

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 (16? ?) 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 (6) and species interactions**
15 **(8, 10?), which shapes the ecological interaction networks**

(7).

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

Community genetics studies (12) have shown that genetic variation in foundation species (13) plays a significant role in defining distinct communities of interacting organisms: such as, endophytes, pathogens, lichens, arthropods, and soil microbes. Multiple studies have now demonstrated that genetic variation influences numerous functional traits (e.g., phytochemical, phenological, morphological) produces a multivariate phenotype (14) that contributes to variation in associated communities (15).

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

Additional work has provided support for the hypothesis that not only does composition vary among genetically distinct

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 genotypes of foundation species, it also impacts the structure
43 of the network of species interactions in these communities
44 (16, 17). Also, work by (18–20) observed consistent patterns
45 of centralized interactions of species modules focused around
46 hubs of plant-fungal interactions. In other words, a small
47 number of plant and fungal symbionts tended to have have
48 disproportionate numbers of interactions with other species
49 and likely are the drivers in determining community assembly,
50 structure and dynamics.

51 More on the importance of ecological networks (9, 11).

52 Add a discussion of DesRoches.

53 From Thompson2014

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

59 Specific hypothesis from Thomspn2014

60 Selection in small webs

61 •

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

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

72 Here, we investigate how genetic variation in a foundation tree species determines the structure of a network of interactions among a community of tree associated lichen species. Using a long-term (20+ years), common garden experiment with replicated individuals of known genetic identity and a naturally established stand of *Populus angustifolia*. We focused on a model community of 9 epiphytic lichen species, as previous research has demonstrated significant compositional responses of epiphytes to genotypic variation (21, 22). In addition, the life-history characteristics of lichens, having highly localized, direct contact interactions and slow population turnover rates, allowed us to assess interactions among lichen species on individual trees. We hypothesize that in natural systems evolution occurs in a community context involving interactions of complex networks of interacting species (2, 16, 17, 23). If correct, we should expect to find that network structure is genetically

99 based in which different plant genotypes support different interaction 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 interaction
103 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

107 I guess I don't want to give the impresison that our communities are necessarily the result of each species evolving into its place in the community on these tree genotypes (although I do understand this as Shuster et al 2006's fundamental explanation for why we see different communities on different genotypes; I don't necessarily agree that this is the only reason we woudl see different communities on dif genotypes). Most of these are pretty generalist lichens, which could be found on other decidous trees in the surrounding city or natural areas. I would look at it more like an assembling of lichen species into unique configurations on genetically different substrates. There may be some selection for different genotype of lichen during the community assembly process but we can't really tell that just by differences in species abundances or coocurnees.

108 I guess to me the evolutionary context that is more directly
109 related to this work is that the tree genotype is a central
110 controller (indeed a sort of hub species in the network) of
111 network structure. By anchoring the lichen network to tree
112 genotype (and variation among networks to variation among
113 tree genotypes) , our study highlights the possibility that
114 natural selection acting on the trees may have an extended
115 consequence for the network structure of organisms living on
116 the trees...the extra thing we add to the field is that we show
117 interaction networks are sensitive to genotype. I doubt the
118 lichens have a direct effect on tree fitness, but favorability of
119 some tree genotypes over others during natural selection will
120 then go on to favor and disfavor certain lichen communities
121 of different network structures. By being sensitive to tree
122 genotype, the lichen community networks are passive riders
123 on the waves of evolutionary dynamics that occur within
124 the tree species they inhabit.**LJL:** I agree that there is
125 a general overarching theme that evolution occurs in
126 a community network context, but I'm not sure that
127 we should state that as our main hypothesis. It seems
128 more that this is a fundamental foundation for our
129 work. The hypothesis is more what we are testing
130 directly, but we don't test this directly.

131 I guess I don't want to give the impresison that our
132 communities are necessarily the result of each species
133 evolving into its place in the community on these tree
134 genotypes (although I do understand this as Shuster
135 et al 2006's fundamental explanation for why we see
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168 our study highlights the possibility that natural selec-
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175 over others during natural selection will then go on to
176 favor and disfavor certain lichen communities of dif-
177 ferent network structures. By being sensitive to tree
178 genotype, the lichen community networks are passive
179 riders on the waves of evolutionary dynamics that oc-
180 cur within the tree species they inhabit.

181 **MKL:** In response to Lamit's comment above, I
182 agree that it is not required that there is co-evolution.
183 Another, perhaps simpler, explanation is that there
184 is variation in environmental filtering of lichen indi-
185 viduals created in part by genetic variation in tree
186 individuals.

187 Materials and Methods

188 The study was conducted along the Weber River, UT
189 (USA), which is a cottonwood (*Populus* spp.) dominated
190 riparian ecosystem. Although two native species, *Popu-*
191 *lus angustifolia* (James) and *Populus fremontii* (S. Watson),
192 occur here and are known to hybridize, only pure or ad-
193 vanced generation backcrosses of *P. angustifolia* were sam-
194 pled. Bark lichens have been extensively studied in this
195 system and provide an ideal system in which to observe
196 and model lichen interaction networks, as their sessile na-
197 ture permits accurate identification of individuals (24).

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

200 **LJL:** If you include the field data, I would suggest
201 changing the topic sentence for this paragraph and adding
202 something about the field data sampling to this paragrph.
203 Note, also that we cored and aged each field tree so we
204 can site the age range of the trees here, to make it clear
205 they are within those of the garden.

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

216 **Bark Lichen Observations.** **LJL:** A total pain in the butt but it
217 might be good just to double check that our exact species
218 that occurred in each quadrat are the same as the species
219 that I found in the composition data. No doubt that I
220 woudl have founrn more species but it might be a good
221 double check of things just to see if they roughly corre-
222 spond. I can do this if you send me your matrix.

223 **LJL:** I think this sentence is good and these lichens
224 tend to be ones that were rarer or really easy to miss.

225 So, the list of species you picked up sounds legit to me.
226 One thing... above you say 14 species but only 9 were
227 found. That is a bit redundant with this next sentence
228 that highlights which species were found and which were
229 not obsered. I would just stick with the second sentence
230 and delete the first sentence.

231 On each tree, presence or absence of each lichen species
232 was assessed in 50 total 1 cm² cells arrayed in a checker-
233 board pattern. Given the small size and sessile nature of
234 lichens, we were able to rapidly assess lichen interactions
235 by quantifying thalli in close contact. Sampling was re-
236 stricted to the northern aspect of the trunk to maximize
237 the abundance of lichen and control for the effect of trunk
238 aspect. Two adjacent 10 cm² quadrats centered at 50 cm
239 and 85 cm from ground level were sampled (Fig 1 A and
240 B). The observed lichen community included (abbreviations
241 are given for species present in study): Xg = *Xanthomendoza galericulata*, Xm = *X. montana*, Ch = *Caloplaca*
242 *holocarpa*, Cs = *Candelariella subdeflexa*, Rg = *Rinodina*
243 *glauca*, Lh = *Lecanora hagennii*, Pm = *Phyciella melanachra*,
244 Pa = *Physcia adscendens*, Pu = *Physcia undulata*. Several
245 other species were not obeserved in the present study but
246 are known to occur in this region: *Phaeophyscia orbicularis*,
247 *Phaeophyscia ciliata*, *Melanelia sublivacea*, *Meanelia*
248 *elegantula*.

249 The cell size and checkerboard sampling pattern was
250 chosen to isolate the individuals in each cell. In a previous
251 survey of lichen thallus size in this common garden,
252 we had observed a median thallus size of 0.12 ± 0.001 cm²
253 (S.E.) (see Supplementary Fig 1). Based on the median
254 thallus size, we expected thalli observed in each cell to gen-
255 erally be spatially independent of thalli present in other
256 cells but exposed to similar micro-environmental condi-
257 tions created by the bark and the location of the sampling
258 area on an individual tree. Therefore, we were confident
259 in treating the cell-wise observations in quadrats as inde-
260 pendent with respect to lichen-lichen interactions.

261 **LJL:** My method was overal visual % cover. I didn't
262 count P/A of rough bark in each square. So, yo might just
263 want to site our Ecology lichen paper as showing rough
264 bark is important, but not cite it as the same method.
265 No doubt the methods should yeild similar results but the
266 approach is slightly different.

267 As bark roughness had previously been shown to be
268 an important, genetically based tree trait impacting bark
269 lichens, we measured the percentage of rough bark on each
270 tree following the methods of (24). Briefly, the number
271 of cells containing disrupted, fissured bark were counted
272 within each quadrat on each tree. The number of rough
273 bark containing cells were then summed and divided by
274 the total number of cells surveyed. This was done for all
275 quadrats on all trees in which lichen communities were
276 also observed.

277 **Lichen Network Modeling and Analysis.** **LJL:** This seems like a
278 key point, one that really makes the study above and be-
279 yond. I would make it clear with the phrasing that in-
280 dividual networks were created for each individual tree
281 sampled, in this way we had replicated networks for each
282 tree genotypes.

283 We used the observations of lichens in the 1 cm² cells
284 on individual trees of *P. angustifolia*. Unipartite networks
285 were generated using the conditional probabilities of each
286 species pair, i.e. the probability of observing one species
287 given an observation of another species $P(S_i|S_j)$, based on
288 the method developed by (25). To calculate conditional
289 probabilities, we quantified the individual probabilities of
290 species occurrences $P(S_i)$ and the joint probability of co-
291 occurrences $P(S_i, S_j)$ using the frequencies of each species
292 and their co-occurrences. We were then able to calcu-
293 late the conditional probabilities of each species pair as
294 $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
295 This yielded a matrix that could possibly be asymmetric, i.e.
296 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another

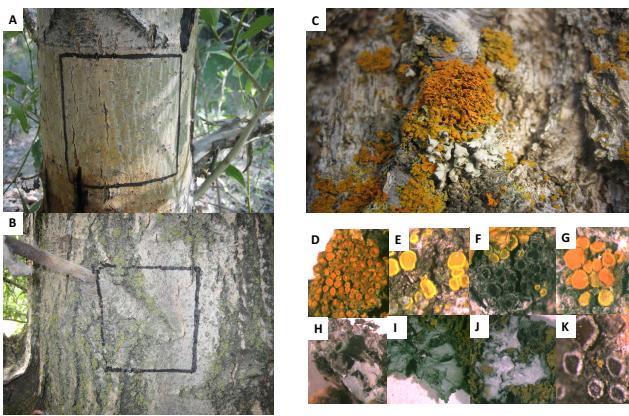


Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees (A and B). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Physciella melanachra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesboung (D-K).

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

LJJ: 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.

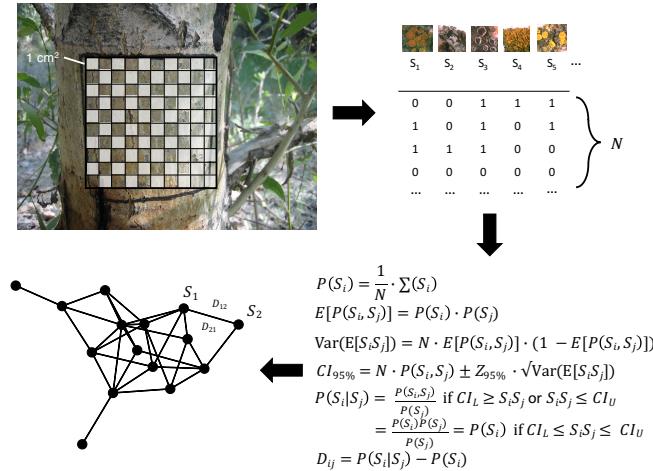


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (25), 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 .

LJJ: 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 op-

384 optimal performance with count data (?). To quantify the
 385 similarity of lichen networks among individual trees, we
 386 calculated the pairwise Euclidean distance of the D interaction
 387 matrices among all pairs of trees.

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

396 For each network, we also calculated two network metrics
 397 that measure different structural aspects. We calculated
 398 the number of interactions or “links” in each network,
 399 which provides a measure of the size of the network
 400 (26?). We also calculated the centralization of each net-
 401 work, which measures the evenness of the distribution of
 402 interactions among the species in the network (?). In
 403 a network with a low level of centralization species have
 404 similar amount of interaction in the network, while a net-
 405 work with a high level of centralization tends to one or
 406 small subset of species that interact with other species.
 407 We used a related function to calculate the centrality of
 408 each species in each network as well. Although there are
 409 many other metrics, see (27), we focus on a subset for the
 410 sake of simplicity and because some metrics are not appro-
 411 priate for our relatively small communities. In particular,
 412 we do not present analysis of the modularity (i.e. the
 413 degree of sub-grouping) because our community has rela-
 414 tively few species to form modules. As with the other re-
 415 sponse variables, the number of links was log-transformed
 416 and centralization scores were square-root transformed to
 417 meet variance and normality assumptions.

418 **LJL:** I suggest deleting the highlighted part. And, just
 419 changing the sentence above it to “...because some metric
 420 (e.g., modularity) are not appropriate...” Too much empha-
 421 sis on caviots will make some readers be uncertain. But,
 422 also, you can save some space that way.

423 We have made all code and data available online. Code
 424 is available at github.com/communitygenetics/lcn. Data is
 425 available via the Harvard Dataverse (needs project ID).
 426 The project is also archived via Zenodo at zenodo.com/doi/XXXXXX. All analyses were conducted using the pro-
 427 gramming language R version 3.4.2 (R Development Core
 428 Team 2018).

431 Results

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

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

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

439 **LJL:** Since we already published that tree genotypes
 440 differ in lichen composition, I wonder if we need to say
 441 somewhere in the manuscript why this test was run here.
 442 It seems to me it is important to verify this with a slightly
 443 different sampling method as used in the 2015 paper, and
 444 for this specific set of genotypes. But, then does this test
 445 of composition just become something necessary just in
 446 a methodological variation that justifies the next step of
 447 examining network structure. Something to think about. It
 448 might be that the NMDS should just go in a supplement,
 449 although I do like it here in some ways. It might also
 450 be another approach to put the composition and other
 451 analyses after the network analysis results are presented.
 452 In this way, you could use the composition and results
 453 with vectors to help provide resolution on what is driving
 454 networks to differ among genotypes.

455 **MKL:** Adapt into a table.

456 **TGW:** clarify positive vs negative interactions.

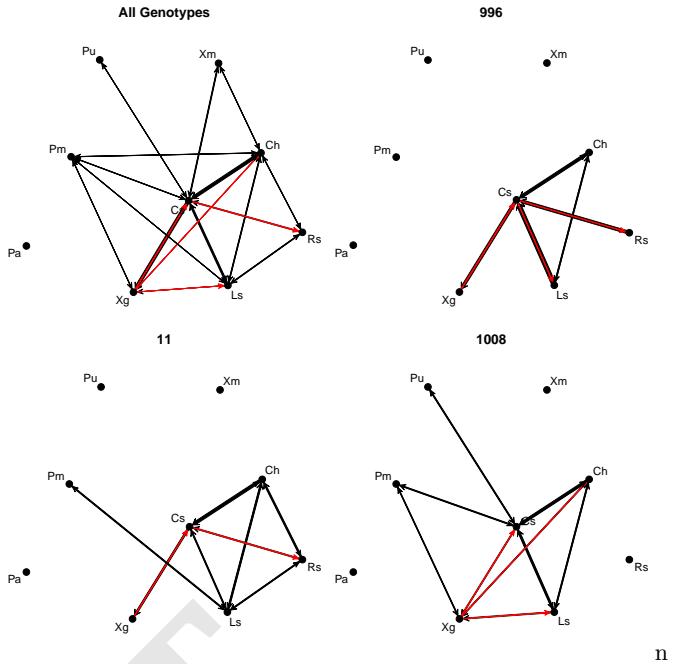


Fig. 3. Lichen networks varied in structure among tree genotypes. Network diagrams of the mean lichen interaction matrices averaged for all trees and for several individual genotypes showing a range of interaction network structure. Directionality (arrowheads) and sign (red = negative, black = positive)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

457 We observed significant lichen network structure. This
 458 structure varied among genotypes (Fig. 3) and lichen
 459 species varied in their importance in the network. *Can-*
 460 *daleriella subdeflexa* was generally the most central species
 461 (i.e. being the most highly connected) having the high-
 462 est average centrality (0.73), followed by *Ca. holocarpa*
 463 (0.54) and *L. hagenii* (0.40). The centralization of the re-
 464 maining species were *R. sp.* (0.18), *X. galericulata* (0.14),
 465 *P. melanochra* (0.08), *X. montana* (0.06) and *Ph. undulata*
 466 (0.02). *Physcia adscendens* was generally not connected
 467 to other species in the networks and had a centralization
 468 score of zero.

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

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

475 **TGW:** Here and in earlier paragraphs, a lot of stats are
 476 presented some of which are significant and some not. For
 477 your topic sentence to be accepted, it seems readers need
 478 to know how many of the stats need to confirm the pattern
 479 and how many would it take to reject. This paragraph has
 480 about 8 stats so need some overarching statement(s). E.g.,
 481 7 of 8 analyses support our overarching hypothesis that ...
 482 Same goes for other such paragraphs such as the 1st and
 483 last paras of the Results.

484 Lichen networks observed on trees of the same geno-
 485 type tended to be more similar in structure. Tree geno-
 486 type significantly predicted the similarity of lichen inter-
 487 action networks (PerMANOVA $R^2 = 0.33795$, $F_{12} =$
 488 2.5379 , $p\text{-value} = 0.0050$) (Fig. 4). Bark roughness (Per-
 489 MANOVA $R^2 = 0.040$, $F_1 = 4.1680$, $p\text{-value} = 0.03770$)
 490 and lichen species richness (PerMANOVA $R^2 = 0.424$, F_1

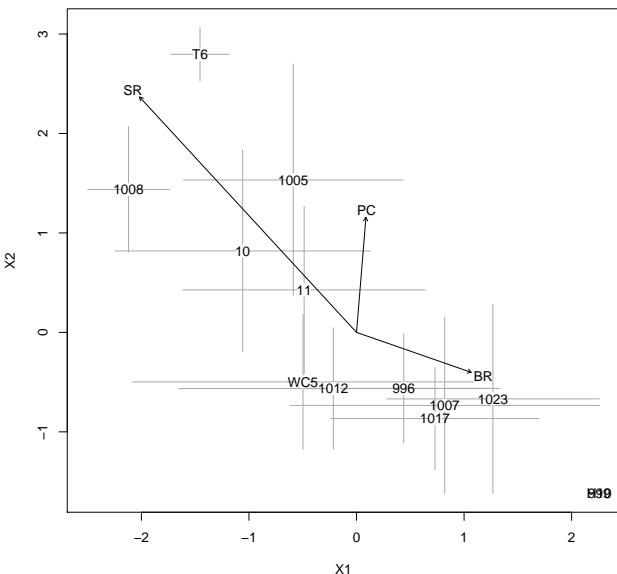


Fig. 4. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.011) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure 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).

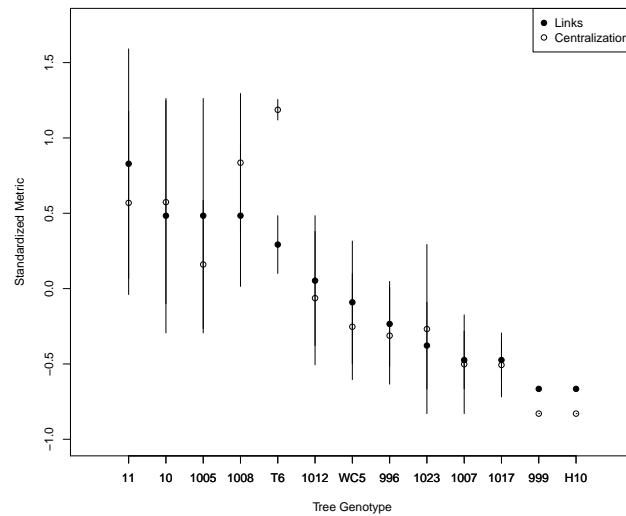


Fig. 5. The impact of tree genotype on lichen network structure was indicative of variation in both the number variation in lichen interactions among species. Plot showing the means (± 1 S.E.) for lichen network metrics, number of links and centralization, for each genotype. Both metrics are presented as standardized scores ($\frac{x-\bar{x}}{\sigma}$).

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

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

Networks were more similar as a result of having sim-

566 communities? We've done this in the past and it has
567 worked, e.g., Randy's genetic similarity rule.

568 Our study provides a window into the genetic underpinnings of an ecological network. We observed
569 significant lichen interaction structure that varied
570 among genotypes of a foundation tree species, nar-
571 rowleaf cottonwood (*P. angustifolia*). We found that
572 a genetically based trait, bark roughness, partially ex-
573 plained the variation in lichen interaction networks.
574 Some of this variation in lichen networks was related
575 to both the overall abundance and species richness of
576 lichen; though, statistically controlling for the effect
577 of genotype on these variables indicates that a signifi-
578 cant portion of the variance in lichen species richness
579 is due to a factor other than tree genotype. By us-
580 ing network metrics, we were also able to probe for
581 specific characteristics of how these networks were re-
582 sponding to tree genotype. We found that both num-
583 ber of links and the centralization of the networks
584 were highly correlated with network similarity and
585 that tree genotype significantly predicted network
586 centrality but only marginally predicted the number
587 of network links. This latter result could be due to the
588 relationship between species richness and the num-
589 ber of links in the network, which were significantly
590 correlated with each other. We also found that bark
591 roughness did not significantly predict either the num-
592 ber of links or the centrality of lichen networks, sug-
593 gesting that bark roughness has some other effect on
594 the structure of the lichen networks. Taken together,
595 these findings support the hypothesis that genotypic
596 variation in a foundation species contributes to the
597 structure of a network of interacting species.

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

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

609 These findings point to the importance of under-
610 standing the community level effects of genetic varia-
611 tion in plant functional traits and highlights the po-
612 tential for indirect effects of genetic variation to prop-
613 agate through networks of interacting species and
614 trophic levels.

615 This work corroborates previous findings of the
616 importance of plant genetics in shaping community
617 structure and ecosystem processes. (?)

618 Altering the structure of interaction networks
619 presents a means for genetic effects to be magnified
620 within the system of interacting species. For example,
621 (16) showed that the genetics based interactions of
622 aphid resistant and aphid susceptible trees resulted
623 in different interaction networks of their associated
624 arthropod communities composed of 139 species. At

625 the scale of ecosystems, trophic networks or food
626 webs direct and control the rates of energy and nu-
627 trient flux (28). Furthermore, in a predator-prey
628 plant study, Smith (29), showed that the interactions
629 among species across trophic levels depended on plant
630 genotype.

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

637 Also,we talk about interaction networks but it is
638 not clear to me if the interactions tend to be positive
639 or negative. Can we get at that with the approach
640 used?

641 TGW: Is there any adaptive component to the tree
642 in having certain lichen communities? e.g., can they
643 feed back to affect tree performance in some way or is
644 this a passive outcome of a trait that affects bark for
645 other adaptive reasons and lichens are passive play-
646 ers that tag along for the ride? I could envision that
647 lichens covering the bark of a tree act as a barrier
648 between insects and pathogens, much like ectomycor-
649 rhizae cover fine roots as a first line of defense by in-
650 vading microorganisms. Uptake of N that gets passed
651 to the tree??

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

656 TGW: I think we need to emphasize the long-term
657 nature of our common garden study as very few com-
658 mon garden studies of lichens likely exist. Any refs
659 on this? If true might want to mention this up front
660 in intro.

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

665 MKL: The effect of bark roughness on network sim-
666 ilarity was primarily genetically based, and there are
667 likely other factors at play.

668 Discussion of network implications for stability
669 with genetics.

670 Although our study was conducted with a commu-
671 nity of lichens, these results should be generalized to
672 other groups of diverse organisms around the world
673 that also exhibit significant genetic signals at the com-
674 munity level (30, 31). In the face of the high de-
675 gree of complexity and potential context dependency
676 of ecological processes, the current study points to
677 the utility of considering the spatial and temporal
678 scales of interactions, as discussed to some in previ-
679 ous studies (32–34). In the present study, we found
680 that community assembly processes, such as environ-
681 mental filtering and species interactions, are geneti-
682 cally based. This is likely due, in part, to the large
683 difference in the differences in size and longevity of
684 the lichen and cottonwood individuals with the trees

688 determining the environment in which the lichen occur. We suggest that future work would be aided by
 689 determining these modules within the biotic community that include species with similar differences in
 690 body-size and time-scales. As heritable variation is
 691 the raw material for natural selection to act upon, a
 692 genetic basis for interaction network structure indicates
 693 evolutionary dynamics should be considered at the
 694 community level and that conserving genetic variation
 695 is important to consider in efforts to restore or
 696 preserve complex species interactions and their associated
 697 ecosystem functions (35). With such findings,
 698 it appears that we are closer to understanding the
 699 evolutionary drivers of Darwin's entangled bank and
 700 the interconnectedness of species in complex communities.
 701

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 707 ships for M.L. and L.L. The Ogden Nature Center staff
 708 helped to maintain the common gardens. Lichen sampling
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 710 Solance Smith.

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Supplementary Materials

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

774
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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

Table 1. Genotypic effects of cottonwood trees on the associated lichen community.

	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.

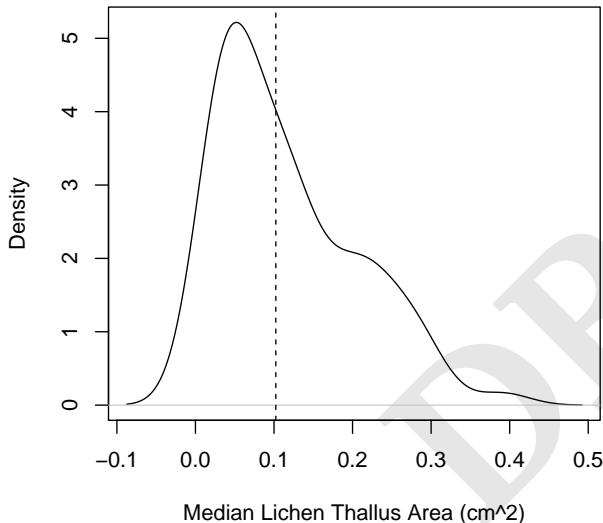


Fig. 1. Density plot of the median lichen thallus area (cm²).

	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)
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.