Evol Ecol (2013) 27:65–81
DOI 10.1007/s10682-012-9582-2

Data across several host species; the one that matches our framework is P. angustifolia, with multiple pops represented.

ORIGINAL PAPER

Herbivore host-associated genetic differentiation depends on the scale of plant genetic variation examined

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Received: 17 March 2012 / Accepted: 14 May 2012 / Published online: 4 June 2012

Abstract Herbivore adaptation to plant genetic variation can lead to reproductive isolation and the formation of host-associated lineages (host-associated differentiation, or HAD). Plant genetic variation exists along a scale, ranging from variation among individual plant genotypes to variation among plant species. Along this scale, herbivores may adapt and diverge at any level, yet few studies have examined whether herbivore differentiation exhibits scaling with respect to host variation (e.g., from genotypes to species). Determining at which level(s) herbivore differentiation occurs can provide insight into the importance of plant genetic variation on herbivore evolution. Previous studies have found strong genetic differentiation in the eriophyid mite, *Aceria parapopuli*, between hybrid *Populus* hosts and parental *Populus* species, but minimal neutral-locus differentiation among individual trees of the same species. We tested whether genetic differentiation in *A. parapopuli* scales with genetic variation in its *Populus* hosts. Using mite ITS1 sequence data collected among host species and among host populations, two key patterns emerged. (1) We found strong differentiation of *A. parapopuli* among *Populus* species, supporting the hypothesis that plant species differences drive reproductive isolation and HAD. (2) We did not find evidence of host-driven genetic differentiation in mites at the level of plant populations, suggesting that this level of plant variation is insufficiently strong to drive differentiation at a neutral locus. In combination with previous studies, we found that HAD occurs at the higher levels of plant genetic variation, but not at lower levels, and conclude that HAD depends on the scale of plant genetic variation examined.

Keywords Host-associated differentiation · Scale-dependence · *Aceria parapopuli* · *Populus*

Electronic supplementary material The online version of this article (doi:10.1007/s10682-012-9582-2) contains supplementary material, which is available to authorized users.

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Introduction

Differentiation of herbivores on alternate plant hosts (host-associated differentiation, or HAD) occurs when herbivore lineages adapt to and diverge in response to plant variation, and is one reason for the astounding diversity in phytophagous arthropod lineages (Via 2001; Dres and Mallet 2002; Stireman et al. 2005; Dickey and Medina 2010). Plant variation occurs along a hierarchical scale from variation among individual plants, among populations, and among plant species, as well as their hybrids, with the potential for herbivore differentiation at any of these levels. Studies of plant-driven HAD have typically been performed at only one of the levels along this scale (Mopper et al. 2000; Stireman et al. 2005; Dickey and Medina 2010). However, research that examines multiple levels simultaneously may be particularly useful because in addition to identifying instances of HAD, it is also important to understand which level(s) (e.g., individuals to species) are important for herbivore evolution.

Different levels of plant genetic variation may be evolutionarily important for different herbivores. For example, some herbivores may show host-related differentiation among plant individuals (Mopper et al. 2000) or among populations within a species (Cogni and Futuyma 2009). Others may show no relationship to plant population variation, but exhibit HAD at the plant species scale (Sword et al. 2005). Still others may reveal no relationship between herbivore and host at any level (Kohnen et al. 2011). As an example, Rank (1992) found population structure in *Chrysomela aeneicollis* leaf beetles at multiple levels, from structure among regional populations, to localities within river drainages, and also among individual willow hosts within localities. Rank (1992) suggested that structure among individual willows was related to drift among small populations, and among drainages and localities to geographical barriers and dispersal limitations; therefore, beetle population structure was not related to variation in its host willows.

The above studies suggest that HAD is influenced by various factors, one of which is likely plant genetic variation. By examining HAD at different levels of plant genetic variation, we can begin to understand which level(s) influence herbivore evolution and ultimately draw conclusions about associated ecological processes and mechanisms. Here, we examine HAD across a range of plant genetic variation from populations to species using cottonwoods and the bud-galling mite, *Aceria parapopuli*.

Aceria parapopuli Kiefer is a widely distributed species that attacks the buds of all North American species of *Populus* (Kiefer 1940; Drouin and Langor 1992; Amrine and Stasny 1994; Baker et al. 1996). Mites form woody, cauliflower-like galls, which can contain hundreds of individuals, and exhibit arrhenotokous parthenogenesis where unmated females can produce haploid males, while fertilized females produce diploid daughters (Helle and Wysoki 1996). Mites crawl along twigs, and windborne dispersal studies (Zhao and Amrine 1997; Bergh 2001) and population genetic studies (Evans et al. 2008) indicate they are capable of long-distance dispersal. *Populus* susceptibility to these mites is genetically based (Kalischuk et al. 1997; McIntyre and Whitham 2003).

Previous work of the bud-galling mite has examined neutral genetic structure between, and adaptation to, hybrid trees v. parental species and among individual hybrid trees (McIntyre and Whitham 2003; Evans et al. 2008). In that work, we found that, *A. parapopuli* was strongly differentiated between hybrid *Populus* and its parental species (Evans et al. 2008), and that mites are locally adapted to hybrid trees (McIntyre and Whitham 2003). Most of the neutral locus genetic variation in *A. parapopuli* was found to occur between hybrid trees and parental species. Furthermore, we found that mites can be locally adapted to individual trees despite evidence of gene flow (Evans et al. 2008). For instance,

only two pairwise comparisons of mite population structure were significant among all pairwise comparisons of mite populations on 10 individual *Populus angustifolia* trees. Among 55 pairwise comparisons of mites on 11 F_1 hybrid trees, 10 were significantly different, and this pattern was related to geographical isolation. Among galls within individual trees, mite galls on F_1 hybrid trees can be different, but those on *P. angustifolia* are not different from one another, and in fact most populations are monomorphic and identical (Evans et al. 2008). Thus, most of the neutral genetic variation in *A. parapopuli* results from differences between hybrids and parental species of *Populus* hosts. Among mites on different *P. angustifolia* individuals there is very little variation, and among mites on individual F_1 hybrid trees, neutral locus differentiation is mostly driven by isolation-by-distance. Mites may still be locally adapted to individual trees, indicating that differences among F_1 hybrid trees may not lead to mite reproductive isolation, but are strong enough to impose local selection on the mites (Evans et al. 2008). Such evidence of selection in the face of gene flow has been observed in a number of other systems (e.g., Chevillon et al. 1995; Mullen and Hoekstra 2008).

These previous findings indicate substantial variation in *A. parapopuli*, and raise the question: At what level of *Populus* variation do we find evidence of *A. parapopuli* reproductive isolation and HAD? To examine how plant variation at multiple levels affects herbivore differentiation and evolution, we investigated population structure in *A. parapopuli* Kiefer (Acari: Eriophyidae) on its *Populus* hosts. An implicit assumption is that variation among plant hosts is greatest among species and decreases as one moves down the scale to the level of individual plant hosts. We have not directly tested this assumption; however, as a conceptual framework, individuals within a classified species are likely to be more similar to one another than individuals of different species. Furthermore, if there is significant population structure, individuals from different populations will be more different from each other than individuals from the same population. Hybridization can result in increased genetic variation within a hybrid swarm (Whitham et al. 1999), and may involve additive, epistatic, and dominant genetic interactions, thus leading to traits that may be intermediate, equal to, or more extreme than the parental species (Fritz 1999). Despite the uncertainty in the quantification of hybrid variation, it represents a level of genetic variation that can drive herbivore evolution (Evans et al. 2008). Here, we collected mites from different *Populus* species and different populations of one host species (*P. angustifolia*). Using data derived from the internal transcribed spacer (ITS1) region in mites, we tested the hypothesis that *Populus* variation at both of these levels drives HAD in *A. parapopuli*. We then tested the overarching hypothesis that HAD is scale-dependent in *A. parapopuli* by assessing evidence of HAD at each level (from species to individuals) by evaluating our current findings (mite HAD among *Populus* species and populations) in the context of previously reported findings (mite HAD among hybrid v. parental species and individual trees; Evans et al. 2008).

Methods

Study system and collections

Aceria parapopuli galls were collected during the summer of 2007 throughout western North America from *P. angustifolia* James, *P. tremuloides* Michaux, *P. fremontii* S. Watson, and *P. deltoides* Marshall (Fig. 1). The latter two, in section *Aigeiros*, are sister taxa based on genetic and phenotypic variation (Eckenwalder 1977, 1984; Ford 2004) but

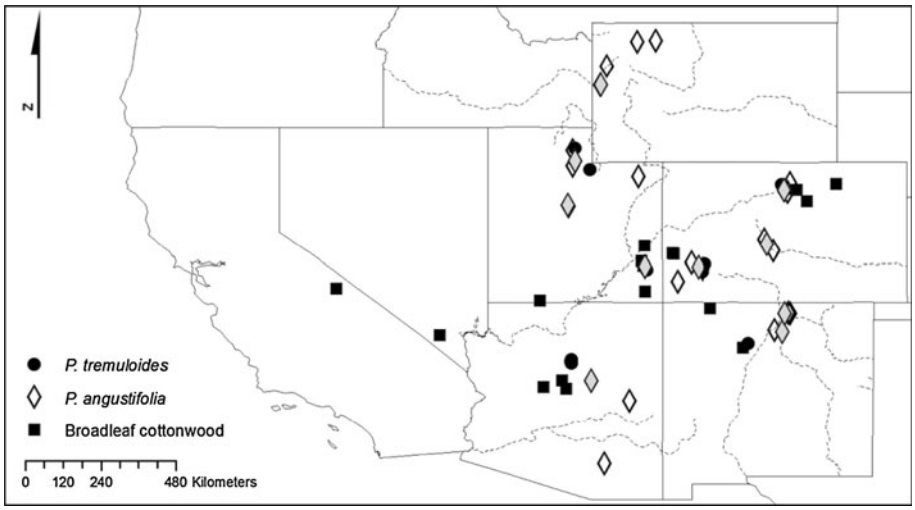

Fig. 1 Sampling location of mites on three species of *Populus* hosts. *Grey diamonds* represent populations of mites sampled from multiple populations of *P. angustifolia* throughout its range. Location abbreviations are as follows: Jack's Canyon, AZ (AZJC), Arkansas R., CO (COAR), Big Thompson R./St. Vrain Cr., CO (COBTSV), San Miguel R., CO (COSM), Rio Pueblo, NM (NMRP), Red R., NM (NMRR), Indian Cr., UT (UTIC), Salt Cr., UT (UTSC), Weber R., UT (UTWR), Snake R., WY (WYSR)

authorities (and preliminary molecular evidence [T. L. M. and L. M. E., unpubl. data]) dispute the classification of broadleaf trees occurring in the four-corners region, and have suggested that those populations in eastern Utah and southwestern Colorado may be hybrids between the two species (Eckenwalder 1977). For this study, we classified all mites from *P. fremontii* and *P. deltoides* as collections from a single "broadleaf" cottonwood host. Hence, our sampling scheme was designed to assess the variation in *A. parapopuli* on each of three host species. We sampled mites on one to two individual hosts per host type at each location from throughout the areas of overlap for each host. We collected (and stored in 95 % ethanol) a total of 18 mites from *P. tremuloides* hosts, 21 mites from broadleaf hosts, and 27 mites from *P. angustifolia* hosts (Fig. 1).

To determine the extent of population structure within a single host species, we sampled intensively from *P. angustifolia*. We collected *A. parapopuli* from 7 to 21 individual *P. angustifolia* trees in each of 10 different rivers (Fig. 1). One mite gall per tree was collected in ethanol. One mite per tree was subsequently used for sequencing the ITS1 region of nuclear ribosomal DNA (see below). Additionally, 3–4 leaves from each tree were collected, dessicated with Drierite (Drierite Co. LTD, Xenia, OH), and stored at room temperature until DNA extraction.

DNA extraction, amplification, and sequencing

To examine population structure of *A. parapopuli* we sequenced the 499 bp ITS1 region from individual mites using the extraction, amplification, and sequencing methods of Evans et al. (2008). Some individuals were heterozygous at multiple positions. Therefore, we determined the gametic phase of haplotypes using PHASE v. 2.1 (Stephens et al. 2001) as implemented in DnaSP (Librado and Rozas 2009). In the collections among *Populus* species, twenty positions were variable, comprising 11 different haplotypes. Among mites

on *P. angustifolia*, we found 9 ITS1 haplotypes comprising 14 variable positions. Ploidy differs between male and female eriophyid mites; males are haploid while females are diploid (Helle and Wysoki 1996). We were unable to determine haploid males from homozygous females as DNA extraction precluded sexing individuals and sexing individuals requires clearing mites for microscopy; therefore, we randomly chose one haplotype per individual (from PHASE output) for analysis so that ploidy level would not bias our results (as in Carew et al. 2004; Evans et al. 2008). We constructed haplotype accumulation curves using the R package *vegan* (Oksanen et al. 2011).

To determine the extent that mite differentiation is determined by genetic variation within a single host species, we compared patterns of population structure between mites and a single host, *P. angustifolia*. Seventeen simple sequence repeat (SSR) loci were chosen from a list of potential loci identified by the *Populus* Genome Project from the *P. trichocarpa* genome sequence (Tuskan et al. 2004, publically available at http://www.ornl.gov/sci/ipgc/ssr_resource.htm). We chose these 17 markers because of their reliable repeat motifs (most are tri- or tetranucleotide repeats), ease of amplification, and distribution throughout the genome. Please see the supplementary material and Table S1 for the primer names and sequences, and PCR methods.

Populus angustifolia reproduces both sexually and vegetatively. Collections from clones were minimized by collecting from stems > 5 m apart, but ramets of the same genet may have been collected. We identified clones using a method similar to Mock et al. (2008), and used 105 unique genotypes for further analyses (see supplementary material for additional details).

Analyses

We tested our hypothesis that HAD is scale-dependent in *A. parapopuli* by examining population structure of mites in relation to its host at each level of plant genetic variation, from species to individuals. To test the hypothesis that differences among *Populus* species have driven HAD in *A. parapopuli*, we used Analysis of Molecular Variance (AMOVA) as implemented in Arlequin v.3.1 (Excoffier et al. 2005).

To address the hypothesis that within host species population structure influences HAD in mites, we compared the population structures of mites on several populations of *P. angustifolia*. We first examined structure within mites and within *P. angustifolia* separately using AMOVA. To examine the role of geographic isolation in population differentiation, we used Mantel tests to determine if pairwise geographic distance between populations was correlated with pairwise $F_{ST}/(1 - F_{ST})$. We tested for a correlation between mite and host pairwise $F_{ST}/(1 - F_{ST})$ when the effect of geographic distance was removed using partial Mantel tests in Arlequin v.3.1.

Although samples of *P. angustifolia* were selected based on morphology, it is possible that advanced backcrossed hybrid individuals were included, as these are often indistinguishable from *P. angustifolia*. If different populations of *P. angustifolia* exhibit different levels of advanced introgression, such advanced introgression could potentially affect herbivore population structure. To test for this potential effect of advanced hybridization, we performed an analysis of admixture in the *P. angustifolia* samples using *structure* 2.3.3 (Pritchard et al. 2000; Falush et al. 2003). We examined the degree of introgression in our *P. angustifolia* samples from other *Populus* species by including samples of *P. fremontii*, *P. deltoides*, and one other *Populus* species (*P. balsamifera*) that occurs in the sample area (*P. fremontii* and *P. deltoides* samples were collected avoiding the area of overlap and potential hybridization mentioned above). We genotyped these samples at all 17 SSR loci

using the methods described above. We used the admixture model with correlated allele frequencies, with the putative species used as LOCPRIOR information. We ran 10 iterations of the program each with 15,000 steps as a burnin period, followed by 20,000 steps through the Gibbs chain for each of K = 1 through K = 14. Visual inspection of chains indicated that convergence was reached and the chains had mixed well. To choose the best K, the ΔK statistic (Evanno et al. 2005) was estimated using Structure Harvester (http://taylor0.biology.ucla.edu/struct_harvest/). *CLUMPP* (Jakobsson and Rosenberg 2007) was used to combine the results of the 10 replicate runs, and *distruct* (Rosenberg 2004) was used to create visual images.

To address possible hybrid origins of the *P. angustifolia* populations, we constructed a tree of the genetic clusters identified in *structure*. We used the net nucleotide distance among the clusters, output from *structure*, and averaged across the 10 runs, and constructed a neighbor-joining tree using the neighbor package in Phylip 3.66 (Felsenstein 2004). We also constructed trees from each of the 10 runs independently, all of which had the exact same tree topology.

To address our overarching hypothesis that *A. parapopuli* HAD is scale-dependent with respect to *Populus* variation, we drew on previously published work (Evans et al. 2008) in addition to the collections and analyses described above. Specifically, we drew from the results and findings of Evans et al. (2008) to assess *A. parapopuli* HAD at the level of plant hybrid v. parental species and at the level of individual plants. Results from those two levels (from Evans et al. 2008) were used in combination with results presented in the current study to examine mite HAD along a scale of *Populus* variation.

Results

Host-associated differentiation of *A. parapopuli* on different *Populus* host species

Aceria parapopuli collected throughout the western United States showed significant differentiation among different *Populus* host species (Table 1). Over 60 % of the molecular variance in *A. parapopuli* ITS1 sequence data was due to differences among host species ($F_{ST} = 0.63$, $p < 0.001$). The haplotype accumulation curve (supplementary material Fig. S1a) began to plateau, which suggests that we sampled the most common haplotypes. Mite haplotype frequencies were quite different among hosts (Table 2), demonstrating that genetically distinct groups of mites are found on different host species.

Pairwise differentiation between mite populations found on different hosts was strong and significant (Table 3). The greatest differentiation was found between mites on

Table 1 Scale of mite F_{ST} at different levels of tree variation

Scale of *Populus* genetic variation level	*Aceria parapopuli*			References
	F_{ST}	Host-driven HAD	Local Adaptation	
Species	0.21–0.74	Yes	–	This study
F_1 hybrid versus parental species	0.66	Yes	Yes	Evans et al. (2008), McIntyre and Whitham (2003)
Population	0.00–0.90	No	–	This study
Individual tree	0.08–0.10	No	Yes	Evans et al. (2008)

Table 2 Frequency of mite ITS1 haplotypes from different host species and from each *P. angustifolia* population

Haplotype	A	B	C	D	E	F	G	H	I	J	K	L	M	P
Populus host species														
P. tremuloides	0.06	0.35	–	0.53	–	–	0.06	–	–	–	–	–	–	–
Broadleaf	–	–	–	–	–	–	0.67	0.05	0.19	0.05	0.05	–	–	–
P. angustifolia	–	–	0.52	–	0.07	0.07	0.04	–	–	–	0.30	–	–	–
P. angustifolia population														
AZJC	–	–	–	–	–	–	–	–	–	–	0.9	–	–	0.1
COAR	–	–	0.43	–	–	0.14	0.07	–	–	–	0.36	–	–	–
COBTSV	–	–	1	–	–	–	–	–	–	–	–	–	–	–
COSM	–	–	0.93	–	0.07	–	–	–	–	–	–	–	–	–
NMRP	–	–	0.33	–	–	0.22	–	–	0.12	–	0.33	–	–	–
NMRR	–	–	0.89	–	–	–	–	–	–	–	–	–	0.11	–
UTIC	–	–	0.36	–	–	–	–	–	–	–	0.55	0.09	–	–
UTSC	–	–	0.92	–	–	–	0.08	–	–	–	–	–	–	–
UTWR	–	–	0.95	–	–	0.05	–	–	–	–	–	–	–	–
WYSR	–	–	1	–	–	–	–	–	–	–	–	–	–	–

Table 3 Pairwise differentiation of *A. parapopuli* on alternate *Populus* hosts

Host Species	*P. tremuloides*	Broadleaf	*P. angustifolia*
P. tremuloides	–	<0.002	<0.002
Broadleaf	0.764	–	<0.002
P. angustifolia	0.246	0.685	–

Pairwise F_{ST} values below diagonal

Pairwise *p* values above diagonal based on 500 permutations

broadleaf hosts and mites on the other two hosts (Table 3). These data support a hypothesis of HAD for mites among different *Populus* species. The fact that this differentiation was found despite geographically overlapping collections indicates that host species, not geographical isolation, drive mite genetic differentiation.

Population differentiation of mites on *P. angustifolia*

Aceria parapopuli found on *P. angustifolia* was strongly genetically structured ($F_{ST} = 0.27$, $p < 0.001$) and haplotype frequency varied greatly among rivers (Table 2). Again, the haplotype accumulation curve began to plateau, though not as much as the samples from different host species (supplementary material Fig. S1b). Among the 131 individuals sequenced, 95 were haplotype C and 24 were haplotype K. Therefore, we sampled the common haplotypes within mites on *P. angustifolia* hosts, as well as many uncommon haplotypes.

Population structure of *A. parapopuli* did not follow an isolation-by-distance pattern, nor did it correspond to geographic regions (Table 4). While there was a significant

Table 4 Pairwise F_{ST} estimates between populations of mites (below diagonal) and *P. angustifolia* (above diagonal)

	AZJC	COAR	COBTSV	COSM	NMRP	NMRR	UTIC	UTSC	UTWR	WYSR
AZJC	–	*0.289*	*0.309*	*0.315*	*0.234*	*0.353*	*0.328*	*0.335*	*0.406*	*0.446*
COAR	*0.240*	–	*0.040*	*0.069*	0.033	0.029	*0.068*	*0.162*	*0.232*	*0.240*
COBTSV	*0.857*	*0.134*	–	*0.057*	*0.074*	*0.082*	*0.061*	*0.121*	*0.198*	*0.211*
COSM	*0.747*	*0.143*	0.000	–	*0.075*	*0.087*	0.008	*0.082*	*0.080*	*0.085*
NMRP	*0.260*	0.000	*0.174*	*0.183*	–	0.024	*0.090*	*0.180*	*0.247*	*0.269*
NMRR	*0.693*	0.057	0.000	0.000	0.080	–	*0.093*	*0.230*	*0.290*	*0.301*
UTIC	*0.205*	0.000	*0.367*	*0.327*	0.024	*0.233*	–	*0.103*	*0.100*	*0.094*
UTSC	*0.674*	0.081	0.000	0.000	*0.109*	0.000	*0.253*	–	*0.062*	*0.066*
UTWR	*0.779*	*0.165*	0.000	0.000	*0.215*	0.002	*0.382*	0.000	–	*0.000*
WYSR	*0.910*	*0.247*	0.000	0.023	*0.326*	0.103	*0.516*	0.049	0.003	–

Comparisons with $p < 0.05$ are in italics

correlation between genetic differentiation and linear distance (Mantel $R = 0.46$, $p = 0.006$; Fig. 2a), the largest pairwise comparisons occurred between Jack's Canyon, AZ and other populations. When this population was removed, the correlation became nonsignificant (Mantel $R = 0.09$, $p = 0.27$; Fig. 2a), suggesting that nearby populations are not more closely related than populations farther apart.

Populus angustifolia also showed significant population structure throughout its range ($F_{ST} = 0.14$, $p < 0.001$), and, unlike the mites, followed an isolation-by-distance pattern and genetic clusters roughly approximated large geographic boundaries. Pairwise F_{ST} estimates between populations ranged from 0.0 to 0.44 (Table 4), and there was a significant correlation between genetic differentiation and linear distance (Mantel $R = 0.74$, $p < 0.001$; Fig. 2b). As with the mites, many of the largest pairwise F_{ST} values occurred in comparisons between Jack's Canyon, AZ and other populations. However, when the Jack's Canyon population was removed, the correlation remained strong (Mantel $R = 0.86$, $p < 0.001$; Fig. 2b), suggesting that the isolation-by-distance pattern is not just driven by one strongly differentiated population.

The *structure* analysis indicated the highest ΔK at $K = 2$ for our *Populus* samples (supplementary material Fig. S2). Using $K = 2$, sections *Aigeros* and *Tacamahaca* were reliably differentiated (data not shown), but species within these sections were not. The next highest ΔK was found at $K = 6$, which differentiated species, as well as three groups in the *P. angustifolia* samples (Fig. 3). The western populations were similar, and those in southwestern CO and southeastern UT populations were closely related. Populations from the eastern slope of CO and NM were also similar, suggesting that natural geographic features affect the population structure of *P. angustifolia*.

Our initial analyses suggested that *P. angustifolia* population structure drove *A. parapopuli* structure. For example, we found a significant correlation between pairwise mite and *P. angustifolia* population structure (Mantel $R = 0.66$, $p = 0.003$; Fig. 4), and a partial Mantel test indicated that the correlation remained strong and significant when geographic distance was controlled for (partial Mantel $R = 0.54$, $p = 0.042$). However, when the Jack's Canyon, AZ site was removed from the analysis, there was no correlation between mite and tree population structure (Mantel $R = 0.012$, $p = 0.44$) even after

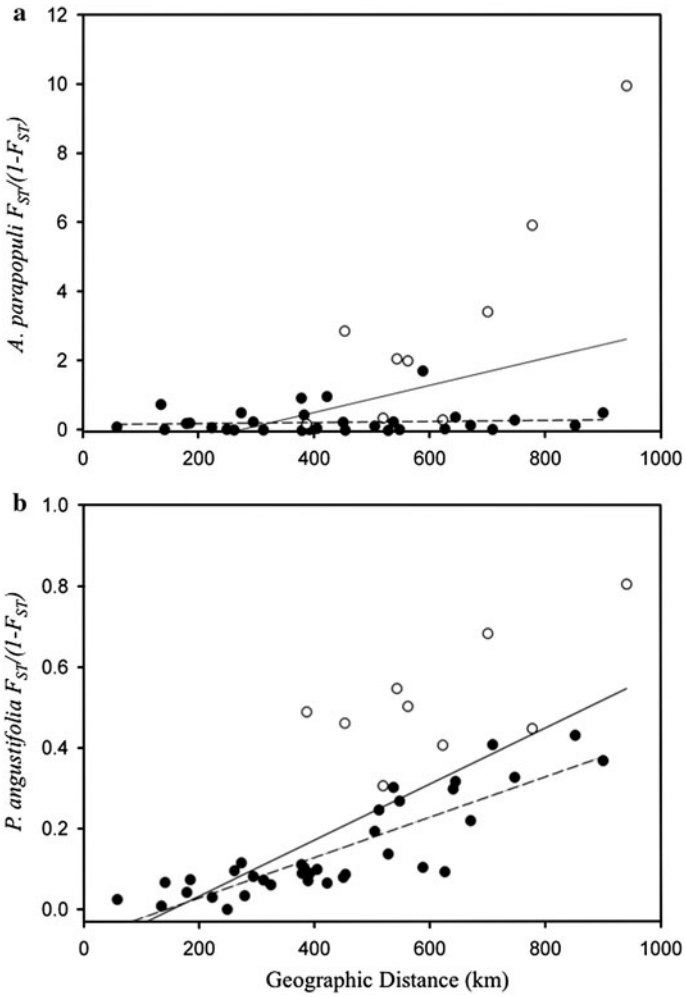

Fig. 2 **a** Relationship between pairwise distance and pairwise genetic differentiation between mite populations on *P. angustifolia*. **b** Relationship between pairwise distance and pairwise genetic differentiation between populations of *P. angustifolia*. *Open circles* represent pairwise comparisons with population AZJC. *Solid line* represents relationship when all populations are included, while *dashed lines* represents relationships when AZJC is removed

controlling for geographic distance (partial Mantel $R = -0.21$, $p = 0.87$). Thus, more similar mite populations were not found on more similar tree host populations. This suggests *A. parapopuli* differentiation is not, in general, affected by population genetic structure *within P. angustifolia*.

Advanced host hybridization and mite differentiation

Although hybridization of trees can drive mite differentiation (Evans et al. 2008) limited evidence of advanced introgression of other *Populus* species within our *P. angustifolia*

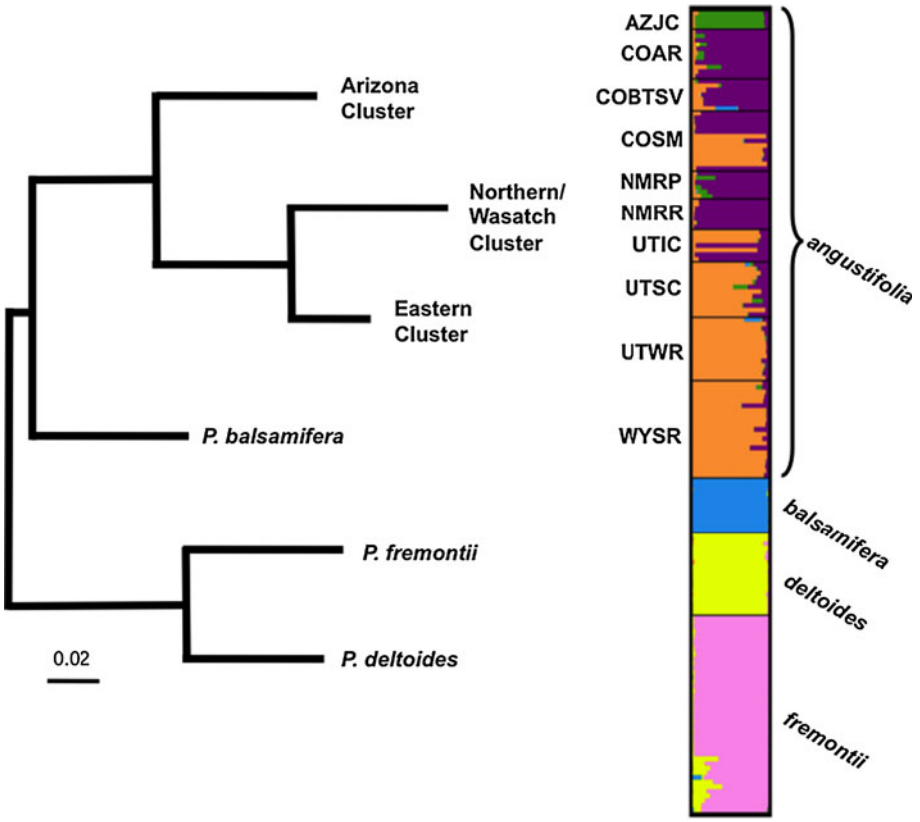

Fig. 3 Visualization of *structure* output when K = 6 for *Populus* and neighbor joining tree based off of net nucleotide distance among the clusters. *Bars* represent individual trees, with the proportion of contribution from a given group within each individual indicated by *different colors*. *Populus* species indicated above bar chart and collection location of *P. angustifolia* indicated below. See Fig. 1 for population abbreviations

samples refutes the hypothesis that differential advanced introgression among rivers drives *A. parapopuli* differentiation. In our *structure* analysis, using K = 6 we found limited evidence of advanced introgression in *P. angustifolia* from the other species. We found few individuals to be admixed with other species, and with so little evidence of introgression we found no systematic differences in introgression among rivers (Fig. 3).

Rather than indicating hybridization differences among *P. angustifolia* populations, the groupings corresponded roughly to populations east and west of the Continental Divide and a third consisting of the Jack's Canyon population along the Mogollon Rim, AZ (the most divergent), with some evidence of admixture among these three *P. angustifolia* groups (Fig. 3). Populations in the center of the species range (San Miguel R., CO and Indian Cr., UT) appear to be a mixture of the eastern and western groups, supporting the hypothesis that geographic features drive population structure in *P. angustifolia*. The three groups clustered together in our distance-based tree based on the net nucleotide distance from *structure*, which did not support a hybrid origin for any of the groups.

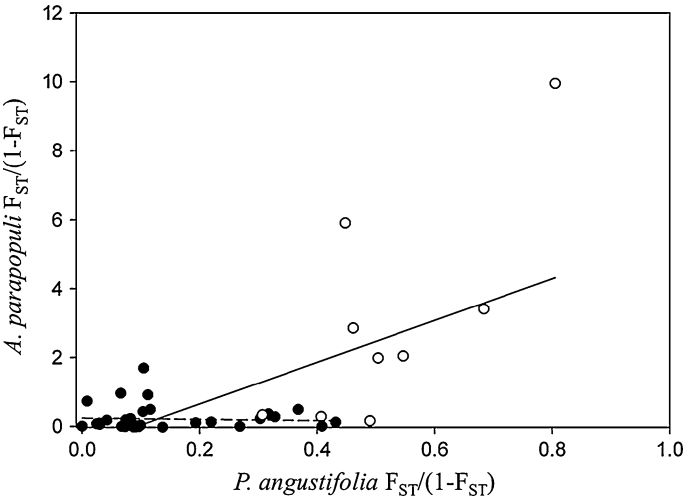

Fig. 4 Correlation between pairwise population differentiation of mite populations and *P. angustifolia* populations. *Open circles* represent pairwise comparisons with population AZJC. *Solid line* represents relationship when all populations are included, while *dashed line* represents relationship when AZJC is removed

Discussion

Mite differentiation among *Populus* species

Aceria parapopuli is significantly differentiated among *Populus* hosts, suggesting that variation among *Populus* species has driven mite genetic differentiation. The strong structure of mites on different *Populus* species (Tables 1, 2, 3) indicates clear differentiation among all hosts. Mites on *P. angustifolia* and *P. tremuloides* were less differentiated from one another than from mites on broadleaf cottonwood hosts; therefore, these mites may be more recently diverged, or there may be limited, but ongoing gene flow between them.

Current mite differentiation cannot be due to geographical isolation because of the overlap in host species' ranges (Fig. 1). For example, from single sites, we collected differentiated mites from *P. angustifolia* and *P. tremuloides* within meters of one another. Therefore, we conclude that adaptation of *A. parapopuli* to different species of *Populus* has likely driven reproductive isolation and subsequent HAD in mites at a level consistent with diagnoses of species in other arthropods ($F_{ST} = 0.16$–0.65; Nason et al. 2002; Abbot and Withgott 2004; Blair et al. 2005; Evans et al. 2008).

Population structure of *P. angustifolia* and mites

In contrast to the strong influence that *Populus* species have on mite differentiation, within one tree species we find little evidence for a corresponding impact of tree genetic structure on mite genetic structure. Differentiation within *P. angustifolia* is moderate ($F_{ST} = 0.14$), and populations appear to cluster along major geographic boundaries or river basins (Table 4; Fig. 3). Such strong genetic structuring is uncommon in forest trees (including *Populus*), typically characterized by large effective population sizes, high gene flow, and

low structure (Hamrick et al. 1992; Hamrick and Godt 1996; Slavov and Zhelev 2010). However, the *structure* analysis found three major divisions within *P. angustifolia* corresponding to major geographical divisions (one corresponding to populations east of the Continental Divide, one corresponding to the western populations, and Jack's Canyon, AZ). The isolated, montane canyons where *P. angustifolia* is found may also promote such structure. Thus, the strong structure seen in *P. angustifolia* is not unexpected given the large sampling area and geographic divides.

Mites are also strongly structured among populations, but we found no clear clustering related to geographical features and no evidence of isolation by distance when the most isolated population (Jack's Canyon, AZ) was removed (Fig. 2a). The haplo-diploid reproduction of eriophyid mites and their fast generation time of $\sim$2 weeks (Helle and Wysoki 1996) possibly results in populations founded by a very small number of migrant female mites through wind dispersal, with subsequent rapid drift leading to strong population differentiation unrelated to distance or geographic features. Thus, chance dispersal, founder effects, and random drift are likely the most important forces creating mite population genetic structure within a host species.

Despite population structure in both mites and *P. angustifolia*, we found little evidence to support the hypothesis that tree population genetic structure influences mite genetic structure. The strong correlation between mite and tree F_{ST} was lost when Jack's Canyon, AZ, a clear outlier, is removed and there was no general pattern (Fig. 4). Though correlations between species have been found in plant-enemy (Jerome and Ford 2002a, b) and plant-mutualist (Anderson et al. 2004) systems, such patterns may not be a general phenomenon, with other studies finding no relationship in population structure between interacting species. For example, Ren et al. (2008) found that differentiation in the sumac aphid, *Schlechtendalia chinensis*, is unrelated to its *Rhus chinensis* hosts, and Michalakis et al. (1993) found that the weevil, *Larinus cynarae,* and its thistle host plant, *Onopordum illyricum*, appear to have different patterns of population structure.

The Jack's Canyon, AZ population of mites is strongly differentiated, which may reflect a combination of factors. First, it is isolated and the only population sampled from the Mogollon Rim, a region surrounded by vast stretches of desert unsuitable to *P. angustifolia* hosts. Such isolation of *A. parapopuli* may result in little gene flow with mites in other regions, and subsequent population differentiation. Second, *P. angustifolia* from Jack's Canyon, AZ is also strongly differentiated from other regions. If mites perform best on natal trees, as shown by Evans et al. (2008), immigrating mites may have low success rates resulting in mite isolation due to host differences. Finally, at this site, the paired differentiation of mites and their hosts may reflect a combination of these two factors. The geographic isolation of the Mogollon Rim may have facilitated the biological isolation of the mites with respect to their hosts.

Advanced host hybridization and mite differentiation

While Evans et al. (2008) and McIntyre and Whitham (2003) showed the importance of F_1 hybridization on mite evolution, we found no evidence that differences in advanced hybridization among *P. angustifolia* populations influence mite population structure. *P. angustifolia* hybridizes with three different *Populus* species throughout our collection range (Eckenwalder 1984), but we found little evidence of advanced hybridization in our collections of *P. angustifolia* and no systematic differences among trees from different rivers (Fig. 3). Furthermore, none of the genetic clusters identified in our *structure* analysis appeared to be of hybrid origin (Fig. 3). Therefore, while differences between F_1 hybrid

trees and their parental species appear to drive mite evolution (Evans et al. 2008), mite genetic differentiation among rivers (see above) is not related to the degree of advanced introgression or different sources of introgression into *P. angustifolia* among rivers.

Mite differentiation along a scale of *Populus* variation

In this study and in Evans et al. (2008), we have examined *A. parapopuli* differentiation at multiple levels of *Populus* genetic variation, from differences among *Populus* species to differences among individual trees (Table 1). Along this scale, we find that genetic variation in *A. parapopuli* at a single neutral locus is related to higher levels of host variation (among species and hybrids v. parental species), but not at lower levels (among populations within a species and individual trees). Therefore, our findings support the hypothesis that *A. parapopuli* HAD is scale-dependent. This suggests that the conclusion of host-driven reproductive isolation (HAD) can depend on the level of host genetic variation (species to individuals) examined in a given study.

Our findings reflect the effects of host variation on adaptation leading to reproductive isolation and HAD in *A. parapopuli*. McIntyre and Whitham (2003) performed transfer experiments to show that mites from F_1 hybrid trees are locally adapted to those F_1 hybrids over *P. angustifolia* trees. Thus, adaptation to natal host type at the hybrid v. parental species level has occurred alongside neutral-locus genetic differentiation, demonstrating the influence of tree hybridization on mite evolution. Alternatively, Evans et al. (2008) found that local adaptation to individual trees occurred without neutral locus differentiation, suggesting that variation among individual host plants may not be strong enough to drive reproductive isolation, but it is strong enough to impose selection on the mites and result in local adaptation even in the presence of ongoing gene flow. Although we find that HAD is scale-dependent in this study, our findings only reflect the influence of host genetic variation on reproductive isolation and associated neutral locus differentiation. This does not rule out the possibility of host-driven selection at lower levels of tree variation, only that such effects are not strong enough to result in reproductive isolation in the face of gene flow. Such adaptation with gene flow has been found in other systems (e.g., Chevillon et al. 1995; Mullen and Hoekstra 2008).

Marker methods and caveats

Our conclusions are based on a single locus, ITS1. Intra-individual variation in ITS1 has been observed (e.g., Buckler et al. 1997), but if this exists in our sample it would only increase the total variance, not the partitioning of that variance via AMOVA. There is little genomic information available for eriophyid mites (Cruickshank 2002), but ITS1 is reliably amplified. Primers for mtDNA (Navia et al. 2005) do not reliably amplify in *A. parapopuli* (L. M. E., unpubl. data), and because of the small size of mites ($\sim$150 μm), using non-Acari specific marker methods is unreliable as non-mite DNA is extracted. Attempts at this, via cloning and sequencing of the amplification product, have yielded bacterial, fungal, and *Populus* DNA (L. M. E., unpubl. data). While it is possible that different or no patterns could be found with different neutral loci, we argue that this is unlikely as similar patterns have been found in other eriophyid mites using SSRs and ITS1 (Carew et al. 2004) and nrDNA and mtDNA (Navia et al. 2005), and ITS1 has been successfully used in mite species discrimination (e.g., Fenton et al. 1993, 2000; Amrine et al. 1994).

A second caveat is that different marker systems were used in the interacting species—SSR loci in *P. angustifolia* and ITS1 sequence data for the mites. SSRs are typically more variable than sequence data (Behura 2006); thus, it may be expected that differentiation would be greater in *P. angustifolia* than in *A. parapopuli*. Despite this, we found higher levels of structure in the mites than their tree hosts, indicating that different mutation rates did not limit our ability to observe differentiation. While only one locus was examined in the mites, 17 were used for *P. angustifolia*. Such a difference may lead to contrasting patterns of gene flow; however, as outlined above, we argue that these ITS1 data are consistent with patterns found in other studies using other multiple loci.

Lastly, our sample sizes were not very large. We sampled a total of 66 mites for the analysis among three host species, and 131 mites for the analysis among the 10 *P. angustifolia* populations. Our haplotype accumulation curves (supplementary material Fig. S1) demonstrate that we sampled the common haplotypes in both cases; however, additional rare haplotypes would likely be found with increased sampling. It is unlikely that additional sampling would have changed our conclusions, though, for two reasons. First, among host species, our collections were geographically overlapping, and common mite haplotypes were found at very different frequencies on different hosts, even when geographically near. Second, >90 % of mites found on *P. angustifolia* were of two haplotypes, suggesting that while additional rare haplotypes would be found with more intensive sampling, differences among populations are primarily due to frequency differences in those two haplotypes. This is also consistent with the paucity of variation found by Evans et al. (2008) within mite populations on *P. angustifolia* within one river.

Factors affecting differentiation

The intimacy of the gall-forming habit, arrhenotokous parthenogenesis, and rapid generation time may predispose *A. parapopuli* to differentiation because of the close interaction required to both produce the gall structure and feed on the plant, and because successful lineages with little genetic diversity can diverge rapidly (Stireman et al. 2005; Dickey and Medina 2010). Stireman et al. (2005) found a higher occurrence of HAD among insects with a strong endophagous life history in a study of nine arthropods on *Solidago altissima* and *S. gigantea*. Parthenogenesis has been suggested as influencing HAD in the yellow pecan aphid, *Monelliopsis pecanis*, on pecan and water hickory (Dickey and Medina 2010). This reproductive mode could also strengthen founder effects and drift, resulting in higher population structure as seen among mite populations on *P. angustifolia* hosts. If drift and founder effects overwhelm gene flow, strongly differentiated populations may arise with little observable geographic patterns.

The mechanisms by which *Populus* species and F_1 hybrids drive reproductive isolation in the mites are unclear. An eriophyid pest of *Solanum dulcamara*, *Eriophyes cladophthirus*, feeds by transforming individual cells into nutritive cells on susceptible plants, while cells of resistant plants become necrotic (Westphal et al. 1981). If *A. parapopuli* uses similar mechanisms, then mites on alternate host trees may be unable to transform cells into feeding or galling structures because of differences among plants. Such differences may be strong enough at higher levels of *Populus* variation (species, F_1 hybrids) to drive genetic differentiation, but not at finer levels of variation (tree populations and individuals). Other likely mechanisms include genetic-based differences in defensive chemistry differences (Rehill et al. 2005), sink-source relationships (Compson et al. 2011), phenology (Floate et al. 1993), and/or interactions with hidden ecological players such as endophytes (Bailey et al. 2005) or microbes (Stone and Schonrogge 2003; Kaiser et al. 2010; Medina

et al. 2011). Eriophyid mites are known vectors of viruses (Lindquist et al. 1996), and bacteria and fungi exist within *A. parapopuli* galls, though it is unknown what role these play (Louis Lamit and L. M. E., unpublished data).

Conclusions

It is clear that variation among host plants can lead to isolation and divergence of herbivores (Feder 1998; Berlocher and Feder 2002; Stireman et al. 2005; Dickey and Medina 2010), but more studies of herbivore differentiation are needed that span levels of plant variation from individual plant genotypes to populations to hybrids to species. In one such study, Cogni and Futuyma (2009) tested for local adaptation of *Utetheisa ornatrix* moths to the legume *Crotalaria pallida* at both the local population and regional levels. While differentiation was found among populations, local adaptation was only found at the regional level. In our study, by combining tests of mite differentiation at multiple levels of plant variation we conclude that differences at higher levels of *Populus* genetic variation are strong enough to drive levels of HAD that are consistent with cryptic speciation of *A. parapopuli* lineages. However, tree variation at lower levels does not appear to drive HAD in mites. Therefore, we conclude that HAD is scale-dependent with respect to genetic variation among hosts. Establishing the generality of this hypothesis and associated scale dependence will require more extensive surveys of HAD in multiple species and other plant systems.

Acknowledgments We thank Matt Zinkgraf, Katie Mayer, Alyssa Bennett, Louis Lamit, Tatum Simonson, and the Cottonwood Ecology Group for help in the laboratory and discussion. We thank Rocky Mountain and Grand Teton National Parks for permission to collect samples. This work was supported by NSF-FIBR DEB-0425908 to T. W. and G. A., a Science Foundation Arizona Graduate Student Fellowship and NSF IGERT traineeship and Doctoral Dissertation Improvement Grant to L. E., and Northern Arizona University.

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