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Differentiating genetic and environmental drivers of plant–pathogen community interactions

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Summary

1. Plant genotypic variation can shape associated arthropod and microbial communities locally, as has been demonstrated in controlled common garden experiments. However, the relative roles of plant genetics and the environment in defining communities at larger spatial scales are not well known. The environmental heterogeneity hypothesis maintains that plant genetic effects on associated communities diminish across the landscape as environmental variation predominates. Alternatively, the local adaptation hypothesis argues that plant genetic effects change across landscapes as a result of species interactions being locally adapted. Thus, very different mechanisms could produce similar patterns.

2. Using replicated common gardens located along an elevation and distance gradient, observational studies in the wild, and a greenhouse inoculation experiment, we examined these two non-mutually exclusive hypotheses for *Populus angustifolia* and its fungal leaf pathogen community.

3. Supporting the environmental heterogeneity hypothesis, plant genotypic effects on fungal leaf pathogen communities were two to three times stronger within than among gardens. Consistent with the local adaptation hypothesis, plant genotypic effects on pathogens also varied significantly among gardens (i.e. G × E interaction effect). Observational data from the wild and our greenhouse inoculation experiment unveiled clinal adaptation in plant genetic resistance that is correlated with disease risk along the elevation gradient, but did not support local pathogen adaptation to plants or vice versa.

4. *Synthesis*. While our study found that plant genotype plays a significant role in shaping associated pathogen communities at local and geographic scales, the environment most strongly influenced *P. angustifolia* leaf pathogens at the geographic scale. Plant genetic effects on pathogens were also influenced by the environment, highlighting the potential for environmental (e.g. climate) change to trigger local evolutionary responses in plant–pathogen community interactions.

Key-words: determinants of plant community diversity and structure, disease, foundation species, genotype × environment interactions, local adaptation, pathogen communities, *Populus*, scaling

Introduction

Plant genes have been shown to affect associated species and entire communities of organisms (Maddox & Root 1987; Fritz 1988; Antonovics 1992; Thompson 1997; Agrawal 2003; Whitham *et al.* 2003; Johnson, Lajeunesse & Agrawal 2006; Hughes *et al.* 2008; Adams *et al.* 2011; Zytynska *et al.* 2011; Bernhardsson *et al.* 2013). Genotypic variation within foundation species (sensu Dayton 1972) can be particularly influential for dependent species because they create locally stable conditions for other species (Whitham *et al.* 2006). For example, intraspecific variation within species of *Populus*, foundational trees typical of riparian forests in western North America, strongly influences associated arthropods (Keith, Bailey & Whitham 2010), soil microbes (Schweitzer *et al.* 2008), and leaf pathogens (Busby *et al.* 2013a).

While the importance of plant genotypic variation for associated communities is well-established in common gardens where environmental variation is minimized (i.e. local spatial scales), the relevance of plant genes for communities at larger spatial scales is not well understood. One hypothesis is that abiotic environmental factors (e.g. climate, soils) will become more influential for species and communities at

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geographic spatial scales than biotic factors such as genotypic variation (also referred to as the environmental heterogeneity, or scale-dependent hypothesis) (Menge & Olson 1990; Johnson & Agrawal 2005). In contrast, others maintain that genetic effects change at larger spatial scales due to local adaptation (e.g. Smith *et al.* 2011). In this case, the argument is that as environmental conditions change at geographic scales, so do the selection pressures operating locally on species. As a result, species may be locally adapted (also referred to as the local adaptation hypothesis) (Kawecki & Ebert 2004; Thompson 2005; O'Neill, Hamann & Wang 2008; Hereford 2009; Grady *et al.* 2011). Under both hypotheses, the plant genotypic signal can weaken at larger spatial scales, but for very different reasons: due to increasing environmental effects, or due to evolutionary changes in response to changing conditions. Although these hypotheses are not mutually exclusive, it is important to differentiate between them, as the outcomes affect our interpretation of the role of genetics-based interactions in structuring communities, and the ability of organisms and communities to ecologically or evolutionarily respond to climate change across the landscape.

A standard experimental approach to accept or reject these hypotheses is a replicated common garden study design permitting evaluation of the relative importance of plant genotype (G), environment (E), and their interaction (G × E) for associated communities at local (i.e. within common garden environments) and geographic spatial scales (i.e. among common garden environments). Because both environmental and genetic variation increase with spatial scale (Bell 1992), comparing their relative roles in affecting phenotypic community variation requires sampling genotypes and environments that are representative of the spatial scale of the study (Tack, Johnson & Roslin 2012b). With such sampling, a decline in genotypic effects at the larger, geographic spatial scale, combined with significant environmental effects, would support the environmental heterogeneity hypothesis. Alternatively, a significant G × E interaction effect compensating for decline in the genotypic effect at the geographic scale would be consistent with the local adaptation hypothesis. The few studies on this topic, using associated communities, have been limited to arthropods, and have reported partial support for each hypothesis (Johnson & Agrawal 2005; Tack *et al.* 2010; Smith *et al.* 2011; Bernhardsson *et al.* 2013; Evans *et al.* 2013). Evaluating these hypotheses for a broader range of organisms should allow us to draw general inferences about the spatial scaling of plant genetic and environmental effects on associated communities (Tack, Johnson & Roslin 2012b).

To distinguish between these hypotheses, for two consecutive years (2009–10), we sampled fungal leaf pathogen communities on the same *Populus angustifolia* genotypes that were planted in three common gardens located along an elevation (300 m) and distance (55 km) gradient on the Weber River in Utah, USA (Fig. 1). For one part of our analyses, we used only tree genotypes that originated from a single wild population located near our intermediate-elevation garden. Therefore a significant G × E interaction effect on pathogen communities among common gardens could suggest the possibility of local pathogen adaptation to tree populations and/or to the environment (cf. Clausen, Keck & Hiesey 1948).

Symptom severity for individual pathogens of *Populus* is known to be strongly influenced by plant genotypic variation in resistance (Newcombe & Bradshaw 1996), and a previous study demonstrated that genotypic variation in *P. angustifolia* was a major factor structuring the fungal leaf pathogen communities in a 1-year study conducted in a single common garden environment (Busby *et al.* 2013a). However, the extent to which genetic and environmental factors interact to shape pathogen communities at larger spatial and temporal scales is unknown.

In this study, we first evaluated plant genotypic effects on pathogen community structure (composition and species severities) locally, within the three contrasting common garden environments. We included study year in analytical models to account for temporal (i.e. inter-annual) variability for pathogen community structure (Burdon & Thrall 1999), and the individual tree to account for repeated sampling. Next, we tested the two hypotheses at the geographic scale by combining data from all common garden environments and evaluating the proportion of total phenotypic variation in pathogen

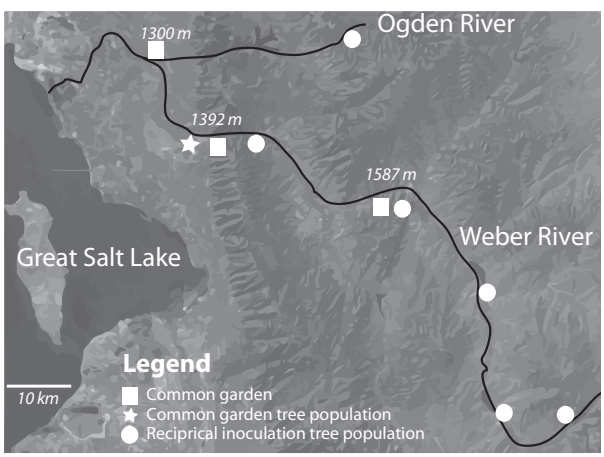

Fig. 1. Map of the study area showing the low, intermediate and high-elevation common gardens (squares), the *Populus angustifolia* population where common garden genotypes originated (star), and the *P. angustifolia* populations where seed and pathogen inoculum were collected for the reciprocal inoculation experiment (circles).

community structure explained by plant genotype (V_G), environment (V_E), and their interaction ($V_{G\times E}$). Again, we included study year and tree in models. Finally, to test the possibility that local pathogen adaptation contributes to $V_{G\times E}$ we conducted a reciprocal inoculation greenhouse experiment using seedlings from six *P. angustifolia* populations located along the same elevational gradient, and inoculum from a single common pathogen species collected in three of those populations. Experimental evidence that pathogen populations are locally adapted to tree populations would support the local adaptation hypothesis.

Materials and methods

STUDY SYSTEM

Our study was conducted in the Wasatch Mountains in north-central Utah, where narrowleaf cottonwood, *P. angustifolia,* occurs along upper reaches of the Weber River. This is a relatively arid region where opportunities for pathogens to infect hosts, and affect host fitness, may be limited. Three common gardens were established along the river at 1300, 1392 and 1587 m (hereafter low, intermediate and high elevation) (Fig. 1). The high-elevation garden was established in 1983, the intermediate in 1988, and the low between 1990 and 1992. By the time of our study (2009–10), trees in all gardens were sexually mature.

The gardens were planted with the same *P. angustifolia* genotypes using cuttings collected from trees growing in natural stands along the stretch of the Weber where gardens are located. Specifically, the five *P. angustifolia* genotypes replicated in all three gardens were collected from a wild population located near the intermediate-elevation garden (Fig. 1). Because the genotypes originated from a single wild population, they are a good spatial match for estimating V_G locally within each garden (Tack, Johnson & Roslin 2012b). However, our across-garden estimate of V_G should be conservative since the genetic sample size is limited. Additional genotypes replicated in only one or two gardens were utilized in within-garden analyses. These genotypes come from two additional tree populations located along the Weber River. In total, we sampled 10 *P. angustifolia* genotypes in the low-elevation garden, 10 genotypes in intermediate-elevation garden, and seven genotypes in high-elevation garden. For our within-garden analyses, we calculated V_G using two different data sets; first, including only the five *P. angustifolia* genotypes that were found in all three gardens, and secondly, including additional genotypes that were found in only one or two gardens. The second analysis could overestimate V_G because the genetic sample size is larger than the environmental sample size.

To characterize environmental differences among gardens, in 2010 we simultaneously measured temperature and relative humidity every 10 min for 2 weeks during the time of peak pathogen symptom severity (August and September) using five to seven HOBO® data loggers (Onset, Bourne Massachusetts, USA) in each garden. We calculated mean daily values over this time period for each parameter. The gardens, in part, conformed to expectations based on their elevation. The high-elevation garden was significantly colder at night (Fig. 2). The low- and high-elevation gardens had similar daytime temperatures and relative humidity (Fig. 2). The intermediate-elevation garden is located in an exposed, windy environment at the mouth of Weber Canyon and was the hottest and driest garden (Fig. 2).

PATHOGEN COMMUNITY

The fungal leaf pathogen community of *P. angustifolia* includes species causing visible symptoms of foliar disease. Busby, Aime & Newcombe (2012) used morphological and DNA sequence data to characterize this community. Common pathogens in the study area are all Ascomycota: *Drepanopeziza populi*, *Phyllactinia populi* and *Mycosphaerella* spp. (orders Helotiales, Erysiphales and Capnodiales, respectively). These taxa are identifiable without magnification. However, at the time of our sampling for the current study, we were unaware of several species of *Mycosphaerella* that are indistinguishable in the field (*Mycosphaerella angustifoliorum* and two undescribed species) (Busby, Aime & Newcombe 2012). Therefore, we were not able to distinguish between species of *Mycosphaerella* for the present study, but all three infect *P. angustifolia*. We also note that these *Mycosphaerella* spp. have recently been moved to the genus Sphaerulina (Quaedvlieg *et al.* 2013), but herein are referred to as *Mycosphaerella*.

Mycosphaerella and *D. populi* are necrotrophic/hemibiotrophic pathogens that kill host tissue and feed on the remains. They are known to cause reduced growth, premature defoliation, shoot and branch death, stem cankers and eventual death in *Populus* (Ostry & McNabb 1986; Ostry 1987). In contrast, *P. populi* is a biotrophic pathogen that feeds on live plant tissue. *Populus angustifolia* genotypes vary significantly in resistance to *Mycosphaerella* and *D. populi* individually, and to this entire community (Busby *et al.* 2013a).

COMMON GARDEN EXPERIMENT

Pathogen community surveys included scoring the severity of damage for all pathogens present on multiple leaves of each tree sampled. In late summer (September 2009, 2010), when foliar pathogens of

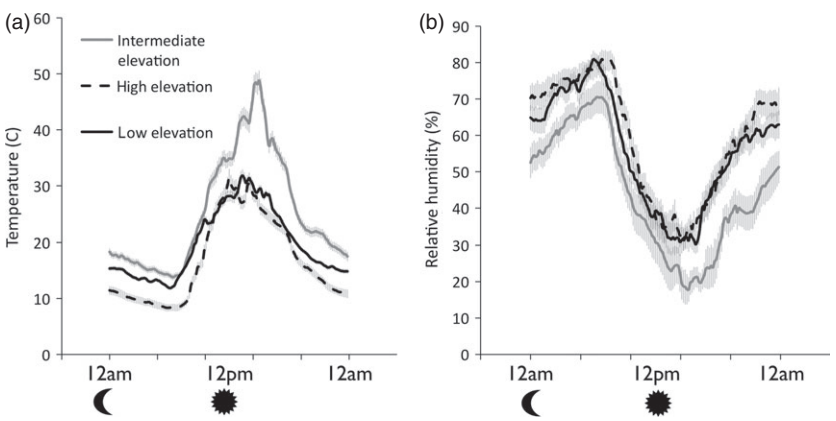

Fig. 2. Average temperature (°C) and relative humidity (%) within common gardens at low, intermediate and high elevations. Bands are standard errors of means for values collected daily for two consecutive weeks in August and September 2010 using HOBO® data loggers.

Populus are at their peak severities, we measured fungal pathogen damage on leaves collected from *P. angustifolia* genotypes in each of the three gardens. In each garden, for 3–10 replicate plants of each *P. angustifoila* genotype (utilizing all the clones available), we estimated tree-level severity for each pathogen by visually quantifying leaf area damaged for 18–24 leaves per plant, standardized by age (leaf plastochron index 3, 4, 5 and 6), collected from six haphazardly selected terminal shoots in the lower canopy. For all leaves, damage for each necrotrophic pathogen was scored on a scale from 0 to 5 reflecting the percentage of leaf area damaged: 0 = no damage, 1 = 1–6%, 2 = 7–12%, 3 = 13–25%, 4 = 26–50% or 5 = >50%. Damage scores were then used to calculate a single weighted damage score (see Method in Dirzo & Domínguez 1995). Due to the absence of necrotrophic tissue caused by the biotrophic pathogen *P. populi*, this pathogen was scored as present or absent at the shoot level in both years.

We also scored damage for unknown pathogens (i.e. those without diagnostic features) using the same categorical scale (Dirzo & Domínguez 1995). Unknown damage accounted for an average of 14% of leaf area damaged. In most cases, we speculate unknown pathogens were *Mycosphaerella* or *D. populi* with immature fruiting bodies. Alternatively, unknown pathogen damage could have been caused by *Fusicladium romellianum*, which occurred infrequently in the study area but did not produce diagnostic characteristics during our surveys.

Traditionally, analysis of ecological communities has utilized presence/absence or abundance data on individual species within communities. Consistent with this approach, we used pathogen symptom severities for individual species as proxies for their relative abundance in the community. Genetic resistance that reduces pathogen colonization (i.e. quantitative resistance) should be inversely correlated with pathogen symptom severity (Geiger & Heun 1989); major genes for resistance to *Mycosphaerella* or *D. populi* are not likely (Newcombe & Bradshaw 1996).

We evaluated the proportion of total phenotypic variation in pathogen community structure (composition and symptom severities) explained by *P. angustifolia* genotype (V_G) within each garden. Study year (Y) and its interactions were included in analytical models to account for temporal (i.e. inter-annual) variability in pathogen community structure. The individual tree, nested within genotype, was included in models to account for repeated sampling. Next, we combined data from all gardens to evaluate the relative importance of plant genotype (V_G), environment (V_E) and their interaction ($V_{G\times E}$) for pathogen community structure (again including study year and tree in models). If genotypic effects were diminished by environmental heterogeneity at the geographic scale, we would expect a decline in V_G, and V_E to outweigh the combined effects of V_G and $V_{G\times E}$. If plant genotypic effects differ among environments but were not diminished, we would expect $V_{G\times E}$ to compensate for declines in V_G.

Statistical analyses were conducted in R v2.14.0 using the vegan packages (R Development Core Team 2008). We used permutational multivariate analysis of variance (PERMANOVA; Anderson 2005) using distance matrices to estimate V_G, V_Y and $V_{G\times Y}$ for pathogen communities within gardens, and V_G, V_E, V_Y, and all interactions among gardens. Our community matrices consisted of columns of pathogen severities, one for each species, excluding unknown species. We fourth-root transformed pathogen symptom severity scores to downweight the effect of highly abundant observations (Anderson 2001). The transformations approximately matched median values for pathogens measured as per cent leaf area damaged (i.e. *D. populi* and *Mycosphaerella*) and proportion of shoots infected (i.e. *P. populi*) ensuring that each species contributed equally to the community analysis. The proportion of phenotypic variation in pathogen communities explained by each factor (e.g. V_G) was calculated as the residual sum of squares divided by the total sum of squares. To test the significance of each factor, we used *F*-tests based on sequential sums of squares from permutations of the raw data (Anderson 2005).

Lastly, we used restricted maximum likelihood (REML) to estimate the variance in individual pathogenic severities explained by the same set of factors both within and among gardens (Conner & Hartl 2004). These analyses were used to aid our interpretation of community results. For these analyses, pathogen symptom severity scores were transformed to meet normality assumptions of REML. We fourth-root transformed *Mycosphaerella* and *D. populi* data to eliminate high-scoring variables while preserving the weights (Clarke 1993); we arcsine-transformed proportional (0–1) *P. populi* data (Zar 1996). The trees were nested within the genotype as a random factor. Wald's test was used to test fixed effects; the likelihood ratio test was used to test the random effect.

RECIPROCAL INOCULATION EXPERIMENT AND OBSERVATIONS IN THE WILD

To explicitly test the local adaptation hypothesis, we next conducted a reciprocal inoculation greenhouse experiment manipulating both plants (seedlings from six *P. angustifolia* populations) and a pathogen (collected from three of those populations) originating along the same elevation gradient where the common gardens are located. We selected *D. populi*, for which $V_{G\times E}$ was significant in the common garden experiment, for the reciprocal inoculation greenhouse experiment. Inoculating with the entire community or the *Mycosphaerella* species complex was not feasible.

We examined two non-mutually exclusive possibilities: (i) plant genetic resistance is locally adapted to pathogen populations, and/or (ii) pathogen populations are locally adapted to plant populations. We compared pathogen performance (i.e. severity of damage on the host plant) with respect to both the elevation gradient and host origin (i.e. local versus foreign). Greater pathogen performance on the local host population than on the foreign host population would be evidence for local pathogen adaptation, while greater pathogen performance on the foreign host population would support local adaptation of tree populations to pathogens (Kaltz & Shykoff 1998). We also evaluated pathogen performance with respect to the elevation gradient to test for a resistance cline (i.e. plants are locally adapted or maladapted to the level of disease risk along the gradient) (Nuismer, Thompson & Gomulkiewicz 2000).

In July 2010, *P. angustifolia* seed was collected from a single female tree in five populations located along the Weber River, and from one population located in a low-elevation stand on the Ogden River, a tributary of the Weber. In these same populations, we assessed the level of disease risk in wild populations by sampling *D. populi* symptom severity on ten haphazardly selected *P. angustifolia* mature trees using the severity index of Dirzo & Domínguez (1995), and collected leaves infected with the pathogen from at least five *P. angustifolia* individuals in three of the six populations: a low (1581 m) and high-elevation (2058 m) population along the Weber River, and the low-elevation Ogden River population (1550 m). Our inclusion of a tree and pathogen population from an adjacent river valley should ensure that our results are not confounded by autocorrelation. Leaves were moist-incubated for 1 week to stimulate asexual spore release. Spores were suspended in deionized water and stored frozen.

Seedlings (half-sibs) were raised in a greenhouse at Stanford University, California. Because *P. angustifolia* flowers are open-pollinated, they should reflect population-level genetic resistance derived from males in the population. In total, we generated an average of 74 seedlings per population (range = 30–140) for each of the six populations. Three-month-old seedlings were inoculated with the pathogen by spraying spore suspensions on leaves, and maintaining moisture on the leaf surface for 12 h. The spore concentration of inoculum solutions was standardized to approximately 7×10^4 mL^{-1}. We inoculated an average of 29 seedlings per host population/pathogen combination (range = 5–56). We collected data on pathogen severity 2 weeks after inoculation. For each individual, we photographed leaves with plastochron index 3, 4 and 5, and used ImageJ (Rasband 1997–2014) to quantify the percentage of leaf area infected. Colour and brightness filters were used to transform photographs into binary, black and white images with pathogen damage in white and healthy leaf material in black. We calculated mean leaf area damaged (%) for each individual, and divided this number by the mean for each inoculum solution. This relative value allowed us to compare average population-level damage across inoculum solutions.

We used analysis of covariance to determine the proportion of variation in relative pathogen symptom severity explained by pathogen and plant populations, their interaction, and elevation. A significant interaction effect would support local adaptation of pathogen or plant populations to the other, and a portion of the G × E interaction from the common garden study could thus be attributed to variation by local adaptation (e.g. Kawecki & Ebert 2004). A significant elevation effect would support a resistance cline. We used analysis of covariance to determine the proportion of variation in observed pathogen damage (in the wild) explained by the elevation gradient. Finally, we used Pearson's product-moment correlation to directly compare population-level pathogen damage in the wild to population-level pathogen damage in the experiment, explicitly testing whether a potential resistance cline is driven by disease risk along the gradient.

Results

PLANT GENOTYPE STRUCTURES PATHOGEN COMMUNITIES LOCALLY

We found that plant genotype explained a significant proportion of the variation in pathogen community structure within each garden (V_G = 0.26–0.36; Table 1). The magnitudes of genotypic effects were approximately the same whether we used five *P. angustifolia* genotypes for our analysis or included the additional genotypes found in only one or two gardens (data not shown). Study year was also significant within each garden, though V_{GxY} was not significant in any garden (Table 1).

Plant genotypic effects on individual pathogens varied among pathogen species and gardens (Table 2). For example, plant genotypic effects on *P. populi* were never statistically significant ($P > 0.05$) because all genotypes were either nearly uniformly infected, or not at all, within individual gardens (Table 2, Fig. 3). Plant genotypic effects on *D. populi* were marginally significant in the low-elevation garden, and significant in the intermediate and high-elevation gardens (Table 2, Fig. 3). In contrast, plant genotypic effects on *Mycosphaerella* were significantly different within each of the three gardens (Table 2, Fig. 3).

Table 1. PERMANOVA results showing the proportion of variance (R^2) in pathogen community structure explained by genotype (V_G), environment (V_E), year (V_Y), and their interactions within each common garden and among common gardens

	d.f.	SS	MS	*F*	R^2	*P*
All gardens						
Genotype (G)	4	2.4	0.6	17	0.12	<0.001
Environment (E)	2	6.1	3.04	85	0.29	<0.001
Year (Y)	1	1.5	1.5	43	0.074	<0.001
G × E	8	2	0.25	7.02	0.096	<0.001
G × Y	4	0.054	0.013	0.38	0.0026	0.97
E × Y	2	0.36	0.18	5.03	0.017	<0.001
Tree	74	5.4	0.073	2.1	0.26	<0.001
G × E × Y	8	0.52	0.064	1.8	0.025	0.007
Residuals	69	2.4	0.035		0.12	
Total	172	21			1	
Low garden						
G	4	1.4	0.35	8.5	0.29	<0.001
Y	1	1.03	1.03	25	0.21	<0.001
G × Y	4	0.18	0.045	1.1	0.036	0.38
Tree	21	1.5	0.073	1.8	0.31	0.03
Residuals	19	0.79	0.042		0.16	
Total	49	4.9			1	
Intermediate garden						
G	4	1.4	0.35	8.5	0.26	<0.001
Y	1	0.67	0.67	16	0.13	<0.001
G × Y	4	0.28	0.07	1.7	0.052	0.11
Tree	23	2.1	0.092	2.2	0.39	0.004
Residuals	22	0.901	0.041		0.17	
Total	54	5.4			1	
High garden						
G	4	1.6	0.39	14	0.36	<0.001
Y	1	0.21	0.21	7.7	0.048	0.002
G × Y	4	0.12	0.029	1.1	0.027	0.403
Tree	30	1.7	0.057	2.1	0.39	0.003
Residuals	28	0.75	0.027		0.17	
Total	67	4.3			1	

SUPPORT FOR ENVIRONMENTAL HETEROGENEITY AND LOCAL ADAPTATION HYPOTHESES

In agreement with the environmental heterogeneity hypothesis, plant genotypic influences on the pathogen community within individual gardens were highest (e.g. V_G ranged from 0.26 to 0.36 within gardens; Table 1), and genetic effects attenuated, but were still significant at the geographic scale (V_G = 0.12 among gardens; Table 1). Among gardens, E explained approximately three times the variation (V_E = 0.3) in pathogen community structure as G (Table 1). However, consistent with the local adaptation hypothesis, a significant $V_{G\times E}$ interaction effect ($V_{G\times E}$ = 0.1) partially compensated for declines in V_G (Table 1). Study year and tree also explained a considerable proportion of the variation in pathogen community structure (Table 1).

At the geographic scale, genotypic effects were significant for *Mycosphaerella* and *D. populi*, but not for *P. populi* (Table 2). $V_{G\times E}$ was significant for *Mycosphaerella* and *D. populi* (Table 2); V_E was highly significant for all pathogens (Table 2). Study year was significant for *D. populi* and *Mycosphaerella*, but not for *P. populi* (Table 2).

Table 2. Restricted maximum likelihood model results for individual pathogen symptom severities within and among common gardens. Wald's test was used to test fixed effects; the likelihood ratio test was used to test the random effect, tree

	All gardens			Low garden			Intermediate garden			High garden		
	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*	χ^2	d.f.	*P*
Mycosphaerella												
Genotype (G)	40	4	<0.001	28	4	<0.001	20	4	<0.001	38	4	<0.001
Environment (E)	47	2	<0.001									
Year (Y)	39	1	<0.001	41	1	<0.001	10	1	0.0013	4.9	1	0.027
G × E	50	8	<0.001									
G × Y	4.7	4	0.32	8.9	4	0.063	4.3	4	0.37	6.5	4	0.16
E × Y	11	2	0.0034									
G × E × Y	15	8	0.056									
Tree	12	1	0.022	0.73	1	0.39	1.1	1	0.29	2.8	1	0.095
Drepanopeziza populi												
Genotype (G)	10	4	0.037	9.1	4	0.059	10	4	0.038	10	4	0.041
Environment (E)	29	2	<0.001									
Year (Y)	11	1	<0.001	4	1	0.046	54	1	<0.001	24	1	<0.001
G × E	22	8	0.0052									
G × Y	7.4	4	0.12	7.5	4	0.11	22	4	<0.001	12	4	0.016
E × Y	4.9	2	0.088									
G × E × Y	20.1	8	0.0101									
Tree	38	1	<0.001	10.9	1	<0.001	20	1	<0.001	0	1	1
Phyllactinia populi												
Genotype (G)	1.4	4	0.83	9.05	4	0.06	6.03	4	0.19	24	4	0.66
Environment (E)	225	2	<0.001									
Year (Y)	2.5	1	0.11	1.7	1	0.2	1.7	1	0.19	0.5	1	0.48
G × E	7.01	8	0.53									
G × Y	2.3	4	0.68	1.2	4	0.87	6.1	4	0.19	1.9	4	0.75
E × Y	0.049	2	0.97									
G × E × Y	4.2	8	0.84									
Tree	13	1	<0.001	0.0082	1	0.93	0	1	1	7.5	1	0.0063

We found no compelling evidence for local pathogen adaptation to tree populations or local adaptation of tree populations to pathogen populations in our greenhouse inoculation experiment (Table 3; Fig. 4). A statistically significant interaction term indicates that the rank order of pathogen damage varied among plant populations (Table 3), but there was no consistent pattern to this variation suggestive of local pathogen adaptation to plants or vice versa.

Observational data from the wild, in combination with the results of the inoculation experiment, yielded evidence that the level of disease risk along the elevation gradient drives clinal adaptation in plant genetic resistance to *D. populi*. In natural stands, pathogen severity, or disease risk, decreased with elevation ($F = 18$, $R^2 = 0.24$, $P < 0.001$; Fig. 4B; Table 3). In the reciprocal inoculation experiment, damage caused by the pathogen, regardless of the origin of the pathogen, increased with the elevation at which plant populations originated ($F = 80$, $R^2 = 0.14$, $P < 0.001$; Fig. 4A; Table 3). These responses to elevation were correlated: plants from low-elevation environments characterized by high pathogen damage were more resistant across all inoculum solutions, whereas plants from high-elevation environments characterized by low pathogen damage were more susceptible across all inoculum solutions ($t = -2.9$, $P = 0.058$, $R^2 = -0.86$). While these results both help to explain significant environmental and plant genetic effects on pathogens observed in the common garden experiment, they do not explain the G × E interaction effect.

Discussion

Many studies in diverse systems have shown that genotypic variation in plant species can strongly influence dependent communities (reviewed by Whitham *et al.* 2012), and we are beginning to better understand how such effects vary across heterogeneous natural landscapes (Thompson 1997; Burdon & Thrall 1999; Craig, Itami & Horner 2007; Soubeyrand *et al.* 2009; Smith *et al.* 2011; Evans *et al.* 2013). For example, separate origins of *Heuchera* polyploidy across the mountains of Idaho differentially shape herbivore and pollinator communities (Thompson 1997). Also, genotypic variation in *P. angustifolia* resistance to aphids influences predation by birds and their top-down effects differently in different environments (Smith *et al.* 2011). These studies demonstrate that genetic effects are not necessarily swamped by environmental heterogeneity at geographic scales (Tack *et al.* 2010). Rather, they can change across biotic and abiotic environmental gradients as a result of local adaptation (Thompson 2005). Our study contributes to this area of research in a novel way by differentiating genetic and environmental drivers of plant–pathogen community interactions at a geographic spatial scale.

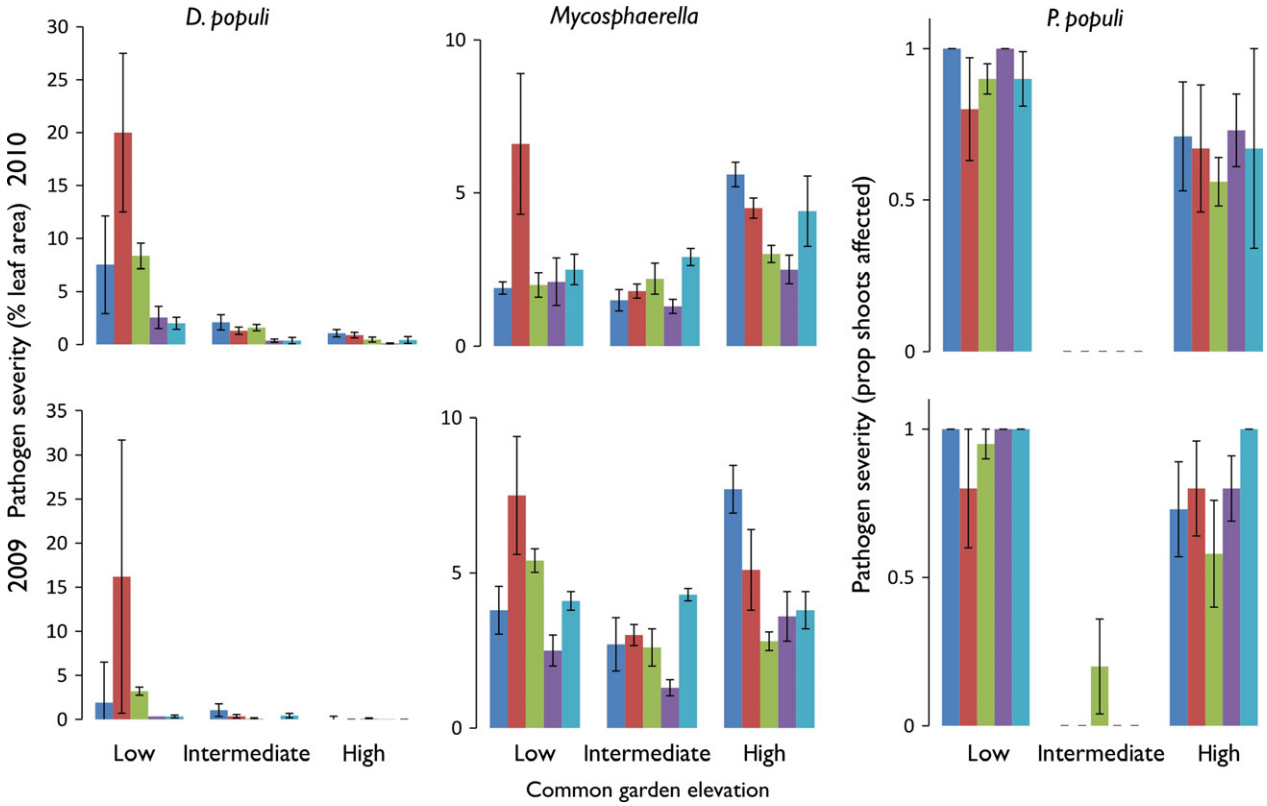

Fig. 3. Plots showing mean pathogen severity for *Drepanopeziza populi*, *Mycosphaerella* and *Phyllactinia populi* in three common garden environments. Different colours represent different host genotypes. Error lines are standard errors of means.

Table 3. ANOVA results showing: (i) proportion of variance (R^2) in *Drepanopeziza populi* severity in natural stands explained by the elevation/distance gradient and tree population, and (ii) variance in *D. populi* severity in the reciprocal inoculation experiment explained by the elevation/distance gradient, tree and pathogen populations, and their interaction

	F	R^2	*P*
Natural stand observations			
Elevation/distance gradient	18	0.24	<0.001
Tree population	4.4	0.18	0.008
Reciprocal inoculation experiment			
Elevation/distance gradient	80	0.14	<0.001
Tree population	8.4	0.075	<0.001
Pathogen population	0.82	NA	0.44
Tree × pathogen interaction	2.3	0.035	0.02

PLANT GENOTYPIC EFFECTS ON PATHOGEN COMMUNITIES AT THE LOCAL SCALE

Pathogen communities have largely been ignored in the community genetics literature (but see Barbour *et al.* 2009b), despite their ecological importance (Ellison *et al.* 2005; Lovett *et al.* 2006). Here, we demonstrate a significant role for plant genotypic variation in structuring fungal leaf pathogen communities within three contrasting environments, even including one location characterized by hot, dry conditions not particularly suitable for pathogen infection and the associated expression of genetic resistance. Our results thus complement and support mounting evidence that foundation plant genotypic variation can play a fundamental role in structuring local communities (Whitham *et al.* 2012). Furthermore, in contrast to previous common garden studies that have potentially overestimated plant genetic effects at local spatial scales by sampling plant genotypes from large spatial scales (Tack, Johnson & Roslin 2012b), our analysis utilized genotypes collected from a single population to ensure that our estimates of local genetic effects are not biased. Contrary to the expectation that including genotypes sampled from larger geographic areas would inflate V_G, our analyses including additional genotypes from two other populations within the study area did not greatly enhance the genetic effect.

While we consistently found significant plant genotypic effects on pathogen communities within individual common garden environments, genotypic effects on individual pathogens varied. For example, in both the intermediate and high-elevation gardens statistically significant genotypic effects were found for *Mycosphaerella* and *D. populi*, but not *P. populi*. In the low-elevation garden, genotype strongly influenced *Mycosphaerella* and was marginally significant for both *D. populi* and *P. populi*. These patterns reflect a geographic mosaic of interactions between plants and pathogens (Thompson 2005). Because these differential plant–pathogen interactions can affect host plant performance, other associated species such as the arthropod community may also be affected (Tack, Gripenberg & Roslin 2012a; Busby *et al.*

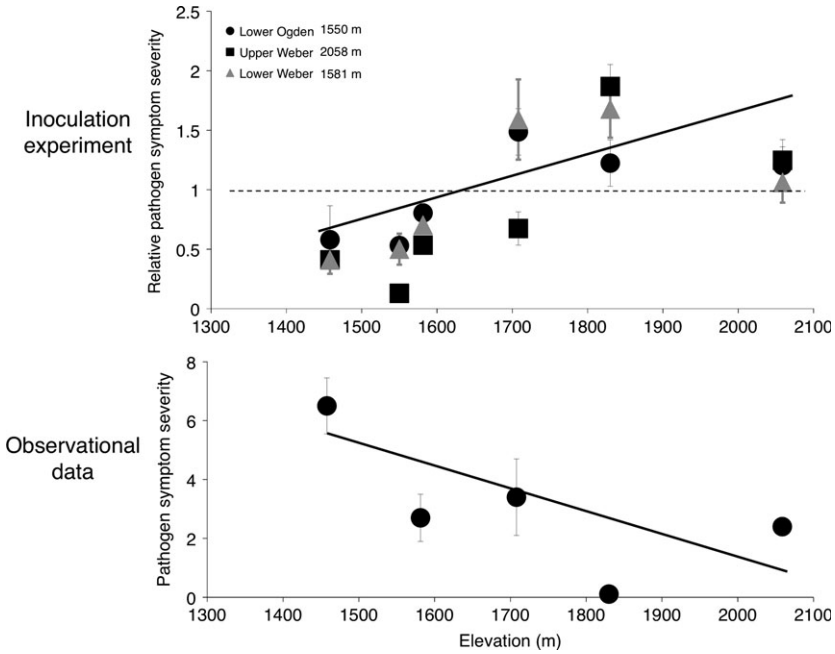

Fig. 4. Top panel shows *Drepanopeziza populi* severity on reciprocally inoculated *Populus angustifolia* populations. Elevation of host populations located along the Weber River is depicted along the *x*-axis. Data points show mean pathogen damage (with standard error) for tree populations inoculated with each of the three inoculum solutions (i.e. low-elevation Weber River 1581 m, high-elevation Weber River 2058 m, and low-elevation Ogden River 1550 m). Values >1 (indicated by a broken line) indicate above-average population-level damage for the inoculum solution; values <1 indicate below-average damage. Bottom panel shows observational data on disease risk in the same natural stands, collected in September 2010.

in press). Such cascading effects will likely differ across environments as disease risk varies.

PLANT GENOTYPIC EFFECTS ON PATHOGEN COMMUNITIES AT THE GEOGRAPHIC SCALE

In the common garden study, we found that plant genotypic effects on pathogen communities declined as spatial scale and environmental heterogeneity increased, supporting the environmental heterogeneity hypothesis (also known as the scale-dependent hypothesis). This result echoes previous studies showing diminished genotypic effects at geographic scales for arthropods (Johnson & Agrawal 2005; Bangert *et al.* 2006; Tack *et al.* 2010), unidentified communities of mycorrhizal fungi (Gehring, Mueller & Whitham 2006), and macrofungal decomposers (Barbour, Storer & Potts 2009a). However, in our study, plant genotypic influences on pathogen communities did not simply diminish at larger spatial sales; they also varied among gardens, which is consistent with the local adaptation hypothesis. Our results thus demonstrate how significant G × E interactions, if not accounted for, can result in underestimating the importance of plant genetic effects for communities at larger spatial scales.

Significant G × E interaction effects on individual pathogens and the pathogen community raised the possibility that pathogen populations are locally adapted to tree populations. Because pathogens typically have shorter generation times and larger population sizes than their hosts, they are expected to be locally adapted to their hosts (Parker 1985; Hoeksema & Forde 2008). However, many studies, including the present study, have not found empirical support for local pathogen adaptation (Kaltz & Shykoff 1998). Possible explanations hinge on spatiotemporal variation in local environments where co-evolutionary dynamics occur. This variation could be explained by pathogen extinction, recolonization, gene flow, variable selection pressure and/or stochasticity, and the scale of genetic variation (Laine 2005; Soubeyrand *et al.* 2009; Tack *et al.* 2012c; Evans *et al.* 2013). Future studies evaluating local pathogen adaption at multiple spatial scales, across a variety of plant–pathogen systems, will be poised to test which factors are most relevant for pathogen local adaptation. In the cottonwood study system, a fully reciprocal common garden design in which tree genotypes are sampled from wild populations adjacent to each garden would allow us to explicitly test for local plant and local pathogen adaptation in the field (Sork, Stowe & Hochwender 1993).

While our inoculation experiment did not support local adaptation by pathogens, it did reveal a resistance cline in the trees driven by disease risk. Trees originating from low-elevation populations characterized by high disease risk were more resistant, while trees originating from high-elevation populations characterized by low disease risk were more susceptible. Resistance clines in this and other plant systems (e.g. Hamilton *et al.* 2013) demonstrate how geographic variation in disease risk can drive adaptive differentiation. Such patterns highlight the potential for local evolutionary responses to other selection pressures. For example, global climate change is predicted to affect pathogen severity and probability of occurrence (Harvell *et al.* 2002; Fisher *et al.* 2012). Our results suggest that in areas where climate change alters disease risk, plants will become maladapted, potentially resulting in both ecological and evolutionary change. Such environmental change should also directly affect plants (e.g. physiologically), making it difficult to accurately predict outcomes for species interactions.

In our common garden experiment, environmental variation among gardens most strongly determined pathogen community structure at the geographic scale. This result is consistent with a long history of research documenting the importance of the abiotic environment for pathogen population dynamics. In our

study, climatic differences among gardens very likely influenced pathogen communities. For example, mildews are dependent on high relative humidity (Burdon & Chilvers 1982) and *P. populi* was almost entirely absent from the dry, and windy intermediate-elevation garden in both years. Likewise, a negative relationship between *D. populi* and garden elevation suggests cold temperatures may limit *D. populi*. In addition to the abiotic environment, biotic environmental effects could have influenced pathogen communities. These include the presence or absence of plant endophytes that affect pathogen resistance; such endophytes are known in *P. angustifolia* (Busby *et al.* 2013b) and warrant further examination.

Conclusions

Many common garden studies have documented the importance of plant genotypic variation for structuring associated communities, but we still lack a clear understanding of plant genotypic effects at a geographic scale. Synthesizing our results from the wild, a replicated common garden field experiment, and a reciprocal inoculation greenhouse experiment, we found that plant genotypic variation can play a significant role in structuring fungal leaf pathogen communities at spatial scales both small and large. At the geographic scale, the environment also influenced plant genetic effects on pathogens. These results are consistent with both the environmental heterogeneity and local adaptation hypotheses, and highlight the potential for environmental change to influence plant–pathogen community interactions.

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