

# Genotypic variation in a foundation tree directs ecological network structure

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This manuscript was compiled on November 14, 2019

1 **Biological evolution occurs in the context of complex networks of**  
2 **interacting species in which natural selection defines the structure**  
3 **of ecological networks. Fundamental to this evolutionary process**  
4 **is the discovery of a genetic basis to ecological network structure.**  
5 **Although previous work has demonstrated that tree genotype con-**  
6 **tributes to interaction network structure at the scale of forest stands,**  
7 **the contribution of tree genetics to localized interaction networks**  
8 **at the scale of individual trees has not yet been explored. To test**  
9 **the degree to which tree genetics can contribute to network struc-**  
10 **ture we conducted quantitative modeling of interaction networks. We**  
11 **constructed networks of epiphytic lichen associated with individual**  
12 **trees that were a part of a long-term experimental common garden**  
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.**

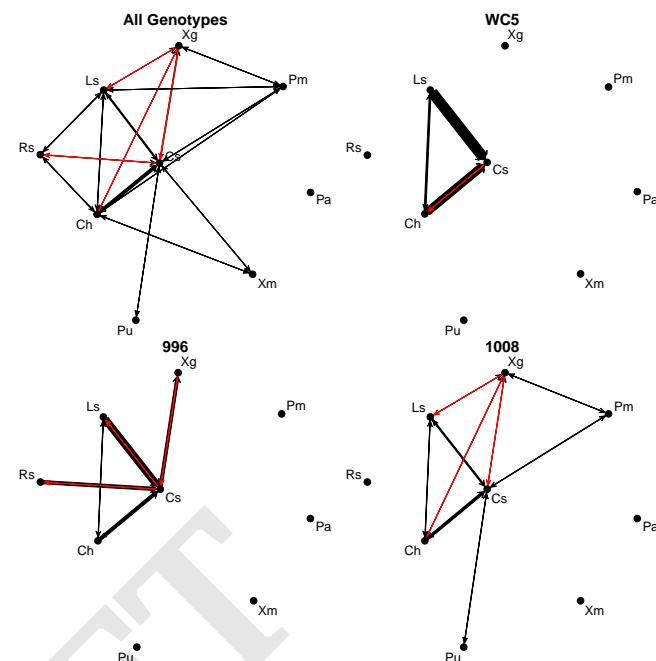
90 Here, we investigate how genetic variation in a  
91 foundation tree species determines the structure of a  
92 network of interactions among a community of tree  
93 associated lichen species. Using a long-term (20+  
94 years), common garden experiment with replicated  
95 individuals of known genetic 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.

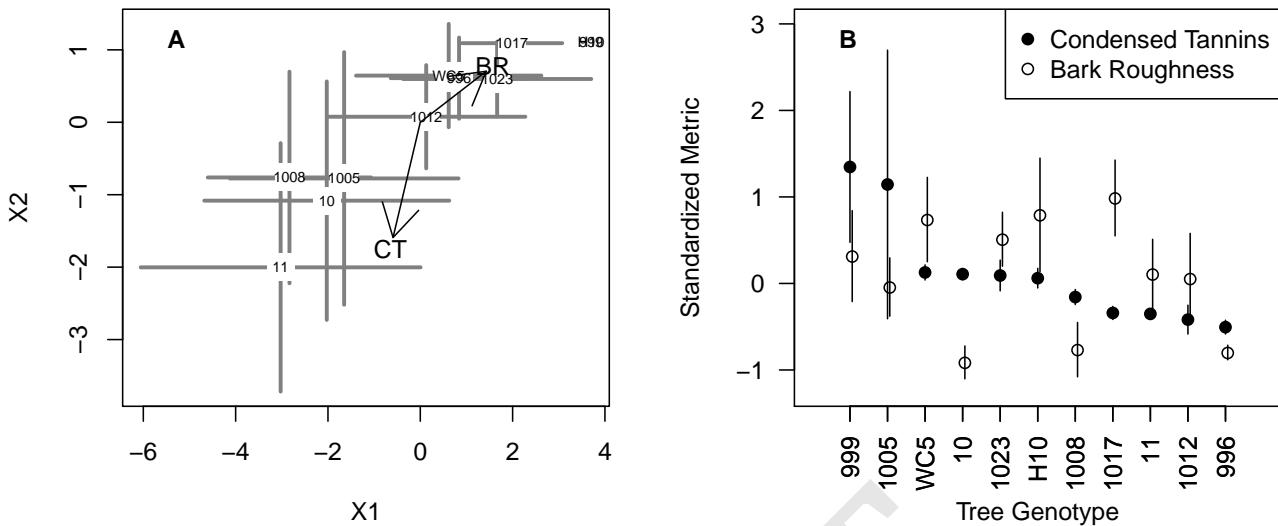


**Fig. 1.** Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

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 (Table 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



**Fig. 2**

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

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

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

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

MKL: Need to add more citations of Lamits or other lichen studies. 207  
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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. 209  
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The study was conducted along the Weber River, UT (USA), which is a cottonwood (*Populus* spp.) dominated riparian ecosystem. Although two native species, *Populus angustifolia* (James) and *Populus fremontii* (S. Watson), occur here and are known to hybridize, only pure or advanced generation backcrosses of *P. angustifolia* were 216  
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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) or not (Mantel R = -0.03, p-value = 0.573).

## Materials and Methods

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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.** L JL: 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.

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

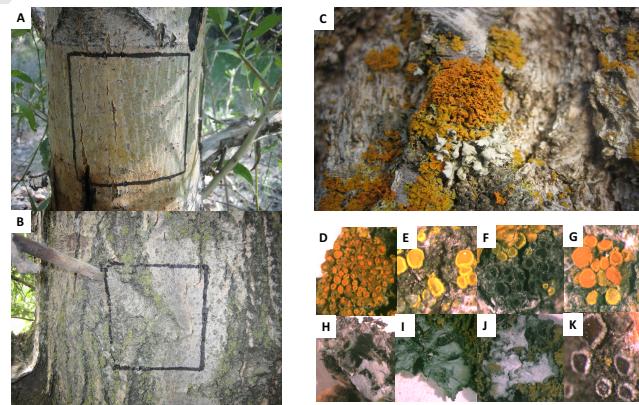
The cell size and checkerboard sampling pattern was chosen to isolate the individuals in each cell. In a previous

survey of lichen thallus size in this common garden, we had observed a median thallus size of  $0.12 \pm 0.001 \text{ cm}^2$  (S.E.) (see Supplementary Fig 1). Based on the median thallus size, we expected thalli observed in each cell to generally be spatially independent of thalli present in other cells but exposed to similar micro-environmental conditions created by the bark and the location of the sampling area on an individual tree. Therefore, we were confident in treating the cell-wise observations in quadrats as independent with respect to lichen-lichen interactions.

L JL: My method was overall 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 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.



**Fig. 3.** The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm<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. Naesbour (D-K).

**Lichen Network Modeling and Analysis.** L JL: This seems like a key point, one that really makes the study above and beyond. I would make it

310 clear with the phrasing that individual networks  
 311 were created for each individual tree sampled, in  
 312 this way we had replicated networks for each tree  
 313 genotypes.

314 We used the observations of lichens in the 1 cm<sup>2</sup> cells  
 315 on individual trees of *P. angustifolia*. Unipartite networks  
 316 were generated using the conditional probabilities of each  
 317 species pair, i.e. the probability of observing one species  
 318 given an observation of another species  $P(S_i|S_j)$ , based on  
 319 the method developed by (20). To calculate conditional  
 320 probabilities, we quantified the individual probabilities  
 321 of species occurrences  $P(S_i)$  and the joint probability  
 322 of co-occurrences  $P(S_i, S_j)$  using the frequencies of each  
 323 species and their co-occurrences. We were then able to  
 324 calculate the conditional probabilities of each species pair  
 325 as  $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$ , based on the axioms of probability.  
 326 This yielded a matrix that could possibly be asymmetric, i.e.  
 327  $P(S_i|S_j)$  does not have to be equal to  $P(S_j|S_i)$ . Another  
 328 important property of this matrix is that the diagonal  
 329 ( $S_{ii}$ ) was equal to one for all species present and zero for  
 330 species that were not observed in any cell.

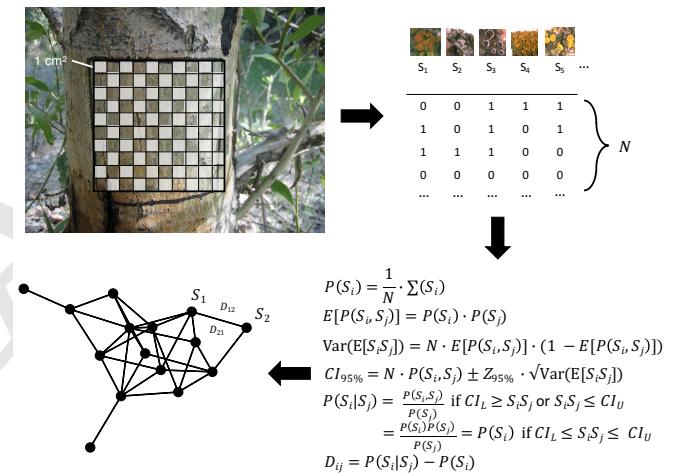
331 **MKL:** regarding Lamit's question about the  
 332 symmetry, the point is that direction of the interaction  
 333 matters. The effect of species A on B can be different from B on A. No the matrix is  
 334 not necessarily triangular (triangular being that  
 335 the matrix either above or below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

337 We then applied an analytical procedure to remove  
 338 non-significant links between species. This procedure  
 339 determines if the joint probability of a species pair  
 340 (i.e.  $P(S_i, S_j)$ ) is different from zero (Fig. 4). Here, a  
 341 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  
 342 frequency of co-occurrences  $E(S_i S_j)$  is the total number  
 343 of cells surveyed ( $N$ ) times the independent probabilities  
 344 of each species  $P(S_i) * P(S_j)$ ,  $Z_{95\%}$  is the Z-score  
 345 for 95% from a Z-distribution and the expected variance  
 346 of  $E(S_i S_j)$  is the total number of cells times the  
 347 expected probability of  $S_i S_j$  and its compliment (i.e.  
 348  $V(S_i S_j) = N * E[P(S_i, S_j)] * (1 - E[P(S_i, S_j)])$ ). If the  
 349 observed number of co-occurrence falls outside of the  
 350 confidence interval, the joint probability  $P(S_i, S_j)$  is de-  
 351 termined to be equal to the product of the individual  
 352 probabilities (i.e.  $P(S_i)P(S_j)$ ), and the conditional prob-  
 353 ability reduces to the individual probability of that species  
 354  $P(S_i)$ . Therefore, unless the co-occurrence of a species  
 355 pair falls outside the confidence interval, the probabili-  
 356 ty that the observation of one species given the other  
 357 is no different than simply observing that species alone.  
 358 This enables us to remove links from a given network by  
 359 re-scaling the resulting conditional probabilities by sub-  
 360 tracting the individual probabilities from the conditional  
 361 probabilities (i.e. how different the conditional probability  
 362 is from the independent probability), which makes any  
 363 species with a non-significant conditional probability zero.

The resulting matrix ( $\mathbf{D} = D_{ij}$ ) can be interpreted as  
 366 how one species impacts another with zero being no effect  
 367 and values less than or greater than zero interpreted as  
 368 negative and positive effects, respectively. Here, we will  
 369 refer to this matrix ( $\mathbf{D}$ ) as an interaction matrix with  
 370 the properties that it can be asymmetric (i.e.  $P_{ij}$  does not  
 371 necessarily equal  $P_{ji}$ ), and the diagonal ( $P_{ii}$ ) is zero (i.e.  
 372 a species does not influence its own probability of being  
 373 observed).

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

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



385 **Fig. 4.** Lichen interaction networks were constructed by conducting field observa-  
 386 tions in 1 cm<sup>2</sup> cells within a 10 cm<sup>2</sup> grid on each tree using a checkerboard pattern  
 387 (grey cells). Thus, a set of  $N$  total cell observations were recorded for each tree  
 388 with the presence or absence of each species recorded for each cell. Applying the  
 389 probability-based network modeling method adapted from (20), we calculated the  
 390 conditional probabilities,  $P(S_i|S_j)$ , for all species pairs and removed (i.e. set equal  
 391 to zero) species pairs whose joint probabilities,  $P(S_i, S_j)$ , were not significant using  
 392 a confidence interval based comparison of their observed co-occurrence frequency,  
 393  $S_i S_j$ , to that expected due to chance alone,  $E[P(S_i, S_j)] = P(S_i)P(S_j)$ , and  
 394  $P(S_i|S_j)$  reduces to  $P(S_i)$ , the observed individual probability of species  $S_i$ .

395 **LJL:** I like the details here. THe one thing is  
 396 that it sort of makes the reader think there is only  
 397 one quadrat on a tree but infact there were two. I  
 398 think you want to make sure to be explicit about  
 399 the two. For analytical purposes, was all the data  
 400 lumped so there was really functionally a 20cm  
 401 by 10cm grid (just split into two pieces). Or, was  
 402 the network made for each of the two grids and  
 403 them averaged or combined in some way? My  
 404 understanding is that it was more the first than  
 405 the latter.

406 **MKL:** Yeah, it was the latter. I'm using two

397 quadrats lumped together. I'll add more text  
398 here to clarify that.

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

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

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

433 For each network, we also calculated two network met-  
434 rics that measure different structural aspects. We cal-  
435 culated the number of interactions or "links" in each  
436 network, which provides a measure of the size of the net-  
437 work citepLau2015, Borrett2014. We also calculated the  
438 centralization of each network, which measures the even-  
439 ness of the distribution of interactions among the species  
440 in the network citepButts2005. In a network with a low  
441 level of centralization species have similar amount of inter-  
442 action in the network, while a network with a high level of  
443 centralization tends to one or small subset of species that  
444 interact with other species. We used a related function  
445 to calculate the centrality of each species in each network  
446 as well. Although there are many other metrics, see (21),  
447 we focus on a subset for the sake of simplicity and be-  
448 cause some metrics are not appropriate for our relatively  
449 small communities. **In particular, we do not present**  
450 **analysis of the modularity (i.e. the degree of sub-**  
451 **grouping) because our community has relatively**

few species to form modules. As with the other re-  
452 sponse variables, the number of links was log-transformed  
453 and centralization scores were square-root transformed to  
454 meet variance and normality assumptions.

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

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

## Discussion

- Genotypic variation can lead to network variation
- Traits could both share or be a causal mechanism with lichen networks. - 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

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

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

- 499 • Thus, it would be possible for genetics to not  
500 only influence other species directly, but also  
501 indirectly by influencing the interactions among  
502 other species.

503 We observed significant lichen interaction struc-  
504 ture that varied among genotypes of a foundation  
505 tree species, narrowleaf cottonwood (*P. angustifo-*  
506 *lia*). We found that a genetically based trait, bark  
507 roughness, partially explained the variation in lichen  
508 interaction networks. Some of this variation in lichen  
509 networks was related to both the overall abundance  
510 and species richness of lichen; though, statistically  
511 controlling for the effect of genotype on these vari-  
512 ables indicates that a significant portion of the vari-  
513 ance in lichen species richness is due to a factor  
514 other than tree genotype. By using network metrics,  
515 we were also able to probe for specific characteris-  
516 tics of how these networks were responding to tree  
517 genotype. We found that both number of links and  
518 the centralization of the networks were highly corre-  
519 lated with network similarity and that tree genotype  
520 significantly predicted network centrality but only  
521 marginally predicted the number of network links.  
522 This latter result could be due to the relationship  
523 between species richness and the number of links  
524 in the network, which were significantly correlated  
525 with each other. We also found that bark roughness  
526 did not significantly predict either the number of  
527 links or the centrality of lichen networks, suggesting  
528 that bark roughness has some other effect on the  
529 structure of the lichen networks. Taken together,  
530 these findings support the hypothesis that genotypic  
531 variation in a foundation species contributes to the  
532 structure of a network of interacting species.

533 **LJJ: I wonder if you need to have so much**  
534 **on richness here. Overall, I think you want to**  
535 **focus on the network responses and patterns**  
536 **among genotype first, and then go into mech-**  
537 **anism later. I think we don't quite have a**  
538 **good mechanism yet so I don't think it needs**  
539 **to come up in the first paragrpah of the dis-**  
540 **cussion.**

541 These findings point to the importance of under-  
542 standing the community level effects of genetic varia-  
543 tion in plant functional traits and highlights the  
544 potential for indirect effects of genetic variation to  
545 propagate through networks of interacting species  
546 and trophic levels.

547 This work corroborates previous findings of the

importance of plant genetics in shaping community  
548 structure and ecosystem processes. citepBangert2008  
549

550 Altering the structure of interaction networks  
551 presents a means for genetic effects to be magni-  
552 fied within the system of interacting species. For  
553 example, (7) showed that the genetics based interac-  
554 tions of aphid resistant and aphid susceptible trees  
555 resulted in different interaction networks of their  
556 associated arthropod communities composed of 139  
557 species. At the scale of ecosystems, trophic net-  
558 works or food webs direct and control the rates of  
559 energy and nutrient flux (22). Furthermore, in a  
560 predator-prey-plant study, Smith (23), showed that  
561 the interactions among species across trophic levels  
562 depended on plant genotype.

563 **LJJ: It could be useful to point out that**  
564 **our findings are not related to trophic inter-**  
565 **actions, which is pretty cool. Also,we talk**  
566 **about interaction networks but it is not clear**  
567 **to me if the interactions tend to be positive**  
568 **or negative. Can we get at that with the ap-**  
569 **proach used?**

570 **TGW: Is there any adaptive component to**  
571 **the tree in having certain lichen communi-**  
572 **ties? e.g., can they feed back to affect tree**  
573 **performance in some way or is this a pas-**  
574 **sive outcome of a trait that affects bark for**  
575 **other adaptive reasons and lichens are pas-**  
576 **sive players that tag along for the ride? I**  
577 **could envision that lichens covering the bark**  
578 **of a tree act as a barrier between insects and**  
579 **pathogens, much like ectomycorrhizae cover**  
580 **fine roots as a first line of defense by invad-**  
581 **ing microorganisms. Uptake of N that gets**  
582 **passed to the tree??**

583 **TGW: might be good to cite papers on**  
584 **competition in lichens or other organizing fac-**  
585 **tors to back up the least expected statement.**  
586 **as epiphytes we might not expect them to**  
587 **care.**

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

593 **MKL: Environmental filtering is evidenced**  
594 **by species richness, but also possibly species**  
595 **interaction varying based on environment as**  
596 **networks varied in terms of sign and magni-**

697 tude as well.

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

## 702 Discussion of network implications for sta- 703 bility with genetics.

704 Although our study was conducted with a commu-  
705 nity of lichens, these results should be generalized  
706 to other groups of diverse organisms around the  
707 world that also exhibit significant genetic signals at  
708 the community level (24, 25). In the face of the  
709 high degree of complexity and potential context de-  
710 pendence of ecological processes, the current study  
711 points to the utility of considering the spatial and  
712 temporal scales of interactions, as discussed to some  
713 in previous studies (26–28). In the present study,  
714 we found that community assembly processes, such  
715 as environmental filtering and species interactions,  
716 are genetically based. This is likely due, in part,  
717 to the large difference in the differences in size and  
718 longevity of the lichen and cottonwood individuals  
719 with the trees determining the environment in which  
720 the lichen occur. We suggest that future work would  
721 be aided by determining these modules within the  
722 biotic community that include species with similar  
723 differences in body-size and time-scales. As heritable  
724 variation is the raw material for natural selection  
725 to act upon, a genetic basis for interaction network  
726 structure indicates evolutionary dynamics should be  
727 considered at the community level and that con-  
728 serving genetic variation is important to consider in  
729 efforts to restore or preserve complex species inter-  
730 actions and their associated ecosystem functions (29).  
731 With such findings, it appears that we are closer to  
732 understanding the evolutionary drivers of Darwin’s  
733 entangled bank and the interconnectedness of species  
734 in complex communities.

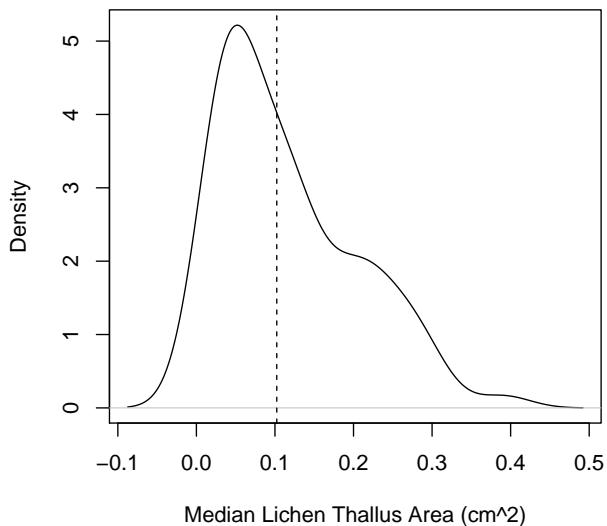
735 **ACKNOWLEDGMENTS.** This work was supported by  
736 the National Science Foundation grant (DEB-0425908)  
737 and Integrative Graduate Research Traineeship (IGERT)  
738 fellowships for M.L. and L.L. The Ogden Nature Center  
739 staff helped to maintain the common gardens. Lichen  
740 sampling was supported by Todd Wojtowicz, Luke Evans  
741 and David Solance Smith.

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702 **Supplementary Materials**

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



**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		