

# Plant genetics predicts intra-annual variation in phytochemistry and arthropod community structure

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## Abstract

With the emerging field of community genetics, it is important to quantify the key mechanisms that link genetics and community structure. We studied cottonwoods in common gardens and in natural stands and examined the potential for plant chemistry to be a primary mechanism linking plant genetics and arthropod communities. If plant chemistry drives the relationship between plant genetics and arthropod community structure, then several predictions followed. We would find (i) the strongest correlation between plant genetic composition and chemical composition; (ii) an intermediate correlation between plant chemical composition and arthropod community composition; and (iii) the weakest relationship between plant genetic composition and arthropod community composition. Our results supported our first prediction: plant genetics and chemistry had the strongest correlation in the common garden and the wild. Our results largely supported our second prediction, but varied across space, seasonally, and according to arthropod feeding group. Plant chemistry played a larger role in structuring common garden arthropod communities relative to wild communities, free-living arthropods relative to leaf and stem modifiers, and early-season relative to late-season arthropods. Our results did not support our last prediction, as host plant genetics was at least as tightly linked to arthropod community structure as plant chemistry, if not more so. Our results demonstrate the consistency of the relationship between plant genetics and biodiversity. Additionally, plant chemistry can be an important mechanism by which plant genetics affects arthropod community composition, but other genetic-based factors are likely involved that remain to be measured.

*Keywords:* community genetics, community structure, condensed tannins, genetic composition, phenolic glycosides, *Populus*

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## Introduction

The notion that plant variation leads to changes in the structure and diversity of arthropod communities is not new; however, our understanding of the level at which

arthropods discriminate among plants has become increasingly refined. While early studies demonstrated insect herbivore choice among different plant species (Southwood 1961; Murdoch *et al.* 1972), more recent studies have found that arthropod herbivores distinguish between different plant species and their hybrids (Whitham 1989; Boecklen & Spellenberg 1990; Fritz *et al.* 1994; Floate & Whitham 1995; Dungey *et al.* 2000; Wimp *et al.* 2005), as

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well as among different plant genotypes within a single species (Fritz & Price 1988; Maddox & Root 1990; Shuster *et al.* 2006; Donaldson & Lindroth 2007). The effects of plant diversity on arthropod herbivores extend to higher trophic levels where changes in prey resources lead to changes in predators and parasitoids among different plant species (Pimentel 1961; Greenstone 1984; Harvey *et al.* 2003), among plant species and their hybrids (Preszler & Boecklen 1994; Eisenbach 1996), and among plant genotypes (Price & Clancy 1986; Stiling & Rossi 1996; Underwood & Rausher 2000; Hare 2002; Bailey *et al.* 2006; Crutsinger *et al.* 2006). Understanding how species discriminate among different plant genotypes has begun to alter the way we view the role of dominant or foundation species (Ellison *et al.* 2005) in shaping arthropod community structure (Whitham *et al.* 2003, 2006). Such discrimination among plants can subsequently affect arthropod diversity and community structure. While an increase in interspecific plant diversity has long been known to affect diversity in the dependent arthropod community (Siemann *et al.* 1998), recent studies have shown that increasing diversity within interbreeding plant systems (i.e. hybrids and single plant species) also leads to an increase in the diversity of the dependent arthropod community (Wimp *et al.* 2004; Reusch *et al.* 2005; Crutsinger *et al.* 2006; Johnson *et al.* 2006; Tovar-Sanchez & Oyama 2006).

Knowledge of the fine-scale associations between arthropods and plants plays an important role in our understanding of the impact of plant genetic diversity on dependent arthropod communities. However, links to the mechanisms that may be driving these relationships remain poorly understood. Phenotypic traits affecting arthropod herbivore communities fall into three primary categories: phenological, mechanical, and chemical, all of which have a genetic basis, but have only rarely been linked to both plant genetics and arthropod community structure (Johnson & Agrawal 2005; Bangert *et al.* 2006). Plant phenology affects both the quantity and quality of resources available to insect herbivores, thus the precise timing of insect eclosion in conjunction with leaf bud break can affect herbivore performance, population density, and evolution (Yukawa 2000; Tikkanen & Julkunen-Tiitto 2003; Abott & Withgott 2004). Plants may also defend themselves from herbivores by erecting physical barriers such as leaf trichomes (Turnipseed 1977; Lambert *et al.* 1995), or internally via changes in leaf toughness (Lucas *et al.* 2000). In addition to mechanical defences, plants defend themselves against herbivores with a wide array of secondary chemicals.

An overwhelming amount of evidence indicates that secondary chemistry plays a major role in the preference of insect herbivores for particular hosts (Ehrlich & Raven 1964; Becerra 1997; Mutikainen *et al.* 2000), and their subsequent performance on those hosts (Rosenthal & Berenbaum 1992; Hwang & Lindroth 1997; Mutikainen *et al.* 2000). Most of these studies have examined the relationship between

plant secondary chemistry and a focal insect species, while fewer studies have examined impacts on larger groups of arthropod herbivores (Feeny 1970; Dungey *et al.* 2000; Abrahamson *et al.* 2003; Forkner *et al.* 2004; Bangert *et al.* 2006a). Even fewer studies have examined the community-wide impacts of secondary chemistry on an arthropod community that encompasses multiple trophic levels and feeding groups. Such an extension is important because insect herbivore response to secondary chemistry can be largely influenced by feeding group of the herbivore (Forkner *et al.* 2004).

Not only does plant secondary chemistry play a large role in structuring arthropod herbivore communities, but it is also influenced by plant genetics (Hamilton *et al.* 2001; Rehill *et al.* 2005), and thus presents a potential mechanistic link between plant genetic variation and arthropod community structure. Genetic variation in *Salix*, *Populus* and *Betula* leads to differences in condensed tannins and other phenolic compounds (Nichols-Orians *et al.* 1993; Hwang & Lindroth 1997; Laitinen *et al.* 2005), which have also been shown to influence populations and communities of arthropod herbivores (Hwang & Lindroth 1997; Forkner *et al.* 2004). However, while plant genotype plays an important role in the production of secondary metabolites, their foliar concentration varies through the season (Osier *et al.* 2000; Riipi *et al.* 2002; Rehill *et al.* 2006), with some chemicals decreasing and others increasing in concentration. Therefore, measuring the links between host-plant genotype, secondary chemistry, and arthropod community structure through the season becomes important as these relationships change through time.

As plant genetics have been shown to play an important role in the production of secondary chemistry (Hamilton *et al.* 2001), and changes in plant chemistry play a predominant role in arthropod host preference, plant chemistry could provide the critical mechanism by which plant genetics affects arthropod community composition. While numerous studies have documented the relationship between host-plant genetics and arthropod community composition (e.g. Dungey *et al.* 2000; Hochwender & Fritz 2004; Wimp *et al.* 2004, 2005), understanding the pathway by which plant genetics affects arthropod communities, not simply the beginning and endpoints, will be crucial in our ability to form predictive hypotheses across diverse systems. Likewise, when we solely examine the effects of plant chemistry on arthropod composition without taking genetics into account, we may not have a complete understanding of the relative importance of genetic vs. environmental factors underlying differences in chemistry, and these two drivers of plant chemistry may have different temporal effects on arthropod communities.

We studied a naturally hybridizing cottonwood system to test the effects of plant genetics on chemistry and the associated arthropod community. We sought to determine

the relative strength of the relationship between plant genetics, chemistry, and an arthropod community that spans multiple trophic levels and feeding groups (253 species in total) by addressing the following hypotheses: (1) Plant chemistry will form an intermediate link between plant genetics and arthropod community composition. We predicted the strongest relationship would exist between plant genetics and plant chemistry, followed by plant chemistry and arthropod community composition. We predicted the weakest relationship between plant genetics and arthropod community composition. (2) The strength of the relationship between the arthropod community and plant chemistry will change as the season progresses. Understanding the strength of these links is important as they provide a mechanistic explanation for the relationship between plant genetics and arthropod community structure.

## Materials and methods

### Garden surveys

We surveyed foliar chemistry and the arthropods that naturally colonized a common garden (planted in 1991) using clones from each of the four cottonwood cross types (*Populus fremontii*, Fremont cottonwood; *Populus angustifolia*, narrowleaf cottonwood) and their natural hybrids ( $F_1$  and backcross hybrids) found growing naturally along the Weber River, Utah, USA. The backcross hybrids found in the garden represented the full range of backcrosses in nature, that is from trees that are genetically similar to their  $F_1$  parents to trees that are genetically similar to their narrowleaf parents. At the time of our experiments in 2003, the trees were ~12 years old, ~12 m tall, and had reached reproductive maturity. Pure or hybrid status of trees in the common garden was verified using restriction fragment length polymorphism (RFLP) analysis (Keim *et al.* 1989; Martinsen *et al.* 2001). Martinsen *et al.* (2001) used 35 species-specific probe-restriction enzyme combinations to screen 550 trees that were randomly selected throughout the Weber River and represented in the common garden. These markers represented fixed polymorphisms between Fremont and narrowleaf cottonwoods. While Martinsen *et al.* (2001) used these data to determine the rate of Fremont marker introgression into the narrowleaf genome, we used the same data to determine the pure and hybrid status of individual genotypes. In our common garden, we chose blocks that contained each of the four cottonwood cross types (Fremont cottonwood,  $F_1$  hybrid, backcross hybrid, and narrowleaf cottonwood). Trees were blocked for age, sex, and position along an irrigation gradient to reduce microsite effects. To control for potential differences in leaf biomass among cross types that could affect arthropod abundance and diversity, we standardized the amount of leaf biomass surveyed per tree using the allometric

relationship established between cottonwood stem diameter and leaf biomass (Fischer *et al.* 2002), and trees within a block were surveyed at the same time of day. Common arthropod species were visually surveyed, and new species were collected and identified for 10 trees on each of the four cross types (40 trees). Visual arthropod surveys were performed so that we could resample the same trees multiple times during the course of the growing season without disturbing the extant community. Based on previous species accumulation curves, we surveyed each tree for a minimum of 20 min. Arthropods were keyed and verified to species or classified to morphospecies based on previous observations of life cycle, mating individuals, and/or large morphological differences among individuals within a genus or family. All individuals that had been observed to participate in the cottonwood arthropod community were recorded. Trees were sampled three times (June, July, and August) throughout the course of the cottonwood-growing season and a total of 170 arthropod species were observed (Table S9, Supplementary material).

### Stand surveys

To compare results from our common garden trees with trees growing in the wild, we sampled 77 cottonwoods (39 Fremont, 20  $F_1$ , 18 backcross) growing along the Weber River that were located within a 13-km hybrid zone. Pure narrowleaf cottonwoods are rare in the hybrid zone (Wimp *et al.* 2005); therefore, our backcross classification represents backcrosses and the rare narrowleaf trees in hybrid zone which are collectively referred to as backcrosses. Trees were chosen haphazardly and buds from these trees were collected for amplified fragment length polymorphism (AFLP) analysis to assess the relationship between cottonwood genetic diversity, foliar chemistry, and arthropod diversity. AFLP marker protocols followed that of Vos *et al.* (1995) with modifications from Travis *et al.* (1996; see Wimp *et al.* 2004 for details). All polymorphic bands were scored, and markers that exhibited the dominant allele in less than 5% of the individuals were discarded from the analysis. A total of 47 AFLP marker loci were used in our statistical analyses (see below). Using the methods described above under 'Common Garden Surveys', we surveyed arthropods three times (June, July, and August) during the growing season and a total of 246 arthropod species were observed (Table S9).

### Chemical analyses

We collected leaves for chemical analysis at the same time (June, July and August) as trees were surveyed for arthropods in both the Ogden Nature Center common garden and the natural stands along the Weber River. We removed the fifth leaf from the current year's growth at the

petiole-blade junction from four to five branches (15–20 leaves) that were at roughly the same insertion point as the branch that was surveyed for arthropods. Leaf samples were immediately frozen between blocks of dry ice. Leaves were lyophilized and ground in a Wiley Mill to pass a 40-mesh screen and stored at  $-20^{\circ}\text{C}$  until chemical analyses could be performed.

We quantified nitrogen, the phenolic glycosides salicortin and HCH-salicortin, and condensed tannins because these chemicals have been shown to be ecologically important (Mattson 1980; Palo 1984; White 1984; Hemming & Lindroth 1995; Lindroth & Hwang 1996; Hwang & Lindroth 1997; Osier *et al.* 2000; Osier & Lindroth 2001). HCH-salicortin concentrations are detectable only in Fremont (*P. fremontii*) and  $F_1$  (*P. fremontii*  $\times$  *angustifolia*) cottonwood trees (Rehill *et al.* 2005). Foliar concentrations (percentage of dry weight) of each chemical were used to calculate the chemical composition and Euclidean distance between individual trees for use in Mantel tests.

Nitrogen was quantified using an elemental analyser (LECO) with glycine *p*-toluenesulphonate as a reference standard. Condensed tannins were determined by the acid butanol assay (Porter *et al.* 1986) after leaves were extracted in 70% acetone with 1 mM ascorbate at  $4^{\circ}\text{C}$ . Narrowleaf cottonwood condensed tannins were purified following methods similar to Hagerman & Butler (1994) and used as the standard. Foliar phenolic glycosides were quantified using high performance thin layer chromatography (Lindroth *et al.* 1993). Purified salicortin and HCH-salicortin from cottonwood leaves (Rehill *et al.* 2005) were used as standards.

### Statistical analyses

Four previous years of survey have demonstrated unremarkable differences in arthropod species richness and abundance among crosstypes (Wimp *et al.* 2004, 2005), but striking differences in arthropod composition that scale-up to accurately predict arthropod diversity at the level of a cottonwood stand (Wimp *et al.* 2004). We therefore used compositional analyses to examine the relationships between plant genetics, chemistry, and the arthropod community, as arthropod response to host-plant genetics in our study system is primarily compositional in nature. Arthropod community, chemical, and genetic compositions among tree types, in both common gardens and natural stands, were graphically analysed using nonmetric multidimensional scaling (NMDS; Kruskal 1964; Minchin 1987; Clarke 1993). NMDS has successfully been used to analyse compositional data among treatments (Dungey *et al.* 2000; Wimp *et al.* 2004, 2005; Bangert *et al.* 2006). Similarity matrices were constructed for the arthropod data using the Bray–Curtis coefficient and Euclidean distance matrices were constructed for the chemical and genetic data (Legendre & Legendre 1998). Differences among tree types were

quantified with analysis of similarity (ANOSIM) on the compositional matrices, which is analogous to an *F*-test with *P* values determined through a randomization procedure of 999 randomizations (Warwick *et al.* 1990). Multiple comparisons in ANOSIM were made using a sequential Bonferroni correction (Rice 1989). We then performed similarity percentages (SIMPER, Clarke & Warwick 2001) to determine the percent contribution that each arthropod species made to the overall dissimilarity among different cross types. SIMPER was only performed between crosstypes that were found to be significantly different in arthropod composition as determined by ANOSIM. To examine whether changes in arthropod community composition were correlated with changes in ecologically important chemicals (i.e. salicortin, HCH-salicortin, and condensed tannins), we used indirect gradient or vector analysis. Vector analysis determined the maximum correlation between the levels of salicortin, HCH-salicortin, and condensed tannins and the configuration of points (i.e. the arthropod community found on the same trees) in the ordination. Significance was determined using 1000 random permutations of the data to determine if the observed vector fit was significantly different than that because of chance alone (Minchin 1987; Faith & Norris 1989).

While NMDS and subsequent analyses may help us to visualize patterns among plant genetics and chemistry, plant chemistry and arthropod composition, and plant genetics and arthropod composition, such analyses do not account for the potential covariance between genetics and chemistry that may account for changes in arthropod composition. Covariance between genetics and chemistry in affecting arthropod community composition also demonstrates the importance of chemistry in mediating the relationship between plant genetics and arthropod composition. Therefore, to address our initial hypothesis that evaluates tree chemical composition as a potential mechanistic link between genetic and arthropod composition (hypothesis 1), partial Mantel tests were used (analogous to partial correlations; Legendre & Fortin 1989; Fortin & Gurevitch 1993; Legendre 1993; Manly 1997; Legendre & Legendre 1998; Vellend 2004). We used partial Mantel tests because they are useful in distinguishing the relationships among correlated variables. The chemical data were natural log transformed before the calculation of the matrices to improve linearity. The Mantel procedure is used for multivariate compositional data and does not separate out each component of the associated composition, but rather treats one of these matrices as an entire unit.

## Results

### *Cross type differences in chemistry and arthropods*

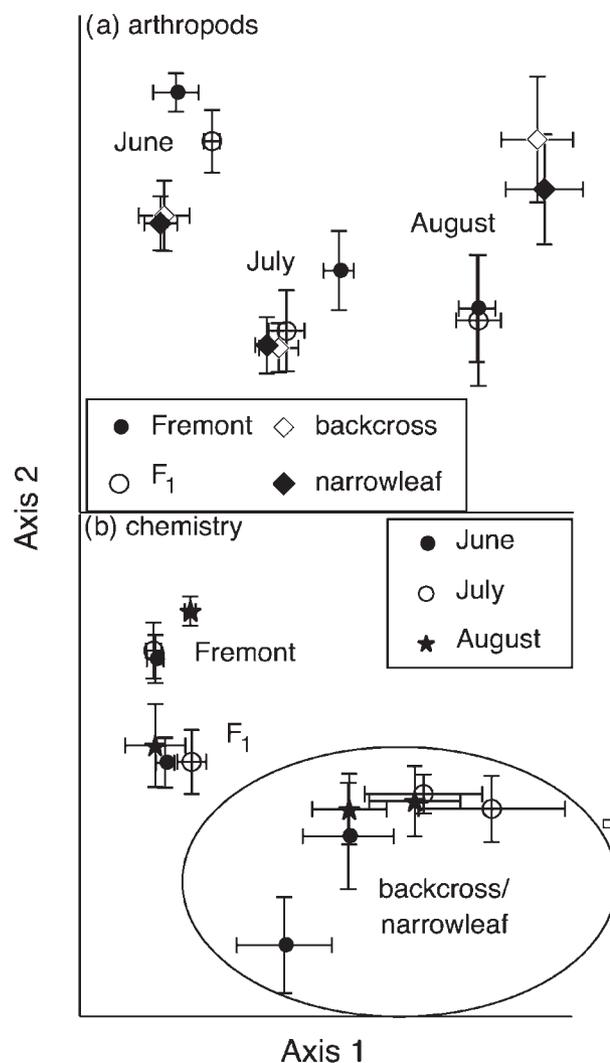
Variation in genetic composition among cottonwood cross types was highly significant (Table 1) suggesting that the

**Table 1** Overall ANOSIM demonstrating compositional differences in plant genetics (RFLP & AFLP), arthropods, and plant chemistry among the different cottonwood crosstypes

	Overall $r$	$P$ value
Common Garden		
RFLP	0.735	< 0.0001
Arthropods	0.495	< 0.0001
Chemistry	0.352	< 0.0001
Natural Stands		
AFLP	0.840	< 0.0001
Arthropods	0.403	< 0.0001
Chemistry	0.479	< 0.0001

cross-type categories based on leaf morphology represent accurate genotypic classes. In the garden, where RFLP data were required to distinguish between the backcross and pure narrowleaf classes, the genetic compositions between these two cross types were marginally different ( $r = 0.08$ ,  $P = 0.054$ ). The community and chemical compositions between these two cross types were not different (community: all  $p$  range 0.53–0.98; chemistry: all  $p$  range 0.34–0.84). Because both the narrowleaf and backcross cross types exhibited the same relationship with the other cross types, we report comparisons among only Fremont,  $F_1$ , and backcross for the garden data (see Tables S1, S2, Supplementary material). Thus, combining the backcross and rare narrowleaf tree (Wimp *et al.* 2005) in the natural stands into a single category ('backcross') does not change the interpretation of the community and chemical differences among the cross types.

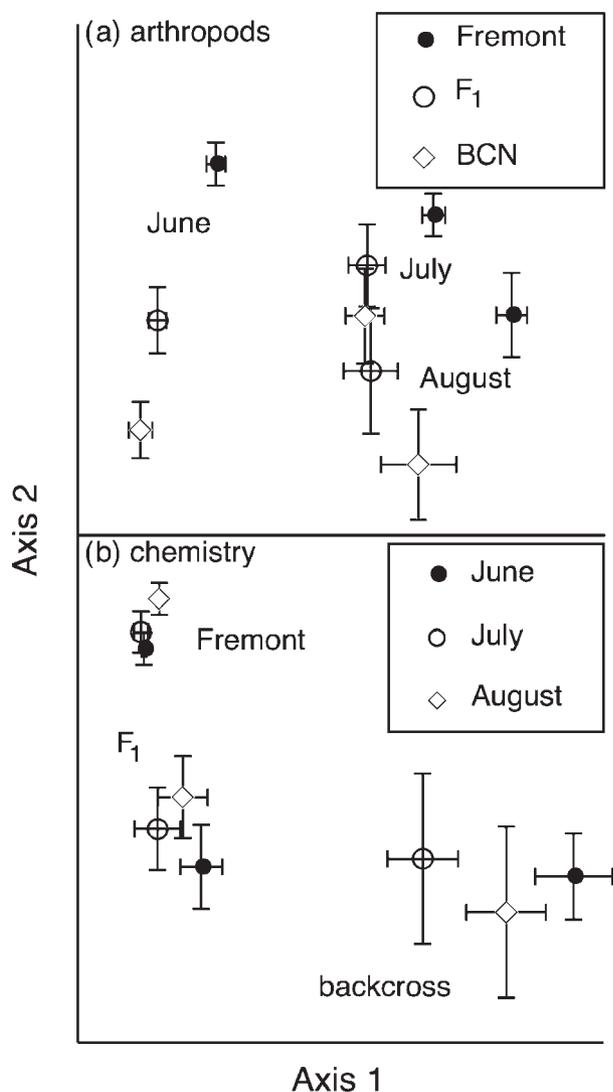
Comparisons of arthropod and chemical compositions among the cross types exhibited similar patterns in the common garden and the natural stands (Tables S1–S4, Supplementary material; Figs 1 and 2). We did not find differences in arthropod community composition or chemistry between backcross and narrowleaf cross types in the garden. We did find significant differences in arthropod community composition among Fremont,  $F_1$  and backcross/narrowleaf cross types across months, demonstrating intraseasonal variation (Table S1; Fig. 1a). Using SIMPER, we found that leaf- and stem-modifying arthropods (*Pemphigus betae*, *Aceria parapopuli*, and *Paraleucoptera albella*; Table S5, Supplementary material) made the greatest contribution to differences in arthropod composition among crosstypes. Likewise, in the garden, we did not find significant differences in chemical composition between backcross and narrowleaf tree types or between Fremont and  $F_1$  with a sequential Bonferroni correction. Chemical composition in the garden was different between both the Fremont/ $F_1$  group and the backcross/narrowleaf group during all months, but tended not to exhibit temporal changes (Table S2; Fig. 1b). When we used vector analysis



**Fig. 1** Results from NMDS ordinations showing arthropod composition (a) and chemical composition (b) through the season in the common garden. Error bars represent  $\pm 1$  standard error.

to determine the correlation between arthropod composition and ecologically important plant chemicals (i.e. salicortin, HCH-salicortin, and condensed tannins), we found that all three secondary chemicals were correlated with changes in arthropod composition early in the growing season, but their importance declined through time and was eventually lost at the end of the season (Table S7, Supplementary material).

In the natural stands, similar patterns were observed but they were more discrete. The arthropod communities were different among cross types and months, demonstrating temporal turnover (Table S3; Fig. 2a). Similar to the common garden, leaf- and stem-modifying arthropods (*P. betae*, *A. parapopuli*, and *Lepidosaphes ulmi*) made the greatest



**Fig. 2** Results from NMDS ordinations showing arthropod composition (a) and chemical composition (b) through the season in the natural stands. Error bars represent  $\pm 1$  standard error.

contribution to differences in arthropod composition among cross types in the natural stands (Table S6, Supplementary material). Interestingly, there was a large degree of overlap in species that made the greatest contribution to differences in community structure among crosstypes for both the common garden and natural stands (Tables S5, S6). Stand chemical compositions were significantly different among cross types during all months, but showed little temporal change (Table S4; Fig. 2b). However, Fremont cottonwoods showed a change in chemical composition from June to August, corresponding to a nearly 25% decrease in both foliar phenolic glycosides and foliar nitrogen content. When we once again used vector analysis to determine the correlation between plant chemicals and arthropod composition, we found: salicortin was never

significantly correlated with arthropod composition, HCH-salicortin was significantly correlated with arthropod composition early in the season but not at the end of the season, and condensed tannins were consistently correlated with arthropod composition throughout the season (Table S7).

#### Seasonal relationships among genes, arthropods, and chemistry

Partial Mantel tests quantified the relationships between all pairwise comparisons of plant genetics, plant chemistry, and arthropod composition while controlling for the third factor (upper right triangle of Tables 2 and 3; Fig. 3). The relationship between plant genetics and chemistry (controlling for the effect of arthropods) was consistent between the garden and natural stands and this relationship was much stronger than the other two relationships. The other two relationships (genetics and arthropods; chemistry and arthropods) showed variable patterns and the relative strength of all of these relationships changed through the season. In general, the correlations between variables became weaker as the season progressed (Tables 2 and 3; Fig. 3). In the common garden, the relationship between plant genetics and arthropod community composition was significant only in June (Fig. 3). This pattern contrasted with results in the natural stands where the relationship between plant genetics and arthropods remained significant across all 3 months (Fig. 3). This difference between the common garden and natural stands may be due to

**Table 2** Partial Mantel  $r$ -values for the relationships between the genetic, chemical, and arthropod compositions in the common garden (after Legendre 1993)

	Genes	Chemistry	Arthropods
June			
Genes	—	0.456**	-0.272***
Chemistry	0.493***	—	-0.133*
Arthropods	-0.098 NS	-0.156*	—
July			
Genes	—	0.531***	-0.117 NS
Chemistry	0.540***	—	-0.134 NS
Arthropods	-0.046 NS	-0.161*	—
August			
Genes	—	0.380***	-0.051 NS
Chemistry	0.377***	—	-0.111 NS
Arthropods	-0.059 NS	-0.131*	—

Partial Mantel  $r$ -values for the entire arthropod community are reported in the upper right triangle and  $r$ -values for the arthropod community without the stem and leaf modifiers are in the lower left triangle of the table.  $P$  values for the partial Mantel tests are based on 999 randomizations (\*\*\*) represents  $P \leq 0.001$ . Chemistry data were LN transformed, before the calculation of the distance matrices, to improve linearity. LN, natural log; NS, not significant.

**Table 3** Partial Mantel  $r$ -values for the relationships between the genetic, chemical, and arthropod compositions in the natural stands (after Legendre 1993)

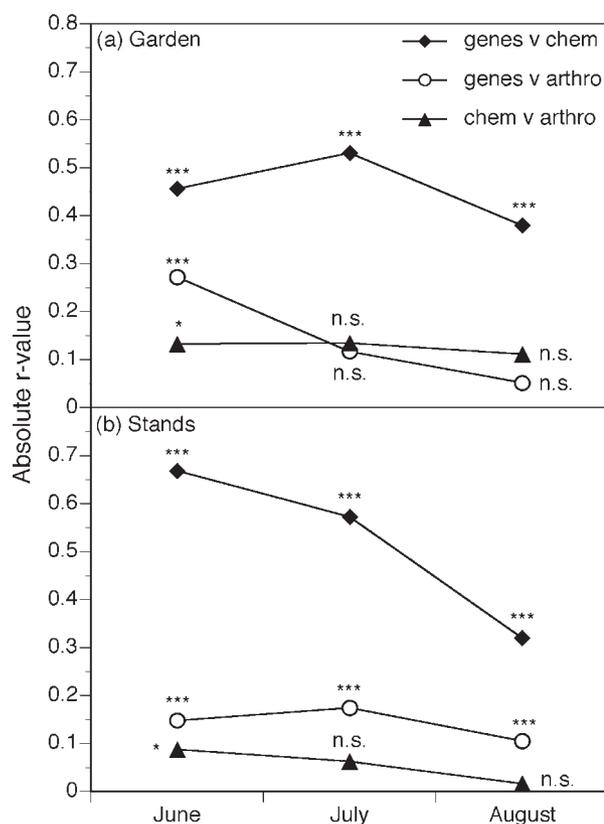
	Genes	Chemistry	Arthropods
June			
Genes	—	0.669***	-0.148***
Chemistry	0.685***	—	-0.087*
Arthropods	-0.044 NS	-0.083*	—
July			
Genes	—	0.573***	-0.175***
Chemistry	0.593***	—	-0.062 NS
Arthropods	0.068**	0.003 NS	—
August			
Genes	—	0.320***	-0.105***
Chemistry	0.326***	—	-0.016 NS
Arthropods	-0.058*	0.053 NS	—

Partial Mantel  $r$ -values for the entire arthropod community are reported in the upper right triangle and  $r$ -values for the arthropod community without the stem and leaf modifiers are in the lower left triangle of the table.  $P$  values for the partial Mantel tests are based on 999 randomizations (\*\*\*represents  $P \leq 0.001$ ). NS, not significant.

differences in the molecular techniques used to characterize cottonwood genetic composition. In the natural stands, we used AFLP techniques which cover a larger proportion of the cottonwood genome relative to the RFLP techniques used in the garden. Additionally, there may be differences in water availability between the garden and natural stands. Because the cottonwoods in the common garden are growing further away from the river, they begin to exhibit signs of water stress earlier than trees growing in the natural stands (G.M. Wimp, personal observation). Thus, environmental variation may begin to trump genetic variation as the major factor affecting arthropod community composition late in the cottonwood growing season. Additionally, the relationship between chemistry and arthropod community composition also became weaker over time and was significant only in June in both the garden and natural stands (Fig. 3). This may be partly due to the fact that highly toxic chemicals such as phenolic glycosides declined by as much as 25% over the course of the season, making plant chemistry a less important factor to arthropods later in the season. Differences in plant chemistry may therefore be more important to arthropod choice and colonization early in the season, whereas environmental factors such as biotic interactions and drought stress may become important later in the cottonwood growing season.

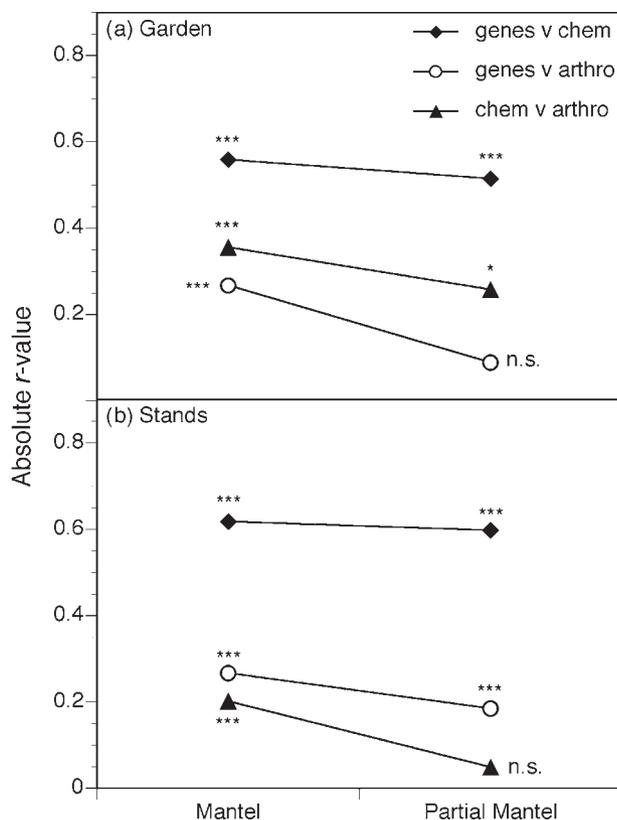
#### *Plant chemistry as a link between genetics and arthropod communities*

If chemistry is the critical link between plant genetics and arthropod community composition, then we expect to see



**Fig. 3** Partial Mantel correlations between genetic markers (genes) and chemical composition (chem), genetic markers and arthropod composition (arthro), and chemical and arthropod composition in the garden (a) and the natural cottonwood stands (b) across the season.

a stronger relationship between chemistry and arthropods than between plant genetics and arthropods (hypothesis 1). When the data were analysed as annual means for chemistry and arthropod community compositions, hypothesis 1 was supported for the garden data but not in the natural stands (Fig. 4). In the garden, the relationship between arthropods and plant genetics became nonsignificant when chemistry was controlled with a partial Mantel test (Fig. 4a), which demonstrates covariance between plant genetics and chemistry and supports our hypothesis that plant genetics is an intermediate link between plant chemistry and arthropod composition. In the natural stands, the relationship between arthropods and plant genetics was stronger than that between chemistry and arthropods (Fig. 4b). The relationship between chemistry and arthropods became nonsignificant when plant genetics was controlled with the partial Mantel test, suggesting that there are other important genetically based factors driving the relationship that were not measured (Fig. 4b). When we analysed these data at the finer scale of intra-annual



**Fig. 4** Mantel and partial Mantel results of the mean arthropod community and chemical compositions for the entire season. When the community is analysed as a single seasonal average, the results fit the genetic similarity rule for the common garden and the natural stands appear to be an exception to this rule. Because these factors are autocorrelated, the partial Mantel results are weaker than the simple correlations revealing the actual strength of these relationships. Because the genetic vs. arthropod relationship becomes nonsignificant in the partial Mantel test in the garden, this suggests that chemistry is a potential intermediate link between genes and arthropods. Likewise, for the chemical vs. arthropod relationship in the garden and natural stands, this suggests that there are other important genetically based factors.

variation, we found that in the common garden, the partial Mantel correlations were strongest between arthropods and genetics in June (Table 2; Fig. 3a). In the natural stands, the relationship between genetics and arthropods was consistently stronger than the relationship between chemistry and arthropods (Table 3; Fig. 3b). The different patterns found in the common gardens relative to the natural stands may also be due to the timing of our surveys. Trees in the common garden were sampled 2 weeks before trees in the natural stands, and we may not have captured important patterns found in the common garden for the natural stands before phenolic glycosides

began to decline. Additionally, arthropod abundance is higher in the common gardens relative to the natural stands early in the season and demonstrates a much steeper decline late in the growing season (Table S8, Supplementary material). Such changes in the arthropod species pool could affect composition leading to difference in the strength of the response exhibited by trees in the common garden relative to the natural stands.

The importance of chemistry as the primary mechanism driving the relationship between plant genetics and arthropod community structure may also be largely influenced by the patterns exhibited in the leaf- and stem-modifying arthropods. Leaf- and stem-modifying arthropods are correlated with phytochemistry (Bangert *et al.* 2006), rely on plant phenology (Abott & Withgott 2004), and are often numerically dominant members of the arthropod community found on cottonwoods. When the leaf- and stem-modifying arthropods are removed from the analysis, the relative effects of plant genetics on the rest of the arthropod community generally become weaker (lower left triangles of Tables 2 and 3). This suggests that the leaf- and stem-modifying members of the arthropod community strongly influence these patterns. These results further indicate that while plant chemistry plays an important role in the composition of the leaf- and stem-modifying arthropod community in the garden (cf. upper and lower triangles in Table 2), other genetically based traits influence host use by leaf-modifiers, as evidenced by the stronger correlations between arthropods and genes relative to arthropods and chemistry in the wild (cf. upper and lower triangles in Table 3). Therefore, additional genetically based traits that influence the composition of leaf and stem modifiers are affecting overall patterns of arthropod community composition in both the natural stands and in the common garden.

## Discussion

We hypothesized that plant chemistry would form an intermediate link between plant genetics and arthropod community composition (hypothesis 1), with the strongest correlations found between plant genetics and plant chemistry, then plant chemistry and arthropods, and the weakest correlation between plant genetics and arthropods. We found the strongest correlation between plant genetics and plant chemistry, and this relationship remained strong through time in both the common garden and natural stands. When we analysed the relationships between genetic, chemical, and arthropod similarity averaged across the growing season, we found chemistry formed an intermediate link between plant genetics and arthropod composition in the common garden, but not in the wild. Examining these relationships at a finer, intra-annual scale, we found chemistry to be an intermediate link between plant genetics and free-feeding arthropods in the common

garden, and early season in the wild. However, the inclusion of leaf- and stem-modifying arthropods in the analysis altered these patterns such that plant chemistry was not an intermediate link between plant genetics and arthropods in either the common garden or in the wild. Thus, while our results generally support our first hypothesis for free-living arthropods, we find that plant chemistry is no longer an intermediate link between plant genetics and arthropods when we include the leaf and stem modifiers at a finer scale. Therefore, plant chemistry does not provide a mechanistic explanation for the relationship between plant genetics and arthropod community structure in the most specialized and sedentary group of arthropods within a growing season. Our data also provide support for our hypothesis (hypothesis 2) that the relationship between the arthropod community and plant chemistry would change as the season progressed. The following subsections discuss the patterns found under hypothesis 1 in greater detail.

#### *The plant genetics/chemistry link*

The role of plant genetics in the production of secondary metabolites has been demonstrated through numerous studies (e.g. Fajer *et al.* 1992; Marquis 1992; Keinanen *et al.* 1999). Indeed, across plant taxa, plant genotype accounts for 50–100% of the phenotypic variation in the production of plant secondary metabolites (Hamilton *et al.* 2001). Our study found that there was a significant correlation between plant genetic composition and chemical composition, both in the common garden and the natural stands through time. We conclude that plant genetics plays an important role in the production of secondary metabolites in the cottonwood system, and the relationship remains important seasonally and across environments.

#### *The plant chemistry/arthropod link*

Our data do not entirely support hypothesis 1 (Fig. 3), for two reasons. First, the leaf and stem modifiers were partially responsible for the patterns we found in the community and their composition was more strongly related to plant genetics than to plant chemistry when compared to other arthropod trophic levels and feeding groups. Plant chemistry is known to play a large role in plant choice by galling arthropods (e.g. Abrahamson *et al.* 2003). Therefore, we either (i) did not capture plant chemistry at the critical time that these arthropods were making decisions about oviposition and gall initiation, or (ii) plant chemistry is only one mechanism driving their association with specific plant genotypes. Other genetically mediated plant traits, such as phenology (e.g. Yukawa 2000), may play a crucial role in plant choice by leaf and stem modifiers (Abott & Withgott 2004). Using experimental crosses of the same

parental tree species as the current study, Woolbright *et al.* (in review) identified three quantitative trait loci (QTL) for the timing of leaf flush in the spring that could affect leaf- and stem-modifying arthropods. Similarly, Floate *et al.* (1993) found that phenological differences in leaf flush among trees in natural stands altered the feeding and subsequent fecundity of female beetles (*Chrysomela confluenta*) by as much as 600%. Additionally, since gall initiation requires precise timing in order to take advantage of undifferentiated tissue that is in a stage of rapid growth (e.g. Abott & Withgott 2004), plant phenology may be as or more important than plant chemistry in determining leaf- and stem-modifying arthropod preferences.

Second, while the relationship between plant genetics and chemistry remains consistently strong through the season, the relative importance of chemistry to the associated arthropods changes through the season. This may be because some of the most toxic chemicals found in the plant (such as phenolic glycosides) declined by as much as 25% as the season progressed, which might increase the number of generalists that are able to take advantage of the different plant genotypes. Many of the most specialized arthropods found on cottonwoods (i.e. leaf and stem modifiers) that use plant secondary metabolites as cues for oviposition and gall initiation (e.g. Abrahamson *et al.* 2003) are primarily early-season feeders in the cottonwood system and complete their development by early July. We may therefore have seen an increase in generalization through time as the relative concentration of toxic secondary metabolites in the cottonwoods decline.

Measuring other plant chemicals could also improve our ability to explain the patterns between arthropod community composition and plant chemistry. For example, chemicals other than N, phenolic glycosides (PG) and condensed tannins (CT) that also vary seasonally (e.g. carbohydrates) can have important effects on the arthropod community (Riipi *et al.* 2005). Herbivores may also respond to visual and nonvolatile cues that differ among cottonwood cross types, but are not related to the production of secondary metabolites.

#### *The plant genetics/arthropod link through genetic similarity*

Although numerous studies have found arthropod diversity to be positively influenced by increased genetic diversity among different plant species (Southwood 1961; Murdoch *et al.* 1972; Siemann *et al.* 1998), plant species and their hybrids (Boecklen & Spellenberg 1990; Fritz *et al.* 1994; Floate & Whitham 1995; Dungey *et al.* 2000), and within a plant species (Fritz & Price 1988; Maddox & Root 1990; Reusch *et al.* 2005; Bangert *et al.* 2006b; Crutsinger *et al.* 2006; Johnson *et al.* 2006), our study demonstrates a potential mechanism for this diversity. The partial Mantel

results indicate that when plants are more genetically similar, their arthropod communities are likewise similar, whereas plants that are less related have arthropod communities that are less similar. In other words, plant genetic diversity increases arthropod diversity (Wimp *et al.* 2004), with different arthropod species keying-in to particular cottonwood genotypes, partially as a result of genetically based plant chemistry. In fact, the relationship between plant genetics and arthropods remains consistently significant throughout the season in the natural stands (a 13-km hybrid zone), where environmental variation among stands is likely to be large. In the common garden, the relationship between plant genetics and arthropods is significant early in the growing season and becomes nonsignificant through time. The stronger correlation between genetics and the arthropod community in the wild relative to the common garden could be related to molecular techniques; the AFLP markers used in the natural stands cover a larger portion of the cottonwood genome relative to the RFLP markers used in the common garden. Our results also demonstrate that the relationship between plant genetics and arthropod diversity is strongly affected by a group of specialist arthropods (i.e. leaf and stem modifiers) that must manipulate the host-plant cottonwood to form their domicile.

We expect leaf and stem modifiers to be a very specialized group with respect to plant genotype, as they require specific genotypes for oviposition and gall initiation (e.g. Moran & Whitham 1990). In fact, we found that leaf and stem modifiers were largely responsible for differences in arthropod community composition among crosstypes. That we did not find a correlation between plant genetics and arthropod composition in the common garden and for one sample date in the wild when we removed the leaf- and stem-modifying arthropods from the analysis indicates that specialization on cottonwood hosts by members of this feeding group drives many of the patterns we see between host-plant genetics and arthropods in this system. Therefore, including both leaf modifiers and free feeders in a single community analysis may give a strong result that could be an artefact of other important genetically based factors not accounted for in the model.

### *Conservation implications*

In our study, we found that genetically similar cottonwood trees supported similar arthropod communities, whereas cottonwoods that were relatively dissimilar in genetic composition also had arthropod communities that were more dissimilar. These findings have important implications for riparian restoration and habitat conservation. First, over \$14 billion have been spent on riparian restoration since 1990 by nonprofit and government organizations in the continental USA (Bernhardt *et al.* 2005). Planting

genetically diverse stocks of cottonwoods in these areas should increase arthropod species diversity and potentially the species diversity of vertebrates who feed on these arthropods, resulting in improved restoration success. Second, when choosing the size of a habitat that is needed to support dependent species, genetic diversity in plant resources should also be considered. By choosing areas that maximize genetic diversity in the dominant or foundation species, we are also conserving their interactions with dependent species, thereby maximizing the total number of species that will be protected in our restoration and conservation efforts (e.g. Bangert *et al.* 2005).

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The work described in this manuscript is part of a collaborative effort to understand the mechanisms driving Arthropod community responses to plant genetics. G. M. Wimp, R. K. Bangert and T. G. Whitham are concerned with Arthropods and community analyses. S. Wooley, B. Rehill, and R. L. Lindroth study the effects of plant phytochemistry on Arthropod communities. W. P. Young, G. D. Martinsen and P. Keim examine the genetic structure of cottonwood hybrid zones.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1** ANOSIM analysis for the Garden arthropod data.

**Table S2** ANOSIM analysis for the Garden chemistry data.

**Table S3** ANOSIM analysis for the natural stand arthropod data.

**Table S4** ANOSIM analysis for the natural stand chemistry data.

**Table S5** Percent contribution of different arthropod species to compositional dissimilarities among crosstypes in the common gardens as measured by SIMPER.

**Table S6** Percent contribution of different arthropod species to compositional dissimilarities among crosstypes in the natural stands as measured by SIMPER.

**Table S7** Indirect gradient analysis results for data from the common garden and natural stands across all months of study.

**Table S8** Arthropod species richness and abundance according to crosstype in the common garden and natural stands for all months of study.

**Table S9** Arthropod species list for surveys in both the common garden and natural stands.

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