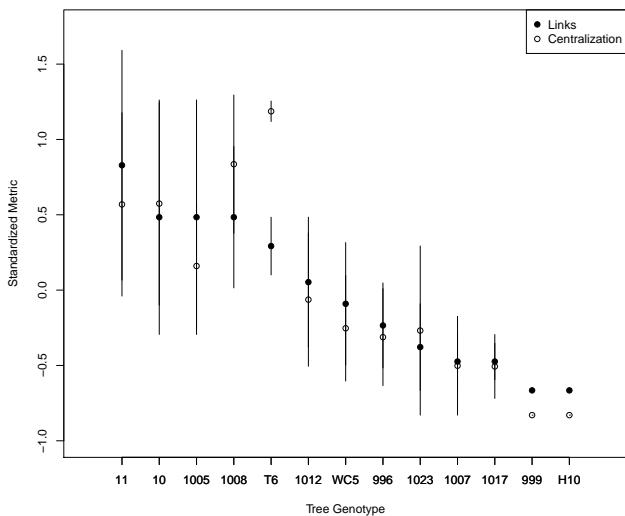
beginning of the previous paragraph, and then move that paragraph to being the first in the Results section.

**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.

Lichen networks observed on trees of the same genotype tended to be more similar in structure. Tree genotype significantly predicted the similarity of lichen interaction networks (PerMANOVA  $R^2 = 0.33795$ ,  $F_{12} = 2.5379$ ,  $p\text{-value} = 0.0050$ ) (Fig. 4). Bark roughness (PerMANOVA  $R^2 = 0.040$ ,  $F_1 = 4.1680$ ,  $p\text{-value} = 0.03770$ ) and lichen species richness (PerMANOVA  $R^2 = 0.424$ ,  $F_1 = 44.5034$ ,  $p\text{-value} = 9.999e-05$ ) were significant predictors of lichen network similarity, while total lichen cover was a weak, marginally significant predictor (PerMANOVA  $R^2 = 0.032$ ,  $F_1 = 3.3573$ ,  $p\text{-value} = 0.06779$ ). However, after controlling for the effect of tree genotype on lichen network similarity, only species richness was a significant predictor of lichen network similarity (PerMANOVA  $R^2 = 0.300$ ,  $F_1 = 33.3755$ ,  $p\text{-value} = 0.00001$ ), and neither bark roughness (PerMANOVA  $R^2 = 0.019$ ,  $F_1 = 2.0858$ ,  $p\text{-value} = 0.14699$ ) nor lichen cover (PerMANOVA  $R^2 = 0.019$ ,  $F_1 = 2.1504$ ,  $p\text{-value} = 0.14409$ ) were significant predictors (Supplementary Tables ?? and 4). Community similarity was not correlated with network similarity (Mantel Spearman  $p = 0.092$ ,  $p\text{-value} = 0.09500$ ).

Bark roughness predicted lichen communities. Percent rough bark varied significantly among genotypes (REML  $R^2 = 0.378$ , RLRT = 10.69,  $p\text{-value} = 0.0001$ ), as did total lichen cover (REML  $R^2 = 0.172$ , RLRT = 2.9627,  $p\text{-value} = 0.0375$ ). However, lichen species richness did not show a significant response to genotype (REML  $R^2 = 0.0981$ , RLRT = 1.0001,  $p\text{-value} = 0.1366$ ). Community composition was also affected by tree genotype (PerMANOVA  $R^2 = 0.243$ ,  $F_{12} = 1.8221$ ,  $p\text{-value} = 0.0029$ ). In addition, community composition was correlated with bark roughness (PerMANOVA  $R^2 = 0.172$ , RLRT = 2.9627,  $p\text{-value} = 0.0375$ ).

461  $R^2 = 0.039$ ,  $F_1 = 3.7408$ ,  $p\text{-value} = 0.0064$ ), lichen cover (Per  
 462 MANOVA  $R^2 = 0.342$ ,  $F_1 = 32.8482$ ,  $p\text{-value} = 0.0001$ ) and lichen  
 463 species richness (PerMANOVA  $R^2 = 0.069$ ,  $F_1 = 6.5958$ ,  $p\text{-value} =$   
 464  $0.0002$ ). However, after controlling for the effect of tree genotype  
 465 on community composition, bark roughness did not significantly  
 466 predict community composition (PerMANOVA  $R^2 = 0.011$ ,  $F_1 =$   
 467  $= 0.9938$ ,  $p\text{-value} = 0.3841$ ) but lichen cover (PerMANOVA  $R^2 =$   
 468  $= 0.236$ ,  $F_1 = 21.2661$ ,  $p\text{-value} = 0.0001$ ) and lichen species richness  
 469 (PerMANOVA  $R^2 = 0.054$ ,  $F_1 = 4.9036$ ,  $p\text{-value} = 0.0011$ ) were  
 470 still significantly correlated with lichen composition (Supplementary  
 471 Tables 1 and 2).



**Fig. 5.** 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 ( $\pm 1$  S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented as standardized scores ( $\frac{x-\bar{x}}{\sigma}$ ).

Networks were more similar as a result of having similar numbers of interactions and distribution of interactions. The number of links (PerMANOVA  $R^2 = 0.392$ ,  $F_1 = 72.4348$ ,  $p\text{-value} = 0.001$ ) and network centrality (PerMANOVA  $R^2 = 0.309$ ,  $F_1 = 57.0440$ ,  $p\text{-value} = 0.001$ ) were highly correlated with network similarity. Tree genotype significantly predicted network centrality (REML  $R^2 = 0.202$ , RLRT = 2.7801,  $p\text{-value} = 0.04012$ ) but marginally predicted the number of links (REML  $R^2 = 0.170$ , RLRT = 2.0484,  $p\text{-value} = 0.065$ ) (Fig. 5). Total cover was correlated with the number of links (ANOVA  $F_1 = 6.867$ ,  $p\text{-value} = 0.0114$ ) and centrality (ANOVA  $F_1 = 8.093$ ,  $p\text{-value} = 0.0063$ ). Lichen species richness was also correlated with the number of links (ANOVA  $F_1 = 29.436$ ,  $p\text{-value} = 0.000015$ ) and centrality (ANOVA  $F_1 = 39.488$ ,  $p\text{-value} < 0.000001$ ). Bark roughness, however, did not significantly predict either the number of links (ANOVA  $F_1 = 2.897$ ,  $p\text{-value} = 0.0946$ ) or the centrality (ANOVA  $F_1 = 2.591$ ,  $p\text{-value} = 0.1134$ ) of lichen networks (Supplementary Tables 5 and 6).

## Discussion

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

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

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

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

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

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

Overall, I think you want to focus on the network responses and patterns among genotype first, and then go into mechanism later. I think we don't quite have a good mechanism yet so I don't think it needs to come up in the first paragraph of the discussion.

These findings point to the importance of understanding the community level effects of genetic variation 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. (?)

Altering the structure of interaction networks presents a means for genetic effects to be magnified within the system of interacting species. For example, (1) 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 (25). Furthermore, in a predator-prey-plant study, Smith (26), showed that the interactions among species across trophic levels depended on plant genotype.

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? L JL: 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?

T GW: 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??

T GW: 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.

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

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

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

Discussion of network implications for stability with genetics.

Although our study was conducted with a community of lichens, these results should be generalized to other groups of diverse organisms around the world that also exhibit significant genetic signals at the community level (27, 28). In the face of the high de-

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

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## Supplementary Materials

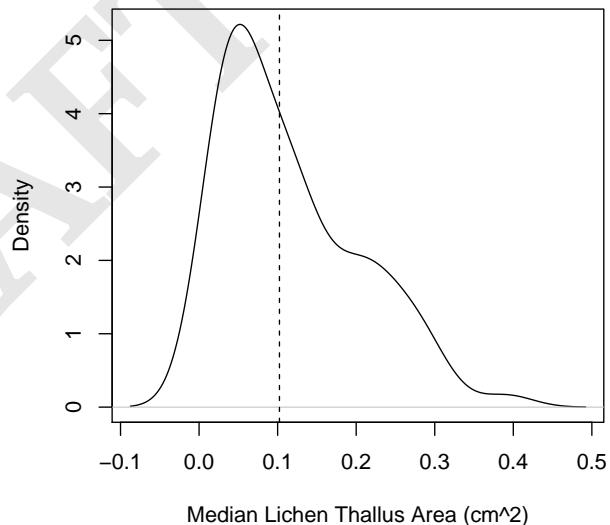
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692 TGW: I know you commented about not talking about H2 in the text, but since you have the data, why not? 718  
719 All heritability findings only apply for the environment or 720 common garden they were measured in as does the rest of 721 the findings presented in this paper. 722

Response	H2	R2	p-value
Percent Rough Bark	0.37835	0.37835	3e-04
Network Centrality	0.20166	0.20166	0.04076
Percent Lichen Cover	0.17279	0.17279	0.0367
Number of Network Links	0.17016	0.17016	0.06602
Lichen Community Composition	0.16093	0.24287	0.0029
Lichen Species Richness	0.09815	0.09815	0.1405
Lichen Network	0.06252	0.29111	0.0094
Network Modularity	0.05731	0.05731	0.2809

712 **Table 1. Genotypic effects of cottonwood trees on the associated**  
lichen community.  
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**Fig. 1.** Density plot of the median lichen thallus area ( $\text{cm}^2$ ).

	Df	SumOfSqs	R2	F	Pr(>F)
BR	1	0.44	0.04	3.74	0.0064
PC	1	3.86	0.34	32.85	0.0001
SR	1	0.78	0.07	6.60	0.0002
Residual	53	6.23	0.55		
Total	56	11.31	1.00		

**Table 1. PerMANOVA Pseudo-F Table showing the predictors of community similarity.**

	Df	SumOfSqs	R2	F	Pr(>F)
geno	12	2.75	0.24	1.82	0.0029
BR	1	0.12	0.01	0.99	0.3841
PC	1	2.67	0.24	21.27	0.0001
SR	1	0.62	0.05	4.90	0.0011
Residual	41	5.15	0.46		
Total	56	11.31	1.00		

**Table 2.** PerMANOVA Pseudo-F Table showing the predictors of community similarity.

	Df	SumOfSqs	R2	F	Pr(>F)
BR	1	61.42	0.04	4.17	0.0377
PC	1	49.47	0.03	3.36	0.0678
SR	1	655.76	0.42	44.50	0.0001
Residual	53	780.96	0.50		
Total	56	1547.61	1.00		

**Table 3.** PerMANOVA Pseudo-F Table showing the predictors of network similarity.

	Df	SumOfSqs	R2	F	Pr(>F)
geno	12	450.52	0.29	2.69	0.0094
BR	1	29.11	0.02	2.09	0.1470
PC	1	30.01	0.02	2.15	0.1441
SR	1	465.78	0.30	33.38	0.0001
Residual	41	572.18	0.37		
Total	56	1547.61	1.00		

**Table 4.** PerMANOVA Pseudo-F Table showing the predictors of network similarity.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
BR	1	102.25	102.25	2.78	0.1016
PC	1	239.57	239.57	6.50	0.0137
SR	1	956.96	956.96	25.98	0.0000
Residuals	53	1952.23	36.83		

**Table 5.** ANOVA F Table showing the predictors of the number of network links.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
BR	1	3.77	3.77	2.17	0.1463
PC	1	6.46	6.46	3.72	0.0590
SR	1	56.48	56.48	32.55	0.0000
Residuals	53	91.95	1.73		

**Table 6.** ANOVA F Table showing the predictors of network centralization.

	Df	SumOfSqs	R2	F	Pr(>F)
L	1	1330.80	0.86	734.67	0.0010
Cen	1	118.99	0.08	65.69	0.0010
Residual	54	97.82	0.06		
Total	56	1547.61	1.00		

**Table 7.** PERMANOVA Pseudo-F Table showing the predictors of network similarity.