Functional Ecology

British Ecological Society

Functional Ecology 2015 doi: 10.1111/1365-2435.12409

Multiple plant traits shape the genetic basis of herbivore community assembly

Matthew A. Barbour*[,1], Mariano A. Rodriguez-Cabal[1,2], Elizabeth T. Wu[3], Riitta Julkunen-Tiitto[4], Carol E. Ritland[5], Allyson E. Miscampbell[5], Erik S. Jules[3] and Gregory M. Crutsinger[1]

[1]*Department of Zoology, University of British Columbia, 2212 Main Mall, Vancouver, British Columbia V6T 1Z4, Canada;* [2]*INIBIOMA, CONICET, Universidad Nacional del Comahue, Quintral 1250, Bariloche, Rio Negro, Argentina;* [3]*Department of Biological Sciences, Humboldt State University, 1 Harpst St., Arcata, CA 95521, USA;* [4]*Department of Biology, University of Eastern Finland, P.O. Box 111, FIN-80101 Joensuu, Finland; and* [5]*Department of Forest and Conservation Sciences, University of British Columbia, 2424 Main Mall, Vancouver, British Columbia, V6T 1Z4 Canada*

Summary

1. Community genetics research has posited a genetic basis to the assembly of ecological communities. For arthropod herbivores in particular, there is strong support that genetic variation in host plants is a key factor shaping their diversity and composition. However, the specific plant phenotypes underlying herbivore responses remain poorly explored for most systems.

2. We address this knowledge gap by examining the influence of both genetic and phenotypic variation in a dominant host-plant species, *Salix hookeriana*, on its associated arthropod herbivore community in a common garden experiment. Specifically, we surveyed herbivore responses among five different arthropod feeding guilds to 26 distinct *S. hookeriana* genotypes. Moreover, we quantified the heritability of a suite of plant traits that determine leaf quality (e.g. phenolic compounds, trichomes, specific leaf area, C : N) and whole-plant architecture, to identify which traits best accounted for herbivore community responses to *S. hookeriana* genotype.

3. We found that total herbivore abundance and community composition differed considerably among *S. hookeriana* genotypes, with strong and independent responses of several species and feeding guilds driving these patterns. We also found that leaf phenolic chemistry displayed extensive heritable variation, whereas leaf physiology and plant architecture tended to be less heritable. Of these traits, herbivore responses were primarily associated with leaf phenolics and plant architecture; however, different herbivore species and feeding guilds were associated with different sets of traits. Despite our thorough trait survey, plant genotype remained a significant predictor of herbivore responses in most trait association analyses, suggesting that unmeasured host-plant characteristics and/or interspecific interactions were also contributing factors.

4. Taken together, our results support that the genetic basis of herbivore community assembly occurs through a suite of plant traits for different herbivore species and feeding guilds. Still, identifying these phenotypic mechanisms requires measuring a broad range of plant traits and likely further consideration of how these traits affect interspecific interactions.

Key-words: architecture, arthropods, community genetics, herbivory, leaf quality, *Salix hookeriana*, secondary metabolites

Introduction

For over two decades, researchers studying plant–herbivore interactions have been interested in how host-plant genetic variation affects associated arthropod communities. Early work by Fritz & Price (1988) with willow (*Salix lasiolepis*) and Maddox & Root (1987, 1990) with goldenrod (*Solidago altissima*) demonstrated that different plant genotypes can host unique combinations of herbivore species. Since then, greenhouse experiments, common garden studies and field observations from a variety of host-plant systems have provided further evidence that plant genetic

*Correspondence author. E-mail: barbour@zoology.ubc.ca

variation is an important driver of herbivore community assembly (reviewed in Whitham *et al.* 2012). Nevertheless, the specific traits mediating herbivore responses to different host-plant genotypes remain unclear, as most studies neglect to screen plant phenotypes in sufficient detail (Hughes *et al.* 2008; Hersch-Green, Turley & Johnson 2011). Consequently, we are lacking a mechanistic understanding of the role host-plant genetic variation plays in the assembly of herbivore communities for most systems.

Identifying the specific host-plant characteristics that shape herbivore community composition can be a complex task. A single herbivore species is often correlated with multiple plant traits (Agrawal 2005; Agrawal & Fishbein 2006), and different herbivore species within a community may exhibit divergent responses to the same traits (Agrawal 2004, 2005; Agrawal & Fishbein 2006). For example, studies of common milkweed (*Asclepias syriaca*) have shown that latex and trichomes negatively affect chewing herbivores, whereas these same traits are either ineffective (latex) or positively (trichomes) associated with sap-sucking insects (Agrawal 2004, 2005; Agrawal & Fishbein 2006). Furthermore, traits other than those related to leaf quality (e.g. secondary metabolites, trichomes, leaf C : N) are often overlooked in plant–herbivore studies, but warrant further consideration. For example, aspects of plant architecture (e.g. biomass, height, branching complexity) can vary within host-plant species and also have strong effects on insect herbivores, particularly in woody plants (Carmona, Lajeunesse & Johnson 2011; Crutsinger *et al.* 2014). Therefore, we need studies that screen plant traits in detail at both the leaf and whole-plant level to understand the mechanisms underlying herbivore community responses (Hughes *et al.* 2008; Hersch-Green, Turley & Johnson 2011).

Identifying the plant phenotypes mediating herbivore responses is also a critical step towards a mechanistic understanding of plant–herbivore eco-evolutionary dynamics (Hersch-Green, Turley & Johnson 2011). For example, we know that temporal changes in the genetic composition of host-plant populations can directly affect the abundance of associated consumer species (Agrawal *et al.* 2013). Yet, predicting these consequences will require us to move beyond simply identifying plant genotype–herbivore associations to research that characterizes: (i) herbivore responses to host-plant traits; and (ii) the magnitude of variation and heritability of these plant phenotypes (Geber & Griffen 2003). From there, we can build a mechanistic understanding of the genetic basis to herbivore community assembly as well as make predictions about the cascading effects of host-plant evolution on the species that feed upon them. To date, such a comprehensive examination is lacking for the majority of host-plant study systems (but see Agrawal 2005; Johnson *et al.* 2009).

In this study, we used a large common garden experiment to examine arthropod herbivore community responses to genetic and phenotypic variation in the dominant host-plant species, *Salix hookeriana*. Specifically, we sought to address three questions: (i) How do herbivore communities respond to host-plant genotype? (ii) How heritable are different host-plant traits? (iii) Which plant traits account for herbivore community responses to host-plant genotype?

Materials and methods

STUDY SYSTEM

Salix hookeriana (coastal willow) is a deciduous shrub (<8 m) that occurs along the Pacific coast ranging from northern California to Alaska. This willow species grows primarily in meadows, floodplains and coastal dunes and is generally restricted to <100 m elevation (Argus 2013). As with other willows, *S. hookeriana* is dioecious and reproduces both sexually (wind and insect pollination; e.g. Sacchi & Price 1988) and asexually through vegetative growth (Argus 2013).

The genus *Salix* has been a model system for examining the role of host-plant genetics in shaping plant–herbivore interactions (e.g. Fritz & Price 1988; Hochwender & Fritz 2004) for a number of reasons. First, willows support a diverse community of arthropods that include many different feeding guilds, such as gallers, leaf miners, leaf chewers, xylem and phloem feeders (Roche & Fritz 1997; Sipura 1999). Secondly, there can be considerable genetic variation within willow populations (Brunsfeld, Soltis & Soltis 1991), with different genotypes displaying extensive phenotypic differences in morphology (Fritz & Price 1988) and phenolic chemistry (Nichols-Orians, Fritz & Clausen 1993). Finally, preference and performance of individual herbivore species has already been linked to some willow traits in other species (Matsuki & MacLean 1994; Björkman, Dalin & Ahrné 2008; Boeckler, Gershenzon & Unsicker 2011), which provides an informative background for *S. hookeriana*.

COMMON GARDEN

In February 2009, we established a common garden experiment consisting of clones from 27 different individuals of *S. hookeriana* ('willow' hereafter) at Humboldt Bay National Wildlife Refuge (HBNWR) (40°40′53″N, 124°12′4″W) near Loleta, California, USA. We haphazardly chose willow individuals (13 males, 14 females) from a single population growing locally around Humboldt Bay in both riparian areas (23 of 27) and dune swales (four of 27) and subsequently genotyped them using microsatellite markers (see Molecular Methods below). We propagated clonal replicates of each individual using 25 cm cuttings that had been soaked in water for 2 weeks and planted directly into the ground in 2 ha of a former cattle pasture at HBNWR. We planted cuttings in a completely randomized design with 25 replicates per willow individual (27 individuals × 25 replicates = 675 willows total), and cuttings spaced 3 m apart in a 45 m × 135 m grid. Each cutting was surrounded by a 1 × 1 m square of heavy-duty weed cloth to prevent vegetation growth in the immediate area. A 2·5-m tall fence was built around the experiment to exclude deer and cattle. Willows in our garden began flowering in February and reached their peak growth in late July to early August. During this study, willows had reached 2–3 m in height.

MOLECULAR METHODS

To confirm that willow individuals were genetically unique, we genotyped each individual using two microsatellite loci, SB80 and SB194 (Barker *et al.* 2003). Polymerase chain reaction (PCR) amplifications were performed in 10 μL reaction volumes

containing 5 ng DNA, 1 pmol each of forward and reverse primers, 0·5 pmol M13 IRD-labelled primer, 200 μM dNTP (New England Biolabs, Ipswich, MA, USA), 1× Paq5000 PCR buffer (Agilent Technologies Canada Inc., Toronto, ON, Canada), 1 U Paq5000 DNA polymerase (Agilent Technologies Canada Inc.) and 2·0 mM $MgSO_4$. Cycling conditions were 94 °C/2 min, 35 cycles of 94 °C/40 s, 54 °C/1 min, 72 °C/1 min and 72 °C/10 min. The PCR products were analysed on a LiCor 4200 automatic sequencer using 5·5% polyacrylamide gels (KBplus; LiCor Biotechnology, Lincoln, NE, USA) and scored using RFLPscan (LiCor Biotechnology) due to their tetraploidy. Of the 27 individuals collected, 26 were found to be genetically unique and were used in this study (13 males, 13 females; Table S1, Supporting information).

HOW DO HERBIVORE COMMUNITIES RESPOND TO HOST-PLANT GENOTYPE?

Sampling

In July 2011, we used two techniques to sample the herbivore community on about five randomly chosen individuals of each of the 26 genotypes (n = 132, range = 4–7 for each genotype). For mobile herbivores, we vacuumed the entire crown of each willow using a modified leaf blower/vacuum (Craftsman 25 cc 2-cycle; Sears Holding Corporation, Hoffman Estates, IL, USA) with a fine insect net attached. We brought samples to the laboratory immediately where we counted each individual and identified them to species or morphospecies under a dissecting scope. For sedentary herbivores, we visually surveyed the entire shrub for different species of galls (leaf and stem) and leaf mines. All herbivores were further assigned to one of the following feeding guilds: gallers, leaf miners, leaf chewers, xylem feeders and phloem feeders. To score damage from leaf chewers, we haphazardly selected five shoots per plant. Starting with the first fully expanded leaf on each shoot, we visually assigned damage scores to every other leaf for six leaves. We scored each leaf to one of 11 damage categories based on percentage leaf area removed (PLAR) (0, 1–5, 5–10, 10–20, 20–30…90–100%). The same observer (MAB) scored all damage to maintain consistency across samples. We averaged damage scores for each shoot and then for all six shoots to obtain a single estimate of PLAR per replicate willow.

Analyses

To examine how the herbivore community responded to willow genotype, we used separate one-way ANOVAs (with 'STATS' package in R; R Core Team 2013) to test for differences in the following responses: total richness, abundance, rarefied richness (using individual-based rarefaction; Gotelli & Colwell 2001), evenness (^1E = exp(Shannon entropy)/richness; Tuomisto 2012) and PLAR. Total richness, abundance and PLAR were log-transformed, and evenness was logit-transformed prior to analysis to improve normality and reduce heteroscedasticity. We also used separate generalized linear models (GLMs) to test for differences in abundance of several herbivore species and feeding guilds among genotypes (with 'MASS' package in R). GLMs were appropriate because they account for response variables with non-normal distributions and heteroscedasticity that were not improved by transformations (O'Hara & Kotze 2010). To test for differences in community composition among willow genotypes, we normalized our community data (site-by-species matrix) using the chord transformation (sum of squared species relative abundances equal to one for each sample; Legendre & Gallagher 2001) and conducted a redundancy analysis (RDA, 1000 permutations; with 'VEGAN' package in R). RDA is analogous to an ANOVA on pairwise community dissimilarity values. Lastly, we calculated Pearson's r (with 'PSYCH' package in R) to determine whether individual species and feeding guilds exhibited correlated responses among willow shrubs (phenotypic correlations, n = 131) and genotypes (genetic correlations, n = 26). Phenotypic correlations were estimated using the abundance (or damage for PLAR) of each species or feeding guild observed on each shrub, whereas genetic correlations were estimated from the mean abundance (or damage for PLAR) of each species or feeding guild found on each genotype.

HOW HERITABLE ARE DIFFERENT HOST-PLANT TRAITS?

We measured 40 different plant traits that have been linked to herbivore preference and performance on willows and other host-plant species (Lawton 1983; Matsuki & MacLean 1994; Cornelissen *et al.* 2003; Björkman, Dalin & Ahrné 2008; Barbehenn & Constabel 2011; Boeckler, Gershenzon & Unsicker 2011). These traits were grouped into two larger categories encompassing leaf quality (36 traits) and plant architecture (four traits).

Leaf quality

Phenolics are among the most abundant secondary metabolites in leaves of species within the family Salicaceae (Palo 1984) and have been shown to influence the preference and performance of several species of leaf-chewing beetles (Family: Chrysomelidae) and sawflies (Family: Tenthredinidae) that specialize on willows (e.g. Tahvanainen, Julkunen-Tiitto & Kettunen 1985; Roininen & Tahvanainen 1989). We measured seven different types of phenolic compounds: condensed tannins (two types), salicylates (eight types), phenolic acids (eight types), flavones (seven types), flavonols (three types), flavanones (eriodictyol 7-glycoside) and flavanonols (two types). To measure phenolics, we collected two fully expanded and undamaged leaves from about five shrubs of each genotype (n = 140, range = 4–7) in early August of 2012. Leaves were stored in paper coin envelopes and allowed to air-dry at room temperature until they could be analysed (Julkunen-Tiitto & Sorsa 2001). Leaf samples were then ground dried and extracted with 100% methanol prior to high-performance liquid chromatography (HPLC) (Agilent, Series 1100; Agilent Technologies, Waldbronn, Germany) analysis of salicylates, phenolic acids and flavonoids (Nybakken & Julkunen-Tiitto 2013). We identified phenolic metabolites by comparing their retention times and UV spectrum to standards (Table S2). After HPLC runs, we quantified condensed tannin content using redissolved methanol extracts (soluble condensed tannins) and dried extraction residue (insoluble condensed tannins) (Nybakken & Julkunen-Tiitto 2013). Methodological details for leaf phenolic processing and extraction are given in Nybakken & Julkunen-Tiitto (2013).

In addition to our extensive characterization of the phenolic profiles of different genotypes, we measured other putatively important traits that could shape leaf quality for herbivores, including specific leaf area (SLA), water content, trichome density, percentage carbon (C) and nitrogen (N), and C : N. For SLA, water content and trichome density, we excised a single fully expanded and undamaged leaf from an average of five replicates of each genotype in July 2012 (n = 137, range = 4–7). We placed leaf samples into separate plastic bags within a cooler and immediately brought them back to the laboratory. We then weighed leaves to obtain fresh mass (g), digitally scanned them to measure leaf area (mm^2) using IMAGEJ (Abràmoff, Magalhães & Ram 2004) and oven-dried them at 60 °C for 72 h to obtain dry weight (g) (Cornelissen *et al.* 2003). We calculated SLA as leaf area/dry weight (Cornelissen *et al.* 2003). Leaf water content was calculated as the (fresh weight − dry weight)/dry weight (Munns &

PrometheusWiki Contributors 2010). To measure trichome density, we counted the number of trichomes along an 11 mm × 1 mm transect in the centre of the leaf, halfway between the leaf edge and the mid-vein, under a dissecting scope. To measure percentage C and N, we collected 10 fully expanded and undamaged leaves from the outer crown of an average of five replicates of each willow genotype in July 2010 (n = 130, range = 4–6). Leaves were air-dried and grounded to a fine powder using a ball mill (Mixer/Mill 8000D, SPEX SamplePrep; Metuchen, NJ, USA). Subsamples of each material were then analysed for percentage C and N on an elemental analyser (NC 2500; Carlo-Erba, Milan, Italy) using acetanilide (10·36% N and 71·09% C) as a reference standard. Shrubs sampled for percentage C and N did not correspond with the same replicates sampled for other plant traits; therefore, we used the mean values for each genotype for calculating phenotypic correlations with other plant traits (further details in Analyses below) and for use in multiple regression analyses (further details in Which plant traits account for herbivore community responses to host-plant genotype?).

Architecture

Plant architectural traits included plant size, plant height, foliage density and fractal dimension (an index of architectural complexity). We measured architectural traits by setting up a white tarp (5·5 m by 7·6 m) as a backdrop behind an average of five replicates per genotype (n = 132, range = 4–7) in late July 2011. We then took a photograph on a tripod with a standard focal length (no zoom) from a standardized position (4 m distance, facing SW direction). Using IMAGEJ, we first removed shadows created by the foliage and then converted photographs to black-and-white images. We estimated plant height as the vertical height of the shrub in each image and plant size as the total two-dimensional area (m^2) covered by the shrub in each image using a known scale. We calculated foliage density using plant size divided by the minimum convex hull area of the plant. The minimum convex hull represents a connected series of straight segments convexly enclosing all of the foreground pixels in our plant images (Fig. S1, Supporting information). To calculate fractal dimension, we used the box-counting method incorporated in the FracLac plugin for IMAGEJ. Fractal dimension is an index of complexity that measures how detail in a pattern changes with the scale of measurement. This architectural trait is also known to display heritable variation among *Populus* hybrids (Bailey *et al.* 2004) and can influence the abundance and size distribution of arthropods on plants (Morse *et al.* 1985).

Analyses

We used separate restricted maximum likelihood (REML) models to test for differences in plant traits among willow genotypes (with 'NLME' package in R). We specified plant genotype as a random effect in all models and evaluated its significance using a likelihood ratio test. We did not include plant sex in our model because exploratory analyses showed that it was only weakly associated with a couple of salicylate compounds that were unimportant in affecting herbivore responses. Traits were transformed as needed to improve normality and reduce heteroscedasticity. To calculate the broad-sense heritability of plant traits, we used the equation: $H^2 = V_G/V_P$, where V_G is the total genotypic variance among clones and V_P is the total phenotypic variance, calculated as the sum of the residual and genetic variance (Lynch & Walsh 1998). Broad-sense heritability values range between 0 and 1, where values close to zero indicate low heritability (i.e. the trait is strongly influenced by the environment) and values close to 1 indicate high heritability (i.e. the trait is strongly controlled by underlying genetic variation). We also calculated phenotypic (range of n = 115–140 shrubs) correlations (Pearson's r) between all plant traits. We explored phenotypic trait correlations (Fig. S2) to determine how to mitigate the effects of plant trait multicollinearity on multiple regression analysis (further details in Which plant traits account for herbivore community responses to host-plant genotype?).

WHICH PLANT TRAITS ACCOUNT FOR HERBIVORE COMMUNITY RESPONSES TO HOST-PLANT GENOTYPE?

We used multiple regression analyses to identify the host-plant traits that best accounted for herbivore community responses; however, we first had to mitigate the effects of multicollinearity. We used three different methods to reduce multicollinearity. For leaf phenolic chemistry, we conducted separate principle components analysis (PCA, with 'LABDSV' package in R) on the following groups of highly correlated compounds (Fig. S2 and Table S3): salicylates/condensed tannins, phenolic acids, flavones/flavonols (flavonoids) and flavanones/flavanonols (miscellaneous flavonoids). Performing separate PCAs allowed us to interpret the relationships between different classes of phenolic compounds and the herbivore community. Prior to PCA, we first transformed phenolics as necessary to linearize correlated relationships and then standardized each trait (mean = 0, SD = 1) to give them each equal weight in the analysis. We used scree plots and tables of variable loadings to select representative principal components (Table S3). When certain pairs of traits were highly correlated with each other ($0{\cdot}4 < |r| < 0{\cdot}8$), we used the residuals from a linear regression of the two traits as a new predictor variable that was no longer correlated with the other trait (Graham 2003). These trait pairs included plant size and height ($r = 0{\cdot}59$, $P < 0{\cdot}001$), plant size and foliage density ($r = 0{\cdot}47$, $P < 0{\cdot}001$), as well as SLA and water content ($r = 0{\cdot}60$, $P < 0{\cdot}001$). In two cases, pairs of traits scaled closely with one another ($|r| > 0{\cdot}80$), so we retained the trait that had a more intuitive ecological interpretation and discarded the other. Therefore, we retained plant size instead of fractal dimension ($r = 0{\cdot}85$, $P < 0{\cdot}001$) and kept C : N instead of N content ($r = -0{\cdot}97$, $P < 0{\cdot}001$). The three different methods we used to reduce multicollinearity resulted in 12 predictor variables. Leaf quality traits included salicylate/tannin PC1, phenolic acid PC1-2, flavonoid PC1-2, miscellaneous flavonoids PC1 (Table S3), water content, SLA residuals and C : N. Plant architectural traits included plant size, height residuals and foliage density residuals. Finally, we conducted variance inflation factor (VIF) analysis on these 12 predictor variables (with 'CAR' package in R) to calculate how much of the variance of an estimated regression coefficient is increased due to collinearity. All VIF values were <1·8, indicating that multicollinearity had only a minor influence on our subsequent multiple regression analyses (Dormann *et al.* 2013).

Using this subset of predictor variables, we used multiple regression with forward model selection to identify the key traits accounting for herbivore responses. We restricted these analyses to herbivore responses that varied significantly among willow genotypes ($P < 0{\cdot}05$). We used the forward model selection approach advocated by Blanchet, Legendre & Borcard (2008), which prevents inclusion of spurious variables (i.e. inflated Type 1 error) and overestimation of explained variance (i.e. R^2). This method first tested whether the full model, which included all 12 predictor variables, was significant ($P < 0{\cdot}05$). We then proceeded with forward model selection using two stopping criteria: (i) $P < 0{\cdot}05$ for including a variable in the model and (ii) the adjusted R^2 calculated on the full model. Whenever forward selection identified a variable that brought one or the other criterion over the fixed threshold, the variable was rejected, and the procedure stopped. To assess the relative importance of each predictor variable in the final model, we calculated the change in explanatory variance when a variable was removed (ΔR^2, with 'ROCKCHALK'

package in R). After identifying a final model, we then used sequential sum-of-squares (i.e. Type 1 SS) to test whether including genotype as a factor still had a significant effect. If it did, this indicated that we either did not identify all of the relevant plant traits or our study failed to capture some other important interaction (e.g. competition or predation) mediated by plant genotype.

Results

HOW DO HERBIVORE COMMUNITIES RESPOND TO HOST-PLANT GENOTYPE?

Community-level

Total herbivore abundance ($F_{25,105} = 1{\cdot}64$, $P = 0{\cdot}044$; Fig. 1a) and community composition ($F_{25,105} = 1{\cdot}62$, $P = 0{\cdot}001$; Fig. 1c) exhibited strong responses to willow genotype, whereas herbivore richness ($F_{25,105} = 1{\cdot}33$, $P = 0{\cdot}162$; Fig. 1b), rarefied richness ($F_{25,105} = 1{\cdot}11$, $P = 0{\cdot}348$) and evenness did not ($F_{25,105} = 1{\cdot}40$, $P = 0{\cdot}123$). Herbivore abundance varied 3·5-fold among clones, ranging from an average of 24 to 84 individuals between the most disparate genotypes (Fig. 1a). Willow genotype explained 27·3% of the variance in community composition (Fig. 1c), with differences driven primarily by two leaf miners (weevil *Tachyerges salicis*, moth *Caloptilia* sp.), two leaf gallers (midge *Iteomyia salicisverruca*; mite *Aculops tetanothrix*) and a xylem feeding leaf hopper (Cicadellidae nymph sp. 1) (Fig. S3). Of these species, the two leaf miners (*T. salicis*, $\chi^2_{25,105} = 80{\cdot}62$, $P < 0{\cdot}001$; *Caloptilia* sp., $\chi^2_{25,105} = 56{\cdot}62$, $P < 0{\cdot}001$) and two gallers (*I. salicisverruca*, $\chi^2_{25,105} = 63{\cdot}62$, $P < 0{\cdot}001$; *A. tetanothrix*, $\chi^2_{25,105} = 54{\cdot}73$, $P = 0{\cdot}001$) varied between 3·7- and 10-fold in their abundance among willow genotypes, whereas Cicadellidae nymph sp. 1 exhibited only a marginally significant response ($\chi^2_{25,105} = 35{\cdot}96$, $P = 0{\cdot}072$). While *Caloptilia* and *I. salicisverruca* exhibited a positive phenotypic (i.e. shrub level) correlation ($r = 0{\cdot}20$, $P = 0{\cdot}020$; Table 1), no species pairs displayed correlated responses among the different willow genotypes (Table 1).

Table 1. Pearson correlations (r) of dominant herbivore species occurring on *Salix hookeriana*

	*Tachyerges**	*Caloptilia*†	*Iteomyia**	*Aculops**
*Tachyerges salicis**	1	0·04	−0·08	−0·11
Caloptilia sp.†	*0·15*	1	**0·20**	0·00
*Iteomyia salicisverruca**	*−0·30*	*−0·11*	1	0·09
*Aculops tetanothrix**	*−0·07*	*0·01*	*0·11*	1

Italicized values below the diagonal represent genetic correlations ($n = 26$), while values above the diagonal are phenotypic correlations ($n = 131$).
Statistically significant correlations ($P < 0{\cdot}05$) are indicated in boldface type.
*log($x + 1$) transformed.
†Square-root transformed.

Feeding guilds

As with total herbivores and individual species, the abundance of most herbivore feeding guilds varied by several fold among willow genotypes (Fig. 2a–f). For example, leaf chewer ($\chi^2_{25,105} = 41{\cdot}62$, $P = 0{\cdot}020$; Fig. 2a) and phloem feeder ($\chi^2_{25,105} = 41{\cdot}16$, $P = 0{\cdot}022$; Fig. 2c) abundance varied 9·8- and 14-fold among genotypes, respectively, but xylem feeders displayed only a marginally significant response ($\chi^2_{25,105} = 36{\cdot}39$, $P = 0{\cdot}066$; Fig. 2b). PLAR also varied 6·3-fold, ranging from an average of 4·7% to 29·6% leaf area removed among genotypes ($F_{25,105} = 2{\cdot}80$, $P < 0{\cdot}001$; Fig. 2d). Finally, galler and leaf

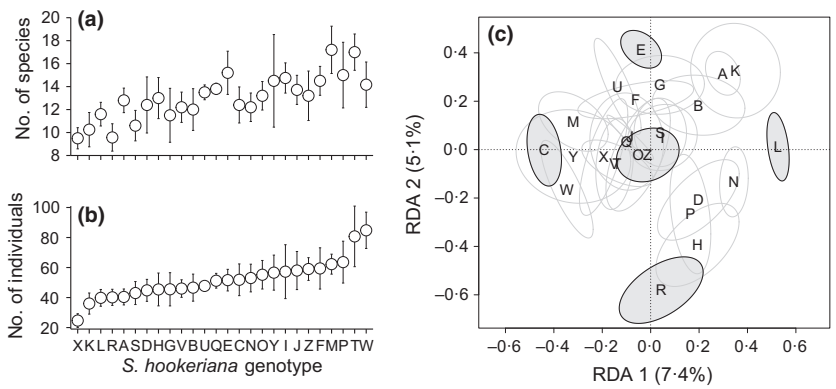

Fig. 1. Herbivore community responses to 26 different genotypes of *Salix hookeriana* growing in a common garden. Community-level variables included (a) total richness, (b) total abundance and (c) an ordination of community composition based on Euclidean distances of chord-transformed community data in which each axis represents the percentage variance explained by the corresponding axis from redundancy analysis (RDA). For (a) and (b), genotypes are ordered based on total herbivore abundance, with circles and error bars representing means and SEs, respectively. For (c), the position of each letter corresponds to the centroid for each genotype and the ellipses represent the SE of the centroid's position. The ellipses of five of 26 genotypes are highlighted to illustrate the differences in herbivore community composition along these axes.

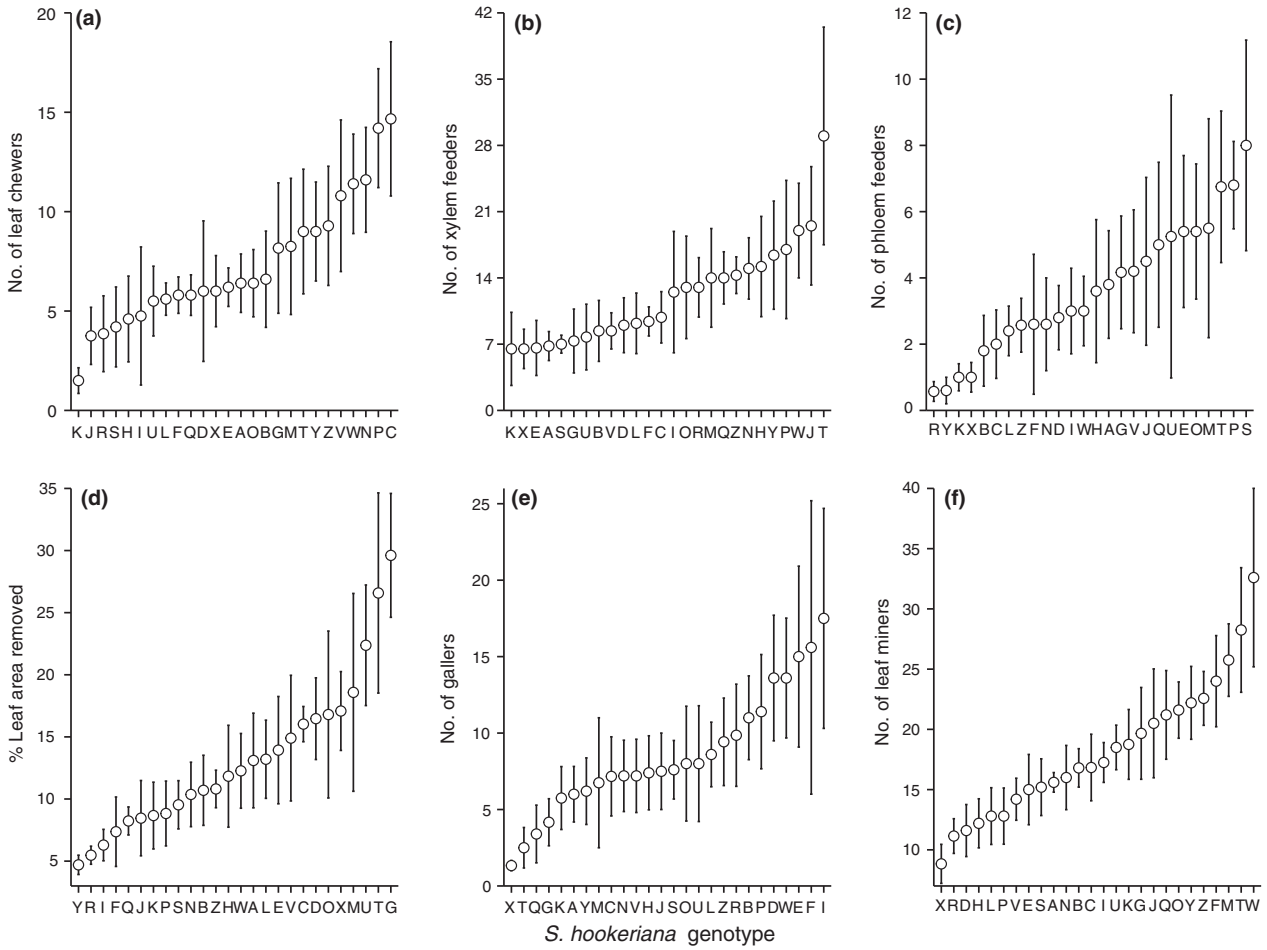

Fig. 2. Herbivore feeding guild and percentage leaf area removed (PLAR) responses to 26 different genotypes of *Salix hookeriana*: (a) leaf chewers, (b) xylem feeders, (c) phloem feeders, (d) PLAR, (e) gallers and (f) leaf miners. Circles and error bars represent means and SEs.

miner abundance differed by 13·1-fold ($\chi^2_{25,105} = 44{\cdot}06$, $P = 0{\cdot}011$; Fig. 2e) and 3·7-fold ($\chi^2_{25,105} = 82{\cdot}06$, $P < 0{\cdot}001$; Fig. 2f) among genotypes, respectively.

Several of these guild-level responses exhibited significant phenotypic correlations. For example, leaf chewers were positively correlated with both phloem feeders ($r = 0{\cdot}26$, $P = 0{\cdot}003$) and leaf miners ($r = 0{\cdot}31$, $P < 0{\cdot}001$; Table 2). Phloem feeders were also positively correlated with leaf miners ($r = 0{\cdot}19$, $P = 0{\cdot}031$; Table 2). In contrast, gallers were negatively correlated with PLAR ($r = -0{\cdot}20$, $P = 0{\cdot}021$; Table 2). Despite the handful of significant phenotypic correlations, no guilds were genetically correlated (Table 2).

Table 2. Pearson correlations (r) of herbivore guild abundances and percentage leaf area removed (PLAR)

	PLAR*	L. chewer†	P. feeder†	Galler†	L. miner*
PLAR*	1	0·08	0·15	**−0·20**	−0·01
Leaf chewer†	*0·24*	1	**0·26**	0·11	**0·31**
Phloem feeder†	*0·37*	*0·30*	1	−0·03	**0·19**
Galler†	*−0·35*	*−0·06*	*0·03*	1	0·15
Leaf miner*	*0·04*	*0·20*	*0·22*	*−0·07*	1

Italicized values below the diagonal represent genetic correlations ($n = 26$), while values above the diagonal are phenotypic correlations ($n = 131$).
Statistically significant correlations ($P < 0{\cdot}05$) are indicated in boldface type.
*log transformed.
†log($x + 1$) transformed.

HOW HERITABLE ARE DIFFERENT HOST-PLANT TRAITS?

Leaf quality

Leaf quality traits displayed a remarkable amount of variation among willow genotypes and were highly heritable (mean $H^2 = 0{\cdot}72$; Fig. 3a–c; Table S4). For example, genotypes varied 3·3- and 88·5-fold in total condensed tannins and total salicylates, with broad-sense heritability values of 0·61 ($\chi^2_1 = 67{\cdot}75$, $P < 0{\cdot}001$) and 0·68 ($\chi^2_1 = 92{\cdot}40$, $P < 0{\cdot}001$; Fig. 3a), respectively. Similarly, willow genotypes varied 8·3- and 2·3-fold in concentration of total phenolic acids ($H^2 = 0{\cdot}88$, $\chi^2_1 = 202{\cdot}92$, $P < 0{\cdot}001$; Fig. 3b) and

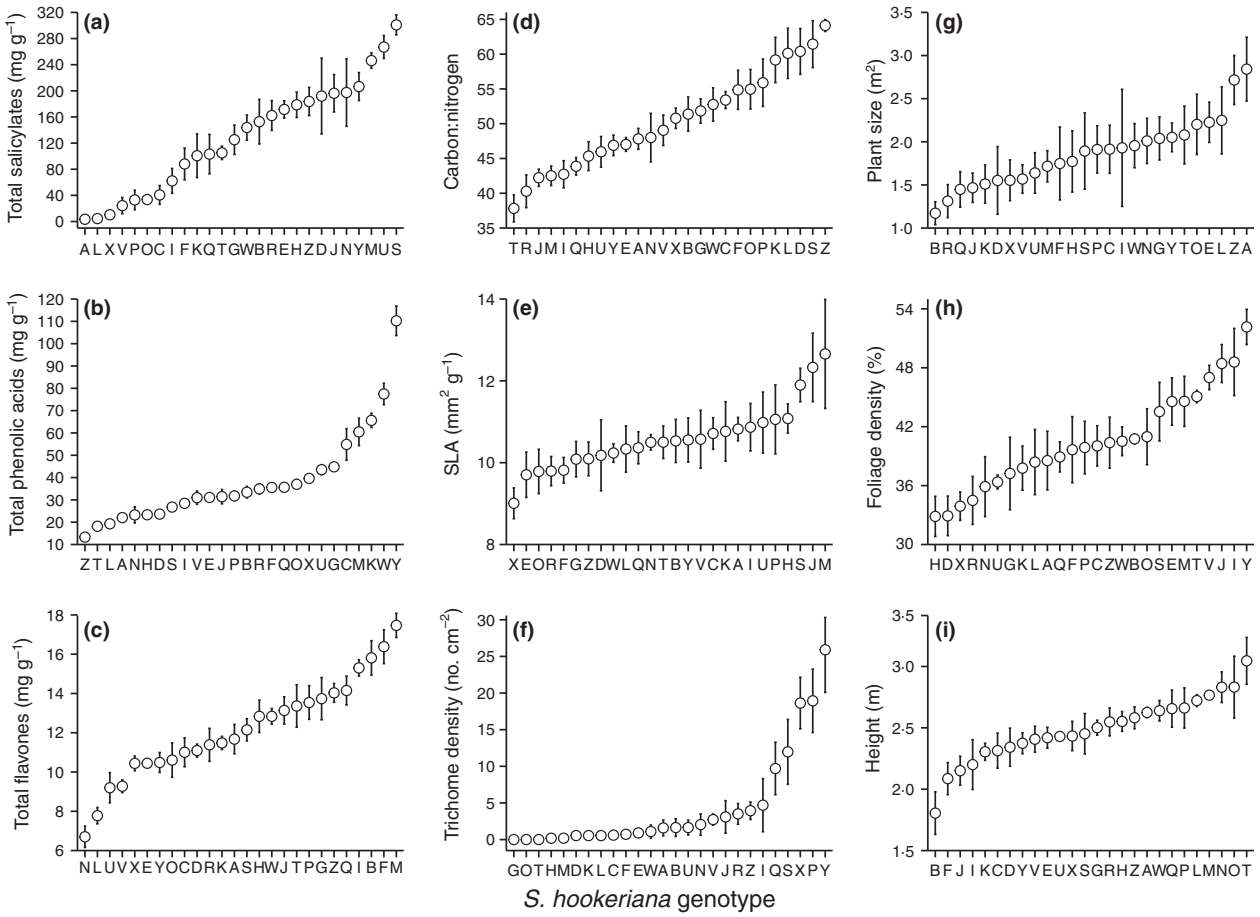

Fig. 3. Variation in plant traits among 26 genotypes of *Salix hookeriana*. Leaf quality traits (a–f): (a) total salicylates, (b) total phenolic acids, (c) total flavones, (d) carbon : nitrogen, (e) specific leaf area (SLA) and (f) trichome density. Plant architecture traits (g–i): (g) plant size, (h) foliage density and (i) plant height. Circles and error bars represent means and SEs, respectively.

total flavones ($H^2 = 0{\cdot}70$, $\chi^2_1 = 92{\cdot}94$, $P < 0{\cdot}001$; Fig. 3c), both of which exhibited high degrees of heritability. Leaf trichome density was also both highly variable (25·9-fold among genotypes) and heritable ($H^2 = 0{\cdot}62$, $\chi^2_1 = 77{\cdot}58$, $P < 0{\cdot}001$; Fig. 3f). Although leaf C : N only varied 1·7-fold among genotypes, it was highly heritable ($H^2 = 0{\cdot}61$, $\chi^2_1 = 64{\cdot}03$, $P < 0{\cdot}001$; Fig. 3d). In contrast to the other leaf quality traits, both SLA ($\chi^2_1 = 5{\cdot}19$, $P = 0{\cdot}023$, Fig. 3e) and leaf water content ($\chi^2_1 = 14{\cdot}41$, $P < 0{\cdot}001$) varied 1·4-fold among genotypes and displayed relatively low heritability values of 0·15 and 0·27, respectively.

Architecture

Variability and heritability of plant architecture was low relative to most leaf quality traits (mean $H^2 = 0{\cdot}27$; Fig. 3g–i; Table S4). Plant size varied 2·4-fold among genotypes with a corresponding heritability of 0·15 ($\chi^2_1 = 5{\cdot}41$, $P = 0{\cdot}020$; Fig. 3g). In comparison, foliage density ($H^2 = 0{\cdot}38$, $\chi^2_1 = 25{\cdot}65$, $P < 0{\cdot}001$; Fig. 3h) and plant height ($H^2 = 0{\cdot}38$, $\chi^2_1 = 23{\cdot}77$, $P < 0{\cdot}001$; Fig. 3i) varied 1·6- and 1·7-fold among genotypes, but were more than twice as heritable as plant size.

WHICH PLANT TRAITS ACCOUNT FOR HERBIVORE COMMUNITY RESPONSES TO HOST-PLANT GENOTYPE?

Community-level

Leaf phenolic chemistry and plant size tended to be the best predictors of herbivore community responses (Table 3). For example, total herbivore abundance was best explained by variation in plant size, trichome density and flavonoid PC2 (Table 3). Specifically, larger plants with fewer trichomes and negative loadings on flavonoid PC2 hosted more herbivore individuals. Herbivore community composition was influenced by plant size and a different set of leaf phenolics (phenolic acid PC1-2 and miscellaneous flavonoids PC1), but these traits did not fully explain the effect of willow genotype (Table 3).

Plant traits corresponding to individual herbivore species responses did not always match the traits that explained overall community composition (Table 3). For example, the leaf mining weevil, *T. salicis*, was more abundant on large shrubs with dense foliage, positive loadings on phenolic acid PC1, but low leaf water content

(Table 3). The leaf mining moth, *Caloptilia* sp., had higher abundances on larger shrubs with negative loadings on flavonoid PC1 (Table 3). The leaf galling midge, *I. salicisverruca*, did not vary with phenolic chemistry, but had higher abundances on larger shrubs with higher leaf C : N (Table 3). In contrast, the leaf galling mite, *A. tetanothrix*, was more abundant on plants with negative loadings on phenolic acid PC2 (Table 3). Despite finding several significant herbivore–trait associations, genotype was maintained as a significant predictor of all herbivore species in our trait analyses, with the exception of *A. tetanothrix*.

Feeding guilds

As with most herbivore community responses, feeding guilds were principally linked with leaf phenolic chemistry and plant architecture (Table 3). For example, leaf chewers were more abundant on larger plants (Table 3). Phloem feeders were also more abundant on larger plants, but also responded positively to taller plants and those that had high SLA and negative loadings on phenolic acid PC1 (Table 3). The full trait models for PLAR and galler abundance were not significant, suggesting that unmeasured plant traits may be underlying their response to different willow genotypes. In contrast, phenolic acid PC1 and PC2, as well as all three architecture traits explained 33·4% of the variance in leaf miner abundance. Specifically, plants with greater architectural complexity (larger, taller and denser foliage), positive loadings on phenolic acid PC1 and negative loadings on phenolic acid PC2 hosted more leaf miners (Table 3).

Discussion

Our results demonstrate that host-plant genetic variation is a key factor shaping *S. hookeriana*'s associated arthropod herbivore community in a willow population in northern California. Total herbivore abundance, community composition, as well as individual species and feeding guilds exhibited strong responses to different willow genotypes.

Table 3. Results from multiple regression and redundancy analyses, after forward model selection, of herbivore responses to plant traits of *Salix hookeriana* growing in a common garden. Additionally, we tested whether willow genotype continued to be a significant predictor of herbivore responses after accounting for the variation explained by plant traits (Genotype *P*)

Response	Variable	coef (±SE)	*P*	ΔR^2	Total R^2	Genotype *P*
Herbivore abundance*	Plant size*	0·53 ± 0·09	<0·001	0·208	0·292	0·074
	Flavonoids PC2	−0·11 ± 0·03	0·001	0·078		
	Trichome density†	−0·09 ± 0·04	0·018	0·037		
Community composition‡	Phenolic acids PC1		0·032	0·017	0·113	0·008
	Phenolic acids PC2		0·001	0·034		
	Plant size*		0·001	0·023		
	Miscellaneous flavonoids PC1		0·019	0·019		
	Flavonoids PC2		0·044	0·016		
Tachyerges salicis†	Phenolic acid PC1	0·11 ± 0·03	0·001	0·078	0·212	0·049
	Foliage density residuals	2·64 ± 1·09	0·017	0·043		
	Plant size*	0·36 ± 0·16	0·023	0·039		
	Water content	−0·60 ± 0·27	0·031	0·035		
Caloptilia sp.§	Plant size*	0·70 ± 0·19	<0·001	0·137	0·212	0·037
	Flavonoids PC1	−0·12 ± 0·04	0·001	0·081		
Iteomyia salicisverruca†	Plant size*	0·48 ± 0·19	0·013	0·051	0·116	<0·001
	C : N	0·03 ± 0·01	0·02	0·044		
Aculops tetanothrix†	Phenolic acid PC2	−0·18 ± 0·04	<0·001	0·142	0·142	0·304
Leaf chewer abundance†	Plant size*	0·79 ± 0·16	<0·001	0·177	0·177	0·119
Phloem feeder abundance†	Plant size*	0·68 ± 0·18	<0·001	0·105	0·201	0·633
	SLA residuals	0·16 ± 0·06	0·012	0·048		
	Phenolic acids PC1	−0·08 ± 0·04	0·031	0·035		
	Height residuals	0·58 ± 0·28	0·040	0·032		
PLAR*	Full trait model		0·128		0·164	
Galler abundance†	Full trait model		0·209		0·147	
Leaf miner abundance†	Plant size*	0·41 ± 0·09	<0·001	0·120	0·334	0·052
	Foliage density residuals	2·34 ± 0·7	0·001	0·070		
	Phenolic acids PC2	−0·10 ± 0·03	0·001	0·075		
	Phenolic acids PC1	0·05 ± 0·02	0·011	0·041		
	Height residuals	0·37 ± 0·16	0·026	0·031		

PLAR, percentage leaf area removed; SLA, specific leaf area.
*log transformed.
†log(x + 1) transformed.
‡Redundancy analysis on chord-transformed herbivore community data (site-by-species matrix) with significance evaluated after 1000 permutations of the data.
§Square-root transformed.

These differences corresponded with extensive phenotypic variation in leaf quality and plant architecture; however, there was no single trait that explained herbivore community responses. Rather, there was a range of host-plant traits that were associated with different herbivore species and feeding guilds.

HOW DO HERBIVORE COMMUNITIES RESPOND TO HOST-PLANT GENOTYPE?

Our study highlights that a genetic basis to arthropod herbivore community composition occurs via differential responses among guilds and species, a result that has been demonstrated in a variety of host-plant systems (Whitham *et al*. 2012). Although species- and guild-level abundances varied among *S. hookeriana* genotypes, none of these responses were correlated among genotypes. Similarly, Roche & Fritz (1997) with *Salix sericea* found little evidence for genetically correlated responses among the 12 species of galling, leaf mining and leaf folding herbivores they examined. Work in other host-plant systems has observed strong genetic correlations among herbivore species (Maddox & Root 1990), as well as correlations that vary from year-to-year (Johnson & Agrawal 2007). The absence of genetic correlations in our study could be indicative of different herbivore species responding to different suites of plant traits. Alternatively, the magnitude of correlated responses measured in our study may have been dampened by naturally occurring competitive interactions among herbivores or predation, or both (Leimu & Koricheva 2006). Either way, this lack of genetic correlation suggests that selection for resistance traits imposed by these herbivores on *S. hookeriana*, and possibly other *Salix* sp., is independent of one another. Given that herbivore communities are often highly heterogeneous in space and time (Lewinsohn, Novotny & Basset 2005), species turnover in *S. hookeriana*'s diverse herbivore assemblage could result in highly variable selection pressures on many different plant traits. This explanation may contribute to why *Salix* sp. often exhibit considerable genetic and phenotypic variation within natural populations (Fritz & Price 1988; Brunsfeld, Soltis & Soltis 1991; Nichols-Orians, Fritz & Clausen 1993).

HOW HERITABLE ARE DIFFERENT HOST-PLANT TRAITS?

Salix hookeriana genotypes varied in all traits that we measured; however, the magnitude of variation among genotypes was much greater for leaf phenolics and trichome density compared to other leaf quality traits or plant architecture. Moreover, leaf quality traits had 2·7-fold higher broad-sense heritability values (mean $H^2 = 0{\cdot}72$) compared to plant architectural traits (mean $H^2 = 0{\cdot}27$) in *S. hookeriana*, a pattern primarily driven by leaf phenolic chemistry. While broad-sense heritability values tend to overestimate the capacity for evolution, these relative differences in plant trait heritability may be quite general. For example, a meta-analysis by Geber & Griffen (2003) found that the mean heritability of plant secondary chemistry was more than two times greater than the heritability of plant morphology, phenology and vegetative performance traits. This pattern may have important implications for community genetics research in plant–herbivore systems, especially when there is considerable plant phenotypic variation. For example, traits under weaker genetic control (i.e. low heritability) will be strongly influenced by the environment in which a host plant is growing. If herbivores cue in on weakly heritable traits (e.g. plant size), predicting community responses will be difficult without explicitly incorporating environmental variation. Of the plant–herbivore genotype-by-environment (G × E) studies that have been done (e.g. Garibaldi, Kitzberger & Chaneton 2011; Silfver *et al*. 2014), much of this work has examined a limited number of traits (similar to the purely genetic studies), environments and spatial scales (Tack, Johnson & Roslin 2011). Consequently, integrating detailed trait screenings within G × E studies should be a priority for future research.

WHICH PLANT TRAITS ACCOUNT FOR HERBIVORE COMMUNITY RESPONSES TO HOST-PLANT GENOTYPE?

Recently, the primacy of plant secondary metabolites in mediating host-plant resistance to arthropod herbivores has been questioned (Carmona, Lajeunesse & Johnson 2011). In concordance, we found that plant size tended to explain nearly twice the variation (mean $\Delta R^2 = 0{\cdot}105$) in herbivore responses compared to any single axis of phenolic variation (mean $\Delta R^2 = 0{\cdot}057$). This result is consistent with a recent meta-analysis demonstrating a positive relationship between the architecture of woody plants and arthropod herbivores (Carmona, Lajeunesse & Johnson 2011). This result also corresponds with predictions from the plant vigour hypothesis (Price 1991), which states that herbivores prefer either larger modules (e.g. shoots, leaves) within plants or larger plant individuals instead of smaller ones, due to increased resource availability. There are many other potential explanations though. For example, plant size was positively correlated with plant height, architectural complexity (fractal dimension) and foliage density. Therefore, larger plants are likely more apparent to herbivores (Castagneyrol *et al*. 2012), provide habitat heterogeneity that decreases predator foraging efficiency (Kareiva & Sahakian 1990) and buffer microclimate conditions (Raghu, Drew & Clarke 2004). Partitioning these causal mechanisms will require future manipulative experiments that hold plant size constant while varying other architectural traits. Nevertheless, we did find independent and positive relationships between plant height or foliage density and the abundance of certain herbivore species (*Tachyerges salicis*) and feeding guilds (leaf miners and phloem feeders), suggesting that plant apparency and

habitat heterogeneity may contribute to genetic variation in host-plant susceptibility.

Another possible explanation for the relatively weak role of leaf phenolic chemistry compared to plant architecture is that the dominant herbivores in our study all specialize on members of the genus *Salix*. *A priori*, we would expect that specialist herbivores would be less sensitive to variation in the most abundant secondary metabolites, since they would have had to evolve some degree of physiological tolerance to these chemicals. In line with this, we found little correspondence between herbivore responses and salicylates and condensed tannins, which were the most abundant secondary metabolites in our leaf samples. Instead, we found that when phenolic compounds did show a relationship with herbivores, they were the less abundant ones (e.g. flavonoids and phenolic acids). Consequently, our results suggest that screening a range of secondary metabolites, above and beyond the most abundant compounds, is necessary for understanding herbivore community assembly.

Despite our detailed characterization of willow phenotypes, the traits we measured did not fully explain the effect of willow genotype on the herbivore community (Table 3), suggesting that other unmeasured traits could be relevant. For example, we did not measure shoot length, which has been identified as one of the best predictors of abundance for a few species of willow-galling sawflies, presumably because more vigorous growing shoots have higher resource availability (Price 1991). We also measured traits at the peak of the growing season, thereby neglecting potential differences in phenology among willow genotypes – an important suite of traits in other systems (Johnson & Agrawal 2005). In addition to unmeasured plant traits, our study focused on community composition, thereby neglecting the diverse competitive and predatory interactions that are likely going on throughout this community. For example, Fritz (1990) showed that the strength of competition between gall-inducing sawflies varied among genotypes of *Salix lasiolepis*. The size and toughness of stem galls induced by the galling sawfly, *Euura lasiolepis*, on *S. lasiolepis* is determined by plant genotype, which in turn affects parasitoid attack rates (Craig, Itami & Price 1990). Thus, a comprehensive understanding of host-plant genetic effects on herbivore communities may also require incorporating interactions among species both within and between trophic levels.

Conclusions

Our research provides several insights into the trait-based mechanisms mediating herbivore community responses to host-plant genetic variation. First, there is emerging evidence from our study and others that plant secondary chemistry tends to be more heritable than plant architectural traits (Geber & Griffen 2003). However, in woody plant systems, plant architecture appears to be a dominant and predictable driver of herbivore community responses, relative to the more idiosyncratic effects of plant secondary chemistry (Carmona, Lajeunesse & Johnson 2011). Since environmental variation is more likely to shape variation in plant architecture (because it is less heritable), it will be particularly important for future work in woody plant systems to explicitly incorporate plant responses across variable environments to understand herbivore community assembly. Next, herbivore responses were not correlated among genotypes, likely because individual herbivore species and feeding guilds are cueing in on different suites of plant traits. These uncorrelated responses also imply separate genetic control of resistance to these species and the lack of potential for multispecies selection on the same resistance traits (Fritz & Simms 1992). Thus, studies should consider a range of traits and partition herbivore species and guild responses to host-plant genotypes. Finally, the direct effects of plant traits on herbivores are likely not the only pathways by which genetic variation structures herbivore communities. Incorporating similar detailed comparisons of competitive or predatory interactions will be an important step for building a mechanistic understanding of the genetic basis to community assembly and the eco-evolutionary dynamics between plants and herbivores.

Acknowledgements

We thank E. Nelson and the staff of Humboldt Bay National Wildlife Refuge (U.S. Fish and Wildlife Service) for facilitating experimental logistics and establishing the fence. J. Bair, G. Argus and P. Haggard assisted with plant and arthropod identifications. L. Mackas-Burns, D. Thomson, S. Sorsa, N. Ask, L. Wu, J. and K. Knisley and numerous undergraduates from Humboldt State University assisted with the fieldwork. M. Johnson, C. Hochwender, R. Zerebecki and C. Greyson-Gaito provided valuable comments on the manuscript. M. A. Barbour was supported by a BRITE Fellowship and a Four-Year Fellowship from the University of British Columbia. G. M. Crutsinger was supported by the Miller Institute for Basic Research in Science, as well as a NSERC Discovery grant.

Data accessibility

Data and R scripts for replicating all analyses are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b1296 (Barbour *et al.* 2015).

References

Abràmoff, M.D., Magalhães, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, **11**, 36–42.

Agrawal, A.A. (2004) Plant defense and density dependence in the population growth of herbivores. *The American Naturalist*, **164**, 113–120.

Agrawal, A.A. (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research*, **7**, 651–667.

Agrawal, A.A. & Fishbein, M. (2006) Plant defense syndromes. *Ecology*, **87**, S132–S149.

Agrawal, A.A., Johnson, M.T.J., Hastings, A.P. & Maron, J.L. (2013) A field-experiment demonstrating plant life-history evolution and its eco-evolutionary feedback to seed predator populations. *The American Naturalist*, **181**, S35–S45.

Argus, G.W. (2013) *Salix* (eds Jepson Flora Project) URL http://ucjeps.berkeley.edu/cgi-bin/get_IJM.pl?tid=42833 [accessed 2 November 2013].

Bailey, J.K., Bangert, R.K., Schweitzer, J.A., Trotter, R.T. III, Shuster, S.M. & Whitham, T.G. (2004) Fractal geometry is heritable in trees. *Evolution*, **58**, 2100–2102.

Barbehenn, R.V. & Constabel, C.P. (2011) Tannins in plant–herbivore interactions. *Phytochemistry*, **72**, 1551–1565.

Barbour, M.A., Rodriguez-Cabal, M.A., Wu, E.T., Julkunen-Tiitto, R., Ritland, C.E., Miscampbell, A.E. *et al.* (2015) Data from: multiple plant traits shape the genetic basis of herbivore community assembly. Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.b1296

Barker, J.H.A., Pahlich, A., Trybush, S., Edwards, K.J. & Karp, A. (2003) Microsatellite markers for diverse *Salix* species. *Molecular Ecology Notes*, **3**, 4–6.

Björkman, C., Dalin, P. & Ahrné, K. (2008) Leaf trichome responses to herbivory in willows: induction, relaxation and costs. *The New Phytologist*, **179**, 176–184.

Blanchet, F.G., Legendre, P. & Borcard, D. (2008) Forward selection of explanatory variables. *Ecology*, **89**, 2623–2632.

Boeckler, G.A., Gershenzon, J. & Unsicker, S.B. (2011) Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry*, **72**, 1497–1509.

Brunsfeld, S.J., Soltis, D.E. & Soltis, P.S. (1991) Patterns of genetic variation in *Salix* section Longifoliae (Salicaceae). *American Journal of Botany*, **78**, 855–869.

Carmona, D., Lajeunesse, M.J. & Johnson, M.T.J. (2011) Plant traits that predict resistance to herbivores. *Functional Ecology*, **25**, 358–367.

Castagneyrol, B., Lagache, L., Giffard, B., Kremer, A. & Jactel, H. (2012) Genetic diversity increases insect herbivory on oak saplings. *PLoS ONE*, **7**, e44247.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E. *et al.* (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.

Craig, T.P., Itami, J.K. & Price, P.W. (1990) The window of vulnerability of a shoot-galling sawfly to attack by a parasitoid. *Ecology*, **71**, 1471–1482.

Crutsinger, G.M., Rodriguez-Cabal, M.A., Roddy, A.B., Peay, K.B., Bastow, J.L., Kidder, A.G. *et al.* (2014) Genetic variation within a dominant shrub structures green and brown community assemblages. *Ecology*, **95**, 387–398.

Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G. *et al.* (2013) Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 027–046.

Fritz, R.S. (1990) Variable competition between insect herbivores on genetically variable host plants. *Ecology*, **71**, 1208–1211.

Fritz, R.S. & Price, P.W. (1988) Genetic variation among plants and insect community structure: willows and sawflies. *Ecology*, **69**, 845–856.

Fritz, R.S. & Simms, E.L. (1992) *Plant Resistance: Ecology, Evolution and Genetics*. University of Chicago Press, Chicago, Illinois, USA.

Graham, M.H. (2003) Confronting multicollinearity in ecological multiple regression. *Ecology*, **84**, 2809–2815.

Garibaldi, L.A., Kitzberger, T. & Chaneton, E.J. (2011) Environmental and genetic control of insect abundance and herbivory along a forest elevational gradient. *Oecologia*, **167**, 117–129.

Geber, M.A. & Griffen, L.R. (2003) Inheritance and natural selection on functional traits. *International Journal of Plant Sciences*, **164**, S21–S42.

Gotelli, N.J. & Colwell, R.K. (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. *Ecology Letters*, **4**, 379–391.

Hersch-Green, E.I., Turley, N.E. & Johnson, M.T.J. (2011) Community genetics: what have we accomplished and where should we be going? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **366**, 1453–1460.

Hochwender, C.G. & Fritz, R.S. (2004) Plant genetic differences influence herbivore community structure: evidence from a hybrid willow system. *Oecologia*, **138**, 547–557.

Hughes, A.R., Inouye, B.D., Johnson, M.T.J., Underwood, N. & Vellend, M. (2008) Ecological consequences of genetic diversity. *Ecology Letters*, **11**, 609–623.

Johnson, M.T.J. & Agrawal, A.A. (2005) Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology*, **86**, 874–885.

Johnson, M.T.J. & Agrawal, A. (2007) Covariation and composition of arthropod species across plant genotypes of evening primrose (*Oenothera biennis*). *Oikos*, **116**, 941–956.

Johnson, M.T.J., Agrawal, A.A., Maron, J.L. & Salminen, J.-P. (2009) Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. *Journal of Evolutionary Biology*, **22**, 1295–1307.

Julkunen-Tiitto, R. & Sorsa, S. (2001) Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. *Journal of Chemical Ecology*, **27**, 779–789.

Kareiva, P. & Sahakian, R. (1990) Tritrophic effects of a simple architectural mutation in pea plants. *Nature*, **345**, 433–434.

Lawton, J.H. (1983) Plant architecture and the diversity of phytophagous insects. *Annual Review of Entomology*, **28**, 23–39.

Legendre, P. & Gallagher, E.D. (2001) Ecologically meaningful transformations for ordination of species data. *Oecologia*, **129**, 271–280.

Leimu, R. & Koricheva, J. (2006) A meta-analysis of genetic correlations between plant resistances to multiple enemies. *The American Naturalist*, **168**, E15–E37.

Lewinsohn, T.M., Novotny, V. & Basset, Y. (2005) Insects on plants: diversity of herbivore assemblages revisited. *Annual Review of Ecology, Evolution, and Systematics*, **36**, 597–620.

Lynch, M. & Walsh, B. (1998) *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, Massachusetts.

Maddox, G.D. & Root, R.B. (1987) Resistance to 16 diverse species of herbivorous insects within a population of goldenrod, *Solidago altissima*: genetic variation and heritability. *Oecologia*, **72**, 8–14.

Maddox, G.D. & Root, R.B. (1990) Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. *Ecology*, **71**, 2115–2124.

Matsuki, M. & MacLean, S.F. Jr (1994) Effects of different leaf traits on growth rates of insect herbivores on willows. *Oecologia*, **100**, 141–152.

Morse, D.R., Lawton, J.H., Dodson, M.M. & Williamson, M.H. (1985) Fractal dimension of vegetation and the distribution of arthropod body lengths. *Nature*, **314**, 731–733.

Munns, R. & PrometheusWiki Contributors (2010) Plant water content and relative water content. URL http://www.publish.csiro.au/prometheuswiki/tiki-pagehistory.php?page=Plant water content and relative water content&preview=6 [accessed 6 November 2013].

Nichols-Orians, C.M., Fritz, R.S. & Clausen, T.P. (1993) The genetic basis for variation in the concentration of phenolic glycosides in *Salix sericea*: clonal variation and sex-based differences. *Biochemical Systematics and Ecology*, **21**, 535–542.

Nybakken, L. & Julkunen-Tiitto, R. (2013) Gender differences in *Salix myrsinifolia* at the pre-reproductive stage are little affected by simulated climatic change. *Physiologia Plantarum*, **147**, 465–476.

O'Hara, R.B. & Kotze, D.J. (2010) Do not log-transform count data. *Methods in Ecology and Evolution*, **1**, 118–122.

Palo, R.T. (1984) Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. *Journal of Chemical Ecology*, **10**, 499–520.

Price, P.W. (1991) The plant vigor hypothesis and herbivore attack. *Oikos*, **62**, 244–251.

R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Raghu, S., Drew, R.A.I. & Clarke, A.R. (2004) Influence of host plant structure and microclimate on the abundance and behavior of a tephritid fly. *Journal of Insect Behavior*, **17**, 179–190.

Roche, B.M. & Fritz, R.S. (1997) Genetics of resistance of *Salix sericea* to a diverse community of herbivores. *Evolution*, **51**, 1490–1498.

Roininen, H. & Tahvanainen, J. (1989) Host selection and larval performance of two willow-feeding sawflies. *Ecology*, **70**, 129–136.

Sacchi, C.F. & Price, P.W. (1988) Pollination of the arroyo willow, *Salix lasiolepis*: role of insects and wind. *American Journal of Botany*, **75**, 1387–1393.

Silfver, T., Rousi, M., Oksanen, E. & Roininen, H. (2014) Genetic and environmental determinants of insect herbivore community structure in a *Betula pendula* population, *F1000Research*, **3**, 1–13.

Sipura, M. (1999) Tritrophic interactions: willows, herbivorous insects and insectivorous birds. *Oecologia*, **121**, 537–545.

Tack, A.J.M., Johnson, M.T.J. & Roslin, T. (2011) Sizing up community genetics: it's a matter of scale. *Oikos*, **121**, 481–488.

Tahvanainen, J., Julkunen-Tiitto, R. & Kettunen, J. (1985) Phenolic glycosides govern the food selection pattern of willow feeding leaf beetles. *Oecologia*, **67**, 52–56.

Tuomisto, H. (2012) An updated consumer's guide to evenness and related indices. *Oikos*, **121**, 1203–1218.

Whitham, T.G., Gehring, C.A., Lamit, L.J., Wojtowicz, T., Evans, L.M., Keith, A.R. *et al.* (2012) Community specificity: life and afterlife effects of genes. *Trends in Plant Science*, **17**, 271–281.

Received 6 July 2014; accepted 8 December 2014
Handling Editor: Julia Koricheva

Supporting Information

Additional Supporting information may be found in the online version of this article:

Table S1. Microsatellite loci used to genotype clones of *Salix hookeriana* in a common garden experiment.
Table S2. List of non-tannin phenolic compounds found within leaves of *Salix hookeriana* with corresponding wavelength, retention time and response factor.
Table S3. Results from principal component analysis (PCA) on correlation matrix of different groups of phenolic compounds identified from leaves of *Salix hookeriana* in a common garden experiment.
Table S4. Range of plant trait variation among 26 genotypes of *Salix hookeriana* and results from restricted maximum likelihood models and broad-sense heritability analyses of plant traits.
Fig. S1. Diagram of minimum convex hull calculated using IMAGEJ to quantify foliage density.
Fig. S2. Heatmap of phenotypic trait correlations (Pearson's r, sample size range = 115–140) for *Salix hookeriana* measured in a common garden experiment.
Fig. S3. Ordination of herbivore community response to *Salix hookeriana* genotype with key herbivore species labeled.

