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TREE HYBRIDIZATION AND GENOTYPIC VARIATION DRIVE CRYPTIC SPECIATION OF A SPECIALIST MITE HERBIVORE

Luke M. Evans,[1,2] Gerard J. Allan,[1] Stephen M. Shuster,[1] Scott A. Woolbright,[1] and Thomas G. Whitham[1]

[1]Department of Biological Sciences, the Environmental Genetics and Genomics Laboratory and the Merriam-Powell Center for Environmental Research, Northern Arizona University, PO Box 5640, Flagstaff, Arizona 86011

[2]E-mail: lme36@nau.edu

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Few studies have investigated the roles that plant hybridization and individual plant genotype play in promoting population divergence within arthropod species. Using nrDNA sequence information and reciprocal transfer experiments, we examined how tree cross type (i.e., pure *Populus angustifolia* and *P. angustifolia* × *P. fremontii* F_1 type hybrids) and individual tree genotype influence host race formation in the bud-galling mite *Aceria parapopuli*. Three main findings emerged: (1) Strong genetic differentiation of mite populations found on pure *P. angustifolia* and F_1 type hybrids indicates that these mites represent morphologically cryptic species. (2) Within the F_1 type hybrids, population genetic analyses indicate migration among individual trees; however, (3) transfer experiments show that the mites found on heavily infested F_1 type trees perform best on their natal host genotype, suggesting that genetic interactions between mites and their host trees drive population structure, local adaptation, and host race formation. These findings argue that hybridization and genotypic differences in foundation tree species may drive herbivore population structure, and have evolutionary consequences for dependent arthropod species.

KEY WORDS: ***Aceria parapopuli*, cryptic speciation, foundation species, host race formation, hybridization, *Populus*.**

Studies of plant–insect interactions have greatly enhanced our understanding of the factors that drive evolution in arthropods (Mopper 1996; Dres and Mallet 2002; Blair et al. 2005; Stireman et al. 2005). Although plant hybridization has not been linked to race formation in herbivorous arthropods, it has played a key role in the evolution of plant species, and may account for the origin of 30–70% of angiosperms (Grant 1971; Stace 1987). Because hybridization is believed to be an important mechanism for the evolution of many plant groups, its effects may also impact the evolution of host-dependent arthropod species. Although several studies have investigated the role plant hybridization plays in the distribution of arthropods (e.g., Whitham 1989; Floate et al. 1993; Whitham et al. 1994, 1999; Gange 1995; reviewed in Fritz 1999), few have addressed how hybridization influences race formation and evolution in arthropods. Floate and Whitham (1993) argued that naturally occurring hybridization in plants could result in host shifts and speciation in arthropods and pathogens, although there has been debate on the importance of these "hybrid bridges" for arthropod diversification (Roderick and Gillespie 1998; Pilson 1999). Despite many studies that have identified host-associated genetic differentiation of herbivores on different plant species (e.g., Gotoh et al. 1993; Carroll et al. 1997; Feder 1998; Via 1999; Groman and Pellmyr 2000; Ruiz-Montoya et al. 2003; Abbot and Withgott 2004; Leebens-Mack and Pellmyr 2004; Blair et al. 2005; Svensson et al. 2005; Diegisser et al. 2006a,b), and extensive reviews on the topic (Via 2001; Berlocher and Feder 2002; Dres and Mallet 2002), studies of plant hybridization and arthropod race formation are lacking.

Additionally, the importance of individual hosts on the genetic structuring and adaptation of arthropod populations has received little attention, despite the potential individual hosts have for driving arthropod evolution (Mopper 1996). Karban (1989)

provided convincing evidence for host races of thrips on individual trees through reciprocal transplant studies. Rank (1992) identified genetic differentiation in the montane leaf beetle at the host population level, as well as among individual trees within populations, although this was in part driven by geographic isolation. Adaptive deme formation has also been found in leafminers on individual oak trees within the dispersal range of the adult moths (Mopper et al. 1995, 2000). Recently, plant genotype has been shown to influence the survival of hybrid and parental host races of *Eurosta solidaginis*, indicating that plant genotype can affect herbivore evolution and speciation (Craig et al. 2007). Furthermore, although plant genotype can drive arthropod population structure (Mopper 1996), no studies have investigated how both plant hybridization and genotypic differences among individual plants influence genetic differentiation and local adaptation in arthropods.

Here we examine how hybridization and genotypic differences within pure and hybrid cross types of *Populus fremontii* × *P. angustifolia* affect the population genetic structure and local adaptation of a common herbivore, *Aceria parapopuli* Kiefer (Acari: Eriophyidae). Specifically, we ask whether tree hybridization and genotypic variation within the tree cross-types result in levels of genetic differentiation and adaptation in *A. parapopuli* that are consistent with host race formation. Cottonwoods are foundation riparian species (species that structure a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes [Dayton 1972; Ellison et al. 2005]) that have strong effects on dependent arthropod communities and ecosystem processes (Whitham et al. 2003, 2006). Cottonwoods naturally hybridize throughout North America (Eckenwalder 1984a,b) and hybrid zones range in size from a few kilometers to 1000 km (T. G. Whitham, unpubl. data). Hybrid cottonwoods occur in the fossil record 12 million years ago (Eckenwalder 1984c), and hybrid speciation has been documented in cottonwoods (Smith and Sytsma 1990). Thus, there has been ample opportunity for these hybrids to affect the evolution of dependent specialized species such as *A. parapopuli*, which is widely distributed and has been described as a single species that attacks the buds of North American *Populus* species and their hybrid derivatives (Kiefer 1940; Drouin and Langor 1992; Amrine and Stasny 1994; Baker et al. 1996). These mites form woody, cauliflower-like galls that can remain active for up to a decade (L. M. Evans, pers. obs.). Mites disperse by crawling among branches within a tree and are wind-dispersed among trees (Sabelis and Bruin 1996). Windborne dispersal studies of eriophyoid mites indicate that they are capable of long-distance dispersal (Zhao and Amrine 1997; Bergh 2001) and cottonwood susceptibility to these mites is genetically controlled (Kalischuck et al. 1997; Whitham et al. 1999; McIntyre and Whitham 2003). For example, using observations in a common garden and mite-transfer experiments, McIntyre and Whitham (2003) found that successful gall formation, probability of population extinction, and the intrinsic rate of population increase, *r*, are strongly affected by cottonwood genotype. Furthermore, Whitham et al. (1999) and McIntyre and Whitham (2003) found evidence of pronounced differences in mite performance genotypes among F_1 type genotypes, but not among pure *P. angustifolia*. Gall density, population persistence, and *r* were much higher on the F_1 genotypes than on *P. angustifolia* genotypes. These studies, however, did not address genetic differentiation or local adaptation of *A. parapopuli* among different host tree species, hybrids, or genotypes.

Our observations on the pervasive effects of cottonwood hybridization and genotypic variation on the survival and fitness of *A. parapopuli* lead to three hypotheses: (1) populations of mites will be genetically differentiated on different cottonwood cross types and individual genotypes within cross types. We also predicted that there would be higher differentiation within the F_1 cross type in which population density and persistence is higher and where greater variation in mite performance on individual genotypes has been found (McIntyre and Whitham 2003); (2) if cottonwood cross type and genotype within cross types influence genetic differentiation in mites, then we expected geographic distance to have little to no effect on mite differentiation; and (3) interaction between cottonwoods and mites would result in the highest performance of mites on natal genotypes in transfer experiments. We tested these hypotheses by analyzing data derived from nrDNA sequences of the internal transcribed spacer I (ITS1) region in *A. parapopuli* and by performing fully reciprocal transfer experiments of mites on individual tree genotypes in performance tests.

Methods

STUDY SITE AND COLLECTIONS

Along the Weber River near Ogden, UT, *P. fremontii* and *P. angustifolia* naturally hybridize within a 13 km hybrid zone, forming F_1 hybrids that unidirectionally backcross toward *P. angustifolia* (Keim et al. 1989; Martinsen et al. 2001). In a survey of 1444 *P. angustifolia*, *P. fremontii*, and their F_1 type hybrids along the Weber River, UT, Whitham et al. (1999) showed that 45% of F_1 type hybrid cottonwoods and approximately 13% of *P. angustifolia* were attacked, but no *P. fremontii* are attacked (Fig. 1). Furthermore, of those that were attacked, the abundance of *A. parapopuli* was approximately 60-fold greater on F_1 type hybrids than on *P. angustifolia* (Fig. 1). Within the hybrid zone, large numbers of galls are found on F_1 type hybrid cottonwoods, but few pure *P. angustifolia* cottonwoods are attacked. Progressing farther upstream well into the pure *P. angustifolia* zone, the abundance of mite galls on *P. angustifolia* increases, but is still very low relative to that observed on F_1 type hybrids in the hybrid zone. For

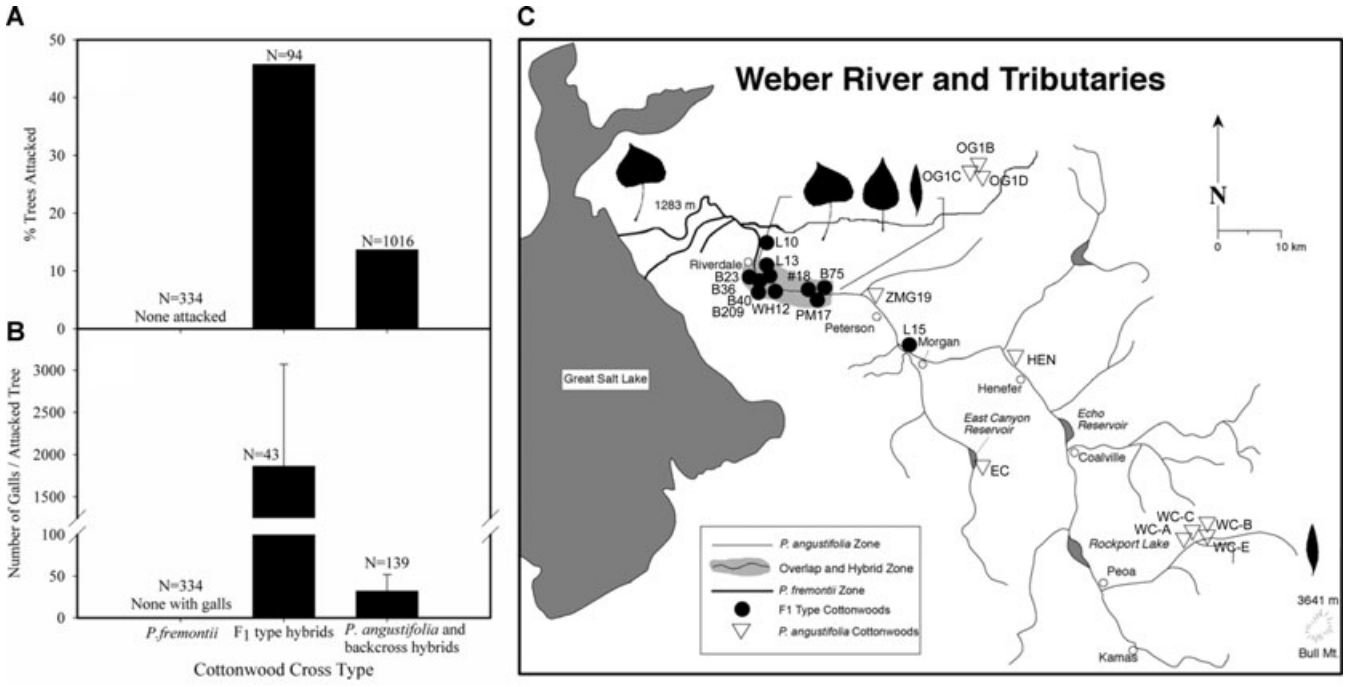

Figure 1. **(A) Mites attack substantial proportions of the *P. angustifolia* and F_1 type hybrid cottonwood populations, but no *P. fremontii* are attacked. (B) Of those trees that are attacked, the number of galls found on F_1 type hybrid cottonwoods is ~60-fold greater than on *P. angustifolia.* Error bars are 1 SEM. Numbers above bars represent the number of trees surveyed. (A and B adapted from Whitham et al. 1999) (C) Map of the Weber River showing the locations of cottonwood trees, where mites were collected as well as the location of the hybrid zone (Map adapted from Martinsen et al. 2001). Tree genotype names are shown next to symbols.**

this reason, we collected galls from F_1 type hybrid cottonwoods primarily in the hybrid zone. Galls from *P. angustifolia* were primarily collected in the pure *P. angustifolia* zone upstream, and no galls were collected from *P. fremontii* (Fig. 1). One to three galls were collected from each of 11 F_1 type hybrid cottonwoods and 10 *P. angustifolia* cottonwood trees, resulting in the collection of 43 individual galls. Leaf morphology was used to determine the cross-type category for individual trees (Floate and Whitham 1995; McIntyre and Whitham 2003). The latitude and longitude of each tree was recorded. Collections from F_1 type trees were up to 31.7 km apart, collections from *P. angustifolia* trees were up 74.2 km apart, and there was some geographical overlap between the two groups.

DNA EXTRACTION AND SEQUENCING

We used sequence information from the ITS1 region of nrDNA to examine population differentiation of *A. parapopuli*. Although intraindividual variation has been observed in nrDNA (e.g., Buckler et al. 1997; Parkin and Butlin 2004), the ITS1 region has been used extensively in phylogenetic reconstructions and population genetic studies of arthropods (for acarology reviews, see Navajas and Fenton 2000; Cruickshank 2002). If intraindividual variation at ITS1 does exist, however, it would increase the genetic variance but not affect the partitioning of that variance in analysis of molecular variance (AMOVA). Importantly, ITS1 has been used to successfully examine population structure and species status in other eriophyid species (Carew et al. 2004; Fenton et al. 1993, 1995, 1997, 2000; Navia et al. 2005a) and is consistent with a morphologically based reclassification of the *Cecidiphyopsis* spp. complex on *Ribes* (Amrine et al. 1994).

Collected galls were stored in 100% ethanol at −20°C until mites were extracted. To extract the mites from the galls, each gall was finely chopped using a razor in a weigh boat filled with ethanol. Two to nine mites from each gall were removed using a fine-tipped pipettor, each placed into its own 0.5-mL microcentrifuge tube, and stored at −20°C until DNA extraction. The procedure was observed under a microscope. Whole genomic DNA was extracted using the DNeasy Micro Kit (Qiagen, Valencia, CA) following the protocol for laser microdissected tissues, modified by incubating the mites in proteinase solution at 56°C overnight rather than 3 h. DNA was recovered by adding 20 μl Buffer AE onto the spin column membrane.

The ITS1 region of nrDNA from 100 mites from F_1 hosts and 105 mites from *P. angustifolia* hosts was amplified using Acari-specific primers and methods modified from Carew et al. (2004). PCR amplifications were carried out in 50 μl volumes, with 10 μl template DNA, 0.2 mM dNTPs, 2.5 units Taq polymerase, 0.5 μM each primer (forward: AGAGGAAGTAAAAGTCGTAACAAG, and reverse: GCTGCGTTCTTCATCGATACTCG), 1× PCR Buffer, and 1.5 mM $MgCl_2$. Cycling conditions consisted of

the following: one cycle at 95°C (3 min); 12 cycles of touchdown at 95°C (20 sec), 55°C decreasing to 43°C (30 sec, decreasing 1°C each cycle), 72°C (1 min); 24 cycles at 95°C (20 sec), 43°C (30 sec), 72°C (1 min); and one cycle at 72°C (10 min). PCR products were visualized on a 1% agarose gel using 5 μl of product. The remaining product was purified using polyethylene glycol precipitation (20% PEG, 1.5 M Tris).

Separate forward and reverse cycle sequencing reactions were performed for each individual mite using Big Dye version 3.1 Terminator Cycle Sequencing kits (Applied Biosystems Inc., Foster City, CA), with 1 μl purified PCR product (∼15-20 ng DNA) used as template. The cycle sequencing product was purified using an isopropanol precipitation. Sequencing reactions were run on an ABI 3730×l DNA Analyzer (Applied Biosystems, Inc.). Sequence information was obtained for both forward and reverse strands. Forward and reverse sequences for each individual mite were compiled using the Seqman program (DNAStar; Lasergene, Madison, WI). All sequences were aligned using Clustal X (Thompson et al. 1997). Of the 499-bp region amplified, there were 12 variable positions, and these 12 positions were used for data analysis. Sequences of all unique haplotypes have been deposited in GenBank (accession numbers: EF641814-EF641835).

Given that some individuals were heterozygous at multiple positions, PHASE version 2.1 (Stephens et al. 2001) was used to determine the gametic phase of haplotypes. A burnin of 1000 iterations followed by 1000 iterations each with 10 steps, using the MS model was used. Five independent runs using five separate random number seeds produced exactly the same results. Male eriophyid mites are haploid whereas females are diploid (arrhenotokus parthenogenesis [Helle and Wysoki 1996]); thus, determining haploid males from homozygous females from the sequence information is problematic, and DNA extraction precluded sexing individuals. We therefore randomly chose (from the PHASE ver. 2.1 output) one haplotype from each individual so that ploidy level of individuals would not bias analyses (sensu Carew et al. 2004).

DATA ANALYSIS

AMOVA was used to test for population differentiation of mites among cottonwood cross types and among genotypes within cross types using GenAlEx version 6 (Peakall and Smouse 2006). Subsequently, each cross type was analyzed separately using AMOVA to test for genetic differentiation of mites among cottonwood genotypes within cross types and among galls within genotypes within each cross type. Pairwise ϕ_{PT} estimates were calculated between mite populations on all cottonwood genotypes among both cross types. To control for the inflated alpha involved with multiple testing, we identified a P-value threshold ($P = 0.032$) equivalent to a false discovery rate q-value of 0.05 using QVALUE (Storey 2002). GenAlEx version 6 was used to perform a Mantel test between pairwise population ϕ_{PT} and geographical distance to test for the effect of geographical isolation on mite population differentiation among all cottonwood genotypes, and separate Mantel tests were performed within each cross type.

Cavalli-Sforza's chord distance was used to calculate pairwise genetic distances based on ITS haplotype frequencies between mite populations found on individual tree genotypes using the Gendist program (Phylip, ver. 3.6, Felsenstein 2004). This distance assumes no mutation (appropriate with low sequence divergence among mites) and does not assume equal population sizes (appropriate with varying gall densities; Fig. 1) (Felsenstein 1984, 2004). Using the Neighbor program in Phylip (Felsenstein 2004), a neighbor-joining tree (Saitou and Nei 1987) was constructed, and each node was evaluated with 10,000 bootstrap permutations using the Seqboot program, with the consensus tree obtained from Consense (Phylip, ver. 3.6).

RECIPROCAL TRANSFER EXPERIMENTS

In 2005 and 2006 we tested mite adaptation to five F_1 type hybrid cottonwood genotypes by transferring mites in a fully reciprocal manner from each genotype to its natal genotype as well as every other genotype. No transfer experiments were performed among *P. angustifolia* or *P. fremontii* genotypes due to the low abundance of galls on the parental host species (see above; Fig. 1). In each experiment, cuttings with and without galls were taken from each tree genotype and planted in 1-gallon pots at the Northern Arizona University greenhouse facilities. In 2005, five F_1 hybrid cottonwood genotypes that were heavily infested by *A. parapopuli* were propagated. All trees were within 6.14 km of one another. One donor cutting with galls was potted in the same pot as, and in contact with, two recipient cuttings with no galls (both recipient cuttings were of the same genotype; two were used to mitigate mortality), and left undisturbed so that mites could transfer on their own (sensu Goolsby et al. 2005). In combining both preference and performance, this experiment evaluates the overall potential for differential survival (i.e., successful gall formation) among genotypes. To eliminate preference from the design and test only for performance, we repeated our experiment in 2006 with minor changes in methodology: one recipient cutting (with no galls) was planted in a 1-gallon pot, and at budbreak one detached, but live gall was tied onto the cutting. As the gall dried out, mites were forced to move in a no-choice manner. The same tree genotypes and mite populations were used in both experiments with the exception of two from the 2005 experiment, which had no additional galls. Two alternate cottonwood genotypes with similar gall densities in the field were therefore used in the 2006 experiment. Ten replicates of each transfer were used in the 2005 experiment, whereas 20 were used in the 2006 experiment, although replicates in which cuttings had died were removed from

the experiment, leaving unequal sample sizes (Table 3). In the greenhouse, pots were arranged randomly within donor mite population blocks and blocks were separated by at least 0.6 m. All cuttings were surveyed for the presence of galls five months after budbreak. The surveys were completed within 4 days. The successful transfer of mites was defined as the presence of at least one gall on the recipient cutting (on at least one of the two, same genotype, recipient cuttings in a replicate in the 2005 experiment).

Our goal was to determine whether mite populations were locally adapted to individual cottonwood genotypes. If local adaptation had occurred, we predicted that mites' relative transfer success would be highest on their natal cottonwood genotype, and lower on nonnatal tree genotypes. Because there were five tree genotypes and five mite populations, each comparison of a given mite population's relative fitness across all five tree genotypes represented a single experiment, whose total sample size equaled the number of mite galls of a given population that were placed on each of the five tree genotypes.

We used two statistical approaches to test the hypothesis that mite populations were locally adapted to individual cottonwood genotypes. First, to provide an overall measure of local adaptation between "natal" and "nonnatal" hosts in each experiment, we used Fisher's exact test to examine the numbers of successful transfers onto natal versus nonnatal tree genotypes for each experiment. Second, we used the method of Shuster and Wade (1997) to examine the relative fitness, w_{ijk}, of the *k*th mite population on each *j*th tree genotype in the *i*th experiment, as

$$w_{ijk} = t_{ijk}/T_{ik}, \quad (1)$$

where t_{ijk} is the absolute transfer rate of the *k*th mite population on the *j*th tree genotype in the *i*th experiment, and T_{ik} is the average transfer rate for the *k*th mite genotype over all *j* tree genotypes in the *i*th experiment. Here, each absolute transfer rate, t_{ijk}, equaled $a_{ijk}/\Sigma a_{ijk}$, where a_{ijk} equaled the number of successful transfers for the *k*th mite population on the *j*th tree genotype in the *i*th experiment, and Σa_{ijk} equaled the total number of experimental trials for the *k*th mite population over all tree genotypes in the *i*th experiment.

In each test, a relative fitness, w_{ijk}, of 1 indicated that members of a mite population on a given tree genotype survived in proportion to their numbers at the start of the experiment. Deviations in relative fitness above or below 1 indicated that a mite population's survival on a given tree genotype was above or below its expected survival, relative to its success on other tree genotypes in that experiment. To test whether the relative fitness of the *k*th mite population on the *j*th cottonwood genotype in the *i*th experiment, deviated significantly from 1, we calculated the standardized difference, $S_{d(ijk)}$, between p_{ijk}, (the proportion of transfers of the *k*th mite population to the *j*th cottonwood genotype of transfers to all cottonwood genotypes in the *i*th experiment), and p'_{ijk}, (the proportion of all successful transfers for the *k*th mite population) for each trial in each experiment. Thus, $S_{d(ijk)} = (p'_{ijk} - p_{ijk})/[p_{ijk}\,(1-p_{ijk})/N]^{1/2}$, with N equal to the total number of successful transfers for the *k*th mite population in the *i*th experiment (Shuster and Wade 1997).

The significance for each standardized difference was determined by comparing the value of $S_{d(ijk)}$ to a standard normal distribution (Shuster and Wade 1997). Although the fitness of each mite genotype was examined repeatedly (i.e., on five tree genotypes within each experiment), our design prevented pseudoreplication because the fitness of each mite genotype on its natal tree genotype was assessed relative to that mite genotype's fitness on all other tree genotypes in each experiment. Because there were no repeated tests among experiments of particular mite genotypes on particular tree genotypes, each standardized difference test was independent of all others. We therefore used $\alpha = 0.05$ as our criterion for each *k*th standardized difference, on each *j*th tree, within each *i*th experiment.

Results

ACERIA PARAPOPULI GENETIC DIFFERENTIATION

In support of our hypothesis that mites found on different cross types are genetically distinct, we found significant differentiation of mites between F_1 type hybrid cottonwoods and *P. angustifolia* cottonwoods ($\phi_{RT} = 0.662$, $P = 0.001$; Table 1). Furthermore, comparisons of mite populations on individual tree genotypes supported this result, with the majority of F_1 versus *P. angustifolia* comparisons highly differentiated ($\phi_{PT} > 0.5$, $P < 0.05$; Table 2). The Neighbor-joining tree consisting of the 44 mite galls also

Table 1. Hierarchical ϕ statistics of *Aceria parapopuli* population differentiation among cottonwood hosts. Significance tests are based on 999 permutations.

Level	Percentage of variance	ϕ statistic		*P*
Between F_1 and *P. angustifolia* cross types				
Among cross types	66%	ϕ_{RT}	0.662	0.001
Among genotypes/cross type	6%	ϕ_{PR}	0.177	0.001
Within genotypes	28%			
Within each cross type				
F_1 populations only				
Among genotypes	8%	ϕ_{RT}	0.083	0.038
Among galls/genotypes	24%	ϕ_{PR}	0.260	0.001
Within galls	68%			
P. angustifolia populations only				
Among genotypes	10%	ϕ_{RT}	0.103	0.063
Among galls/genotypes	1%	ϕ_{PR}	0.009	0.403
Within galls	89%			

Table 2. Pairwise population ϕ_{PT} estimates of mite populations on all cottonwood genotypes (both F_1 and *P. angustifolia* cross types). ϕ_{PT} values below diagonal. Probability values based on 9999 permutations are shown above diagonal. Significant comparisons after controlling for the false discovery rate (i.e., $q < 0.05$) are in bold.

	F_1 type hybrid cottonwood mite populations											*P. angustifolia* mite populations									
	#18	B209	B23	B36	B40	B75	L10	L13	L15	PM17	WH12	EC	HEN	OG1B	OG1C	OG1D	WC-A	WC-B	WC-C	WC-E	ZMG19
#18	–	0.346	0.076	0.302	0.089	0.348	0.440	0.435	0.162	0.076	**0.012**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.041	**0.000**	**0.000**	**0.001**
B209	0.000	–	0.351	0.335	0.107	0.241	0.398	0.458	0.139	0.048	0.068	**0.000**	**0.001**	**0.001**	**0.000**	**0.000**	**0.000**	0.037	**0.001**	**0.002**	**0.010**
B23	0.099	0.000	–	0.068	0.391	**0.031**	0.329	0.352	**0.008**	**0.001**	0.251	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.002**	**0.000**	**0.000**	**0.001**
B36	0.000	0.000	0.115	–	0.105	0.340	0.476	0.509	0.262	0.124	**0.014**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.059	**0.000**	**0.000**	**0.001**
B40	0.092	0.132	0.000	0.097	–	0.057	0.265	0.236	**0.017**	**0.003**	0.375	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.000**	**0.003**	**0.000**	**0.000**	**0.001**
B75	0.000	0.092	**0.227**	0.000	0.213	–	0.373	0.173	0.142	0.150	**0.015**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.117	**0.000**	**0.001**	**0.002**
L10	0.000	0.000	0.000	0.000	0.000	0.000	–	1.000	0.101	0.073	0.306	**0.002**	**0.007**	**0.008**	**0.001**	**0.001**	**0.002**	0.070	**0.005**	**0.009**	**0.018**
L13	0.046	0.032	0.000	0.041	0.000	0.219	0.000	–	0.107	0.107	0.301	0.089	**0.002**	0.477	0.047	**0.002**	0.054	0.032	0.476	0.469	0.445
L15	0.195	0.431	**0.693**	0.190	**0.739**	0.235	0.586	0.864	–	0.283	**0.012**	**0.010**	**0.009**	0.040	**0.007**	**0.018**	**0.007**	0.380	**0.026**	**0.031**	0.054
PM17	0.179	0.381	**0.592**	0.159	**0.609**	0.184	0.456	0.614	0.000	–	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.138	**0.000**	**0.000**	**0.004**
WH12	**0.171**	0.209	0.015	**0.172**	0.000	**0.310**	0.023	0.000	**0.763**	**0.669**	–	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.001**	**0.000**	**0.000**	**0.001**
EC	**0.597**	**0.833**	**0.821**	**0.606**	**0.875**	**0.731**	**0.922**	0.000	**0.961**	**0.792**	**0.831**	–	0.460	1.000	1.000	0.497	1.000	0.042	1.000	1.000	1.000
HEN	**0.523**	**0.732**	**0.743**	**0.535**	**0.775**	**0.605**	**0.772**	**0.864**	**0.818**	**0.690**	**0.766**	0.000	–	0.353	0.454	0.356	0.466	0.055	0.381	0.352	0.275
OG1B	**0.539**	**0.778**	**0.790**	**0.549**	**0.846**	**0.659**	**0.886**	0.000	0.939	**0.732**	**0.812**	0.000	0.000	–	1.000	0.083	1.000	0.185	1.000	1.000	1.000
OG1C	**0.649**	**0.872**	**0.849**	**0.658**	**0.899**	**0.786**	**0.944**	0.000	**0.973**	**0.837**	**0.850**	0.000	0.017	0.000	–	0.490	1.000	**0.020**	1.000	1.000	1.000
OG1D	**0.510**	**0.707**	**0.727**	**0.520**	**0.748**	**0.569**	**0.731**	**0.817**	**0.748**	**0.628**	**0.753**	0.014	0.000	0.000	0.044	–	0.483	0.108	0.100	0.082	0.231
WC-A	**0.640**	**0.866**	**0.844**	**0.649**	**0.895**	**0.777**	**0.940**	0.000	**0.971**	**0.830**	**0.847**	0.000	0.011	0.000	0.000	0.039	–	**0.024**	1.000	1.000	1.000
WC-B	0.178	0.352	**0.501**	0.184	**0.494**	0.152	0.337	0.492	0.209	0.168	**0.577**	0.289	0.168	0.200	**0.369**	0.092	**0.355**	–	0.081	0.188	0.309
WC-C	**0.555**	**0.795**	**0.799**	**0.566**	**0.854**	**0.680**	**0.898**	0.000	**0.946**	**0.750**	**0.817**	0.000	0.000	0.000	0.000	0.000	0.000	0.226	–	1.000	1.000
WC-E	**0.539**	**0.778**	**0.790**	**0.549**	**0.846**	**0.659**	**0.886**	0.000	**0.939**	**0.732**	**0.812**	0.000	0.000	0.000	0.000	0.000	0.000	0.200	0.000	–	1.000
ZMG 19	**0.499**	**0.732**	**0.769**	**0.511**	**0.825**	**0.603**	**0.851**	0.000	0.916	**0.686**	**0.799**	0.000	0.000	0.000	0.000	0.000	0.000	0.135	0.000	0.000	–

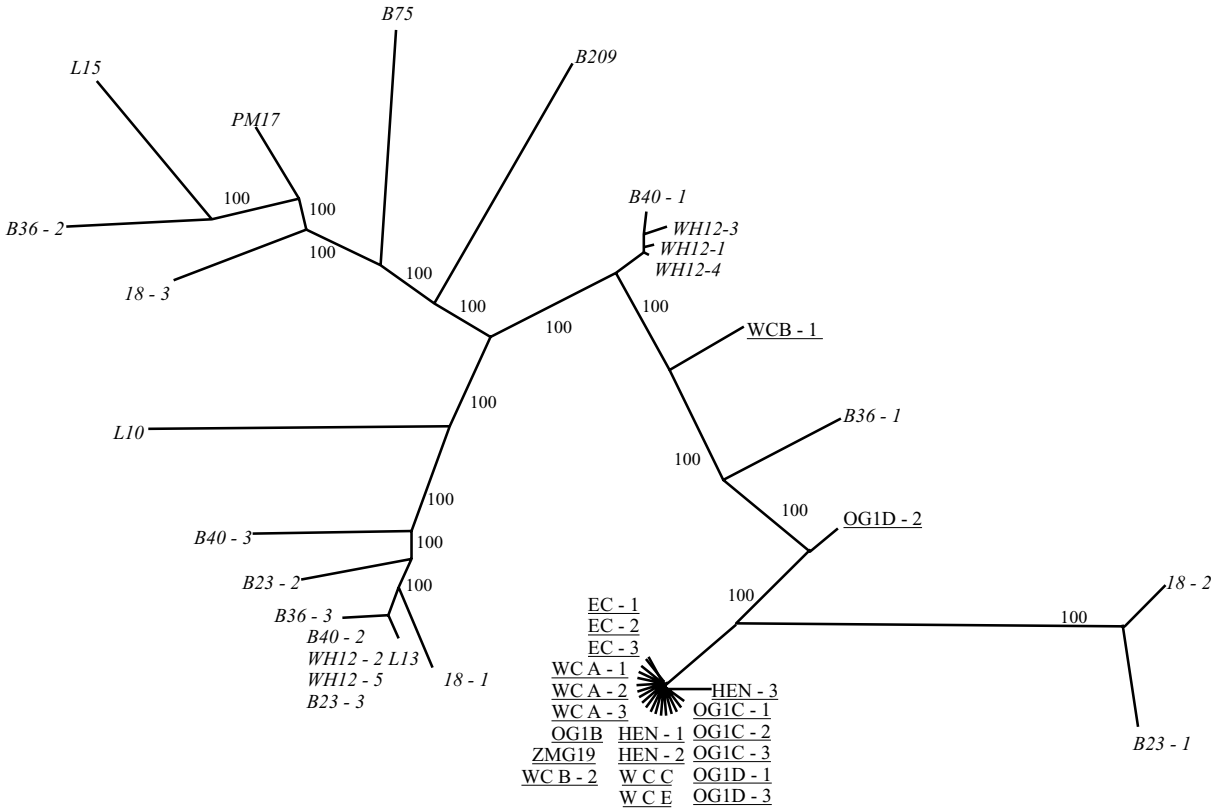

Figure 2. Neighbor-Joining tree of mite populations within individual cottonwood galls based on Cavalli-Sforza chord distances shows extensive differentiation between mite populations on narrowleaf cottonwood (underlined) and F_1 hybrid cross types (italics) and among mite populations on F_1 type trees, but little differentiation among mite populations on *P. angustifolia* trees. Names represent the tree genotype followed by the gall number. Numbers at nodes indicate the bootstrap values at that node; only nodes with greater than 50% bootstrap support are shown.

reveals two distinct clusters, one corresponding to F_1 hybrid hosts and the other to *P. angustifolia* hosts (Fig. 2). Although there is some overlap, mite galls from the two cross types are generally separated and nodes leading to these two groups are supported by bootstrap values of 100%. In combination, these findings suggest that cottonwood cross type drives population differentiation in this herbivore.

In addition to between cross-type differences, we also found moderate differentiation of mite populations among individual tree genotypes within cross types ($\phi_{PR} = 0.179$; Table 1), supporting our hypothesis that tree genotype will structure mite populations at the level of individual trees. When each cross type was analyzed separately, patterns were similar with modest differentiation among tree genotypes, but significance differed between the host cross types (among F_1 genotypes: $\phi_{PR} = 0.083$, $P = 0.038$; among *P. angustifolia* genotypes: $\phi_{PR} = 0.103$, $P = 0.063$; Table 1). Pairwise comparisons of mite populations found on individual trees support these results. Many comparisons of populations among F_1 hybrid trees were significant, although differentiation among *P. angustifolia* mite populations appear to be driven by one genotype, WC-B (Table 2).

At the finest scale of analysis, we also found strong genetic differentiation of mites among galls within individual F_1 cottonwood genotypes ($\phi_{PR} = 0.260$), but not within *P. angustifolia* genotypes ($\phi_{PR} = 0.009$; Table 1). This is mirrored by the phylogenetic analysis, which indicates that galls on individual F_1 hybrid trees are separated by long branch lengths with 100% bootstrap support, but the majority of galls on *P. angustifolia* genotypes cluster together in one polytomy (Fig. 2). Within both cross types, the majority of variation was within galls, indicating that mites within galls are genetically different (Table 1). The relatively small number of mites sampled from each gall may have biased these values upward; however, they are much larger than the differentiation found among genotypes, which suggests that much of the genetic differentiation observed at the ITS1 locus results from migration, founder effects, and drift.

GEOGRAPHIC ANALYSES

The Mantel test indicates a small, but significant effect of geographic distance on population differentiation among all host genotypes (both F_1 hybrids and *P. angustifolia*; Mantel $R = 0.283$, $P = 0.001$, respectively; Fig. 3A). Within the F_1 cross type, there

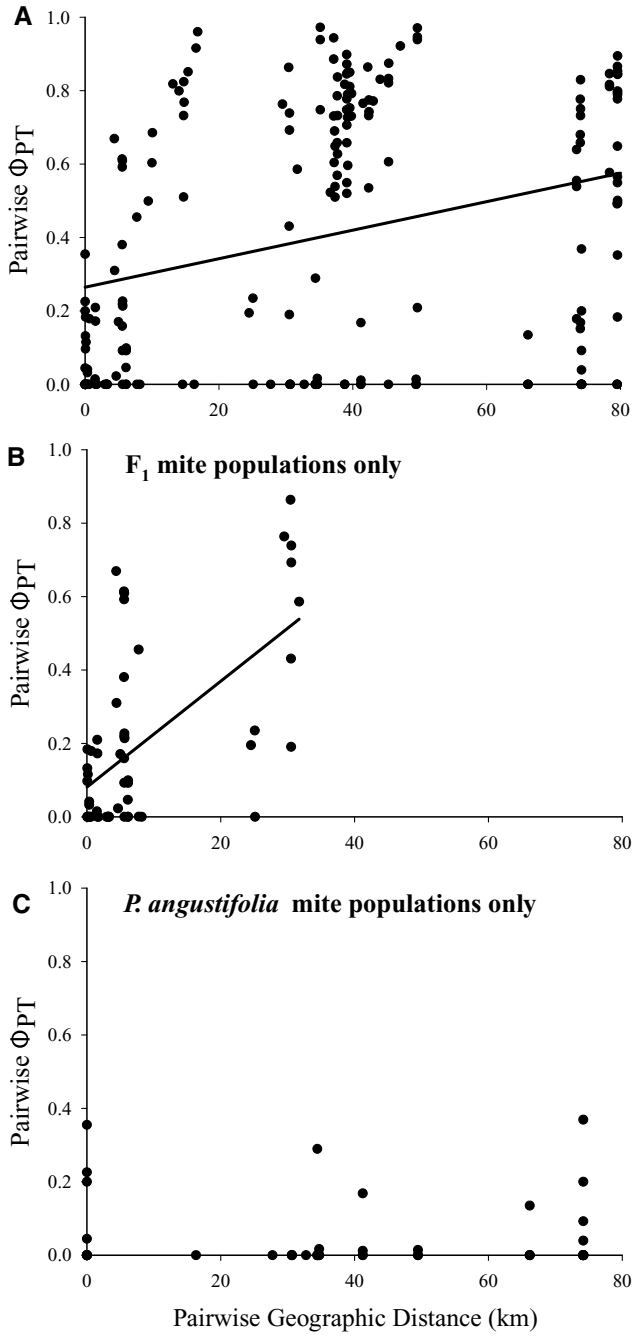

Figure 3. Mantel test showing a significant effect of pairwise geographic distance on pairwise population differentiation (ϕ_{PT}) among (A) all cottonwood genotypes and (B) among F_1 type cottonwood genotypes only. Conversely, there is no relationship between pairwise ϕ_{PT} and geographic distance between mite populations on all *P. angustifolia* genotypes (C).

was a significant effect of geographic distance on population differentiation (Mantel $R = 0.569$, $P = 0.001$; Fig. 3B). Thus, geographic isolation appears to be driving patterns of population differentiation among mites within the F_1 cross type. Conversely, within the *P. angustifolia* cross type in which geographic distances are about 2.5 times greater than those observed with F_1 hybrids, we found no effect of geographic distance on mite population differentiation (Mantel $R = -0.097$, $P = 0.167$; Fig. 3C). Geographic isolation does not appear to be driving population differentiation among mite populations on *P. angustifolia*.

MITE RECIPROCAL TRANSFER EXPERIMENTS

Two independent sets of experiments support our local adaptation hypothesis that mites exhibit greater survival on their natal hosts. In the first experiment that does not differentiate between preference and performance (2005 test), mites transferred significantly more frequently to their natal tree in three of five cases (Fisher exact test; $P < 0.05$; Table 3). In a fourth case, the frequency of successful transfer was highest onto the natal tree genotype; however, this trend was not statistically significant. Similarly, in our second test of performance only (i.e., 2006), in three of five cases mites transferred more frequently onto the natal tree genotype than the nonnatal; however, this was statistically significant ($P < 0.05$) in only one case (Table 3). These results show that some mite populations are adapted to their natal host cottonwood trees.

We found one tree genotype (18) to be extremely susceptible to infestation by all mite populations. In fact, in the 2005 test the highest transfer rate was onto this genotype for three of the five mite populations and in the 2006 test for all five mite populations (Table 3). As the extreme susceptibility of tree genotype 18 may have driven or concealed patterns, we removed this genotype and its mite population and reanalyzed both experiments. After removing tree 18, three of four mite populations showed significantly higher transfer rates onto the natal than the nonnatal genotypes in both experiments (Fisher's exact test; $P < 0.05$; Table 3). Together, both tests indicate that mite populations are locally adapted to their natal tree genotypes and that some tree genotypes can be considered to be universally susceptible.

Our alternative analyses of the standardized differences (S_d) of transfer rates yielded similar results in both experiments (Table 3). Importantly, they suggest that the relative transfer rate of mite populations covaries with natal tree genotype and shows significant variation in suitability of individual cottonwood genotypes as hosts for the mite populations of individual trees. Together, the two independent experiments suggest that some mite populations are locally adapted to their natal cottonwood genotypes, and represent tree genotype-associated host races.

Discussion

MITE GENETIC DIFFERENTIATION AND CRYPTIC SPECIATION IN RESPONSE TO PLANT HYBRIDIZATION

Aceria parapopuli has been described as a single species on North American *Populus* hosts (Kiefer 1940; Drouin and

Table 3. The standardized difference, S_d, and their significance values, between the successful proportion of a mite populations' attempted transfers to a cottonwood genotype and the population's proportion of all successful transfers for both the preference test and performance test. We repeated the analysis with and without tree genotype 18 due to its extreme susceptibility. *FET p indicates the *P*-value of the Fisher's exact test testing if significantly more mite galls were initiated onto "natal" over "nonnatal" tree genotypes for each mite population.

Mite population	Tree genotype	No. of successful transfers	No. of attempted transfers	All tree genotypes and mite populations			Tree genotype and mite population 18 removed		
				*FET p	S_d	*P*	*FET p	S_d	*P*
2005 transfer experiment									
18	18	7	10	0.003	2.69	0.004			
	B23	3	9		0.26	0.397			
	B40	1	10		−1.26	0.104			
	PM17	3	10		0.05	0.480			
	WH12	0	9		−1.80	0.036			
B23	18	6	9	0.190	2.83	0.002			
	B23	3	10		0.39	0.348		1.41	0.079
	B40	1	10		−1.04	0.149	0.026	−0.47	0.319
	PM17	1	11		−1.17	0.121		−0.59	0.278
	WH12	1	9		−0.90	0.184		−0.34	0.367
B40	18	2	10	0.006	1.12	0.131			
	B23	1	10		0.00	0.500		0.33	0.371
	B40	2	10		1.12	0.131	0.012	1.67	0.048
	PM17	0	10		−1.12	0.131		−1.00	0.159
	WH12	0	10		−1.12	0.131		−1.00	0.159
PM17	18	3	5	0.870	2.90	0.002			
	B23	1	4		0.61	0.271		1.00	0.159
	B40	1	4		0.61	0.271	0.870	1.00	0.159
	PM17	0	5		−0.88	0.212		−0.76	0.224
	WH12	2	5		1.64	0.051		2.27	0.012
WH12	18	1	10	0.014	−0.38	0.352			
	B23	1	9		−0.26	0.397		−0.34	0.367
	B40	3	7		2.20	0.014	0.019	2.10	0.018
	PM17	0	9		−1.24	0.108		−1.32	0.093
	WH12	2	4		2.01	0.022		1.91	0.028
2006 transfer experiment									
18	18	17	21	<0.0001	3.73	0.001			
	B209	8	19		0.40	0.345			
	B23	5	20		−1.00	0.159			
	L10	5	20		−1.00	0.159			
	WH12	1	17		−2.33	0.010			
B209	18	17	20	0.162	4.24	0.000			
	B209	9	19		1.01	0.156		2.80	0.003
	B23	5	18		−0.58	0.281	0.0004	0.65	0.258
	L10	2	19		−2.02	0.022		−1.21	0.113
	WH12	0	18		−2.79	0.019		−2.27	0.012
B23	18	15	16	0.513	5.42	0.000			
	B209	3	19		−1.21	0.113		0.48	0.316
	B23	4	16		−0.31	0.378	0.019	1.63	0.052
	L10	1	14		−1.66	0.049		−0.64	0.261
	WH12	0	15		−2.30	0.021		−1.56	0.059

Continued.

Table 3. Continued.

Mite population	Tree genotype	No. of successful transfers	No. of attempted transfers	All tree genotypes and mite populations			Tree genotype and mite population 18 removed		
				*FET p	S_d	P	*FET p	S_d	P
L10	18	18	18	0.201	2.93	0.002			
	B209	12	19		0.59	0.278		1.49	0.068
	B23	6	20		−1.65	0.050	0.024	−1.07	0.142
	L10	13	20		0.74	0.230		1.68	0.047
	WH12	3	19		−2.54	0.006		−2.11	0.017
WH12	18	19	19	0.851	2.44	0.007			
	B209	10	17		−0.13	0.448		0.54	0.295
	B23	9	17		−0.48	0.316	0.528	0.15	0.440
	L10	8	20		−1.37	0.085		−0.79	0.215
	WH12	9	17		−0.48	0.316		0.15	0.440

Langor 1992; Amrine and Stasny 1994; Baker et al. 1996). However, the strong population differentiation (Tables 1 and 2) and phylogenetic separation (Fig. 2) among mites on *P. angustifolia* and those on F_1 type hybrid trees suggests that these mites form host-associated races and are likely morphologically cryptic species, similar to the species complex of *Cecidophyopsis* mites found on *Ribes* (Fenton et al. 1995, 1997, 2000). Our estimate of mite population differentiation between F_1 hybrids and *P. angustifolia* ($\phi_{RT} = 0.662$) is well within the range of genetic differentiation described for cryptic species (Dobbler and Farrell 1999; Nason et al. 2002; Abbot and Withgott 2003; Blair et al. 2005; Diegisser et al. 2006a) and higher than most comparative estimates of population differentiation for host races (e.g., $F_{ST} = 0.012$, McPheron et al. 1988; $F_{ST} = 0.07$, Groman and Pellmyr 2000; $F_{CT} = 0.080$, Leebens-Mack and Pellmyr 2004; $F_{ST} = 0.033$–0.381, Ruiz-Montoya et al. 2003; $\phi_{ST} = 0.116 - 0.853$, Stireman et al. 2005; and $\phi_{ST} = 0.19$, Svensson et al. 2005).

It is important to note that our conclusions are based on ITS1 alone, and the possibility exists that other markers (e.g., mtDNA) may yield alternative results. However, we argue that this is unlikely because previous studies have found congruent evidence from multiple loci for eriophyid mites (e.g., microsattelites and ITS1 [Carew et al. 2004], nrDNA and mtDNA [Navia et al. 2005a]), and ITS1 has been successfully used in species discrimination (e.g., Fenton et al. 1993, 2000). One caveat is that some morphological F_1 trees may be early generation backcrosses to *P. angustifolia*, and some morphological *P. angustifolia* may be complex, advanced backcrosses (McIntyre and Whitham 2003). Despite this, differences between the two morphological groups are great and the conclusions presented are unlikely to be affected. In combination with transfer experiments showing four- to five-fold differences in the survival rates of mites on pure and hybrid hosts (McIntyre and Whitham 2003), our molecular genetic findings support the hypothesis that hybridization in a foundation tree species can influence the population differentiation and evolutionary trajectory of a dependent herbivore.

Different plant species represent different environments that can drive herbivore adaptation and reproductive isolation (Berlocher and Feder 2002) and hybrid plants often differ from their parental species in phenotypic traits (Rieseberg and Ellstrand 1993). In the cottonwood system examined here, previous studies have found that *Populus* hybrids and pure species differ in their defensive chemistry, tree architecture, and phenology, which, in turn, has strong effects on the distribution and/or performance of dependent arthropod, microbial, and vertebrate communities (reviewed in Whitham et al. 2006). Thus, we hypothesize that differences in budbreak phenology and defensive chemistry between the two cross types have driven adaptation and incipient speciation in *A. parapopuli*. Other species also appear to have genetically differentiated in response to hybridization. Moran and Whitham (1988) found that the gall-producing aphid, *Pemphigus betae*, exhibits life-cycle differences between the *P. angustifolia* zone and the hybrid zone. Reciprocal transfer experiments support the hypothesis that these life-cycle differences are genetically based (Moran and Whitham 1988).

If hybridization can drive the evolution of dependent organisms (Floate and Whitham 1993), then we expect mites on different *Populus* species and hybrids to be genetically differentiated even when host populations are geographically near to one another. Preliminary data based on the ITS1 region indicate that mites found on the same *Populus* species are genetically similar even when separated by hundreds of kilometers, while mites found on different *Populus* species and hybrids are genetically distinct, even when found in close proximity (L. M. Evans, unpubl. data). Furthermore, these preliminary data suggest that in the Weber River, UT, mites on F_1 hybrid cottonwoods are derived from those on *P. angustifolia*. Further testing of these hypotheses in other rivers with independent hybridization events is needed

for generalization, but our results argue that hybridization in cottonwoods along the Weber River has been a key factor driving genetic differentiation and cryptic speciation in *A. parapopuli*.

ALTERNATIVE HYPOTHESES

Although the observed patterns are consistent with host-mediated population differentiation in *Aceria*, geographical isolation and elevational effects constitute valid alternative explanations. Mites collected from F_1 type hybrid cottonwoods were primarily from the hybrid zone of the Weber River, whereas mites collected from *P. angustifolia* were primarily from the pure *P. angustifolia* zone (see Keim et al. 1989; Martinsen et al. 2001). This geographical and elevational isolation of the two groups could explain the observed population differentiation in the mites.

Two lines of evidence contradict these hypotheses. First, mite populations from F_1 type cottonwood L15 and *P. angustifolia* ZMG19 overlap geographically (Fig. 1). Phylogenetically, the L15 mite population clusters with the F_1 mite group, whereas the ZMG19 mite population clusters with the *P. angustifolia* group (Fig. 2), and the pairwise ϕ_{PT} value is high (0.916, Table 2). Differences in population structure would not be observed if geographical and elevational differences were solely responsible for these patterns. Second, the experiments of McIntyre and Whitham (2003) demonstrate that mites from an F_1 genotype perform much better on F_1 than *P. angustifolia* trees when grown in a common environment. We are currently conducting reciprocal transplant studies to test for local adaptation of mite populations to hybrid cross types within a greenhouse setting, thereby removing possible environmental effects.

An alternative interpretation of the differentiation of mites on the cross types is that mites on hybrid cottonwoods are similar to those found on *P. fremontii* and are simply tracking introgressing *P. fremontii* genes, and that hybridization per se is not driving differentiation. The absence of mites on *P. fremontii* in the Weber River, however, argues against this. Replicated studies in multiple hybrid systems will aid in distinguishing these alternatives, but preliminary data suggest that mites on hybrid cottonwoods are differentiated from those on pure species in multiple rivers.

CONTRASTS BETWEEN MITE GENETIC DIFFERENTIATION AT A NEUTRAL LOCUS AND LOCAL ADAPTATION

We found moderate genetic differentiation of mites among different genotypes within cross types (Tables 1 and 2). Within the F_1 cross type, geographical isolation contributes to the population structure of *A. parapopuli*. Conversely, we found no effect of geographical distance on mite population differentiation within the *P. angustifolia* populations across a much greater area (Fig. 3). Additionally, within the F_1 type hybrids, the variance attributable to differences among galls within individual genotypes was three times greater than that among genotypes (Table 1) and there was strong separation of individual galls phylogenetically (Fig. 2). This suggests that the effect of genotype on neutral marker genetic differentiation of mite populations is relatively weak, and that differentiation within the F_1 cross type is more likely attributable to migration of mites, founder effects, and genetic drift.

Given the reproductive mode of eriophyid mites (haplodiploid parthenogenesis; Helle and Wysoki 1996), one expects little to no variation within galls (Fenton et al. 1995, 1997, 2000; Kumar et al. 1999, Carew et al. 2004, Navia et al. 2005a). However, within each cross type the majority of variation (68-89%) was within galls (Table 1), which is strong evidence for considerable dispersal among galls and among trees.

This finding, using a neutral marker, that migration, genetic drift, and founder effects structure mite populations contrasts strongly with our two independent reciprocal transfer experiments, which demonstrate a strong effect of genotype on the local adaptation of mite populations (Table 3). Although migration can lead to homogenization of populations (Slatkin 1987), selection imposed by different habitats can drive divergence of populations (Schluter 2000). Furthermore, Nosil et al. (2005) found that selection against maladapted immigrants may be a mechanism leading to reproductive isolation and incipient speciation. Strong selection can drive local adaptation of arthropods despite considerable gene flow inferred from neutral loci (Chevillon et al. 1995; Mullen and Hoekstra 2008). Our tests of local adaptation were performed in a greenhouse setting, removing some sources of potential divergent selection, such as predation (Denno et al. 1990), making these tests conservative. This is consistent with the findings of other studies that plant genotype can affect herbivore evolution (Mopper 1996; Craig et al. 2007). Thus, our contrasting results suggest that despite migration among mite populations inferred from a neutral locus, selection for adaptation to individual plant genotypes and against maladapted immigrants is strong enough to drive the evolution of *A. parapopuli*.

An alternative hypothesis is that differential performance of mite populations on individual genotypes results from environmental conditioning (Agrawal et al. 2002). We see this as an unlikely explanation for our results because our metric of performance was survival. The effects of conditioning seem to be less likely when organisms cannot survive on alternate hosts (e.g., Via 1986, 1991). If mites are unable to survive on trees, we find it doubtful that mites could become conditioned to trees. Second, transferred mites likely included inseminated females, which in a study of *Aceria guerreronis* composed 5–10% of the population (Navia et al. 2005b). Newly laid eggs or hatched larvae on the recipient cuttings in our study would not have been exposed to conditioning by the maternal host genotype.

It is interesting to note that patterns of genetic variation of *A. parapopuli* appear to match patterns of expected genetic

variation in their host trees. Although this study did not quantify cottonwood genetic variation, previous studies based on RFLPs (Whitham et al. 1999) showed greater genetic variation in hybrid cottonwoods than in their associated parental species. Thus, patterns of genetic differentiation and variation in population ecology characteristics (McIntyre and Whitham 2003) suggest that mites closely track genetic variation in their cottonwood hosts.

IMPLICATIONS FOR COMMUNITY GENETICS AND CONSERVATION

This study shows that plant hybridization and genotypic variation among individual plants may have evolutionary consequences for herbivores. Two important implications arise from this. First, local adaptations among species are necessary conditions for assessing the potential for community evolution (Shuster et al. 2006; Whitham et al. 2006). Genetic interactions with *Populus* appear to have evolutionary consequences for *A. parapopuli*, and the results of numerous studies of this nature can be used to investigate the concepts of community genetics and evolution in foundation-driven communities (e.g., Stireman et al. 2005). Second, as plant hybridization can drive the evolution of plant species (Grant 1971; Stace 1987) and some of their dependent herbivores (Moran and Whitham 1988; Floate and Whitham 1993; this study), conservation of hybrid plants can be important for maintaining and perpetuating plant and arthropod diversity (Whitham et al. 1991). Our findings of morphologically cryptic species of mites found on pure and hybrid cottonwoods argue for the conservation of genetic diversity in foundation plants and their naturally occurring hybrids.

CONCLUSIONS

As a galling arthropod, *A. parapopuli* is intimately tied to its host trees. Our results strongly suggest morphologically cryptic species occur on different cottonwood cross types. Phenotypic differences among trees could potentially drive the observed adaptation and genetic differentiation of *A. parapopuli*, although we presently lack the empirical studies to support any one mechanism. Nevertheless, host-associated population structure is one postulate for host race formation, and local adaptation of herbivore populations to trees can result in host-associated population structure (Via 2001; Dres and Mallet 2002). Although studies of other river systems are required for generalization, our results argue that hybridization and genotypic differences in *Populus* species and their naturally occurring hybrids have played an important role in the evolutionary diversification of *A. parapopuli*.

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