Genotype variation in bark texture drives lichen community assembly across multiple environments

L. J. LAMIT,1 M. K. LAU, R. REESE NESBORG, T. WOJTOVICZ, T. G. WHITHAM, AND C. A. GEHRING

Department of Biological Sciences and Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona 86011 USA

Abstract. A major goal of community genetics is to understand the influence of genetic variation within a species on ecological communities. Although well-documented for some organisms, additional research is necessary to understand the relative and interactive effects of genotype and environment on biodiversity, identify mechanisms through which tree genotype influences communities, and connect this emerging field with existing themes in ecology. We employ an underutilized but ecologically significant group of organisms, epiphytic bark lichens, to understand the relative importance of 

Populus angustifolia
(narrowleaf cottonwood) genotype and environment on associated organisms within the context of community assembly and host ontogeny. Several key findings emerged. (1) In a single common garden, tree genotype explained 18–33% and 51% of the variation in lichen community variables and rough bark cover, respectively. (2) Across replicated common gardens, tree genotype affected lichen species richness, total lichen cover, lichen species composition, and rough bark cover, whereas environment only influenced composition and there were no genotype by environment interactions. (3) Rough bark cover was positively correlated with total lichen cover and richness, and was associated with a shift in species composition; these patterns occurred with variation in rough bark cover among tree genotypes of the same age in common gardens and with increasing rough bark cover along a ~40 year tree age gradient in a natural riparian stand. (4) In a common garden, 20-year-old parent trees with smooth bark had poorly developed lichen communities, similar to their 10-year-old ramets (root suckers) growing in close proximity, while parent trees with high rough bark cover had more developed communities than their ramets. These findings indicate that epiphytic lichens are influenced by host genotype, an effect that is robust across divergent environments. Furthermore, the response to tree genotype is likely the result of genetic variation in the timing of the ontogenetic shift from smooth to rough bark allowing communities on some genotypes to assemble faster than those on other genotypes. Organisms outside the typical sphere of community genetics, such as lichens, can help address critical issues and connect plant genotype effects to long-established streams of biological research, such as ontogeny and community assembly.

Key words: common garden; community assembly; genotype; heritability; lichen; ontogeny; Populus angustifolia; structural equation modeling; Weber River, Utah, USA.

INTRODUCTION

A primary goal of the field of community genetics is to understand how genotypic differences among individuals within species influence communities of interacting taxa (Whitham et al. 2006). By focusing on one of the fundamental units important to biodiversity and evolution, the genotype, community genetics has the potential to unite the fields of community ecology, evolutionary biology, and genetics (Antonovics 1992, Whitham et al. 2006, Wade 2007). However, critics argue that several strides need to be taken before community genetics can be considered a mature discipline. First, the role of genetic variation relative to other factors shaping ecological communities needs to be more fully elucidated. Some researchers have argued that the influence of genotype may be relatively unimportant compared to the effect of environmental variation in natural settings (Hersch-Green et al. 2011, Tack et al. 2012). Second, patterns documented in community genetics research need greater integration with foundational ecological research themes such as community assembly and succession, lines of research that have been major foci of ecology since its inception (e.g., Cowles 1899, Cooper 1923, Tansley 1935). How does accounting for genotype improve our understanding of these ecological themes? Third, because of its roots in plant–enemy interactions, community genetics initially focused on arthropod responses to plant genotype (e.g., Johnson and Agrawal 2005, Wimp et al. 2007). To understand the broad effects of plant genotype on biodiversity, research needs...
to fully incorporate the diverse array of other plant-associated organisms.

Epiphytic lichens are model organisms with which to advance the field of community genetics. First, lichens are diverse, widespread, and provide habitat and food for a range of other organisms from microbes to vertebrates (Andre 1985, Maser et al. 1985, Bates et al. 2011, 2012). Second, trophic disconnection from their hosts may cause lichens to be sensitive to a different suite of plant traits, and more likely to exhibit neutral responses to plant genotype relative to antagonistic organisms that feed directly on the plant, such as herbivorous arthropods. Lichens therefore represent a robust test of the generality of the extended effects of plant genotype on associated communities. Third, epiphytic lichens are stationary, with slow dynamics within the discrete boundaries of trees. These traits allow for large sample sizes in studies, monitoring of the same individuals over many years (e.g., Shriver et al. 2012), and clear documentation of interactions between lichens (e.g., Stone 1989). Fourth, there is a preexisting body of knowledge on epiphytic lichen community assembly and succession (see Ellis 2012). This work often utilizes gradients in tree or branch age as space-for-time substitutions (e.g., Stone 1989, Ellis and Coppins 2007, Johansson et al. 2007, Gjerde et al. 2012), which on a smaller scale are similar to studies with terrestrial plant dynamics on soil age gradients (e.g., Cooper 1923, Jumpponen et al. 2012).

Epiphyte community dynamics take place on living trees with genetically based phenotypes; genetic variation in host traits may therefore influence lichen community assembly and succession (Ellis and Coppins 2007, Lamit et al. 2011). Many tree species exhibit a developmental shift in bark texture from smooth to coarse and furrowed with age (Borger 1973, Hoffman and Boe 1977, Johansson et al. 2007). Variation in bark texture among genotypes of the same age (e.g., Lamit et al. 2011) indicates that the timing of the ontogenetic shift from smooth to rough is under genetic control, and lichens may be influenced by this genetic variation in bark ontogeny. Furthermore, environmental conditions can modulate the expression of genetically variable plant traits, and these genotype by environment (G x E) interactions can affect plant-associated communities (e.g., Orians and Fritz 1996, Johnson and Agrawal 2005, Davies et al. 2014). The relative and interactive effects of genotype and environment on ecological communities are still poorly understood (Hersch-Green et al. 2011, Tack et al. 2012, but see Busby et al. 2014). Consideration of the relative importance of genotype vs. environment to bark traits that influence lichens, as well as their interactions, is required for a complete understanding of epiphytic lichen community assembly.

Our aim is to understand the role that genetic variation in a foundation tree species plays in shaping epiphytic lichen communities. We hypothesize that lichen communities vary among tree genotypes growing in a common garden. Because the environmental context may influence lichens and their interactions with trees, our next step is to understand the relative strengths of environment and tree genotype on lichen communities, and the influence of the environment in modulating the effect of tree genotype. Therefore, we hypothesize that strongly contrasting environments (common gardens in a favorable and more severe environment) differentially influence lichen community structure and interact with tree genotype to create G x E interaction effects on lichen communities. To identify the mechanism by which tree genotype influences lichens, we examine their response to variation in bark roughness, with the hypothesis that variation in lichen communities among tree genotypes is due to genetic variation in bark texture. Results from addressing the first hypotheses suggest that rough bark facilitates the colonization of lichens, and we hypothesize that variation in lichen communities among tree genotypes is a consequence of communities assembling faster on genotypes with rougher bark than communities on genotypes with smooth bark. We test this hypothesis by comparing patterns of community response to different tree genotypes with those of community assembly on gradients of tree age. The latter involves comparisons along a ~40-year-old tree chronosequence, and between paired parent trees and offspring ramets (root suckers) in a common garden. Addressing these hypotheses will clarify the relative influence of genotype and environment, within the context of host ontogeny, on plant-associated organisms, and add the dimension of community genetics to our understanding of community assembly.

METHODS

Study system and field sites

Populus angustifolia is a foundation species of middle-to-upper elevation (~1300–2500 m in our study region) riparian habitats of intermountain western North America (Eckenwalder 1984). This study utilized P. angustifolia common gardens propagated from cuttings collected at random, within a 105-km stretch of riparian habitat along the Weber River, Utah, USA (linear distance between farthest collection sites, ~80 km; Appendix A). One garden was established at the Ogden Nature Center (ONC garden: 41.248146°N, 111.998830°W; elevation, 1302 m; area, ~1.2 ha; trees spaced 4–7 m apart) in 1991, near the lower distribution of P. angustifolia along the Weber and Ogden rivers. The second garden (Pit garden: 41.133445°N, 111.901660°W; elevation, 1394 m; area, ~1.6 ha; trees spaced 4–7 m), was established in 1988, at a ~15-km linear distance from the ONC garden. At both gardens, genotypes were characterized using 35 codominant RFLP markers, and verified as P. angustifolia without significant introgression from the partially sympatric P. fremontii (Martin-sen et al. 2001, Lamit et al. 2011b). Lichens were also sampled on naturally established P. angustifolia growing...
along the Weber River (Uintah natural stand chronosequence: 41.138655° N, 111.944475° W; elevation, 1362 m; site area, ~93.5 ha). Trees at the Uintah stand exhibited *P. angustifolia* morphology but were not characterized with DNA markers. All sites were located in a region with an estimated mean annual precipitation of 440 mm/yr (Bridgeland et al. 2010) and average growing season (April–October) air temperature of ~10°C (Lojewski et al. 2009).

The Pit and ONC gardens represent two distinct environments. The Pit garden receives regular, intense wind due to the movement of air from high elevations in the east into the desert to the west through the adjacent Weber River canyon. Trees in the ONC garden do not experience these winds. Relative to the ONC garden, the Pit garden has a lower water table (depth: Pit, >2 m; ONC, ~1.3 m), faster-draining soil, lower relative humidity and higher air temperature in the growing season, and an understory containing several plants indicative of arid conditions (e.g., *Artemisia tridentata* and *Opuntia* sp.; Bridgeland et al. 2010, Busby et al. 2014). Stressful conditions have reduced the long-term tree growth rate at Pit to nearly half that at the ONC garden (Lojewski et al. 2009), which has resulted in a closed canopy at the ONC garden and an open canopy at the Pit garden (canopy cover [mean ± SE]: ONC, 78.4% ± 20.5%; Pit, 12.4% ± 8.4%; M. K. Lau unpublished data). Furthermore, a number of studies utilizing these two gardens found large environmental effects on tree traits, soil properties, associated communities, and ecosystem processes (e.g., Smith et al. 2011, Pregitzer et al. 2013, Busby et al. 2014).

**Statistical analysis**

Linear models with random effects were fit using restricted maximum likelihood (REML; Shaw 1987), where random effects were tested with likelihood ratios tests and fixed effects with Wald chi-square tests via packages lme4 (Bates et al. 2014), RLRsim (Scheipl et al. 2008), and car (Fox and Weisberg 2011) in R 2.8.1 (R Development Core Team 2011). Univariate regressions were also conducted in R. Distance-based permutation MANOVA (PERMANOVA) and regression (DISTLM) were conducted in Primer 6.1.15 with PERMANOVA+1.0.5 (PRIMER-E, Plymouth, UK). PCord 5.10 (MJM Software, Gleneden Beach, Oregon, USA) was used for nonmetric multidimensional scaling (NMDS) and vector analyses. Structural equation modeling (SEM) was conducted in AMOS 19.00 (AMOS Development, Meadville, Pennsylvania, USA). Significance was set at α = 0.05.

**Hypothesis 1: Lichen community structure varies among tree genotypes**

Lichens were sampled on 18 *P. angustifolia* genotypes (two to nine trees per genotype, 76 trees total; Appendix A) at the ONC garden, in May 2010. Cover of each species was quantified with a hand lens in four 10 × 10 cm quadrats, two on the north and south sides of each tree at 45–55 cm and 80–90 cm above the soil surface. Cover values from all quadrats on a tree were averaged. For all data sets in this study, lichens were identified using a variety of keys, including McCune and Geiser (1997) and Nash et al. (2002, 2004, 2007). The effect of tree genotype on species richness and total cover was tested with REML-based linear models, and variance components were used to estimate broad-sense heritabilities (\(H^2\): the proportion of total variance attributable to genotype). Richness was log-transformed and total cover was square-root-transformed. Community composition was examined using PERMANOVA and visualized with NMDS using Bray-Curtis dissimilarity. Prior to composition analyses, percent covers of each species were fourth-root-transformed to down-weight the influence of abundant taxa (Anderson et al. 2008). Vectors for each lichen species, total cover, and richness were fit to the NMDS ordination, which represent the linear relationship of a variable through ordination space (McCune and Grace 2002).

**Hypothesis 2: Strongly contrasting environments differentially influence lichen community structure and interact with tree genotype to influence lichens**

Lichens were examined on the same 10 *P. angustifolia* genotypes in the Pit (three to nine trees per genotype, 57 trees total) and ONC gardens (three to nine trees per genotype, 51 trees total). This subset of genotypes originated from a ~37-km stretch of riparian habitat along the Weber River (~32-km linear distance). Trees at the Pit garden were sampled in May 2010 and 2011, while the ONC garden trees were a subset of those used to address hypothesis 1. Methods for sampling Pit were the same as those used at the ONC garden. The combined data set contained 10 genotypes, 7–15 trees per genotype, and 108 trees total, and will be referred to as the G × E data set. Due to the slow dynamics of lichen communities, we did not expect the length of the survey period or slight offset of garden ages (ONC, 19 yr; Pit, ~22 yr) to affect our results. If these factors did influence our patterns, the most likely outcome would be to confound and inflate the garden effect, which was unlikely given our results.

Three components of community structure were examined. The effect of tree genotype on total lichen cover and species richness was analyzed with a mixed model fit with REML, containing the fixed effect of garden and the random effects of tree genotype and genotype × garden interaction. Richness was log-transformed, and total lichen cover was square-root-transformed before analysis. Community composition was examined with PERMANOVA, treating genotype and the genotype × garden interaction as random effects, and garden as a fixed effect. PERMANOVA was conducted on a fourth-root-transformed matrix with Bray-Curtis distance, using Type III sums of squares, and pseudo-F ratios obtained by permuting.
residuals of reduced models (Anderson et al. 2008). For community composition, we also compared the effects of genotype, garden, and their interaction using the square root of the variance component. Variance components from PERMANOVA are expressed in squared units of dissimilarity (Bray-Curtis in this case, which are scaled as a percentage in PRIMER), and the square root of a variance component represents the average percentage of community dissimilarity among units of a factor (e.g., genotypes, gardens; Anderson et al. 2008). NMDS, performed with Bray-Curtis dissimilarity, was used to visualize multivariate composition, and vectors of each lichen species, total cover, and richness were fit to the ordination.

To examine the robustness of our test of hypothesis 2, these analyses were repeated with a reduced data set containing only genotypes propagated from a single cottonwood stand adjacent to the Pit garden (six genotypes, 7–15 trees per genotype, 70 trees total). Our first test, with the full G × E data set, examined lichens on genotypes collected from approximately twice the linear distance that the gardens were from each other (32-km linear distance for genotype collection, but only 14 km between gardens), which may bias toward detecting greater tree genotype effects than environmental effects on lichen communities, and create a garden with much higher genetic diversity than would be found in an equivalently sized natural *P. angustifolia* stand (Tack et al. 2012). Therefore, the second set of analyses using a set of genotypes originating from a single small *P. angustifolia* stand ensured that the effect of genotype was not inflated by trees being propagated from a larger spatial range than the scale of a single common garden, but was also conservative in estimating genotype effects because the distance between gardens created a much greater environmental gradient than existed within the single natural stand of trees from which the genotypes originated.

**Hypothesis 3: Bark texture is an important mechanism by which tree genotype affects lichens**

*Populus* and many other tree taxa exhibit bark surfaces ranging from smooth to coarsely textured and furrowed, and we focused on bark texture because of its potential importance to epiphytes. We categorized smooth bark as periderm tissue lacking three-dimensional surface attributes, whereas rough bark was any bark with three-dimensional structure and was either rhytidiome or periderm transitioning to rhytidiome (Borger 1973). Importantly, no trees had the deeply furrowed bark typical of old *Populus* trees. Rough bark cover was estimated in the four quadrats where lichens were measured, and averaged for each tree. The effect of genotype on rough bark cover was examined using the full ONC garden data set, while the G × E data set was used to examine the response of rough bark cover to tree genotype, garden, and their interaction, using REML methods described for hypotheses 1 and 2, respectively.

A two-step approach was taken to statistically link tree genotype, bark texture, and lichens. First, a vector of rough bark cover was fit through the NMDS ordination of the full ONC garden and G × E data sets. These ordinations were rotated to align the rough bark cover vectors with their first NMDS ordination axes.

The second analysis to address hypothesis 3 used SEM. SEM allows for the testing of a multivariate hypothesis about the causal structure of a network of interacting variables. Our interest was to determine if rough bark cover represented the tree phenotype acting as a mechanism through which tree genotype affects lichen composition. Only observations from genotypes present in both gardens (i.e., the G × E data set) were used. Lichen composition was represented in the model by the NMDS axes from the rotated ordination performed to address hypothesis 2, as in Antoninka et al. (2009). Because tree genotype was categorical, it was modeled as a composite variable using binary dummy variables (Grace 2006). Our hypothesized a priori model included (1) a direct effect of genotype on rough bark cover (the garden effect on rough bark was not included because it was insignificant in univariate analyses, see *Results*), (2) direct paths from rough bark cover to both sets of NMDS axis scores, and (3) a direct path from garden to both sets of NMDS axis scores. Additionally, an undirected path between the two NMDS axes was used to account for their covariance. A garden × genotype interaction was not included because univariate analyses indicated that this effect was not important (see *Results: Hypothesis 2*). Correspondence between the hypothesized model structure and the data (i.e., model fit) was tested with a maximum likelihood chi-square test and the root mean square error of approximation (RMSEA).

**Hypothesis 4: Variation in lichen communities among tree genotypes is a consequence of communities assembling faster on genotypes with rougher bark**

**Test 1: Patterns of lichen community assembly along a tree age chronosequence mirror lichen variation among tree genotypes of the same age.**—From May to December 2012, lichens and rough bark were quantified in quadrats on 32 trees at the Uintah natural stand chronosequence using the same methods as for the common gardens. Tree ages were estimated from annual growth rings in cores collected with an increment borer at ~15 cm above the soil. The relationships of rough bark cover with tree age, species richness, and total lichen cover were tested with linear regression. The effect of rough bark on lichen composition was tested with DISTLM, using Bray-Curtis dissimilarity on a fourth-root-transformed matrix (excluding three young trees lacking lichens). The fourth-root-transformed community matrix was ordinated with NMDS, fit with vectors of rough bark cover, tree age, total lichen cover, species
richness, and all individual species, and rotated to align the vector of rough bark cover with axis 1.

We used SEM to establish that rough bark cover of chronosequence trees had a direct effect on lichen composition, acting independently of the direct influence of tree age. The hypothesized a priori model included (1) a path from tree age to rough bark, (2) a path from rough bark to composition, and (3) a path from tree age to composition. Lichen composition was represented by NMDS axis 1 from the ordination described in the previous paragraph; axis 2 was not included because it represented only 2.5% of the variation in composition. The a priori model contained no degrees of freedom to test model fit because all variables were linked by paths. To assess the appropriateness of this model, we examined the significance of each individual path. We also tested two reduced models, one lacking the path from bark roughness to lichen composition, and the other lacking the path from tree age to composition. Because model fit indices (see Methods: Hypothesis 3) primarily indicate whether or not important paths are included in a model, poor fit of reduced models provides indirect evidence in support of the full model.

Test 2: communities on smooth-barked adult trees are poorly developed and similar to their juvenile ramets, while communities on rough-barked adult trees are more developed and dissimilar to their ramets.—To directly contrast lichen communities on adult parent trees and juvenile offspring, lichens were sampled in December 2012 on adult tree and juvenile ramet pairs in the ONC common garden (32 pairs from 15 genotypes, one to four pairs per genotype; Appendix A). Sample size was limited because not all trees had naturally occurring ramets. Parents were ~21.5 yr old and sampling was restricted to ramets approximately half the age of the parents (mean ramet age = 10.8 yr, range = 9.3–11.9 yr; estimated using an equation relating ramet growth rings and basal circumference, data not shown). Ramets were small in diameter, so covers of lichens and bark roughness were sampled in 2 cm wide × 100 cm tall transects, originating at 10 cm above the soil surface, on the north and south side of ramets and adult trees. Cover values were averaged from each transect, for each parent and ramet. Ramet stems had an average 1.37-m (range: 0–3.75 m) distance from the trunk of the parent tree and were always located closer to the parent than to any neighbor. Our extensive observations on leaf phenology (which is strongly genetically based) in the garden, and belowground connection between parents and ramets revealed through past excavations, made us confident that ramets were offspring of their paired parent trees. Species richness and total lichen cover differences were calculated as the parent minus the ramet value, and for composition, Bray-Curtis dissimilarity between a parent and ramet was calculated from a fourth-root-transformed community matrix. The relationship between the difference in rough bark cover and the difference in each community variable between parent and ramet pairs was examined using mixed linear models fit with REML. In addition to the difference in rough bark cover between parent-ramet pairs, models contained basal ramet circumference (to account for slight variation in ramet age), the linear distance between each parent and ramet, and tree genotype as a random effect (to avoid pseudo-replication because some genotypes had multiple trees in the data set), however, only the effect of parent–ramet rough bark difference was tested.

RESULTS

Hypothesis 1: Lichen community structure varies among tree genotypes

Lichen communities varied among tree genotypes in the ONC garden, supporting hypothesis 1. Tree genotype explained over 30% of the variance in total cover (df = 1, $\chi^2 = 8.685, P = 0.001, H^2 = 0.33$, range of genotype means = 0.86–18.73%) and 20% of the variance in species richness (df = 1, $\chi^2 = 3.815, P = 0.025, H^2 = 0.20$, range of genotype means = 1.33–6.00 species). Nearly one-fifth of the variation in composition was due to differences among tree genotypes ($F_{17,58} = 1.885, P = 0.007, H^2 = 0.18$). The community consisted of nine species, and was dominated by Xanthomendoza galericulata, followed by Candelariella subdeflexa (Appendix B). In general, trees with low lichen cover were only colonized by X. galericulata. NMDS axis 1 explained the majority of variation in the data (83.3%), and all species increased in abundance from left to right along this axis (Fig. 1; Appendix B).

Hypothesis 2: Strongly contrasting environments differentially influence lichen community structure and interact with tree genotype to influence lichens

Hypothesis 2 was not fully supported. Tree genotype significantly influenced total lichen cover and species richness, and there was no evidence of a garden effect or genotype × garden interaction (Table 1, Fig. 2a, b). In contrast, tree genotype and garden influenced species composition; communities were ~13% dissimilar among genotypes and ~10% dissimilar between gardens, on average (square root of variance components: genotype, 13.09%; garden, 9.69%; genotype × garden, 4.83%; Table 1, Fig. 2c). Results for all variables did not change with the reduced data set containing genotypes propagated from a single location (square root of variance components for composition: genotype, 14.72%; garden, 9.97%; genotype × garden, 7.59%; Table 1). The majority of species were found in both gardens, and X. galericulata was the most abundant (Appendix B). When a tree had low lichen cover, it was typically only colonized by X. galericulata. Even when combining data from contrasting garden environments, the majority of variation in composition was represented by NMDS axis 1 (65.9%; Fig. 2c), which primarily represented variation among genotypes, and many of
Hypothesis 3: Bark texture is an important mechanism by which tree genotype affects lichens.

Rough bark cover was influenced by genotype. Genotype explained ~50% of the variance in rough bark cover in the ONC garden (df = 1, $\chi^2 = 22.568$, $P < 0.001$, $H^2 = 0.48$, range of genotype mean cover = 17.56–67.92%). With the G × E data set, genotype also had a strong influence on rough bark cover, and garden and genotype × garden effects were nonsignificant (ONC genotype mean cover = 17.56–67.92%; Pit genotype mean cover = 16.50–63.13%; Table 1). Results were

Table 1. Effect of tree genotype, garden, and their interaction on lichen community variables and bark roughness from the genotype × environment (G × E) data set, with models containing the full set of 10 genotypes and the reduced set of six genotypes propagated from a single riparian stand.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Garden</th>
<th>Genotype × garden</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>v</td>
<td>df</td>
</tr>
<tr>
<td>A) Response variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full data set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lichen cover (%)</td>
<td>1</td>
<td>19.742</td>
</tr>
<tr>
<td>Lichen species richness</td>
<td>1</td>
<td>2.666</td>
</tr>
<tr>
<td>Rough bark cover (%)</td>
<td>1</td>
<td>8.356</td>
</tr>
<tr>
<td>Reduced data set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lichen cover (%)</td>
<td>1</td>
<td>15.518</td>
</tr>
<tr>
<td>Lichen species richness</td>
<td>1</td>
<td>4.330</td>
</tr>
<tr>
<td>Rough bark cover (%)</td>
<td>1</td>
<td>3.241</td>
</tr>
<tr>
<td>B) Response variable</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Full data set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lichen species composition</td>
<td>9, 88</td>
<td>2.674</td>
</tr>
<tr>
<td>Reduced data set</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lichen species composition</td>
<td>5, 58</td>
<td>3.425</td>
</tr>
</tbody>
</table>

Notes: $\chi^2$ are from Wald’s tests for fixed factors (garden) and likelihood ratios tests for random effects (genotype, genotype × garden interaction), while $F$ are pseudo-$F$ ratios from PERMANOVA. PERMANOVA residual degrees of freedom are 88 for the full data set and 38 for the reduced data set. Bolded values are significant at $P < 0.05$. 
similar for the reduced G × E data set of trees propagated from a single location.

In support of our hypothesis, there was a relationship between rough bark cover and lichen composition. The vector of rough bark cover correlated positively with the rotated NMDS axis 1 in the ONC and G × E data sets (see hypotheses 1 and 2; ONC r = 0.330; G × E r = 0.407; Figs. 1, 2c; Appendix B). Furthermore, many lichen species individually increased in cover or occurrence as rough bark cover increased among genotypes (Appendix C).

Structural equation modeling further supported our hypothesis that genetic variation in rough bark is a mechanism linking tree genes to variation in lichen communities (Fig. 3). There was good fit between the data and the hypothesized model, however, two paths did not have significant effects. We removed these paths, and the reduced model maintained good fit (df = 30, χ² = 31.90, P = 0.372; RMSEA = 0.024, P = 0.723). The final model demonstrated two important points. First, a direct path between genotype and NMDS axis 1 was not necessary to obtain good model fit. This suggests that the effect of genotype on lichen composition acted primarily through rough bark cover. Second, the effect of tree genotype and garden acted on independent gradients in the community, with genotype influencing the gradient represented by NMDS axis 1 and garden influencing the gradient associated with axis 2. The net effect of garden on NMDS axis 2 was 0.44 (the path coefficient), whereas the net effect of genotype on NMDS axis 1 was 0.29 (obtained by multiplying the path coefficients from genotype to rough bark, and rough bark to NMDS axis 1: 0.73 × 0.41). Importantly, NMDS axis 1 captured over three times more variation in composition than axis 2 (65.9% vs. 19.8%; see Results: Hypothesis 2); thus the effect of genotype on community composition is likely greater than the garden effect.

Hypothesis 4: Variation in lichen communities among tree genotypes is a consequence of communities assembling faster on genotypes with rougher bark

Test 1: Patterns of lichen community assembly along a tree age chronosequence mirror lichen variation among tree genotypes of the same age.—In support of hypothesis 4, lichen communities showed a relationship with rough bark along the Uintah natural stand chronosequence similar to that among tree genotypes of the same age in the common gardens. Rough bark cover of chronosequence trees increased with age (F₁,30 = 47.96, P < 0.001, R² = 0.62; rough bark = 38.23% ± 27.49% [mean ± SD], range = 2.5–100%; tree age = 21.25 ± 9.65 yr, range = 7–39 yr). As rough bark cover increased, species richness (F₁,30 = 47.61, P < 0.001, R² = 0.567; richness = 4.06 ± 3.05 species, range = 0–10 species) and total cover (F₁,30 = 47.92, P < 0.001, R² = 0.602, total cover = 10.42% ± 13.03%, range = 0–43.75%) increased, and composition shifted (pseudo-F₁,27 = 16.167, P < 0.001, R² = 0.375). The majority of species that occurred on the chronosequence were present in the gardens, and X. galericulata was also dominant on chronosequence trees (Appendix B). The only species on very young trees with smooth bark was X. galericulata, where it occurred on small, isolated rough patches. Mirroring patterns seen in the gardens, rough bark and all species tended to increase along the NMDS axis that explained the majority of variance in composition (NMDS axis 1: 90.1%; Fig. 4a; Appendix B), and individual plots of
species showed that most species increased in cover or occurrence with rough bark cover (Appendix C). Because these trees vary strongly in age, the gradient with bark roughness is the pattern of assembly during community establishment.

Structural equation modeling confirmed that lichen community patterns in the chronosequence were likely driven in part by the direct effect of rough bark (Fig. 4b). All paths in the full model were significant, and the two reduced models fit poorly (model lacking path from rough bark to lichen composition had df = 1, χ² = 4.530, P = 0.033; RMSEA = 0.355, P = 0.040; model lacking path from age to lichen composition had df = 1, χ² = 4.352, P = 0.037; RMSEA = 0.346, P = 0.044). In the full model, the direct effect of tree age on NMDS axis 1 (0.41) was nominally larger than that of age acting through its influence on rough bark on axis 1 (0.80 × 0.42 = 0.36). Despite the important effect of age on composition, rough bark remained a significant factor after controlling for the direct effect of tree age, indicating that variation in communities on the chronosequence was due in part to a unique effect of increasing rough bark.

Test 2: Communities on smooth-barked adult trees are poorly developed and similar to their juvenile ramets, while communities on rough-barked adult trees are more developed and dissimilar to their ramets.—In further support of hypothesis 4, adult trees in the ONC garden with smooth bark were more similar to their ramets than adult trees with rough bark (Fig. 4c). As the difference between parent and ramet rough bark increased, species richness differences increased (mean ± SD = 3.19 ± 2.26, range = 0–7; df = 1, χ² = 6.592, P = 0.010, standardized β = 0.448), and composition similarity decreased (24.20% ± 22.62% Bray-Curtis dissimilarity, range = 0–83.47%; df = 1, χ² = 9.012, P = 0.003, standardized β = −0.450; Fig. 4c). Differences in total lichen cover also increased with increasing bark roughness differences, although marginally (17.15% ± 111.29%, range = 0.13–41.04%; df = 1, χ² = 3.54, P = 0.060, standardized β = 0.308). Rough bark cover of ramets was low and varied little (6.32% ± 4.70%, range = 0–15.00%), while it varied greatly among parent trees (38.13% ± 13.68%, range = 12.50–65.0%). Therefore, variation in the differences in rough bark between parents and ramets (31.80% ± 14.17%, range = 1.25–60.00%) was primarily driven by variation in rough bark cover among parent trees.

**Discussion**

Lichen response to tree genotype

We show that communities trophically disconnected from their hosts are sensitive to differences among tree genotypes. The influence of genotype on epiphyte communities has been examined only a limited number of times (e.g., Zytynska et al. 2011, Davies et al. 2014). For example, genetically similar Brosimum alicastrum trees in a tropical forest host more similar vascular epiphyte communities than genetically dissimilar trees (Zytynska et al. 2011). It is well established that organisms trophically tied to their hosts, such as arthropods, are influenced by plant genotype. Studies with epiphytes are important because they extend the range of taxonomic and functional groups known to be affected by host genotype.

The effects of tree genotype on lichens are strongly associated with genetic variation in bark texture. The importance of genetic variation in physical bark characteristics as habitat for arthropod communities is demonstrated by Barbour et al. (2009), where they show that a largely predaceous spider community responds positively to the exfoliating bark and crevices that differ greatly among geographic races of Eucalyptus globulus. The heterogeneous surface of rough bark may facilitate the establishment and growth of epiphytes, because it catches dispersing propagules, offers microsite shelter from desiccation, and may have increased moisture holding capacity (Lamit et al. 2011a, Adhikari et al. 2012). We recognize that in some habitats, smooth-barked trees can be rich in lichens, and suggest that the benefits of rough bark to lichens may be especially important in arid environments, such as our study sites. Although bark chemistry may also be important to
lichens in some systems, previous work indicates that bark nitrogen and condensed tannin concentrations are not correlated with bark texture or cover of the dominant species in this system, *X. galericulata* (Lamit et al. 2011a). This indicates that the relationship between rough bark cover and the lichen community is not due to confounding effects from these chemical variables, however future studies connecting tree genotype with lichen communities should examine other bark chemical traits, such as pH.

For all variables, the variation in lichen communities explained by tree genotype is greater than that of garden environment. The scale-dependent hypothesis predicts that the effect of plant genotype on associated communities diminishes relative to environmental influences, with expanding geographic scale (see Johnson and Agrawal 2005). Furthermore, the strength of the plant genotype effect on herbivorous arthropods can be inflated when plants in a common garden are propagated from large geographic areas (Tack et al. 2012). In our study, strongly contrasting garden environments do not have a larger effect than tree genotype on lichen communities, and there is no evidence for a diminished effect of genotype when considering genotypes propagated from a single riparian stand compared to a set of genotypes propagated from a 37-km stretch of riparian habitat. Our results indicate that tree genotype effects on lichens may persist in natural habitats that exhibit substantial environmental variation, and underscore the importance of including a greater range of organisms in community genetics studies before generalizing about the relative effects of genotype and environment on plant-associated taxa.

We found no evidence that garden environment modulates the effect of genotype on lichen communities. These findings contrast those of other studies at these gardens that show significant G×E effects on foliar pathogen communities (Busby et al. 2014) and aphid fecundity (Smith et al. 2011). Plant-associated communities can show G×E effects for several reasons, such as when the expression of plant traits that they are sensitive to changes between environments, when different environments contain different species pools, and when community members are locally adapted (Johnson and Agrawal 2005, Busby et al. 2014). The lack of evidence for a G×E effect on the lichen community may be due to a similar species pool at each garden, and because rough bark cover did not exhibit a G×E response. The nonsignificant G×E effect does not rule out the possibility of local adaptation in lichens, since we did not account for lichen genotype or measure lichen traits. We suggest that organisms sensitive to genetically variable traits that are not strongly phenotypically plastic are more likely to exhibit similar responses to the same tree genotype in different environments, in contrast to organisms such as herbivores, which are often sensitive to plant traits that can be plastic and inducible (e.g., secondary chemistry; Heil 2010).

It is noteworthy that the one other study to examine epiphyte community responses to tree genotype and environment found a significant G×E interaction on lichen composition (Davies et al. 2014). The study by Davies et al. (2014) examines epiphytes on genotypes collected from a much larger range than those in our G×E data set, planted in gardens with greater geographic separation. Over a very large geographic scale, many of the factors that produce G×E effects are more likely to express themselves (i.e., reduced gene flow to promote...
local adaptation, changes in species pools, differential plant trait expression). However, environmental heterogeneity within a small region, and even within a single site, can be high in riparian habitats (Naiman and Décamps 1997); our findings suggest that tree G × E effects are not important to lichens at local scales, even when significant environmental heterogeneity is present.

**Epiphytic lichen community assembly and genetic variation in host ontogeny**

We provide several lines of evidence that tree genotype influences the rate at which lichen communities assemble during the beginning of succession. Lichen communities on the chronosequence in a natural stand have greater cover, richness, and a different composition when on older, rougher-barked trees, indicating that changes in bark texture as a young tree ages facilitate community development. However, the timing of the development of rough bark is genetically variable. In the ONC common garden, paired comparisons between 20-year-old adult trees and 10-year-old ramets of the same genotype show that adult trees only have more developed lichen communities than their smooth-barked ramets when the adult has a rougher bark phenotype. Communities of plant-associated organisms frequently accumulate species as young host plants age (e.g., Nara et al. 2003, Johansson et al. 2007, Bengtsson et al. 2012). Our results suggest that the rate of community assembly as plants age can be modulated by plant genetic controls on the expression of phenotypic traits that influence the associated community.

Safe sites are locations with conditions favorable for propagule establishment (Harper et al. 1961, Jumpponen et al. 2012), and bark roughness can be viewed as a genetically based allogenic factor of the substrate, which determines the number of safe sites available for epiphytes. In classic primary succession study systems, such as glacier forefronts and volcanic fields, safe sites offer refuges for plant establishment within matrices of inhospitable young substrate (e.g., Del Moral and Wood 1993, Jumpponen et al. 2012). In our study, smooth-barked trees (due to age and genotype) have a limited number of rough spots, which are typically only colonized by very small *X. galericulata* thalli; species and cover accumulate as rough bark area increases, driving a shift in composition based on safe site availability. Although dispersal and autogenic factors are important drivers of lichen community assembly and succession (Ellis 2012), the genetic basis to the area of the substrate representing safe sites is an additional factor that should be accounted for when understanding epiphyte community dynamics on individual host trees.

Differences in rough bark cover among genotypes of the same age represent genetic variation in the timing of the ontogenetic shift from smooth to rough bark. Genetic variation in ontogeny is known to occur in traits that influence plant interactions with other organisms (e.g., secondary chemistry; Holeski et al. 2012), but the community context of these variations is poorly understood (but see Waltz and Whitham 1997). We show that variation in the timing of the ontogenetic shift in bark texture influences the rate of assembly of an entire community of lichens. Because a large number of diverse species interact with lichens (Andre 1985, Maser et al. 1985, Bates et al. 2011, 2012), the influence of host genotype on lichens likely cascades to an additional suite of species. All multicellular species, from the largest mammals to microscopic fungal hyphae, and from the tallest trees to nonvascular plants (e.g., Nemoto 1956, Williams and Sillett 2007, Hoffman and Arnold 2010, Spor et al. 2011, Wahl et al. 2012), host communities of diverse taxa. All organisms go through developmental changes, and these can affect associated communities (Boege 2005, Berke and Woodin 2008, Muñoz and Zamora 2011, Spor et al. 2011). It is therefore likely that genetic variation in the timing of ontogenetic shifts commonly influences the assembly and succession of diverse communities associated with a wide array of multicellular organisms.

**Conclusions**

By combining multidecadal experimental common gardens, a chronosequence of naturally established trees, structural equation modeling, and an overlooked but ecologically important group of taxa (epiphytic lichens; Ellis 2012), this study helps progress the field of community genetics in at least three important ways. First, the response of lichen communities to tree genotype is robust and consistent across common gardens, where it has a stronger effect on community structure than different common gardens in different environments, no matter whether genotypes originate from a 37-km stretch of riparian habitat or a single stand of trees. Thus, in this system, the community effects of tree genotype appear to be important across a heterogeneous landscape. Second, by using epiphytic lichens, which might be least-expected to be sensitive to host plant genotype, we broadened the scope of communities known to be influenced by plant genetics. Third, by using epiphytic lichens, we were able to connect community genetics to larger themes in biology, such as ontogeny and community assembly. Due to the slow dynamics of lichen community assembly and succession, the cumulative effects of tree genetic, ontogenetic, and age variation over many years are represented in a community snapshot, providing a window into the process of community assembly and succession. Because all epiphytes, and other organisms living on or in another individual, may be influenced by genetically variable host traits, a community genetics approach will help identify previously unappreciated dynamics across a broad spectrum of ecological systems.

**Acknowledgments**

We thank L. Evans, R. Mau, the Gehring Lab, the Cottonwood Ecology Group, and the Ogden Nature Center. Research was supported by National Science Foundation...
Frontiers in Integrative Biological Research grant DEB-0425908, MRI support for the Southwest Experimental Garden Array (DBI-1126840), and Integrative Graduate Education and Research Traineeships to L. J. Lamit, M. K. Lau, and T. Wojtowicz. Additional support was provided by Achievement Rewards for College Scientists scholarships.

LITERATURE CITED


Supplemental Material

Ecological Archives

Appendices A–C are available online: http://dx.doi.org/10.1890/14-1007.1.sm