

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 at
8 the scale of individual trees has not yet been explored. To test the de-
9 gree to which tree genetics can contribute to network structure, we
10 conducted quantitative modeling of interaction network for a commu-
11 nity of epiphytic lichens in a long-term experimental common garden
12 of genotyped trees of a foundation species (*Populus angustifolia*).
13 We found three main results: 1) bark roughness and lichen commu-
14 nities displayed significant responses to tree genotype, 2) tree geno-
15 type strongly contributed to network structure, explaining a third of
16 the variation in lichen interaction networks, and 3) several metrics of
17 interaction network structure varied in response to genotype, includ-
18 ing the number of interactions and centralization. These results sup-
19 port the hypothesis that variation in ecological interaction networks
20 can result from genetically based variation in foundation species.
21 This study opens the possibility for a genetic basis to both direct
22 and indirect interactions among species in complex communities.**

Keyword 1 | Keyword 2 | Keyword 3 | ...

1 **E**volution occurs in the context of complex networks of
2 interacting species. In ecological communities, commu-
3 nity dynamics depend on key interactions (1) that occur in
4 species interaction networks, such as: trophic (2) and mu-
5 tualistic (3) interaction networks. Phylogenetic patterns in
6 ecological networks support the importance of evolutionary
7 processes in shaping species interactions, community structure
8 and ecosystem processes (4–6).

More on ecological networks

10 Community genetics studies (?) have shown that genetic
11 variation in foundation species (7) plays a significant role in
12 defining distinct communities of interacting organisms: such as,
13 endophytes, pathogens, lichens, arthropods, and soil microbes.
14 Multiple studies have now demonstrated that genetic variation
15 influences numerous functional traits (e.g., phytochemical, phe-
16 nological, morphological) produces a multivariate phenotype
17 (8) that contributes to variation in associated communities (9).

18 Additional work has provided support for the hypothesis
19 that not only does composition vary among genetically distinct
20 genotypes of foundation species but it also impacts the struc-
21 ture of the network of species interactions in these communities
22 (? ?). Also, work by (10 ? ?) observed consistent patterns
23 of centralized interactions of species modules focused around
24 hubs of plant-fungal interactions. In other words, a small
25 number of plant and fungal symbionts tended to have have

disproportionate numbers of interactions with other species
26 and likely are the drivers in determining community assembly,
27 structure and dynamics.

28 Here, we investigate how genetic variation in a foundation
29 tree species determines the structure of a network of inter-
30 actions among a community of tree associated lichen species.
31 Using a long-term (20+ years), common garden experiment
32 with replicated individuals of known genetic identity and a
33 naturally established stand of *Populus angustifolia*. We fo-
34 cused on a model community of 9 epiphytic lichens species,
35 as previous research has demonstrated significant compo-
36 sitional responses of epiphytes to genotypic variation (11, 12).
37 In addition, the life-history characteristics of lichen, having
38 highly localized, direct contact interactions and slow popula-
39 tion turnover rates, allowed us to assess interactions among
40 lichen species on individual trees. We hypothesize that in natu-
41 ral systems evolution occurs in a community context involving
42 interactions of complex networks of interacting species (13)?
43 ? ? ? . If correct, we should expect to find that network
44 structure is genetically based in which different plant geno-
45 types support different interaction networks and that these
46 interactions networks can function as indicators of ecological
47 dynamics important for conserving biodiversity. Applying
48 a probability-theory based network modeling approach, we
49 constructed a set of interaction network models for the lichen
50 associated with individual trees. Using these models, we then
51 examined the genetic basis a foundation tree species on the
52 structure of ecological networks.

Materials and Methods

The study was conducted along the Weber River, UT (USA),
55 which is a cottonwood (*Populus* spp.) dominated riparian ecosystem.
56

Significance Statement

Authors must submit a 120-word maximum statement about
the significance of their research paper written at a level under-
standable to an undergraduate educated scientist outside their
field of speciality. The primary goal of the Significance State-
ment is to explain the relevance of the work in broad context
to a broad readership. The Significance Statement appears in
the paper itself and is required for all research papers.

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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58 Although two native species, *Populus angustifolia* (James) and *Pop-*
 59 *ulus fremontii* (S. Watson), occur here and are known to hybridize,
 60 only pure or advanced generation backcrosses of *P. angustifolia* were
 61 sampled in order to avoid the effect of the hybridization between
 62 these two species (?).

63 A common garden was used to isolate the effect of tree genotype
 64 from the effect of the localized microenvironment associated with
 65 each individual and spatial autocorrelation. Asexually propagated
 66 clones of genotyped *P. angustifolia* individuals were obtained from
 67 wild collections and planted randomly in a single field (0.025 km^2)
 68 at the Ogden Nature Center, Ogden, UT in 1992. A total of thirteen
 69 genotypes replicated between 3 and 8 times each, were chosen for
 70 sampling. Genotype names were previously published in (?).

71 **Bark Lichen Observations.** On each tree, presence or absence of
 72 each lichen species was assessed in 50 total 1 cm^2 cells arrayed in
 73 a checkerboard pattern. Given the small size and sessile nature
 74 of lichen, we were able to rapidly assess lichen interactions by
 75 quantifying thalli in close contact. Sampling was restricted to
 76 the northern aspect of the trunk to maximize the abundance of
 77 lichen and control for the effect of trunk aspect. Two adjacent
 78 10 cm^2 quadrats centered at 50 cm and 85 cm from ground level
 79 were sampled (Fig 1 A and B). The bark lichen community in this
 80 system is comprised of fourteen species; however, only 9 species
 81 were observed within our study quadrats (Fig 1 C-K). The observed
 82 lichen community included (abbreviations are given for species
 83 present in study): Xg = *Xanthomendoza galericulata*, Xm = *X.*
 84 *montana*, Ch = *Caloplaca holocarpa*, Cs = *Candelariella subdeflexa*,
 85 Rg = *Rinodina glauca*, Lh = *Lecanora hagenii*, Ls = *Lecanora*
 86 sp., Pm = *Phyciella melanachra*, Pa = *Physcia adscendens*, Pu =
 87 *Physcia undulata*. Several other species were not observed in the
 88 present study but are known to occur in this region: *Phaeophyscia*
 89 *orbicularis*, *Phaeophyscia ciliata*, *Melanelia sublivacea*, *Meanelia*
 90 *elegantula*. Species accumulation curves indicated that communities
 91 in the the common garden were thoroughly sampled and similar in
 92 composition and richness to nearby naturally established cottonwood
 93 stands (Supplementary Materials).

94 The cell size and checkerboard sampling pattern was chosen to
 95 isolate the individuals in each cell. In a previous survey of lichen
 96 thallus size in this common garden, we had observed a median thallus
 97 size of $0.12 \pm 0.001 \text{ cm}^2$ (S.E.) (?). Based on this, we expected
 98 thalli observed in each cell to generally be spatially independent
 99 of the other cells in the quadrat but exposed to similar micro-
 100 environmental conditions created by the bark and the location of the
 101 sampling area on an individual tree. Therefore, we were confident
 102 in treating the cell-wise observations in quadrats as independent
 103 with respect to lichen-lichen interactions.

104 As bark roughness had previously been shown to be an important,
 105 genetically based tree trait impacting bark lichen, we measured the
 106 percentage of rough bark on each tree following the methods of (?).
 107 Briefly, the number of cells containing disrupted, fissured bark
 108 were counted within each quadrat on each tree. The number of
 109 rough bark containing cells were then summed and divided by the
 110 total number of cells surveyed. This was done for all quadrats on
 111 all trees in which lichen communities were also observed.

112 **Lichen Network Modeling and Analysis.** We used the observations of
 113 lichen in the 1cm^2 cells on individual trees of *P. angustifolia*. Uni-
 114 partite networks were generated using the conditional probabilities
 115 of each species pair, i.e. the probability of observing one species
 116 given an observation of another species ($P(A|B)$), based on the
 117 method developed by (14). To calculate conditional probabilities,
 118 we quantified the individual probabilities of species occurrences
 119 ($P(A)$) and the joint probability of co-occurrences ($P(A, B)$) using
 120 the frequencies of each species and their co-occurrences. We were
 121 then able to calculate the conditional probabilities of each species
 122 pair as $P(A|B) = \frac{P(A, B)}{P(B)}$, based on the axioms of probability. This
 123 yielded an asymmetric matrix, that is $P(A|B)$ does not have to be
 124 equal to $P(B|A)$ with a trace equal to the identity matrix (i.e.
 125 $P(A|A) = 1$).

126 Once these network matrices were constructed using the conditional
 127 probabilities of species pairs, we then applied an analytical
 128 procedure to remove non-significant links between species (Fig. 2).
 129 This procedure determines if the joint probability of a species

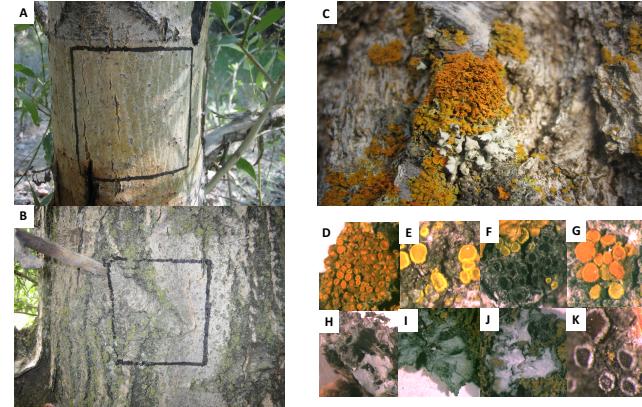


Fig. 1. The communities of bark lichen were observed in a common garden of replicated genotypes of narrowleaf cottonwood trees (*P. angustifolia*) at the Ogden Nature Center (Ogden, UT). Lichen were sampled within a fixed area ($10\text{cm} \times 10\text{cm}$) on individual trees (A and B). (C) a photo 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 main lichen species observed, respectively: *X. montana*, *Candelariella subdeflexa*, *Rinodina* sp., *Caloplaca holocarpa*, *Physcia adscendens*, *Phyciella melanachra*, *Physcia undulata* and *Lecanora hagenii*.

pair (i.e. $P(S_i S_j)$) is different from zero. If the frequency of co-
 130 occurrences ($S_i S_j$) falls outside of the interval ($CI_{95\%}$), the joint
 131 probability is inferred to be non-zero. Here, $CI_{95\%}$ is calculated
 132 as $CI_{95\%} = E[S_i S_j] * Z_{95\%} * \sqrt{V(S_i S_j)}$, where the expected
 133 frequency of co-occurrences ($E(S_i S_j)$) is the total number of cells
 134 surveyed (N) times the independent probabilities of each species
 135 ($P(S_i) * P(S_j)$), $Z_{95\%}$ is the Z-score for 95% from a Z-distribution
 136 and the expected variance of $E(S_i S_j)$ is the total number of cells
 137 times the expected probability of $S_i S_j$ and its compliment (i.e.
 138 $V(S_i S_j) = N * E[P(S_i S_j)] * (1 - E[P(S_i S_j)])$). Since the
 139 conditional probability is calculated using the joint probability as the
 140 numerator, whenever the joint probability is determined to be zero
 141 the conditional probability is determined to also be zero, since
 142 $P(S_i | S_j) = \frac{P(S_i S_j)}{P(S_j)} = \frac{0}{P(S_j)} = 0$.

Statistical Analyses, Software and Data. We used a combination of
 144 parametric and non-parametric, permutation based frequentist statistical
 145 analyses to test for the effects of genetic variation on lichen
 146 communities and their interaction networks.
 147

- REML
- Least squares models
- PerMANOVA
- Mantel tests
- Code and data available via github and zenodo
- ENA -> network metrics

All analyses were conducted using the programming language R version 3.4.2 (R Development Core Team 2018).
 154
 155

Results

Bark roughness and some lichen community characteristics responded to tree genotype. Percent rough bark varied significantly among genotypes (REML RLRT = 10.69, p-value = 3e-04), as did total lichen cover (REML RLRT = 2.9627, p-value = 0.0375) and community composition (PerMANOVA $R^2 = 0.243$, F 12 = 1.8221, p-value = 0.0029). However, lichen species richness did not show a significant response to genotype (REML RLRT = 0.13047, p-value = 0.3134). Community composition was correlated with lichen cover (PerMANOVA $R^2 = 0.236$, F 1 = 21.2661, p-value = 9.999e-05) and richness (PerMANOVA spr.onc $R^2 = 0.054$, F 1 = 4.9036, p-value = 0.0011) after controlling for tree genotype
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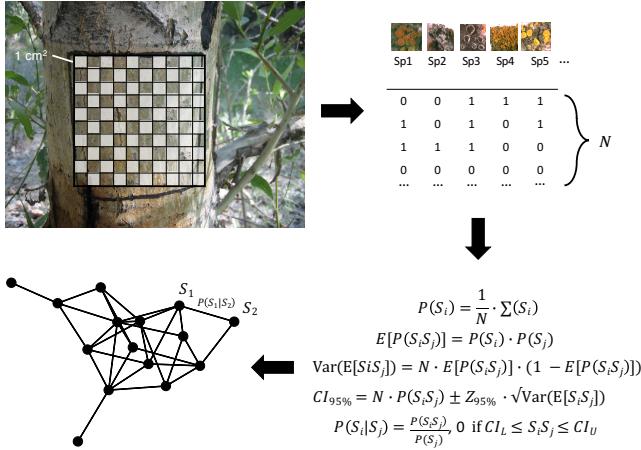


Fig. 2. Lichen interaction networks were constructed by conducting field observations in 1 cm^2 cells within a 10 cm^2 grid on each tree using a checkerboard pattern (grey cells). Thus, a set of N total cell observations were recorded for each tree with the presence or absence of each species recorded for each cell. Applying the probability-based network modeling method adapted from (14), 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, $P(S_iS_j)$, were not significant using a confidence interval based comparison of their observed co-occurrence frequency, S_iS_j , to that expected due to chance alone, $E[P(S_iS_j)] = P(S_i)P(S_j)$.

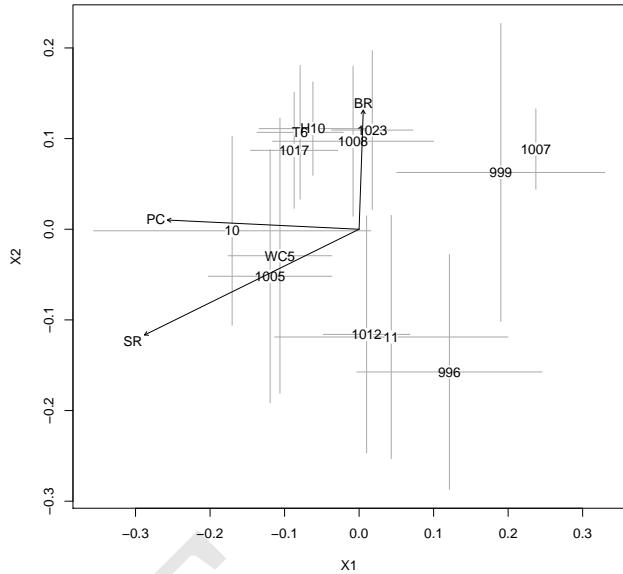


Fig. 3

nor the centrality (ANOVA $F_1 = 2.591$, p-value = 0.1134) of lichen networks. The number of network 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.

Figure: (A) Linkage and centrality by genotype and (B) Total cover and species richness predict L and Cen

Heritability estimates for lichen networks. Table: heritability

- Compare all trait heritabilities

•

Supplementary: Stats tables

Response	Predictor	p-value	H2
Percent Lichen Cover	Tree Genotype	0.0396	0.17
Lichen Species Richness	Tree Genotype	0.1351	0.1
Percent Rough Bark	Tree Genotype	2e-04	0.38
Lichen Network	Genotype	0.0411	0.17
Number of Network Links	Genotype	0.0812	0.15
Network Centrality	Genotype	0.1299	0.12

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

Discussion

- Rehash of results support hypothesis of genetic basis to network structure
- Genotypic environmental filtering leads to altered interaction network structure and potentially dynamics
- Indirect effects of genotypes (G - rough - cover - richness - links - networks)
- Importance of indirect effects and complexity and relevance to IIGEs

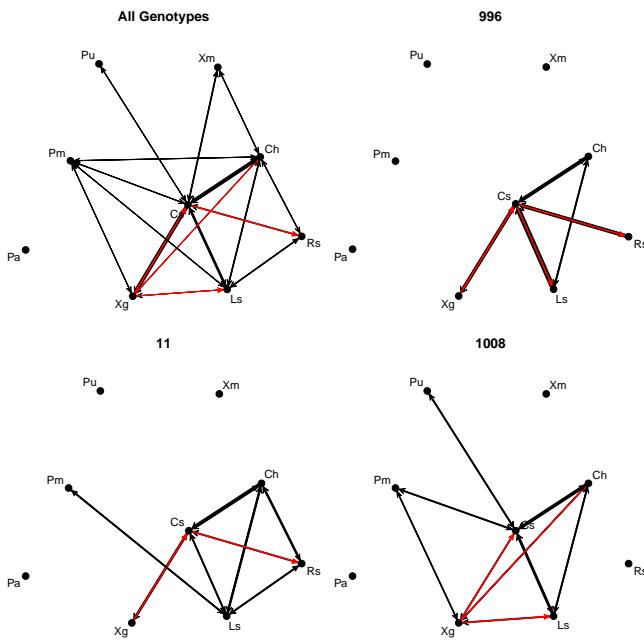


Fig. 4. Significant lichen interaction networks across genotypes.

229 • Conclusion

230 Trait variation + assembly + ecosystem function

231 These findings support the hypothesis that genotypic variation
232 in a foundation species contributes to the structure of a
233 network of interacting species that might be least expected to
234 exhibit such structure.

235 **TGW: MIGHT BE GOOD TO CITE PAPERS
236 ON COMEPTITION IN LICHENS OR OTHER OR-
237 ORGANIZING FACTORS TO BACK UP THE LEAST
238 EXPECTED STATEMENT. AS EPIPHYTES WE
239 MIGHT NOT EXPECT THEM TO CARE.**

240 **MKL: This is a job for Lamit and Rikke.**

241 Several lines of evidence support this conclusion. First, the
242 wild stand showed significant interaction network structure
243 (Fig. 1a and b); and both tree genotype and the genetically
244 based tree trait, bark roughness, was a strong predictor of
245 co-occurrence patterns (Fig. 2a).

246 **TGW: I THINK WE NEED TO EMPHASIZE
247 THE LONG-TERM NATURE OF OUR COMMON
248 GARDEON STUDY AS VERY FEW COMMON
249 GARDEON STUDIES OF LICHENS LIKELY EXIST.
250 ANY REFS ON THIS? IF TRUE MIGHT WANT
251 TO MENTION THIS UP FRONT IN INTRO.**

252 **MKL: Same here. This is a job for Lamit and
253 Rikke.**

254 Second, in a long-term common garden study, network (Fig.
255 1b) structure showed a high degree of similarity to the wild
256 stand network structure (Fig. 1c and d). Third, tree genotype
257 was a significant predictor of SES values (Fig. 2a), displaying
258 significant correlation with a genetically linked trait, bark
259 roughness, both in the common garden (Fig. 2a) and in a
260 naturally established stand of trees (Fig. 2b). Last, both of the
261 bipartite genotype-species networks in the common garden and
262 natural stand displayed significant modularity, suggesting that
263 genotypic variation is leading to the formation of evolutionarily

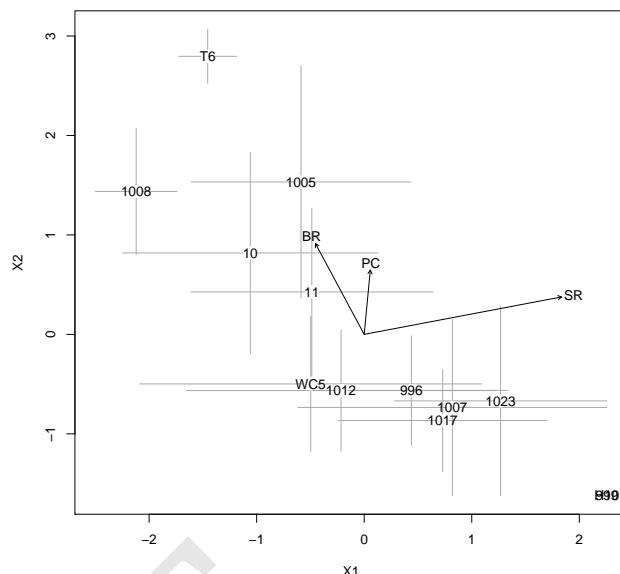


Fig. 5. Significant lichen interaction network structure resulting from tree genotypic variation was observed in the common garden. A) A network diagram showing significant interactions averaged over all trees shown as edges connecting lichen species shown as vertices. B) Genotype centroids (points) of NMDS ordinated lichen networks (± 1 S.E.). Arrows show the magnitude and direction of correlation of the ordinated networks with tree bark roughness (RB), percent lichen cover (PC) and lichen species richness (SR).

264 dynamic compartments within the community. Thus, just as
265 numerous studies have shown that plant genotype can affect
266 species richness, abundance, diversity, and composition and
267 previous work has demonstrated that evolutionary processes
268 shape ecological networks (15, 16), our study includes genetics
269 in an empirical investigation that combines both experimental
270 common garden findings along with studies in the wild that
271 are in close agreement.

272 Our results point to the importance of understanding the
273 community level effects of genetic variation and corroborate
274 previous findings of the importance of plant genetics in shaping
275 community structure and ecosystem processes (6). This study
276 highlights the potential for indirect effects of genetic varia-
277 tion to propagate through networks of interacting species and
278 trophic levels. Altering the structure of interaction networks
279 presents a means for genetic effects to be magnified within the
280 system of interacting species. For example, Keith et al. (2017)
281 showed that the genetics based interactions of aphid resistant
282 and aphid susceptible trees resulted in different interaction
283 networks of their associated arthropod communities composed
284 of 139 species. At the scale of ecosystems, trophic networks or
285 food webs direct and control the rates of energy and nutrient
286 flux (17). Furthermore, in a predator-prey-plant study, Smith
287 (18), showed that the interactions among species across trophic
288 levels depended on plant genotype.

289 **A. Units of evolutionary potential: Moving beyond species
290 pairs.** Although our study was conducted with a community
291 of lichens, these results should be generalized to other groups
292 of diverse organisms around the world that also exhibit signifi-
293 cant genetic signals at the community level (19, 20), although

294 spatial scale of interactions should be considered (21) Bangert
295 et al. 2006. As heritable variation is the raw material for
296 natural selection to act upon, a genetic basis for interaction
297 network structure indicates evolutionary dynamics should be
298 considered at the community level and that conserving ge-
299 netic variation is important to consider in efforts to restore
300 or preserve complex species interactions and their associated
301 ecosystem functions (22). With such findings, it appears that
302 we are closer to understanding the evolutionary drivers of
303 Darwin's entangled bank and the interconnectedness of species
304 in complex communities.

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- 311 1. Fontaine C, et al. (2011) The ecological and evolutionary implications of merging different
312 types of networks. *Ecol. Lett.* 14(11):1170–81.
- 313 2. Bascompte J, Jordano P, Olesen JM (2006) Asymmetric Coevolutionary Networks Facilitate
314 Biodiversity Maintenance 10.1126/science.1123412. *Science* (80-.). 312:431–433.
- 315 3. Rafferty NE, Ives AR (2013) Phylogenetic trait-based analyses of ecological networks. *Ecol-
316 ogy* 94(10):2321–33.
- 317 4. Crutsinger GM (2016) A community genetics perspective: Opportunities for the coming
318 decade. *New Phytol.*
- 319 5. Rezende EL, Lavabre JE, Guimarães PR, Jordano P, Bascompte J (2007) Non-random coex-
320 tinctions in phylogenetically structured mutualistic networks. *Nature* 448(7156):925–8.
- 321 6. Whitham TG, et al. (2006) A framework for community and ecosystem genetics: from genes
322 to ecosystems. *Nat. Rev. Genet.* 7:510–523.
- 323 7. Ellison AM, et al. (2005) Loss of foundation species: consequences for the structure and
324 dynamics of forested ecosystems. *Front. Ecol. Environ.* 3(9):479–486.
- 325 8. Holeski LM, Hillstrom ML, Whitham TG, Lindroth RL (2012) Relative importance of genetic,
326 ontogenetic, induction, and seasonal variation in producing a multivariate defense phenotype
327 in a foundation tree species. *Oecologia* 170:695–707.
- 328 9. Bailey JK, et al. (2009) From genes to ecosystems: a synthesis of the effects of plant
329 genetic factors across levels of organization. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*
330 364(1523):1607–16.
- 331 10. Toju H, Guimarães PR, Olesen JM, Thompson JN (2014) No Title. *Nat. Commun.* 5:5273.
- 332 11. Winfree R, Gross BJ, Kremen C (2011) Valuing pollination services to agriculture. *Ecol. Econ.*
333 71:80–88.
- 334 12. Ztylnska SE, Fay MF, Penney D, Preziosi RF (2011) Genetic variation in a tropical tree
335 species influences the associated epiphytic plant and invertebrate communities in a complex
336 forest ecosystem. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366:1329–1336.
- 337 13. Thompson JN (2013) *Relentless Evolution*. (University of Chicago Press), p. 499.
- 338 14. Araújo MB, Rozenfeld A, Rahbek C, Marquet PA (2011) Using species co-occurrence net-
339 works to assess the impacts of climate change. *Ecography (Cop.)*, 34:897–908.
- 340 15. Guimarães PR, Jordano P, Thompson JN (2011) Evolution and coevolution in mutualistic
341 networks. *Ecol. Lett.* 14(9):877–85.
- 342 16. Moya-Laraño J (2011) Genetic variation, predator-prey interactions and food web structure.
343 *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1425–37.
- 344 17. Borgatti SP, Everett MG (2006) A Graph-theoretic perspective on centrality. *Soc. Networks*
345 28:466–484.
- 346 18. Smith DS, Bailey JK, Shuster SM, Whitham TG (2011) A geographic mosaic of trophic inter-
347 actions and selection: trees, aphids and birds. *J. Evol. Biol.* 24(2):422–9.
- 348 19. Rowntree JK, Shuker DM, Preziosi RF (2011) Forward from the crossroads of ecology and
349 evolution. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 366(1569):1322–8.
- 350 20. Whitham TG, et al. (2012) Community specificity: Life and afterlife effects of genes.
- 351 21. Zook AE, Eklof A, Jacob U, Allesina S (2010) Food webs: Ordering species according to body
352 size yields high degree of intervality. *J. Theor. Biol.* 271(1):106–113.
- 353 22. Evans DM, Pocock MJO, Memmott J (2013) The robustness of a network of ecological net-
354 works to habitat loss. *Ecol. Lett.* 16:844–52.