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THE EFFECTS OF HOST-PLANT GENOTYPE, HYBRIDIZATION, AND ENVIRONMENT ON GALL-APHID ATTACK AND SURVIVAL IN COTTONWOOD: THE IMPORTANCE OF GENETIC STUDIES AND THE UTILITY OF RFLPS

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Abstract.—Using restriction fragment length polymorphisms (RFLPs) we show how host-plant genotype and hybridization in cottonwood, *Populus* sp., affects the attack and survival of the gall-forming aphid, *Pemphigus betae.* Fremont cottonwoods, hybrid F1's and backcross 1's were found to be highly resistant, while backcross 2's, 3's, 4's and pure narrowleafs ranged from highly resistant to highly susceptible with only a few trees being highly resistant. Results from our genetic analysis also suggest that resistance is likely polygenic, not the result of single gene resistance. In addition, genetic analysis showed that studies based upon leaf morphology alone give an inaccurate assessment of host-plant genotype and the extent of hybridization, altering previous views of the relationship between plant hybridization and pest attack. Previous studies assumed that narrowleafs were more resistant than backcross genotypes based upon comparisons of overall levels of resistance between the hybrid zone and the "pure" narrowleaf zone. Results from RFLP analyses, however, show that there are no significant differences in the levels of resistance between backcross genotypes (BC2's–4's) and pure narrowleafs. Furthermore, results show that the "pure" narrowleaf zone is in fact a mixture of pure and backcross genotypes, extending the zone of introgression previously reported. Experiments in combination with RFLP analyses suggest that resistance traits are differentially expressed along an environmental gradient partially explaining the previously reported differences in resistance between these two regions. In light of our results it is clear that genetic studies will be necessary to discern the true relationship between hybridization and pest resistance. Until such studies are widely conducted generalizations regarding the effects of hybridization on the structure and dynamics of pest populations will be premature at best.

Key words.—Fremont cottonwood, narrowleaf cottonwood, *Pemphigus betae,* plant hybridization, *Populus angustifolia, Populus fremontii,* resistance, RFLPs.

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With the recent advent of recombinant DNA technology, and its use in the identification of restriction fragment length polymorphisms (RFLPs), we now have the capability of addressing a number of problems of ecological and evolutionary interest that have proven intractable using conventional methods of investigation. Restriction fragment length polymorphisms have been successfully used to assign parentage to individuals within populations (Wetton et al., 1987; Burke and Bruford, 1987; Quinn and White, 1987; Rabenold et al., 1990), to establish matriarchal ancestry (Avise and Nelson, 1989) to assess genotypic differences within and between populations (Harrison et al., 1987; Baker et al., 1989; Paige et al., 1991) and to measure genetic distance and levels of heterozygosity (Plante et al., 1989). In particular, these techniques circumvent many of the problems associated with electrophoretic (isozyme) studies of natural populations of plants and animals. For example, such genetic studies are often limited by the number of marker loci available. With the development of RFLPs, however, we now have an almost unlimited number of marker loci available. Furthermore, these markers are fully penetrant and unaffected by any form of environmental expression or suppression, examining the DNA directly.

Although RFLP technology is being applied to studies of natural populations of plants and animals with increasing frequency, to date, its use has been limited and sparingly applied. Our use of this technique represents the first time it has been applied to studies of plant–herbivore interactions in a natural system and to plant hybrid zones. This last point is of particular importance in that numerous species hybridize (Stace, 1987) lending wide application to the techniques and uses described herein. In this study, we show how host-plant genotype and hybridization in cottonwood, *Populus* spp.,

affects the attack and survival of a gall-forming aphid, *Pemphigus betae.* We also demonstrate how this technique, in combination with studies of plant hybridization and pest attack, can be used in assessing environmental effects on pest resistance along an environmental cline. The genetic basis of the observed patterns of host-plant resistance are also discussed.

Methods and Materials

Study Site and Organisms

Studies of the interactions of the gall-forming aphid, *Pemphigus betae,* and its cottonwood hosts were conducted along the Weber River drainage north of Salt Lake City, Utah. One tree species, Fremont cottonwood, *Populus fremontii,* occupies lower elevations of riparian habitat and the other, narrowleaf cottonwood, *P. angustifolia,* occupies higher elevations. These two species can be easily distinguished by leaf morphology. Fremont trees have wide leaves, long petioles and a few serrated teeth. In contrast, narrowleaf trees have narrow leaves with short petioles and many small serrated teeth. These two tree populations are connected by a zone of overlap where they interbreed, forming a hybrid swarm.

Previous studies of the nuclear inheritance of this interspecific hybridizing population of cottonwood trees indicated an asymmetry in the frequency of parental genes (Keim et al., 1989; Paige et al., 1991). In individuals making up the hybrid swarm, loci were homozygous for narrowleaf alleles or were heterozygous (both narrowleaf and Fremont alleles were observed). In genetically mixed individuals, however, no homozygous Fremont alleles were found. Thus, hybrid trees represent either F1 hybrids or backcrosses between hybrids and narrowleaf parents. No progeny could be attributed to hybrid–hybrid crosses or backcrosses to Fremont. The most parsimonious explanation for the asymmetric distribution of Fremont and narrowleaf nuclear genes within the hybrid population is likely genetic incompatibility among nuclear genes (see Paige et al., 1991 for a discussion).

Genetic and Morphological Analyses

Host-plant genotype and the extent of hybridization was determined by genetic analysis, using RFLPs (restriction fragment length polymorphisms). The techniques are discussed in detail in Keim et al. (1989). Briefly, high molecular weight DNA was extracted from each of 76 randomly selected trees for genetic analysis (trees were selected from each of three locations; 34 trees from the pure narrowleaf population, 3 trees from the pure Fremont population and 39 trees from the zone of overlap and hybridization). DNAs were cut with restriction enzymes, subjected to electrophoresis in agarose gels, and Southern transferred to charged nylon membranes. Nylon membranes were hybridized to radioactively labelled recombinant DNA clones. Recombinant clones were constructed by ligating total genomic endonuclease-digested DNA, taken from an individual narrowleaf cottonwood, into phage and plasmid vectors to create "libraries" of DNA fragments. Two cloning strategies were used. In one case we digested cottonwood DNA with *Sau*3AI to generate small fragments and then ligated these fragments into the *Bam*HI restriction site of the M13 vector mp9. In the other, we used *Pst*I (a methylation sensitive restriction enzyme) to enrich for single copy DNA (Keim and Shoemaker, 1988). The *Pst*I digested cottonwood DNA was ligated into the *Pst*I restriction site of the plasmid pBS+ (Stratagene Inc.). Both nuclear and cytoplasmic DNA clones were represented in these libraries. In Southern hybridization, cytoplasmic DNA sequences produce a calculated 1,000-fold stronger signal than that obtained with nuclear DNA sequences. This allows the discrimination between nuclear and cytoplasmic sequences. Thus, only nuclear sequence probes from these libraries were used in determining the nuclear genetic constitution of trees. Following hybridization, membranes were washed and then placed against photographic film for autoradiographic exposure. Useful clones, showing a polymorphism between the two parental tree species, were used against individual hybrid or parental trees within the population to determine their pattern of inheritance.

Figure 1 illustrates how these RFLP markers, visualized by autoradiography, are scored and used in determining the genetic constitution of individual cottonwood trees

	TREES					
	F	N	989	11	996	1008
PROBES						
5S	F	N	NF	N	N	N
2	F	N	NF	N	N	N
11	F	N	NF	N	N	NF
13	F	N	NF	N	N	N
23	F	N	NF	NF	N	N
26	F	N	NF	NF	N	N
27	F	N	NF	N	N	N
76-10	F	N	NF	N	N	N
76-11	F	N	NF	NF	N	N
76-20	F	N	NF	NF	N	N
GENETIC CONSTITUTION	P	P	F1	BC2	P	BC4

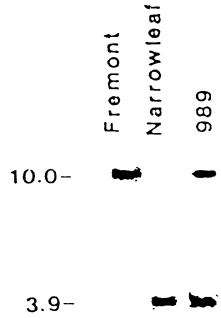

FIG. 1. RFLP analyses allow one to determine the genetic constitution of an individual tree. Each locus (or probe) is scored as either pure narrowleaf (NN), pure Fremont (FF) or hybrid (NF) according to the information derived from the autoradiograph (see below). For example, tree 989 has both narrowleaf and Fremont fragments illustrating the codominant pattern of inheritance and would be scored as NF at this locus. By scoring and accumulating a large number of loci one can determine whether a tree is a pure parental type (100% narrowleaf or 100% Fremont), an F1 hybrid (50% Fremont, 50% narrowleaf) or some complex backcross (e.g., BC1—25% Fremont, 75% narrowleaf; BC2—12.5% Fremont, 87.5% narrowleaf, etc.). Examples of how trees are scored are shown.

within the population; i.e., whether the tree is a pure narrowleaf, a pure Fremont, an F1 hybrid or some complex backcross (a BC1, BC2, BC3, etc.). An average of 13.6 polymorphic markers were used against each tree (1,032 markers on 76 trees), ranging from 5 to 40 markers/tree (see Appendix).

Results also include a single mitochon-

drial DNA marker against 72 of the 76 trees in addition to the nuclear DNA markers used (see Paige et al., 1991 for details of the mitochondrial DNA analysis). Briefly, recombinant clones for mitochondrial analysis were constructed by digesting mitochondrial DNA (extracted from a narrowleaf cottonwood cell suspension tissue culture) with *Hin*dIII and ligating the fragments into the *Hin*dIII restriction site of the plasmid pBS+ (Stratagene Inc.). Probes were screened against DNA from known pure narrowleaf and Fremont cottonwoods (trees that had been screened with between 25 and 35 nuclear markers): Following DNA extraction (from leaf material) and purification, DNA from these trees was digested with restriction enzymes and the fragments were separated according to their molecular size by gel electrophoresis in agarose gels. The separated fragments were transferred to nylon membranes (Southern, 1975) and then hybridized with the radioactively labelled probe, after which the membrane was analyzed by autoradiography. Useful mitochondrial clones showing a polymorphism between the two parental tree species were used against individual hybrid or parental trees within the population to determine their pattern of inheritance.

To assess how accurate leaf morphology is as a predictor of host-plant genotype three measurements on five haphazardly selected leaves from sample specimens of 32 trees were taken (5 pure Fremont, 7 pure narrowleaf, 4 F1 hybrids and 16 backcrosses). Measurements included leaf length, leaf width, and petiole length. A shape variable was created by dividing petiole length by the ratio of leaf length to leaf width.

Host-Plant Resistance

Each spring these cottonwood trees are attacked by the leaf galling aphid (the colonizing stem mother), *Pemphigus betae*. If the stem mother is successful, she is rapidly encapsulated within a gall where up to 300 progeny are produced parthenogenetically (Whitham, 1978). Stem mothers that die during gall initiation leave a small scar as evidence of their failed attempt (Whitham, 1989). Thus, host-plant resistance can be quantified as the survival rate of these aphid colonizers (e.g., of 607 gall attempts recorded for one tree, 310, or 51%, of all aphids survived whereas, of 60 gall attempts recorded on a nearby tree, none survived; Whitham, 1989). Using this approach, host-plant resistance was assessed for each of the 76 trees [resistance data were kindly provided by Dr. T. G. Whitham (Whitham, 1989)]. Resistance data were obtained primarily from transfer experiments and in five cases (trees 993, 1017, 1018, 1023 and 1029), natural colonization events. Natural and experimentally determined survival rates (Whitham, unpubl. data) yield similar results ($r = 0.92$, $N = 19$, $P < 0.00001$; see Appendix for identity of these trees). For transfers, approximately 40 stem mothers were experimentally placed onto each tree and their subsequent survival rates recorded. Stem mothers were taken from overwintering eggs collected from a single source tree within the hybrid zone to eliminate donor effects. Eggs were refrigerated and, when exposed to ambient temperatures, hatched within a few days. These first instar wingless stem mothers were then transferred to trees at bud break. Stem mothers were individually transferred to small branches and a sticky barrier was placed at the base of each branch to prevent emigration; survival rates were recorded about 45 days later (from Whitham, 1989).

To determine whether variation in host-plant resistance is genetically based, sixteen mature trees (see Appendix for identities) with aphid survival rates ranging from 0 to 75% were selected from the hybrid zone (1,381 m in elevation), vegetatively propagated and grown for four years in a common garden (located 1,582 m in elevation). The survival rates of 3,198 aphids transferred to these derivative clones were then compared to the survival rates of 8,458 aphids that had naturally colonized the parental trees (from Whitham, 1989). For transfer experiments, stem mothers were taken from overwintering eggs collected from a single source tree within the hybrid zone, hatched and transferred to trees at the time of bud break. Stem mothers were individually transferred to small branches and a sticky barrier was placed at the base of each branch to prevent emigration; survival rates were recorded about 45 days later (from Whitham, 1989).

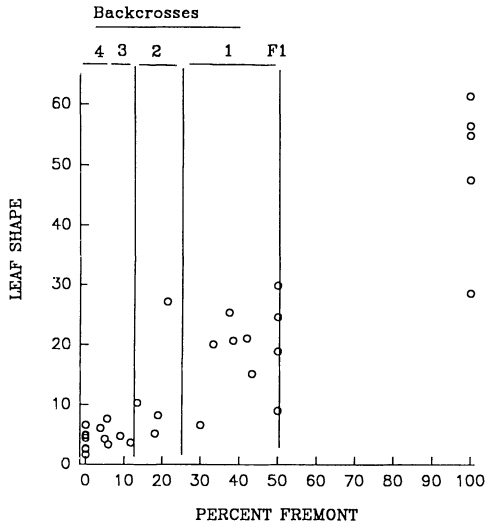

FIG. 2. The relationship between leaf morphology and host-plant genotype. To assess how accurate leaf morphology is as a predictor of host-plant genotype, three measurements on five haphazardly selected leaves from sample specimens of 32 trees were taken. Each point represents the average of the five leaves for each tree. Host-plant genotype was assessed using RFLPs. The leaf shape variable was created by dividing petiole length by the ratio of leaf length to leaf width. All individuals at the extreme left of the abscissa are pure narrowleafs and all individuals at the extreme right are pure Fremonts. Results show that leaf morphology alone gives an inaccurate assessment of host-plant genotype and the extent of hybridization due to extensive overlap, particularly for BC2's, 3's, 4's and pure narrowleafs.

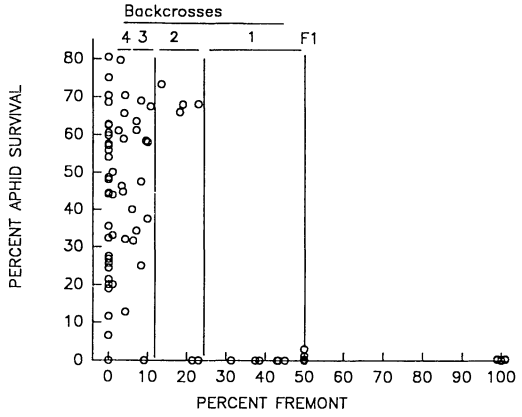

FIG. 3. The relationship between aphid survival and host-plant genotype. Pure Fremont, F1 hybrids and BC1's were highly resistant with only a few aphids (<3%) surviving on hybrid F1's. Backcross 2's, 3's, 4's and pure narrowleafs ranged from highly resistant to highly susceptible with only a few trees being highly resistant. Data are based on 76 tree genotypes.

RESULTS

Genetic and Morphological Analyses

Results from our genetic analysis of this interspecific hybridizing population of cottonwoods show that morphology alone gives an inaccurate assessment of host-plant genotype and the extent of hybridization. For example, it is extremely difficult to distinguish between a pure narrowleaf and a complex backcross (BC2's, BC3's, etc.) to the narrowleaf side based on leaf morphology alone (Fig. 2). RFLP analyses also show that the "pure" narrowleaf zone, originally based on morphology, is in fact a mixture of pure and backcross genotypes, i.e., 41% of the trees are complex backcrosses (BC3's and BC4's).

In addition, results from our genetic analysis show that successful matings only occur between hybrids and narrowleaf trees (see also Keim et al., 1989; Paige et al., 1991). Therefore, hybrids do not breed true and introgression is directional (i.e., no homozygous Fremont alleles were found in a hybrid background; therefore, no trees could be attributed to hybrid–hybrid crosses or backcrosses to Fremont; see appendix data summary).

Host-Plant Resistance

Aphid survival rates on these trees ranged from 0 to 80% (Fig. 3). Such variation in host-plant resistance was also found to be genetically based. When the survival rates of aphids transferred to derivative clones in the common garden were compared to the survival rates of aphids that had naturally colonized the parental trees, a strong positive relationship was found ($r = 0.90$, $N = 16$, $P < 0.001$, $b = 0.98 \pm 0.127$; Whitham, 1989).

Hybridization and Host-Plant Resistance

The relationship between gall-aphid survival and host-plant genotype is shown in Figure 3. The complete absence of *P. betae* on Fremont cottonwood indicates that it is not used as a host. Even when stem mothers were experimentally transferred to these trees they were unable to make galls, ultimately resulting in the deaths of these aphids (Whitham, 1989). Hybrid F1's and backcross 1's were found to be highly resistant

with only a few aphids (<3%) surviving on hybrid F1's. In addition, backcross 2's, 3's, 4's, and pure narrowleafs ranged from highly resistant to highly susceptible with only a few trees being highly resistant. No significant differences in the levels of resistance were found among these last four genotypic classes ($F = 0.498$, $df = 3,57$, $P = 0.685$; average survival on BC2's = 45.9% ± 14.6 standard error of the mean, BC3's = 46.1% ± 6.0, BC4's = 49.3% ± 5.2 and pure narrowleafs = 40.9% ± 4.0).

Discussion

Genetic Basis of Host-Plant Resistance

Studies in which the genetic basis of plant resistance to pest attack is known suggest that relatively simple genetic models could explain our results (Gallun and Khush, 1980; Sage et al., 1986). For example, if alleles conferring resistance differ in the two species, with Fremont alleles acting in a dominant fashion to narrowleaf alleles, the majority of susceptible genotypes (BC2's–BC4's) would arise after initial (F1 and BC1) hybridization events. To test this idea we fit a single gene model (1 locus, 3 alleles) to the data, where, in the narrowleaf population, AA was designated completely resistant, and Aa and aa susceptible to varying degrees. In the Fremont population A′A′ was designated completely resistant (all Fremont are completely resistant to gall aphid attack) with A′ dominant to all alleles in the narrowleaf population. Allele frequencies were calculated directly from the percentages of totally resistant trees in the pure narrowleaf and Fremont populations.

Although the patterns so generated using this model approximate the results obtained from our genetic analysis in a very general sense (in that susceptibility increases with continual backcrossing), the pattern differs significantly from the observed data; i.e., the single gene model fits the observed distribution of resistance genotypes in the narrowleaf and F1 hybrid populations, but breaks down in the BC1 generation. This single gene model predicts that 51.7% of BC1 trees should be resistant and 48.3% should have some level of susceptibility; however, all BC1 trees were found to be resistant (the probability of all 6 BC1 trees being resistant by chance alone is only 0.019, a relatively rare event). Thus, a single gene model appears to be too simplistic indicating that the inheritance of resistance is likely polygenic, i.e., several loci would have to be involved for the majority, if not all, of the BC1's to be resistant.

Host-Plant Resistance: Genetics and Morphology

In previous studies, classification of host-plant genotype based upon morphological traits led to considerable confusion as to the relationship between host-plant genotype and pest resistance. For example, in a previous study it was assumed (Whitham, 1989) that "narrowleafs" were more resistant than backcross genotypes based upon comparisons of overall levels of resistance between the hybrid zone and the "pure" narrowleaf zone (mean aphid survival = 60.7% ± 2.59 versus 38.5% ± 1.99). These genotypic determinations were based solely on morphological traits. However, RFLP analyses reveal that the "pure" narrowleaf zone is in fact a mixture of pure and backcross genotypes; 41% of the trees (14 of 34 trees) were backcross 3's and 4's, extending the zone of introgression previously reported (Whitham, 1989). Only 47.5% of the trees (19 of 40 trees) in the "hybrid" zone were susceptible backcross genotypes (BC2's, BC3's, and BC4's). Furthermore, our results show that there are no significant differences in the levels of resistance between backcross genotypes (BC2's–BC4's) and pure narrowleafs. This pattern is observed both throughout the drainage ($F = 0.498$, $df = 3,57$, $P = 0.685$; average survival on BC2's = 45.9%, BC3's = 46.1%, BC4's = 49.3% and pure narrowleafs = 40.9%) and within each of the zones ("hybrid" zone $F = 0.022$, $df = 2,17$, $P = 0.978$; average survival on BC3's = 60.3%, BC4's = 59.4% and pure narrowleafs = 57.9%; "pure" narrowleaf zone $F = 1.17$, $df = 2,31$, $P = 0.325$; average survival on BC3's = 44.6%, BC4's = 37.7% and pure narrowleafs = 34.0%; BC2's only occur in the "hybrid" zone). Thus, these data do not support the hypothesis that aphid distributions are determined by the distribution of backcross genotypes as previously thought (Whitham, 1989; Paige et al., 1990).

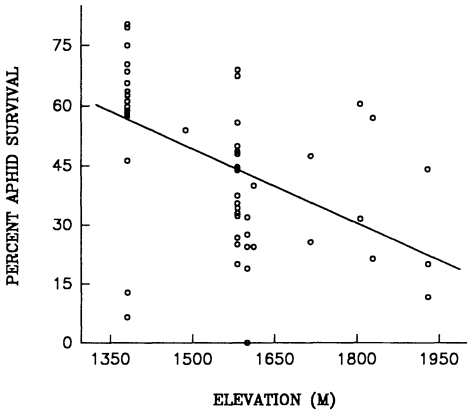

FIG. 4. Correlation between aphid survival on individual cottonwood trees and elevation. As elevation increases, aphid survival declines ($r = -0.50$, $F = 17.07$, $df = 1,52$, $P < 0.00013$). Data are based on 54 trees, including BC3's, BC4's and pure narrowleafs which co-occur along the elevational gradient.

Host-Plant Resistance: Environmental Effects

These results beg the question as to why differences in resistance exist between the two designated zones ("pure" narrowleaf and "hybrid" zones)? RFLP analyses combined with aphid survival data suggest that resistance traits may be differentially expressed along an environmental gradient.

As one proceeds from lower to higher elevation along the Weber River, plants become progressively less susceptible, of course with the exception of pure Fremonts which are totally resistant (Fig. 4, $r = -0.500$, $F = 17.07$, $df = 1,52$, $P < 0.00013$; for BC3's, BC4's and pure narrowleafs which co-occur along the elevational gradient from 1,381–1,930 m). Pure Fremont's, F1's, BC1's and BC2's (which also co-occur with BC3's–BC4's and pure narrowleafs) are restricted primarily to a narrow range (1,329–1,433 m) at low elevation (the "hybrid" zone) and thus were not included in the elevational analysis. This relationship also holds for a given genotype (for BC4's $r = -0.610$, $F = 7.57$, $df = 1,13$, $P < 0.016$, for pure narrowleafs $r = 0.402$, $F = 5.02$, $df = 1,26$, $P < 0.034$, and for BC3's $r = -0.549$, $F = 3.89$, $df = 1,9$, $P < 0.080$, although only marginally significant, the relationship is in the same direction). Although elevational differences in host-plant resistance might seemingly alter the patterns of resistance illustrated in Figure 3, overall patterns remain the same (i.e., BC3's, 4's and pure narrowleafs still vary widely in resistance) when adjustments for differences in elevation are made (data not shown; pure Fremont, F1 hybrids, BC1's and 2's were not included in this analysis because they do not co-occur along the elevational gradient).

To experimentally demonstrate that a change in elevation can alter host-plant resistance, we compared aphid survival data obtained from our previous experiment (see above) in which aphids were transferred to derivative clones (established at 1,582 m in elevation) to the natural survival rates of aphids on parental trees (located 1,381 m in elevation). Results of this experiment showed that aphid survivorship was consistently lower (39% versus 48%, on average) on the derivative clones than on the parental trees (Wilcoxon signed rank test, $Z = 2.55$, $N = 16$, $P < 0.01$). This was not the case when transfers were conducted within the same site in the hybrid zone (48.8% versus 48.0%, experimentally transferred versus natural survival rates, respectively; Wilcoxon signed rank test, $Z = 0.56$, $N = 19$, $P > 0.50$). These results are not likely due to the effects of tree age. Kearsley and Whitham (1989) point out that stecklings (rooted cuttings) maintain the resistance traits of the mature trees from which they are taken. Indirect support also comes from the naturally occurring gradient of resistance; trees occurring along the natural gradient are similar in age yet differ in resistance traits. As predicted, the magnitude of the change in aphid resistance from regression analysis (10%; Fig. 4) is similar to the magnitude of change observed in our transfer experiments (9%) over the same elevational gradient (45% versus 55% from regression analysis, 39% versus 48% from transfer experiments) indicating an environmental influence on resistance trait expression. Overall, regression results (Fig. 4) indicate that for a given genotype resistance may increase by as much as 2.5-fold as one moves to the extremes in elevation, from the "hybrid" zone (1,381 m) high into the "pure" narrowleaf zone (1,930 m). Additional experimental studies (establishment of a common garden at high elevation), however, will be necessary to substantiate this claim.

An alternative hypothesis for the increase in resistance as one proceeds higher in elevation contends that aphids are directly affected by elevational differences in environment (e.g., temperature). Environmental differences, such as temperature, are known to effect patterns of aphid host-alternation (Moran and Whitham, 1988). Such environmental differences may also result in increased mortality as well (aphids were collected from a single source tree and transferred, therefore results are not confounded by potential genotypic effects of site). Furthermore, the effects of environment on both resistance trait expression and aphid survivorship may well work in concert. However, the mechanisms affecting the interaction between host-plant resistance and aphid survivorship along this elevational gradient remain to be determined.

In addition, there may be allelic differences in resistance along the elevational gradient contributing to the observed patterns in insect resistance. In a previous study (Paige et al., 1991) we presented evidence supporting the idea that the narrowleaf population is spreading down the canyon and the Fremont population receding. A hybridization pattern of decreasingly complex backcrosses as one proceeds from higher to lower elevation within the hybrid swarm, a residue of Fremont cytoplasmic DNA within the pure narrowleaf population, and the unidirectional nature of these crosses (i.e., hybrids only backcross with pure narrowleaf; no hybrid by hybrid or hybrid by Fremont crosses occur) are all consistent with such a pattern. Thus, as the narrowleaf population spreads and descends, the zone of overlap and the hybrid swarm also descend. Given these patterns, one might expect a gradient in resistance due to the length of time upon which selection has had to act upon new genetic combinations along the environmental gradient (i.e., a decrease in the number of generations as one moves from higher to lower elevation due to the descent of the pure narrowleaf population and hybrid zone through time). Yet, demonstrating allelic differences associated with insect resistance is, at present, a difficult problem in that we do not have specific markers for insect resistance. We are, however, continuing to add RFLP markers to our data set in hopes of marking resistance genes in the future (see Paige et al., 1990) so that we can directly address this question.

In light of our results it is clear that genetic studies are necessary to discern the true relationship between hybridization and pest resistance. Until such studies are widely conducted generalizations regarding the effects of hybridization on the structure and dynamics of pest populations will be premature at best (Boecklen and Spellenberg, 1990). RFLPs clearly circumvent the pitfalls of making assessments based on morphology alone and allow one to circumvent the problems of conducting extensive crossing experiments on long-lived organisms to assess genetic structure and its effects.

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APPENDIX

Nuclear and mitochondrial genotypes of individual cottonwood trees along the Weber River north of Salt Lake City, Utah. The nuclear genotype of each tree is presented by the number of markers examined and classified as Fremont (F) only, hybrid or heterozygotic (H), or narrowleaf (N). Trees were designated as either pure parental types (all markers homozygous Fremont or narrowleaf), F1 hybrids (all loci heterozygous), or as a backcross (e.g., BC1—25% Fremont, 75% narrowleaf; BC2—12.5% Fremont, 87.5% narrowleaf, etc.). Mitochondrial genotypes are presented as either narrowleaf (N) or Fremont (F). Plants which had only narrowleaf nuclear markers and Fremont mitochondrial DNA were all classed as BC4's but designated as BC4 or greater ("or >," i.e., possibly a more complex backcross).

Tree		Nuclear genotype (F:H:N)	Mitochondrial genotype (N or F)	Cross
A	F	39:00:00	F	F
A	033	16:00:00	F	F
A	9-17	18:00:00	F	F
A	001	16:00:00	F	F
A	012	16:00:00	F	F
A	H14	11:00:00	F	F
A	1994	00:05:00	F	F1
AB	1997	00:05:00	F	F1
A	Hm1	00:17:00	F	F1
ABC	989	00:26:00	F	F1
A	H1	00:12:02	F	BC1
A	H2	00:10:03	F	BC1
A	H6	00:18:02	F	BC1
AB	1981	00:13:02	F	BC1
A	1979	00:09:03	F	BC1
A	1937	00:05:03	N	BC1
A	H3	00:05:08	F	BC2
A	H9	00:03:04	N	BC2
A	H10	00:04:07	N	BC2
A	H12	00:05:06	N	BC2
ABC	11	00:07:19	F	BC2
	2029B	00:05:06	F	BC2
ABC	1007	00:04:17	N	BC3
B	1021	00:01:04	N	BC3
B	1014	00:01:06	N	BC3
	T21	00:03:11	N	BC3
	Ec1	00:01:05	N	BC3
BC	1005	00:01:06	N	BC3
	COAL2	00:01:07	F	BC3
	T23	00:01:04	F	BC3
	T1	00:01:06	N	BC3
	T19	00:01:05	F	BC3
	T18	00:01:05	—	BC3
AC	999	00:01:12	F	BC4
A	48	00:00:16	F	BC4 or >
ABC	1008	00:02:22	N	BC4
	1017	00:01:15	N	BC4
ABC	1019	00:01:11	N	BC4
AC	1023	00:01:19	N	BC4
AB	1025	00:01:14	N	BC4

APPENDIX. Continued.

	Tree	Nuclear genotype (F:H:N)	Mitochondrial genotype (N or F)	Cross
B	1028	00:01:13	N	BC4
	T13	00:00:13	F	BC4 or >
	T20	00:00:13	F	BC4 or >
	Rm5	00:01:12	N	BC4
	He3	00:01:08	N	BC4
	Wc4	00:00:08	F	BC4 or >
	1029	00:01:11	N	BC4
	T11	00:01:13	N	BC4
ABC	996	00:00:23	N	N
AC	13	00:00:16	N	N
A	3200	00:00:16	N	N
A	T15	00:00:22	N	N
BC	1000	00:00:13	—	N
B	1001	00:00:08	N	N
AB	1011	00:00:17	N	N
ABC	1012	00:00:15	N	N
	1018	00:00:16	N	N
	He8	00:00:12	N	N
	RL1	00:00:11	N	N
	Rm6	00:00:15	N	N
	T16	00:00:07	N	N
	T9	00:00:05	N	N
	RL5	00:00:11	N	N
	Rm1	00:00:10	N	N
	T14	00:00:13	N	N
	Ec3	00:00:13	N	N
	Wc1	00:00:12	N	N
B	1006	00:00:05	—	N
	Rm2	00:00:08	N	N
	Rm7	00:00:08	N	N
	T7	00:00:09	N	N
	T22	00:00:08	N	N
	Wc5	00:00:08	N	N
	T2	00:00:05	N	N
	COAL3	00:00:06	—	N
B	993	00:00:06	N	N

[A] Trees used in previous studies (Keim et al., 1989; Paige et al., 1991).
[B] Nineteen trees in which natural survival rates were compared to survival rates of aphids experimentally transferred.
[C] Twelve of sixteen trees (four were not included in our genetic analysis and are not listed) in which natural survival rates were compared to the survival rates of aphids experimentally transferred to derivative clones grown in a common garden at higher elevation.