

Genotypic variation in a foundation tree directs ecological network structure

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

networks | community | genetics | lichen | cottonwood | *Populus* | com-
mon garden

- 1 • Evolution occurs in complex ecosystems
- 2 • Community genetics studies have demonstrated
3 the importance of genetics and evolutionary dy-
4 namics in structuring communities and ecosys-
5 tems
- 6 • Community composition, ecosystem processes
7 have been shown to vary in response to the
8 genetic variation of foundation species
- 9 • Previous studies have examined aspects of net-
10 works but to date, no studies have examined the
11 genetic basis of networks of interacting species
- 12 • Here we examine the genetic basis of network
13 structure on a community of sessile lignicolous
14 (i.e. bark) lichen on cottonwood trees. This

system is ideal for examining for this kind of
study because it:

- 15 1. Lichen do not move among trees, although
16 asexual propagules could move to other
17 trees in the garden
- 18 2. Is not unapproachably complex
- 19 3. Is comprised of distinct individuals that
20 are small enough for an entire community
21 to occur on a single tree but not too small
22 to be quantified easily

- 23 • We hypothesize that trees will vary in some
24 phenotypic traits, such that trees of the same

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.

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27 genotype will tend to have similar traits producing
28 similarities in lichen network structure. To
29 test this hypothesis, we use a common garden
30 of cottonwood trees with lichen communities.

31 **E**volution occurs in the context of complex eco-
32 logical networks. Initially, evolution in a com-
33 munity context was focused on examples of highly
34 co-evolved pairs of species (e.g. Darwin's famous
35 prediction of the Sphinx Moth and Christmas
36 Orchid) citeWade2007. However, studies of dif-
37 fuse co-evolution (*sensu* citeJanzen1980) citeStinch-
38 combe2002, Strauss2007, Dicke2010, geographic mo-
39 saics of co-evolution citeThompson2005 and com-
40 munity genetics citeWhitham2006 have provided an
41 in-road for ecological network approaches citeFor-
42 tuna2009, Keith2017, Lau2016 to illuminate a more
43 complex perspective of the interface between eco-
44 logical and evolutionary dynamics. There is now
45 evidence to support that selection tends to occur
46 among groups of species citeWade2007, Pieterse2007,
47 Dicke2010 favoring the development of small webs
48 citeNuismer2006, Guimaraes2007, Gomez2009 and
49 that genetic variation and phylogenetic relatedness
50 contributes to variation in community assembly (1)
51 and species interactions citeWhitham2006a, Bai-
52 ley2009, Moya-Larano2011, which shapes the eco-
53 logical interaction networks (2).

54 **LJL: I think a key point in the first para-**
55 **graph is to point out that networks are be-**
56 **lieve in part to emerge out of complex evo-**
57 **lutionary interactions. But, to-date, the pat-**
58 **terns observed in networks are primarily only**
59 **on the scale of species interactions and phylo-**
60 **genetic signature. There has yet to be much**
61 **work connecting ecological species networks**
62 **at the scale relevant to the actual workings of**
63 **microevolutionary processes (e.g., the geno-**
64 **type or population level).**

65 **LJL: likewise, studies of networks do not**
66 **have a genetic component**

67 Community genetics studies (3) have shown that
68 genetic variation in foundation species (4) plays a
69 significant role in defining distinct communities of in-
70 teracting organisms: such as, endophytes, pathogens,
71 lichens, arthropods, and soil microbes. Multiple stud-
72 ies have now demonstrated that genetic variation
73 influences numerous functional traits (e.g., phyto-
74 chemical, phenological, morphological) produces a
75 multivariate phenotype (5) that contributes to varia-

tion in associated communities (6).

76
77 **LJL: I think the thing that needs to emerge**
78 **in this paragraph is that even though there**
79 **is some incling that genotype is relevant in**
80 **community networks, as based on our few**
81 **past studies, no study has truely examined**
82 **networks on individual trees.**

83 Additional work has provided support for the hy-
84 pothesis that not only does composition vary among
85 genetically distinct genotypes of foundation species,
86 it also impacts the structure of the network of species
87 interactions in these communities (7, 8). Also, work
88 by (9–11) observed consistent patterns of centralized
89 interactions of species modules focused around hubs
90 of plant-fungal interactions. In other words, a small
91 number of plant and fungal symbionts tended to
92 have disproportionate numbers of interactions with
93 other species and likely are the drivers in determining
94 community assembly, structure and dynamics.

95 More on the importance of ecological networks
96 (12, 13).

97 Add a discussion of DesRoches.

98 From Thompson2014

- 99 • Pairwise interactions are usually influenced by
100 other species
- 101 • Selection favors the development of small webs
- 102 • Evolution of new lifestyles leads to changes in
103 selection on large and small webs

104 Specific hypothesis from Thompson2014

105 Selection in small webs

106 **LJL: If I recall, the Elamo paper just looks**
107 **at genetic correlations between pairwise indi-**
108 **vidual abundances. I would suggest maybe it**
109 **doesn't deserve to be in this 1st paragraph.**
110 **Perhaps it actually should be in the 2nd or**
111 **3rd paragraph, just as a reference that points**
112 **to the potential for genotype to influence net-**
113 **works. Definately our 2015 JOE paper goes**
114 **much further, too, since it has whole com-**
115 **munities being correlated. But, again, I**
116 **would put both of these as citation in the com-**
117 **munity genetics paragraphs (2 of 3) instead**
118 **of the first paragrpah, which focuses on the**
119 **general network lit.**

120 Here, we investigate how genetic variation in a
121 foundation tree species determines the structure of a
122 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 (14, 15). 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 (7, 8, 16, 17). If correct, we should expect to find that network structure is genetically based in which different plant genotypes support different interaction networks and that these interactions networks can function as indicators of ecological dynamics important for conserving biodiversity. Applying a probability-theory based network modeling approach, we constructed a set of interaction network models for the lichens associated with individual trees. Using these models, we then examined the genetic basis of the structure of these ecological networks.

LJL: I agree that there is a general overarching theme that evolution occurs in a community network context, but I'm not sure that we should state that as our main hypothesis. It seems more that this is a fundamental foundation for our work. The hypothesis 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 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 would see different communities on dif genotypes). Most of these are pretty generalist lichens, which could be found on other deciduous 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 coocurneces. I guess to me the evolutionary context that is more direclty 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 anchoring the lichen network to tree genotype (and variation among networks to variation among tree genotypes) , our study highlights the possibility 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 sensitive to genotype. I doubt the lichens have a direct effect on tree fitness, but favorability of some tree genotypes over others during natural selection will then go on to favor and disfavor certain lichen communities of different network structures. By being sensitive to tree genotype, the lichen community networks are passive riders on the waves of evolutionary 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 simpler, explanation is that there is variation in environmental filtering of lichen individuals created in part by genetic variation in tree individuals.

Materials and Methods

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

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

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

222 advanced generation backcrosses of *P. angustifolia* were
223 sampled. Bark lichens have been extensively studied
224 in this system and provide an ideal system in which to
225 observe and model lichen interaction networks, as their
226 sessile nature permits accurate identification of individuals
227 (18).

228 A long-term, common garden experiment was used
229 to isolate the effect of tree genotype from the effect of
230 the localized microenvironment associated with each individual
231 and spatial autocorrelation. Established in 1992,
232 asexually propagated clones of genotyped *P. angustifolia*
233 individuals were obtained from wild collections and
234 planted in fully randomized design at the Ogden Nature
235 Center, Ogden, UT. From the population of established
236 individuals in the common garden, we chose a total of
237 thirteen genotypes, replicated between 3 and 8 times each,
238 for sampling.

239 **Bark Lichen Observations.** LJJ: A total pain in
240 the butt but it might be good just to double
241 check that our exact species that occurred in each
242 quadrat are the same as the species that I found
243 in the composition data. No doubt that I would
244 have found more species but it might be a good
245 double check of things just to see if they roughly
246 correspond. I can do this if you send me your
247 matrix.

248 LJJ: I think this sentence is good and these
249 lichens tend to be ones that were rarer or really
250 easy to miss. So, the list of species you picked
251 up sounds legit to me. One thing... above you
252 say 14 species but only 9 were found. That is a
253 bit redundant with this next sentence that highlights
254 which species were found and which were
255 not observed. I would just stick with the second
256 sentence and delete the first sentence.

257 On each tree, presence or absence of each lichen species
258 was assessed in 50 total 1 cm² cells arrayed in a checker-
259 board pattern. Given the small size and sessile nature
260 of lichens, we were able to rapidly assess lichen interactions
261 by quantifying thalli in close contact. Sampling
262 was restricted to the northern aspect of the trunk to
263 maximize the abundance of lichen and control for the
264 effect of trunk aspect. Two adjacent 10 cm² quadrats
265 centered at 50 cm and 85 cm from ground level were
266 sampled (Fig 1 A and B). The observed lichen community
267 included (abbreviations are given for species present
268 in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
269 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella*
270 *subdeflexa*, Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*,
271 Pm = *Phyciella melanchra*, Pa = *Physcia adscendens*,
272 Pu = *Physcia undulata*. Several other species were not
273 observed in the present study but are known to occur
274 in this region: *Phaeophyscia orbicularis*, *Phaeophyscia*
275 *ciliata*, *Melanelia sublivacea*, *Meanelia elegantula*.

276 The cell size and checkerboard sampling pattern was

277 chosen to isolate the individuals in each cell. In a previous
278 survey of lichen thallus size in this common garden, we
279 had observed a median thallus size of 0.12 ± 0.001 cm² (1
280 S.E.) (see Supporting Information). Based on the median
281 thallus size, we expected thalli observed in each cell to
282 generally be spatially independent of thalli present in
283 other cells but exposed to similar micro-environmental
284 conditions created by the bark and the location of the
285 sampling area on an individual tree. Therefore, we were
286 confident in treating the cell-wise observations in quadrats
287 as independent with respect to lichen-lichen interactions.
288

289 **LJJ: My method was overall visual % cover. I**
290 **didn't count P/A of rough bark in each square.**
291 **So, yo might just want to site our Ecology lichen**
292 **paper as showing rough bark is important, but**
293 **not cite it as the same method. No doubt the**
294 **methods should yeild similar results but the ap-**
295 **proach is slightly different.**

296 We also measured several bark traits for each tree:
297 including, bark roughness and chemistry.
298

299 To quantify bark roughness, the number of cells con-
300 taining disrupted, fissured bark were counted within each
301 quadrat. The number of rough bark containing cells were
302 then summed and divided by the total number of cells
303 surveyed. This was done for all quadrats on all trees in
304 which lichen communities were also observed. We also
305 measured several bark chemistry traits: including, pH,
306 condensed tannins, carbon and nitrogen.
307

308 **MKL: will need Lamit to add the chemistry**
309 **methods.**

310 **MKL: Remove A, then move current B up and**
311 **C below. You can then present current D-K ver-**
312 **tically and potentially increase their size.**

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

320 We used the observations of lichens in the 1 cm² cells
321 on individual trees of *P. angustifolia*. Unipartite networks
322 were generated using the conditional probabilities of each
323 species pair, i.e. the probability of observing one species
324 given an observation of another species $P(S_i|S_j)$, based on
325 the method developed by (20). To calculate conditional
326 probabilities, we quantified the individual probabilities
327 of species occurrences $P(S_i)$ and the joint probability
328 of co-occurrences $P(S_i, S_j)$ using the frequencies of each
329 species and their co-occurrences. We were then able to
330 calculate the conditional probabilities of each species pair
331 as $P(S_i|S_j) = \frac{P(S_i, S_j)}{P(S_j)}$, based on the axioms of probability.
332 This yielded a matrix that could possibly be asymmetric, i.e.
333 $P(S_i|S_j)$ does not have to be equal to $P(S_j|S_i)$. Another
334 important property of this matrix is that the diagonal

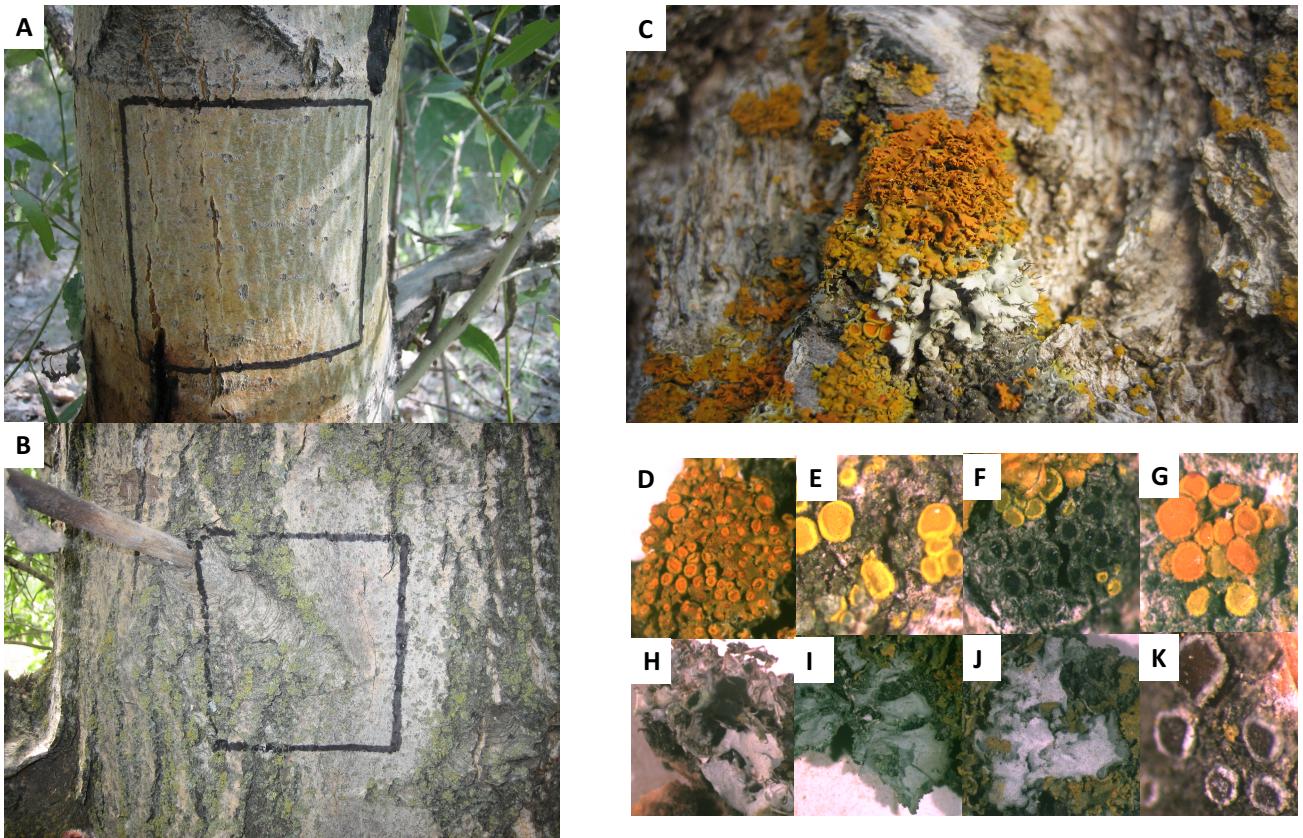


Fig. 1. The communities of bark lichens were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichens were sampled within a fixed area (10 cm^2) on individual trees at two heights, 40cm and 80cm from the ground (A and B, respectively). (C) a photo of a typical community of bark lichen species interacting on the trunk of a cottonwood tree, including one of the more abundant species, *Xanthomendoza galericulata*, in the center. (D-K) shows the other lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanchra*, *Physcia undulata* and *Lecanora hagenii*. Photo Credits: L.J. Lamit (A-C) and R.R. Naesbourg (D-K).

(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. $P(S_i) \dot{P}(S_j)$), 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).

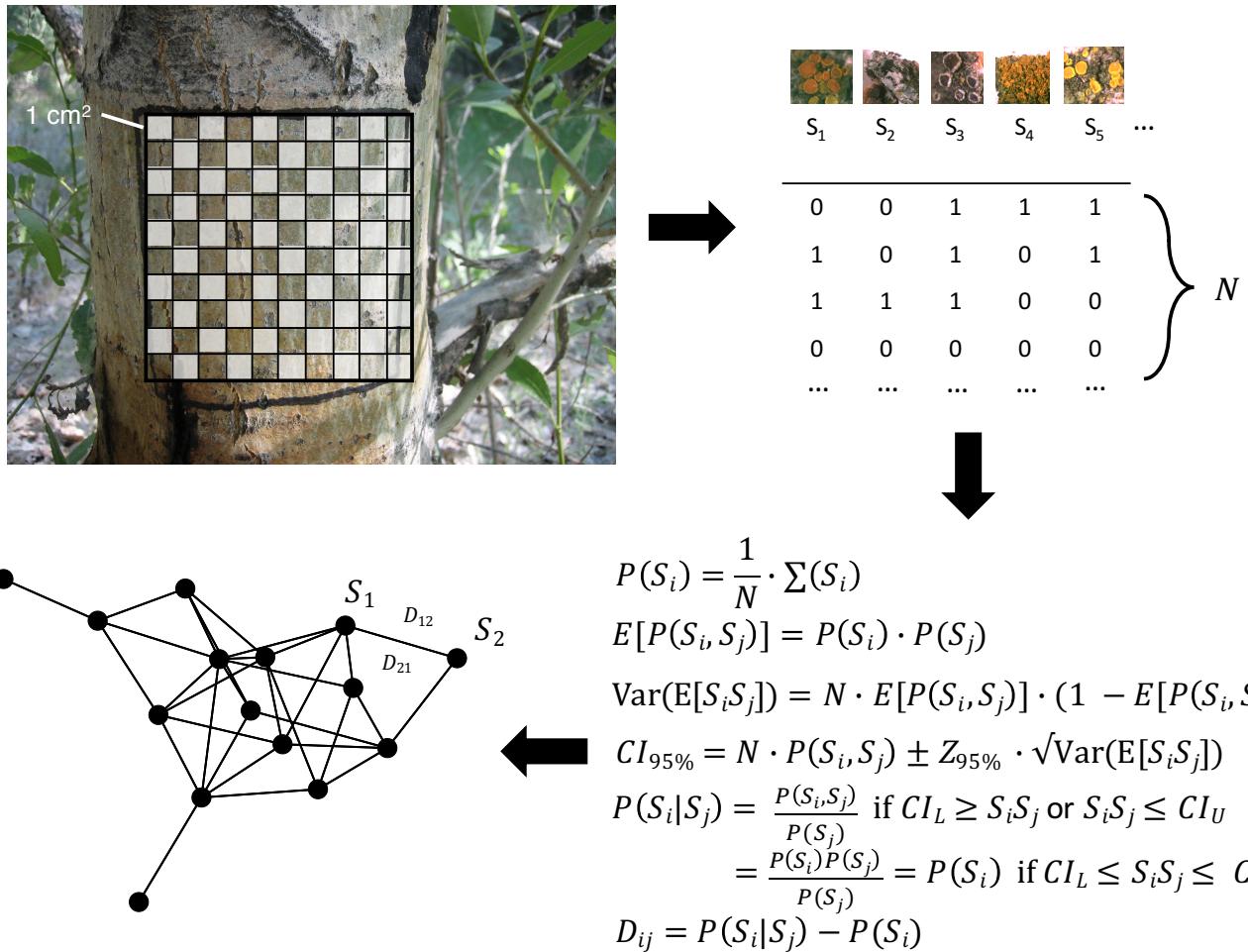


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded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

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

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

388 **LJL:** I like the details here. THe one thing is
 389 that it sort of makes the reader think there is only
 390 one quadrat on a tree but infact there were two. I
 391 think you want to make sure to be explicit about
 392 the two. For analytical purposes, was all the data
 393 lumped so there was really functionally a 20cm
 394 by 10cm grid (just split into two pieces). Or, was
 395 the network made for each of the two grids and

them averaged or combined in some way? My
 396 understanding is that it was more the first than
 397 the latter.

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

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

413 root transformed to meet the assumptions of homogeneity
414 of variance and normality for these tests.

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

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

436 For each network, we also calculated metrics that mea-
437 sure different structural aspects. Although there are many
438 other metrics, for the sake of simplicity we focus on a sub-
439 set that represent several interesting features of network
440 structure (see (21)). We calculated the number of interac-
441 tions or “links” in each network, which provides a measure
442 of the size of the network citepLau2015, Borrett2014. We
443 also calculated the centralization of each network, which
444 measures the evenness of the distribution of interactions
445 among the species in the network citeButts2005. In a net-
446 work with a low level of centralization species have simi-
447 lar amount of interaction in the network, while a network
448 with a high level of centralization tends to have one or
449 small number of species that interact with other species.
450 We used a related function to calculate the centrality of
451 each species (i.e. node level centrality) in each network
452 as well. The modularity of each network was also quanti-
453 fied using a weighted algorithm citeBeckett2016, which
454 measures the degree to which a given network is divided
455 into groups of species more connected to each other than
456 other species. As with the other response variables, the
457 number of links was log-transformed and both modularity
458 and centralization scores were fourth-root transformed to
459 meet variance and normality assumptions.

460 All code and data for the project are openly avail-
461 able online. Code and data are available at github.com/
462 [ecgen/comgen](https://github.com/ecgen/comgen). The project is also archived via Zenodo
463 at zenodo.com/doi/XXXXXX. All analyses were conducted
464 using the programming language R version 3.6.1 (R De-
465 velopment Core Team 2019).

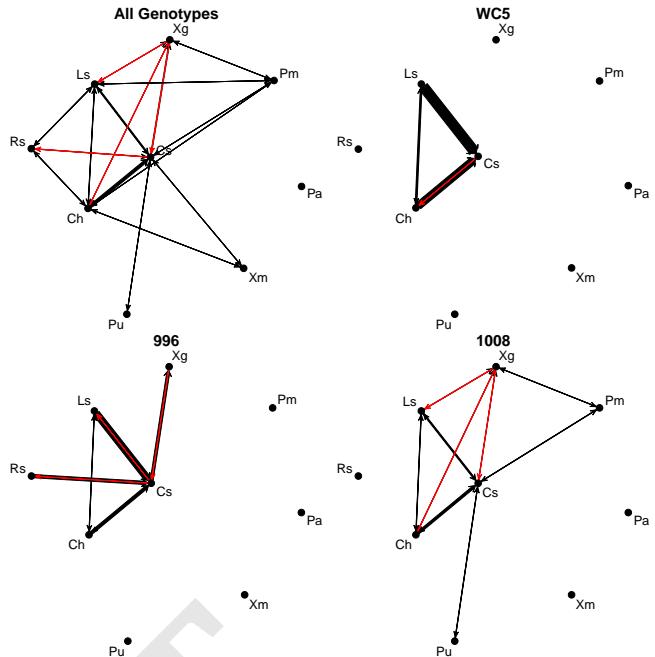


Fig. 3. Lichen networks varied in structure among tree genotypes. Network dia-
grams of the mean lichen interaction matrices averaged for all trees and for several
individual genotypes showing a range of interaction network structure. Directionality
(arrowheads) and sign (red = negative, black = positive) 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 geneti-
cally 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. 4); however, this was not the case for other tree
traits, bark pH and carbon to nitrogen ratio. Also,
none of the lichen network metrics were significantly
predicted by tree genotype, either at the scale of the
entire network (Table 1) or for individual species (see
Supporting Information). Although both showed a
response to tree genotype, bark roughness and con-
densed tannins were not correlated (Pearson's $r =$
 0.084 , $p\text{-value} = 0.556$).

Tree traits and lichen community metrics were cor-
related with lichen networks. The genetically based
traits, bark roughness and condensed tannins were
both significant predictors of network similarity (Ta-
ble 2). Bark C:N ratio was also a significant predictor
of network similarity, but, as shown previously (see

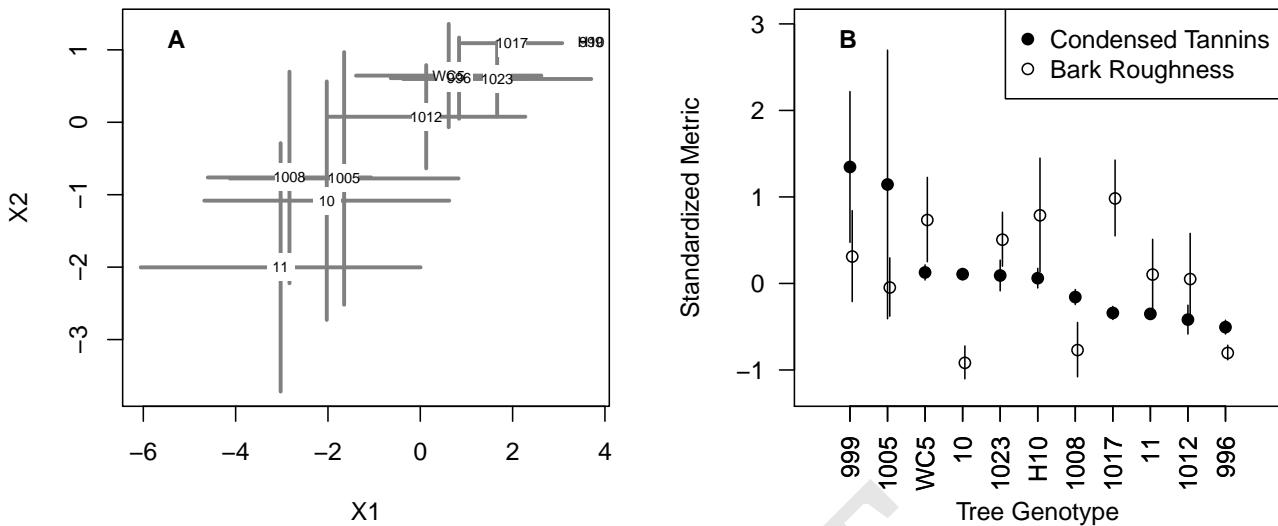


Fig. 4. Tree genotype affected lichen networks and several bark traits. A. The plot shows genotype centroids of NMDS ordinated ($R^2 = 0.999$, stress = 0.008) lichen networks (± 1 S.E.). Centroids that are closer are more similar in the structure of their lichen networks. B. Plot showing the standardized ($\frac{x-\bar{x}}{\sigma}$) means (± 1 S.E.) for the two genetically based tree traits: tree bark roughness and condensed tannin concentration.

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

Table 1. Genotypic effects on the associated lichen community.

	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.

Discussion

- We found:
 - Lichen networks genetically based
 - Several genetically based traits also explained variation in lichen network structure
 - Although correlated with lichen network structure, the network metrics explored were not genetically based
- Genetic response of lichen networks is not a

Table 1), there is not sufficient evidence support a genetic basis for it. Bark pH was not a significant predictor of lichen network similarity (Table 2). The abundance, richness, evenness and diversity of the bark lichen community, although also not predicted by tree genotype, were all significantly correlated with lichen network structure (Table 2). Lichen community composition was not correlated with lichen network similarity, either when species abundances were relativized (Mantel R = -0.09, p-value = 0.139) or not (Mantel R = -0.03, p-value = 0.573).

function of the abundance, richness, diversity⁵¹ or composition of lichen communities and is not⁵² reducible to simple network metrics. This is⁵³ likely the result of having similar interactions⁵⁴ among similar species in networks on trees of the⁵⁵ same genotype. This is difficult to disentangle⁵⁶ with the current set of analyses. Future work⁵⁷ should apply a structural statistical approach,⁵⁸ such as SEM or path analysis.⁵⁹

- Habitat filtering of communities (richness, composition) vs environmental influence on interactions. Likely a combination of both of these factors.
 - Lichen network structure correlated with species richness, evenness and diversity
 - Lichen community composition not correlated with network structure
 - None of these were genetically based
- An important consequence for diversity is that genotypes could be supporting unique communities, even if the composition of the communities is the same among individuals and genotypes.
- Genetic diversity could be influencing the stability of communities through the effects on interactions. Some network structures are likely to be more stable, either in response to disturbance or via self-organized dynamics. Although, none of the metrics examined, such as the number of links, modularity or centrality, showed a genetic signature.
- Important factors to consider in studies of other ecological networks:
 - Relative body size
 - Mobility
 - Reproductive isolation
- Future work should consider the potential influence on evolutionary dynamics of communities
 - Network structure influences network stability
 - Are the communities nested subsets?

TGW: I think window is too vague and this topic sentence needs to be much stronger for

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."⁵⁹⁷

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.⁵⁹⁸

- Genetic assembly rule = similar genetics will have more similar communities⁵⁹⁹
- What we don't know is whether or not these interactions will also lead to similar interactions among other species.⁶⁰⁰
- Thus, it would be possible for genetics to not only influence other species directly, but also indirectly by influencing the interactions among other species.⁶⁰¹

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.

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. citepBangert2008

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

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

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

fine roots as a first line of defense by invading microorganisms. Uptake of N that gets passed to the tree??

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

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

MKL: 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.

MKL: 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.

Bark roughness had previously been shown to be an important tree trait influencing bark lichens (18) that is under strong genetic control (19).

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 (24, 25). In the face of the high degree 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 (26–28). 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 (29). 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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