

## HYBRID POPULATIONS SELECTIVELY FILTER GENE INTROGRESSION BETWEEN SPECIES

GREGORY D. MARTINSEN,<sup>1</sup> THOMAS G. WHITHAM,<sup>1</sup> RICHARD J. TUREK,<sup>2</sup> AND PAUL KEIM<sup>1</sup>

<sup>1</sup>Department of Biological Sciences and The Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, Arizona 86011

E-mail: gregory.martinsen@nau.edu

<sup>2</sup>Department of Mathematics and Statistics, Northern Arizona University, Flagstaff, Arizona 86011

**Abstract.**—Hybrids have long been recognized as a potential pathway for gene flow between species that can have important consequences for evolution and conservation biology. However, few studies have demonstrated that genes from one species can introgress or invade another species over a broad geographic area. Using 35 genetically mapped restriction fragment length polymorphism (RFLP) markers of two species of cottonwoods (*Populus fremontii* × *P. angustifolia*) and their hybrids ( $n = 550$  trees), we showed that the majority of the genome is prohibited from introgressing from one species into the other. However, this barrier was not absolute; Fremont cpDNA and mtDNA were found throughout the geographic range of narrowleaf cottonwood, and 20% of the nuclear markers of Fremont cottonwood introgressed varying distances (some over 100 km) into the recipient species' range. Rates of nuclear introgression were variable, but two nuclear markers introgressed as fast as the haploid, cytoplasmically inherited chloroplast and mitochondrial markers. Our genome-wide analysis provides evidence for positive, negative, and neutral effects of introgression. For example, we predict that DNA fragments that introgress through several generations of backcrossing will be small, because small fragments are less likely to contain deleterious genes. These results argue that recombination will be important, that introgression can be very selective, and that evolutionary forces within the hybrid population to effectively "filter" gene flow between species. A strong filter may make introgression adaptive, prevent genetic assimilation, lead to relaxed isolating mechanisms, and contribute to the stability of hybrid zones. Thus, rather than hybridization being a negative factor as is commonly argued, natural hybridization between native species may provide important genetic variation that impacts both ecological and evolutionary processes. Finally, we propose two hypotheses that contrast the likelihood of contemporary versus ancient introgression in this system.

**Key words.**—Cottonwoods, disequilibrium, hybrid zones, hybridization, introgression, *Populus*, recombination.

Received April 24, 2000. Accepted March 20, 2001.

Interspecific hybridization is an important process in plant evolution—an estimated 50–70% of all angiosperms arose by hybridization (Stace 1987). Hybridization can impact evolution in at least three ways (Stebbins 1959). First, because hybridization is a form of genetic recombination, it generates new genotypes that may exhibit heterosis, especially in novel habitats that are likely to occur at the edge of species' ranges, where hybrid zones commonly form. Second, the stabilization of hybrid populations (often through polyploidy) can result in the formation of new biological species. Finally, if hybrids backcross to the parental species, gene flow can occur across species boundaries (introgression). Introgressive hybridization was first defined as the infiltration of germ plasm of one species into another through repeated backcrossing of hybrids to parental species (Anderson and Hubricht 1938).

Introgression can have various consequences (Arnold 1992). At one extreme, introgression may cause a merging of the hybridizing species. As a result, recent conservation policy discourages hybridization between species, because hybridization is thought to cause "genetic disintegration" (O'Brien and Mayr 1991). At the other extreme, introgression may lead to selection for conspecific mating and increased reproductive isolation (Howard 1986). Introgression may also result in more fit genotypes which are able to colonize new habitats (Lewontin and Birch 1966). Finally, introgression can result in increased susceptibility to pests, such that hybrids may act as "sinks" for pest organisms (Whitham 1989).

Early evolutionary biologists realized that introgression could be a potent evolutionary force (Anderson 1949; Stebbins 1959). However, until recent years the demonstration of

introgression in natural populations has been problematic. It is difficult to detect introgression based on morphological characters because backcross hybrids often closely resemble the parental species. The amount of genetic material from the opposite parent is halved with each successive backcross generation, making introgression difficult to detect even with genetic techniques. In addition, there are at least two alternative explanations for apparent introgression: convergent evolution and symplesiomorphy.

The development of molecular genetic markers has greatly facilitated studies of hybridization and introgression. Unlike morphological characters, molecular markers are independent and have simple modes of inheritance and expression. Also, the number of potentially available genetic markers far exceeds the number of morphological characters. Thus, genetic markers are much more likely to detect low levels of introgression. These markers can be used to study both nuclear and cytoplasmic gene flow. Cytoplasmic markers are non-recombinant, so they both provide evidence of the direction of introgression and help distinguish introgression from convergence and symplesiomorphy. Furthermore, there are several cases where introgression is only detectable using cytoplasmic markers (Rieseberg and Brunfeldt 1992, Rieseberg and Wendel 1993).

The question of whether or not introgression leads to adaptive evolution is fundamental yet rarely addressed (Arnold 1997). Although we have certainly not answered this question, our data suggest that hybrids act as evolutionary filters (see also Key 1968; Harrison 1986), allowing beneficial genes through but preventing introgression of negative genes. The

idea of hybrids as filters or "barriers" (Barton and Bengtsson 1986) is consistent with hybrid zone theory (Barton and Hewitt 1985, 1989; Harrison 1990); however, previous studies that invoked a filter have emphasized introgression of neutral genetic markers (Key 1968; Harrison 1986).

We studied introgression in cottonwoods (*Populus*), which dominate the riparian vegetation throughout much of western North America. These cottonwoods are classified in two sections, and intersectional hybridization in this genus is widespread (Eckenwalder 1984a). Along the Weber River in northern Utah, Fremont (*P. fremontii*) and narrowleaf (*P. angustifolia*) cottonwood naturally hybridize. This system has several features that make it ideal for studies of natural introgression. First, the introgression is unidirectional (Keim et al. 1989), therefore this system is simpler than ones in which hybrids mate with both parental species or with each other. Second, because narrowleaf cottonwood is found at high elevations whereas Fremont cottonwood is found at low elevations, these species show clear environmental and spatial separation. Because these species and their hybrids are limited to stream-side habitats, potential gene flow is linear (i.e., either up or down canyons). This linear gene flow greatly simplifies sampling and the spatial analyses, which would be confounded in other more complex hybridizing systems. Third, we were able to compare rates of introgression in a small zone of sympatry (localized introgression) with introgression rates among allopatric species (dispersed introgression) (Heiser 1973). Finally, there has been extensive work with *Populus* molecular genetics, and there are now over 200 restriction fragment length polymorphism (RFLP) markers for which a linkage map has been constructed (Bradshaw et al. 1994).

The objectives of this study were as follows: (1) document the extent of introgression in the hybrid zone, where hybridization is obviously occurring based on morphological differences among trees (Whitham 1989, Martinsen and Whitham 1994); (2) compare these rates of localized introgression with potential dispersed introgression occurring at higher elevations in the "pure" narrowleaf zone; (3) compare introgression rates of different nuclear (nDNA) markers; and (4) compare nuclear introgression with cytoplasmic introgression using chloroplast (cpDNA) and mitochondrial (mtDNA) markers. With these data, we then address ecological and evolutionary issues associated with introgression, that is, how hybrids might act as genetic filters, the role of fragment lengths in introgression, and the stability of hybrid zones.

#### MATERIALS AND METHODS

Cottonwoods are the dominant tree throughout the drainage of the Weber River. Fremont cottonwood grows at elevations of approximately 1300–1500 m and narrowleaf cottonwood grows at elevations of approximately 1400–2300 m. Where their ranges overlap there is a 13-km-long zone where extensive hybridization occurs. Complex backcross trees closely resembling pure narrowleaf cottonwood occur in the narrowleaf zone, but for the purposes of this study the hybrid zone was defined as the zone of overlap, or sympatry between the two parental species. There are no Fremont cottonwoods or F<sub>1</sub> hybrids in the narrowleaf zone.

We isolated DNA from a total of 550 trees spread throughout the drainage of the Weber River. Trees were haphazardly sampled as follows: 50 at four sites in the pure Fremont zone, 125 at five sites in the hybrid zone, and 250 at 10 sites in the narrowleaf zone (Fig. 1). We sampled an additional 125 trees representing extremes of arthropod or disease resistance/susceptibility or uncommon phenotypes such as F<sub>1</sub> hybrids in the hybrid zone. Early flush leaves were collected, immediately frozen using dry ice, and later lyophilized and ground to a fine powder. Total genomic DNA was isolated using a slightly modified version of the protocol described by Bradshaw and Stettler (1993).

RFLP analyses of individual cottonwood trees used the Southern transfer technique, in which DNA is transferred from an agarose gel to a nylon membrane (blot), followed by molecular hybridization with radioactive DNA markers (probes). Details are given in Keim et al. (1992). The nuclear probes were those described in Bradshaw et al. (1994) and represent independent loci on separate chromosomes or separated by more than 50 cM, the chloroplast probe was from Lindholm and Gustafsson (1991), and the mitochondrial probe was from Paige et al. (1991). Screening blots were composed of DNA from known Fremont, narrowleaf, and F<sub>1</sub> hybrid cottonwoods digested with one of four restriction enzymes (EcoRI, EcoRV, HindIII, or DraI). These screening blots were used to identify probe-restriction enzyme combinations that generated polymorphisms that were species specific (Fig. 2). The same probe was then run against a set of survey blots containing DNA of all 550 trees. Table 1 lists polymorphic restriction fragments for all of the probes used in this study.

Only those markers that showed fixed polymorphisms between narrowleaf and Fremont cottonwood were used to study introgression. Also note that RFLP markers are codominant (e.g., Fig. 2), which is a huge advantage for hybrid studies. Codominant markers (e.g., allozymes, RFLPs) show both alleles of a given locus, allowing for the selection of species specific alleles of the same genetic locus. Dominant markers (most RAPDs and other PCR-based markers) only allow for the visualization of one allele. Assuming the distribution of markers is equal between species, twice as many dominant markers are needed to provide the same amount of information and genetic resolution (Rieseberg and Ellstrand 1993). In addition, use of codominant markers does not require the assumption of Hardy-Weinberg equilibrium for population genetic inference.

Because previous RFLP studies (Keim et al. 1989) showed introgression to be unidirectional (e.g., from Fremont to narrowleaf), we analyzed the data in terms of frequency of the Fremont marker allele. For an individual tree, a score of 1.0 would be homozygous Fremont, 0.0 would be homozygous narrowleaf, and 0.5 would be heterozygous. For every marker, we also computed mean scores for all trees at each of the 19 sites. We used one cpDNA marker, one mtDNA marker, and a total of 33 nDNA markers. All of these markers were diagnostic of the parental species.

Regression analyses were used to model introgression rates for different genetic markers. For the five hybrid zone sites and the 10 narrowleaf zone sites, the mean scores for each site (described above) were regressed against relative dis-

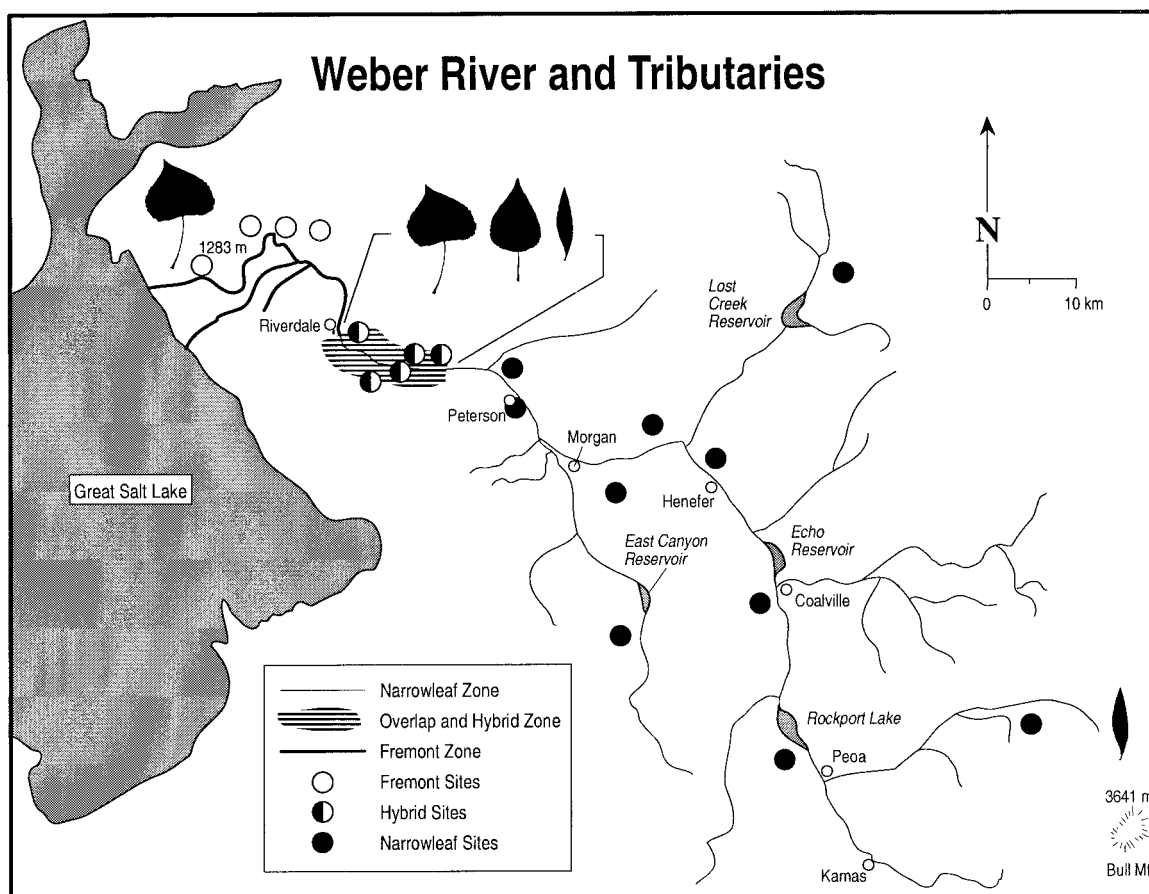


FIG. 1. Map of the ca. 500 km Weber River drainage system in northern Utah, including the 13-km hybrid and overlap zone. The solid black circles show 19 sites where 425 trees were collected at random for genetic analyses. An additional 125 trees were collected in the hybrid zone (see text).

tance from the westernmost hybrid site. To linearize the data and stabilize variances, transformation techniques were used. Specifically, the mean scores were arcsine square root transformed and regressed on the reciprocal of the square of the relative distances of the sites.

Several individual regression equations were compared in order to detect differences in introgression rates between different markers. Because a total of 21 pairwise comparisons were conducted, we employed a Bonferroni adjustment to control the experiment-wise error rate. Individual tests were each done using a significance level of  $\alpha = 0.005$ .

We examined the potential for cytonuclear disequilibrium (i.e., linkage between cytoplasmic markers and nuclear markers). Using a test of binomial proportions, we compared the number of hybrid trees with the Fremont chloroplast haplotype and individual Fremont nuclear markers with the number of trees with Fremont chloroplast DNA and narrowleaf nuclear DNA.

Based on junction theory (Fisher 1953), which was developed to track parental chromosome blocks in inbred populations, later extended to other mating designs for crop plants (Hanson 1959a, b), and recently applied to hybrid zones by Baird (1995), we present a simple illustration of the effects of recombination on size of introgressed fragments under multiple generations of backcrossing. We make two

important assumptions. First, we have assumed that each backcross generation matches Mendelian recombinational expectations. This simplifies the modeling problem to a discrete number of particular centimorgan (cM) size categories (e.g., 50, 25, 12.5, etc.) as opposed to a continuous distribution with average expectations. This assumption does not affect predicted average size because the continuous distribution would still average the same as our discrete categories. Second, we assume that there is no selection for or against particular recombinational products due their gene composition. Given these assumptions, the size of introgressing segments was decreased by postulating a recombination event in the middle of the segment to create two equally sized products. This represents the maximum disruptive event possible and, therefore, predicts the fastest possible disequilibria decay rate (without selection). The frequency and probability of these recombination events is, of course, a function of each segment's size in cM.

We recognize that other authors have generated more complex models. For example, Baird's (1995) model considers the effects of selection, migration, and recombination on linkage disequilibria among multiple loci. In contrast, to explain patterns of gene flow of independent loci, we focus on the effects of recombination alone on segment lengths. Thus, our

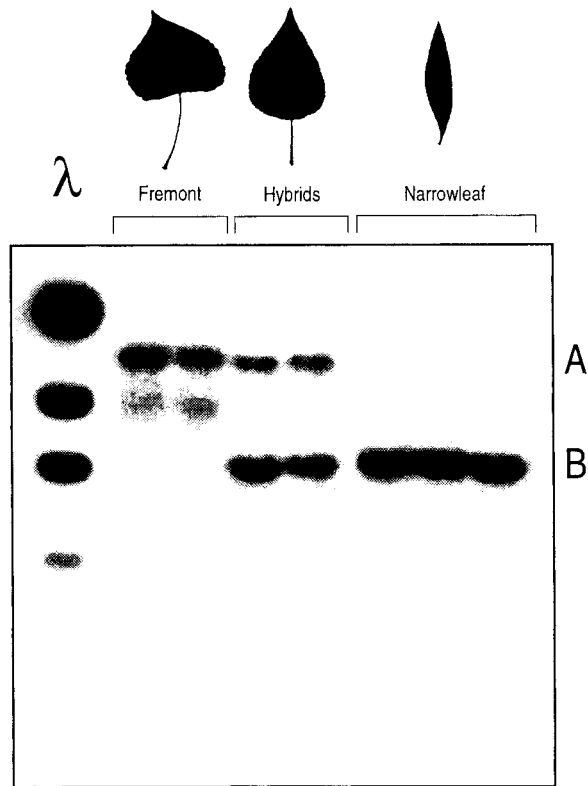


FIG. 2. RFLP data for probe #756.  $\lambda$  phage DNA digested with HindIII was used as a molecular weight standard. Two pure Fremont cottonwoods have band A. Three pure narrowleaf cottonwoods have band B. Two hybrids have both parental bands. Blots such as this were used to identify probe-restriction enzyme combinations that generate polymorphisms. The same probe was then run against all 550 trees.

predicted introgressed fragment sizes are actually a conservative representation of the effects of a hybrid zone filter.

## RESULTS

### *Localized versus Dispersed Introgression*

Twenty-six of the 33 nDNA markers exhibit the pattern depicted in Figure 3A. This pattern corresponds precisely with our morphologically based definition of the hybrid zone (i.e., a zone of overlap of both parental species and their hybrids). Based on these data, all of the trees in the narrowleaf zone are "pure" narrowleaf cottonwood. In other words, the majority of the nuclear markers show no dispersed introgression; introgression of Fremont nDNA into narrowleaf cottonwood is limited to the 13km-long hybrid zone (local introgression).

The amount of introgression in the hybrid zone is much greater than that in the narrowleaf zone (Figs. 3 and 4). Because of the presence of morphologically distinct  $F_1$  hybrids in the hybrid zone, this result was expected. These  $F_1$  hybrids are morphologically so unlike either Fremont or narrowleaf cottonwood that they were originally described as a separate species, *Populus acuminata* (Rydberg 1893). However, our genetic data clearly demonstrate that they are hybrids. All of the hybrids, whether they were morphologically intermediate

TABLE 1. RFLP markers used in this study (nuclear markers from Bradshaw et al. 1994, chloroplast marker from Lindholm and Gustafsson 1991, mitochondrial marker from Paige et al. 1991).

Marker #	Linkage group	Restriction enzyme	Allele fragment size (Kb)	
			Fremont	Narrowleaf
201	D	DraI	1.5	1.0
237	H	EcoRI	8.5	17.0
754	P	EcoRV	16.0	6.0
755	C	EcoRI	3.5	4.0
767	unlinked	DraI	6.3	5.0
789	N	HindIII	1.8	6.0
849	E	EcoRI	5.2	3.0
856	I	DraI	2.2	1.5
867	P	HindIII	13.0	5.0
912	G	DraI	2.0	2.5
993	G	DraI	6.3	4.1
1027	R	EcoRI	7.2	17.0
1045	P	DraI	3.8	4.5
1049	C	EcoRI	4.0	6.8
1054	B	DraI	1.8	1.6
1059	H	HindIII	3.9	3.3
1064	X	EcoRI	5.5	7.5
1085	unlinked	EcoRI	6.5	5.8
1122	D	EcoRI	6.8	9.0
1123	B	EcoRV	7.5	3.6
1140	Y	DraI	2.7	2.5
1145	M	HindIII	6.5	8.0
1187	B	HindIII	2.5	3.5
1253	D	HindIII	1.5	3.0
1254	unlinked	EcoRV	9.4	1.8
1258	C	HindIII	1.2	2.0
1274	unlinked	HindIII	2.0	1.7
1280	unlinked	HindIII	6.5	4.1
1286	N	DraI	4.7	3.5
1298	D	DraI	2.0	3.3
1312	R	EcoRI	3.0	4.2
1317	I	HindIII	2.5	1.8
1326	F	EcoRV	3.6	15.0
H228 (cp)		DraI	2.8	1.0
M10 (mt)		EcoRI	1.5	0.2

$F_1$ 's or backcrosses similar to narrowleaf, contained marker alleles corresponding to Fremont and/or narrowleaf cottonwood; there were no unique marker alleles that would be expected of a different species.

Although nearly 80% of the Fremont nuclear DNA markers were only found in the hybrid zone (e.g., Fig. 3A), seven nDNA markers showed low levels of dispersed introgression into the narrowleaf zone (Fig. 3B–H). Cytoplasmic markers (both chloroplast and mitochondrial) were found at higher levels throughout the narrowleaf zone (Fig. 4). Introgression has occurred at distances up to 100 km from the nearest extant Fremont cottonwood. Although an earlier study demonstrated the presence of some complex backcross hybrids in the narrowleaf zone (Paige and Capman 1993), the greatly increased sample sizes and number of RFLP markers used in the present study enabled us to examine rates of gene flow over the entire 600km long drainage system of the Weber River.

### *Differential Rates of Nuclear Introgression*

Although 26 of 33 nuclear markers showed no dispersed introgression (Fig. 3A shows one example, marker #1280), the other seven markers showed varying rates of dispersed

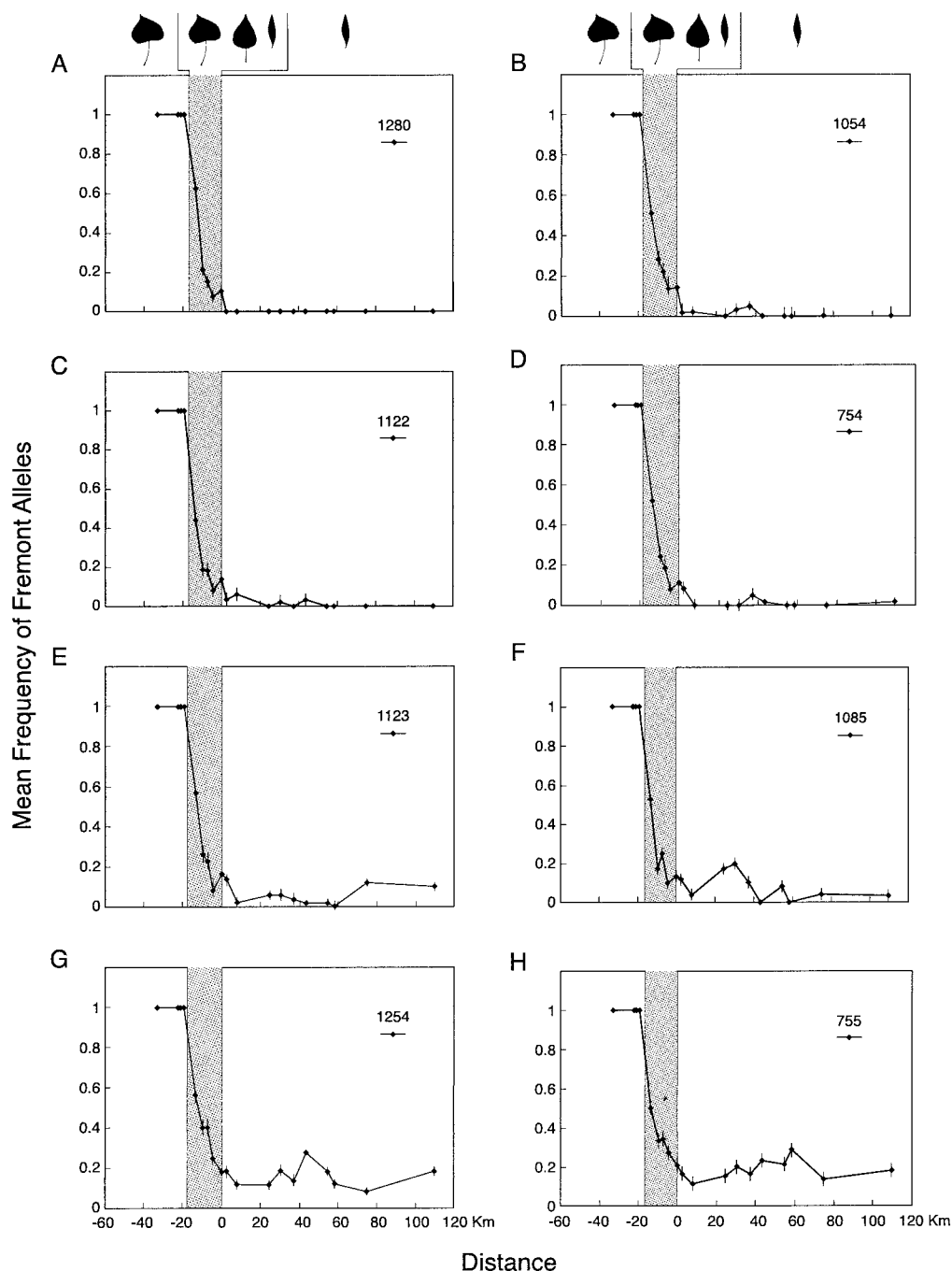


FIG. 3. Rates of introgression for eight individual nDNA markers. Sites are arranged as in Figure 1: four Fremont sites, five hybrid zone sites, and 10 narrowleaf sites. Because introgression is unidirectional (e.g., from Fremont to narrowleaf), we analyzed the data in terms of frequency of the Fremont marker allele. For an individual tree, a score of 1.0 is homozygous Fremont, 0.0 is homozygous narrowleaf, and 0.5 is heterozygous. For every marker, we computed mean scores for all trees at each of the 19 sites; thus, each point represents the mean frequency for 30–60 trees. (A) marker #1280—no introgression beyond the hybrid zone—this pattern was observed for 25 other markers; (B–H) seven other markers show varying degrees of introgression of Fremont nDNA into the narrowleaf zone.

introgression (Fig. 3B–H). Markers #1054, #1122, and #754 are found at low frequencies at only four narrowleaf sites. Markers #1123 and #1085 are present at the highest elevation site furthest from the hybrid zone, but their frequencies are variable. Markers #755 and #1254 are found throughout the Weber River drainage.

Clearly, individual markers introgress at different rates.

Each of 26 markers had no presence at the first narrowleaf cottonwood site and no presence at all subsequent sites in the narrowleaf zone. Each of seven nuclear markers and two cytoplasmic markers were present at the first narrowleaf site, and presence at the first site assured its presence at some further sites in the narrowleaf zone. Thus, it appears that the hybrid zone stands as a filter or barrier to some markers, and

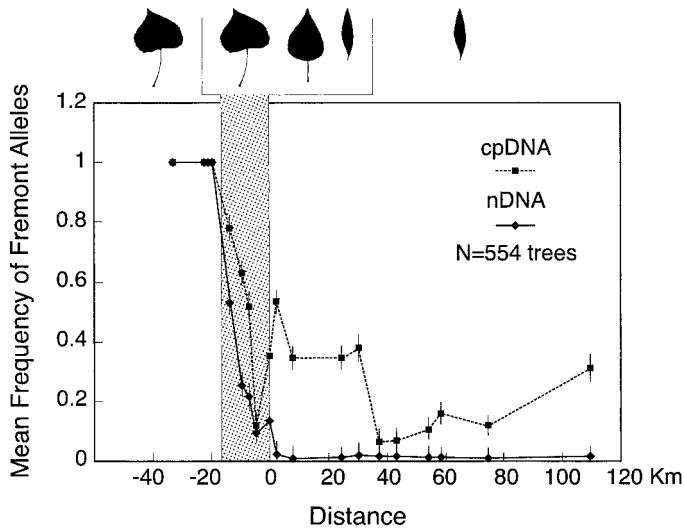


FIG. 4. Nuclear versus cytoplasmic introgression. The mean introgression rate for 33 nDNA markers compared to a cpDNA marker. The mtDNA marker followed the same pattern as the cpDNA marker. Sites arranged as in Figure 1. The distances are measured in kilometers from the easternmost hybrid zone site. As in Figure 3, each point represents the mean score for 30–60 trees.

a marker's presence at the first narrowleaf site beyond the hybrid zone was in some sense a predictor of what was to follow.

Regression analyses established two distinct classes of introgression rates among the seven markers that exhibited dispersed introgression. Each of the seven individual regressions was significant ( $P < 0.001$  in all cases with  $r^2$  values ranging from 0.67 to 0.90). Statistical comparisons of individual regression equations with each other determined two distinct groups. The high-rate introgression group consisted of markers #755 and #1254 (Fig. 3G–H). The other five markers (Fig. 3B–F) made up the low-rate group. There were no significant differences among members within a group, but each member of the high rate group had a regression equation that was significantly different from those of each member of the low-rate group.

Thus, we present evidence for at least three different classes of introgression rates based on nDNA marker alleles. The largest group (e.g., Fig. 3A) shows no introgression of Fremont DNA outside the hybrid zone. Presumably, these markers are associated with genes that negatively affect fitness, and this pattern of introgression supports the idea that the hybrid zone is maintaining reproductive isolation and preventing genetic assimilation between Fremont and narrowleaf cottonwood. A second group of Fremont markers (Fig. 3B–F) makes it past the hybrid zone filter, but these markers are generally found at low frequencies and at a variable number of narrowleaf sites. The third group of markers (Fig. 3G–H) is found throughout the range of narrowleaf cottonwood. Markers that introgress long distances and are found at high frequencies in the narrowleaf zone may be associated with beneficial Fremont genes.

#### *Nuclear versus Cytoplasmic Introgression*

Chloroplast DNA and mitochondrial DNA showed significantly higher rates of introgression than nuclear DNA (Fig.

TABLE 2. Cytonuclear disequilibria. For each of the seven nuclear markers that introgressed into the narrowleaf cottonwood zone, the proportion of trees in the hybrid and narrowleaf zones with the Fremont chloroplast marker and the Fremont nuclear marker was significantly greater than the proportion of trees with the Fremont chloroplast marker and the narrowleaf nuclear marker.

Marker #	$P(F_{cp}, F_n)$	$P(F_{cp}, n_n)$	Z	P
1054	0.712	0.239	7.74	<0.0001
1122	0.833	0.239	8.48	<0.0001
754	0.857	0.233	9.25	<0.0001
1123	0.640	0.215	7.92	<0.0001
1085	0.550	0.240	5.74	<0.0001
1254	0.404	0.234	3.86	<0.0001
755	0.396	0.252	3.30	<0.0005

4) (Patterns of mitochondrial introgression are not presented because they were identical with chloroplast introgression, e.g., there was a one-to-one correspondence between mtDNA haplotype and cpDNA haplotype.). The frequency of the Fremont cpDNA marker was greater than the frequency of the average nuclear marker at 14 of 15 (hybrid zone and narrowleaf zone) sites. Because cytoplasmic genes are haploid and uniparentally inherited, they are much more likely to become fixed in the recipient species and exhibit greater introgression rates than nuclear genes (see Discussion).

Each of the seven dispersed introgression markers exhibited cytonuclear disequilibria: that is, Fremont nuclear markers always showed significant association with Fremont chloroplast genes (Table 2). Such associations provide strong evidence for introgression (as opposed to alternative explanations for two species sharing genes—discussed below) and lend further support to the idea that hybrid zones act as filters.

## DISCUSSION

### *Alternatives to Introgression*

There are two major alternative explanations for apparent introgression: convergent evolution and symplesiomorphy (shared ancestral characters). The use of molecular genetic markers has helped rule out these alternatives. Although genetic markers as a whole are much more valuable than morphological traits in studies of introgression, cytoplasmic markers provide specific additional information about introgression. Cytoplasmic genomes are nonrecombinant, therefore any cytoplasmic marker represents the entire cytoplasmic genetic material. In our system, a single cytoplasmic marker represents both mitochondrial and plastid genomes, because they both appear to be strictly maternally inherited. This uniparental inheritance has been used to determine that biased matings are occurring in the initial stages of introgression (Paige et al. 1991). The use of both nuclear markers and cytoplasmic markers in studies provides even stronger tests for introgression and allows unique insights concerning alternative hypotheses including convergent evolution and symplesiomorphy (Rieseberg et al. 1990, 1991).

The convergent evolution hypothesis revolves around convergent mutations in cytoplasmic and the nuclear genomes of both Fremont and narrowleaf cottonwood that give rise to identical marker alleles. This scenario is unlikely in our study

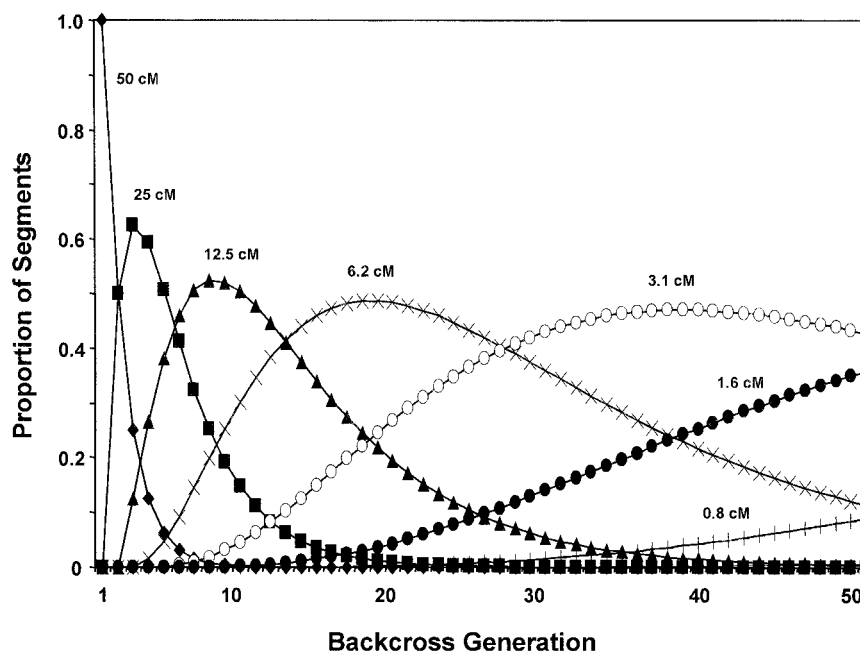


FIG. 5. Genome segment size with introgression. Using recombinational probabilities alone, we modeled the probability of particular sized genomic segments during progressive backcross generations. Predictions for different "perfect" Mendelian segments (50, 25, 12.5 cM, etc.) are labeled. Segments of less than 0.8 cM occur in advanced generations, but remain very infrequent. See also Hansen 1959a, b.

for several reasons. First, we found nine different Fremont markers in the narrowleaf population, requiring convergence to act many times to mimic the effects of introgression. Assuming that mutations affecting restriction enzyme recognition sites are neutral, this would be an incredible coincidence at nine independent genetic loci. In addition, we found both nuclear and cytoplasmic markers of Fremont cottonwood in the narrowleaf population, making this coincidence even less likely. Finally, linkage disequilibrium between cytoplasmic markers and nuclear markers (Table 2) is inconsistent with convergence (Rieseberg and Brunsfeld 1992) and supports the introgression model.

The sympleiomorphy hypothesis (i.e., Fremont marker alleles found in both species represent shared ancestral characters) is equally unlikely for the same and some additional reasons. *Populus fremontii* and *P. angustifolia* do not share a recent common ancestor. The two species are not close relatives within this genus as they belong to different sections that have been distinct for at least 12 million years (Eckewalder 1984b). Thus, the idea that the two species share a highly polymorphic recent common ancestor and then retained these polymorphisms following speciation is dubious.

The Fremont markers that are found in the narrowleaf cottonwood zone show cytonuclear disequilibria; that is, the Fremont nuclear marker alleles are correlated with the Fremont cytoplasmic markers. For each of the seven nuclear markers, the proportion of trees that have Fremont nuclear DNA and Fremont cpDNA is significantly greater than the proportion of trees that have narrowleaf nDNA and Fremont cpDNA. This result further argues against a sympleiomorphic or convergent evolution explanation of our dispersed introgression patterns. Either sympleiomorphy or convergence would have had to have operated independently in both

the nuclear and two cytoplasmic genomes. Only the directional introgression hypothesis is consistent with cytonuclear disequilibria. The nonrandom inheritance of particular nuclear loci with cytoplasmic genomes suggests selective pressure to maintain particular gene combinations (Arnold 1993).

Consequently, hybridization and introgression most likely generated the observed patterns of Fremont DNA in what appear to be pure narrowleaf cottonwoods. Below, we consider some of the consequences of this interspecific gene flow.

#### *Hybrids as Filters*

Our results indicate that hybrid populations act as evolutionary filters, preventing the introgression of most genes but allowing others to introgress throughout the range of the recipient species. Although 79% of our genetic markers showed no dispersed introgression, the other 21% are found in the narrowleaf cottonwood zone. That is, most genes do not pass through the filter, but approximately one-fifth of the genome, possibly including the beneficial genes, does. Also, for those markers exhibiting dispersed introgression, there is significant variation in how far different markers introgress, further suggesting the existence of a selective filter.

The introgression rates of individual markers represent whole segments of the genome and potentially thousands of genes. Following the pioneering work of Hanson (1959a, b), which was extended by Baird (1995), we make several predictions. First, modeling of introgressed genome segments suggests that the size of segments (or blocks) decreases with backcrossing; however, the size of the linkage disequilibrium region diminishes slowly relative to the number of backcross generations (Fig. 5—also see Hanson 1959a, b; Baird 1995). After 10 backcross generations, the average size of intro-

gressed regions is greater than 12 cM. After 25 generations, the expected size is still greater than 5 cM. This suggests that linkage among blocks of introgressing genes will persist for many generations in the recipient population (Rieseberg et al. 1996). Depending upon the genomic recombination frequency and the total number of genes, five to 12 cM could represent hundreds of genes.

Decreased segment size should have important implications for segments carrying genes that negatively affect fitness. Because large segments are more likely to carry such negative genes, we predict that backcrosses carrying large segments would be selected against (also see Rieseberg et al. 1996). Consistent with this hypothesis, selective filtering of the genome appears to be most intense in the very earliest backcross generations (e.g., at the hybrid zone boundary; Figs. 3 and 4) where the average introgressing segment is likely to be 25 cM or larger (Fig. 5). Hanson (1959b) and Baird (1995) made similar predictions using less conservative assumptions. Because selection will act upon the linked genes together, the possibility for negative interactions between introgressing and recipient genomes is great (Barton and Hewitt 1985, 1989). Even if there are many positive acting genes in a segment, a single negative or lethal acting gene will determine the fate of all the linked genes. Thus, a few well dispersed, negative acting genes could prohibit the majority of the genome from introgressing.

Likewise, marker allele introgression rates will be dependent upon their linked genes and their genetic action. Parameters affecting marker introgression rates will include the magnitude of selection on the linked genes and the recombinational distance separating marker loci from particular genes. Given that nearly 80% of the markers in this study displayed only localized introgression (Figs. 3 and 4), negatively acting genes must be common. We expect that neutral and positive acting genes, as well as marker loci, linked to these negatively acting genes will be blocked from introgression unless they are fortuitously released by recombination. The arrangement of genes and their action will have a major impact on the genomic pattern and rates of introgression. A recent study demonstrated the utility of this marker based approach, using genetic mapping to study differential introgression of chromosomal blocks (e.g., a barrier to introgression) in sunflowers (Rieseberg et al. 1999).

Linkage disequilibria in hybrid zones produce the filter, or barrier, and the strength of the filter depends on the opposing effects of selection and recombination (Barton and Hewitt 1985; Harrison 1990; Baird 1995). For example, when selection is weak and cannot prevent the increase of introgressing alleles, recombination breaks up blocks of introgressing genes into smaller and smaller fragments, which are selected against less and less strongly (Barton and Bengtsson 1986). Baird's (1995) suggestion that it might be possible to use block sizes in hybrids as a "molecular clock" to age hybrid zones was followed by Ungerer et al. (1998), who used this approach to estimate the speed of hybrid speciation in an annual sunflower. Our data suggest that, for long lived species, hybrid zone movement (see narrowleaf invasion hypothesis below) may leave a record of numerous generations of backcrossing that, in combination with block sizes, is use-

ful for modeling long term gene flow (compare Figs. 3 and 4 with Fig. 5) and possibly for aging hybrid zones.

Introgression is seldom quantified over a large geographic area, but because of the linear nature of rivers, we are uniquely positioned to measure introgression rates, or distances of "foreign" gene flow from a hybrid/overlap zone, over large areas. Differential introgression rates among our genetic markers suggest that there are different selection pressures for different genomic regions. These differences do not appear to be due to stochastic processes because 26 of 33 Fremont nuclear marker alleles introgress only into the hybrid zone and are not observed in the narrowleaf population, which is consistent with negative selection. In contrast, seven nuclear markers and two cytoplasmic markers introgress long distances into the narrowleaf zone consistent with positive selection or neutrality. Because of the great distances from the present day hybrid zone, many backcross generations have probably occurred (see above), yet, several of these Fremont markers persist at relatively high frequencies (e.g., markers #1254 and #755) throughout the narrowleaf zone. Differential rates of nuclear introgression have been found in diverse taxa such as mice (Hunt and Selander 1973), crickets (Harrison 1986) and irises (Arnold et al. 1990a), where natural selection is assumed to be responsible.

On average, cytoplasmic introgression rates were greater than rates of nuclear introgression (Fig. 4). This result is not unusual, and others have reported that cytoplasmic gene flow exceeds nuclear gene flow with perhaps the best examples in *Helianthus* (Rieseberg et al. 1990, 1991). In the *Helianthus* system, chloroplast gene introgression was approximately 10 times greater than nuclear genes. "Foreign" cytoplasmic markers are sometimes found hundreds of kilometers from the present day hybrid zone, in the absence of "foreign" nDNA. Gyllensten and Wilson (1987) present a dramatic example in Scandinavian mice, but see Marchant et al. (1988) who found nuclear markers introgressed farther than mtDNA in Australian grasshoppers. Such long distance gene flow may reflect ancient introgression and movement of the hybrid zone (see below).

Because cytoplasmic genomes are nonrecombinant, a single marker accurately tracks the entire genetic molecule. In addition, our data indicate that there is strict maternal inheritance of both cpDNA and mtDNA, allowing both cytoplasmic genomes to be monitored with a single marker. The rapid cytoplasmic introgression rates can be attributed to their haploid nature, uniparental inheritance, and the lack of negative or, possibly, positive selection. Even combined, cytoplasmic genomes are relatively small and will contain less than 200 genes. Small genome size decreases the probability of negative gene actions during introgression, especially compared to large nuclear genomic segments found in early hybrids. Despite these cytoplasmic introgressive advantages, we observed two nuclear markers (markers #755 and #1254) with introgression rates comparable to the cytoplasmic rates.

Three distinct nDNA introgression rates are apparent in our data: only-localized introgression, slow-dispersed introgression, and fast-dispersed introgression (Figs. 3 and 5). Negative selection must be acting on the only-localized introgression markers via genetically linked genes. In contrast, the slow-dispersed introgression markers can be considered



neutral or must be only loosely linked to negative gene actions. It is of course possible that the slow-dispersed regions are associated with both negative and positive gene actions and that their reduced introgression rates are determined by the recombination that must break these linkages. Finally, the fast-introgression regions have rates that are comparable to the cytoplasmic genomes in spite of their diploid and biparental inheritance attributes (increased effective population size). Fast-dispersed nDNA introgression regions would seem to be under positive selection to attain these rates.

As other authors have observed (e.g., Stebbins 1959), introgressing genes with positive effects can provide recipient species with evolutionary adaptive shortcuts. The hybrid zone filter provides a mechanism: positive selection acting on the introgressing genes. However, neutral models such as cytonuclear drag could still account for rapid nuclear marker introgression. If there are important genetic interactions between the cytoplasmic and nuclear genomes, selection for these interactions will dictate that some nuclear genomic segments introgress at comparable rates. Rapid, neutral introgression of cytoplasmic genomes would thus result in rapid introgression of particular nuclear regions. Cytonuclear drag is another consequence of a strong filter, because a nonrandom association between cytoplasmic and nuclear markers indicates selection in the hybrid zone (Arnold 1993).

If hybrid populations act as evolutionary filters, there are several important implications. First, species barriers are maintained in the face of hybridization. Hybrids are controversial because of their role in genetic assimilation, but a strong filter would prevent complete assimilation (genetic swamping). Second, a strong filter should prevent the introgression of deleterious genes while allowing introgression of beneficial ones. Thus, hybrid zones are locations of recombination and selection that affect gene flow between species. Finally, our results help explain the existence and long-term persistence of hybrid zones. For example, hybrid cottonwoods have been recorded in the fossil record during the past 12 million years (Eckenwalder 1984b), and Goodfriend and Gould (1996) provide direct fossil evidence of ancient, persistent hybrid zones in *Cerion*. Hybrid zones may act as filters that prevent the introgression of negative genetic effects, while allowing positive introgression.

It is important to differentiate between "natural" hybridization among native species, and "exotic" hybridization events brought about by human disturbances and introductions (e.g., Whitham et al. 1991, Whitham and Maschinski 1996). It is well known that exotic hybridization can lead to genetic assimilation and species loss (Levin et al. 1996). However, natural hybrids are common among many of the most successful and dominant taxa of the world (e.g., oaks, pines, eucalypts), suggesting that hybridization may have played an important role in their evolutionary success rather than the harmful role that is often portrayed in the literature (e.g., O'Brien and Mayr 1991).

#### *Contemporary versus Ancient Introgression*

Heiser (1973) argued that, while localized introgression is relatively common, dispersed introgression is rare. However, the advent of molecular techniques has allowed for the doc-

umentation of many cases of dispersed introgression (Arnold 1992, Rieseberg and Wendel 1993). Localized introgression may depict present-day hybridization, whereas dispersed introgression is representative of past hybridization and subsequent movement of the hybrid zone (Marchant et al. 1988; Arnold et al. 1990b; Dowling and Hoeh 1991; Shaw et al. 1993). Similarly, cytoplasmic introgression, especially in the absence of nuclear introgression, is often used as evidence for ancient hybridization (Rieseberg and Soltis 1991; for an example in *Populus*, see Smith and Sytsma 1990). In this hybrid cottonwood system, we have documented both localized and dispersed introgression of both nuclear and cytoplasmic markers. Seemingly, we have evidence for both contemporary and ancient hybridization, which argues for the long-term stability of cottonwood hybrid zones. Further, because this result raises the issue of spatial versus temporal patterns of gene flow, we propose two opposing hypotheses below.

Chloroplast and, apparently, mitochondrial genomes are maternally inherited in *Populus* (Smith and Sytsma 1990), which requires seed-based dispersal for the extensive introgression that we observed. There are two possible explanations for this phenomenon. Under a "long-distance dispersal" hypothesis, female Fremont trees would mate with male narrowleaf trees in the present day hybrid zone. The resulting seeds are then wind blown up the canyon for various distances, in some cases as great as 100 km, where they germinate, grow, and mate with the local population. Repeated backcrossing by female hybrid trees with male narrowleaf trees would produce the observed backcross trees with Fremont cytoplasmic genomes. Dispersal by seed would seem to be at a distinct disadvantage to dispersal by pollen movement (e.g., Potts and Reid 1988), but the extensive introgression of cytoplasmic DNAs argues that long distance seed dispersal can occur.

The second hypothesis revolves around population succession over thousands of years with a much more limited role for seed and pollen dispersal. Under a "narrowleaf invasion" hypothesis, Fremont populations were more widely distributed in the past, possibly overlapping with narrowleaf cottonwood at higher elevations. The present day pattern of cytoplasmic DNAs would have been generated by these past species distribution patterns. Climate models predict several thermal maxima during the Holocene (Davis 1984), lending support to the idea that Fremont cottonwood may have occurred at higher elevations. The distribution of Fremont populations today is much more restricted than that of narrowleaf (Fig. 1). This observation combined with the fact that narrowleaf cottonwood is acquiring Fremont genes leads to speculation that Fremont may eventually go locally extinct (Keim et al. 1989; Paige et al. 1991). This scenario involves a dynamic hybrid zone that is slowly marching down the canyon, resulting in the eventual elimination of Fremont cottonwood.

Thus, the introgressing species is in greater danger of local extinction than the nonintrogressing species. This is the scenario predicted of compilospecies (Harlan and de Wet 1963), where a widespread species acquires genes from a more restricted relative and may eventually replace it. The narrowleaf invasion hypothesis is supported by the extensive localized introgression (e.g., in the hybrid zone) in this system. Re-

ardless of whether or not its location has shifted, two lines of evidence argue for the long-term persistence of the hybrid zone: (1) the genetic structure of this hybrid population suggests that extensive selection has occurred, and (2) the fitness of the surviving hybrid trees is equal to if not greater than the adjacent parental types (J. Schweitzer, unpubl. data). For the Weber River system, the most plausible scenario is that by acting as a strong filter, the hybrid zone has moved down in elevation and allowed for interbreeding between Fremont and narrowleaf cottonwood while still maintaining them as distinct species. The eventual fate of the Fremont cottonwood population is a matter of some speculation. The hybrid zone may represent the advance and eventual triumph of a superior competitor, or, alternatively, its location may reverse itself, probably a more likely scenario given that hybrid cottonwoods have been around for at least 12 million years.

#### ACKNOWLEDGMENTS

We thank K. Charters, N. Cobb, S. DiFazio, J. Ganey, C. Gehring, P. Price, T. Trotter, R. vanOmmeren, G. Wimp, and two anonymous reviewers for comments on the manuscript. We thank D. Blackett, H. D. Bradshaw, L. Dickson, E. LaHood, K. Floate, J. Jones, A. Martin, V. Oza, L. Rieseberg, and J. Schupp, for advice and assistance. H. D. Bradshaw provided RFLP markers. This work was supported by National Science Foundation grants BSR-9107042, DEB 9311210, DEB 9408009, Department of Energy 94ER61849, United States Department of Agriculture 92-37302-7854, and the Ogden, Utah Nature Center. We are especially grateful to the trees for unwillingly relinquishing a few of their leaves.

#### LITERATURE CITED

- Anderson, E. 1949. Introgressive hybridization. Wiley, New York.
- Anderson, E., and L. Hubricht. 1938. Hybridization in *Tradescantia*. III. The evidence for introgressive hybridization. *Am. J. Bot.* 25:396–402.
- Arnold, J. 1993. Cytonuclear disequilibria in hybrid zones. *Annu. Rev. Ecol. Syst.* 24:521–554.
- Arnold, M. L. 1992. Natural hybridization as an evolutionary process. *Annu. Rev. Ecol. Syst.* 23:237–261.
- . 1997. *Natural Hybridization and Evolution*. Oxford University Press, Oxford, U.K.
- Arnold, M. L., J. L. Hamrick, and B. D. Bennett. 1990a. Allozyme variation in Louisiana irises: a test for introgression and hybrid speciation. *Heredity* 65:297–306.
- Arnold, M. L., B. D. Bennett, and E. A. Zimmer. 1990b. Natural hybridization between *Iris fulva* and *Iris hexagona*: pattern of ribosomal DNA variation. *Evolution* 44:1512–1521.
- Baird, S. J. E. 1995. A simulation study of multilocus clines. *Evolution* 49:1038–1045.
- Barton, N. H., and B. O. Bengtsson. 1986. The barrier to genetic exchange between hybridizing populations. *Heredity* 56:357–376.
- Barton, N. H., and G. M. Hewitt. 1985. Analysis of hybrid zones. *Annu. Rev. Ecol. Syst.* 16:113–148.
- . 1989. Adaptation, speciation, and hybrid zones. *Nature* 341:497–503.
- Bradshaw, H. D., Jr., and R. F. Stettler. 1993. Molecular genetics of growth and development in *Populus*. I. Triploidy in hybrid poplars. *Theor. Appl. Genet.* 86:301–307.
- Bradshaw, H. D., Jr., M. Villar, B. D. Watson, K. G. Otto, S. Stewart, and R. F. Stettler. 1994. Molecular genetics of growth and development in *Populus*. III. A genetic linkage map of hybrid poplar composed of RFLP, STS, and RAPD markers. *Theor. Appl. Genet.* 89:167–178.
- Davis, O. K. 1984. Multiple thermal maxima during the Holocene. *Science* 225:617–619.
- Dowling, T. E., and W. R. Hoeh. 1991. The extent of introgression outside the contact zone between *Notropis cornutus* and *Notropis chryscephalus* (Teleostei: Cyprinidae). *Evolution* 45:944–956.
- Eckenwalder, J. E. 1984a. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections Aigeiros and Tacamahaca. II. Taxonomy. *Can. J. Bot.* 62:325–335.
- . 1984b. Natural intersectional hybridization between North American species of *Populus* (Salicaceae) in sections Aigeiros and Tacamahaca. III. Paleobotany and evolution. *Can. J. Bot.* 62:336–342.
- Fisher, R. A. 1953. A fuller theory of junctions in inbreeding. *Heredity* 8:187–197.
- Goodfriend, G. A., and S. J. Gould. 1996. Paleontology and chronology of two evolutionary transitions by hybridization in the Bahamian land snail *Cerion*. *Science* 274:1894–1897.
- Gyllenstein, U., and A. C. Wilson. 1987. Interspecific mitochondrial DNA transfer and the colonization of Scandinavia by mice. *Genet. Res. Camb.* 49:25–29.
- Hanson, W. D. 1959a. Early generation analysis of lengths of chromosome segments around a locus held heterozygous with backcrossing or selfing. *Genetics* 44:833–837.
- . 1959b. The breakup of initial linkage blocks under selected mating systems. *Genetics* 44:857–868.
- Harlan, J. R., and J. M. J. de Wet. 1963. The compilospecies concept. *Evolution* 17:497–501.
- Harrison, R. G. 1986. Pattern and process in a narrow hybrid zone. *Heredity* 56:337–349.
- . 1990. Hybrid zones: windows on evolutionary processes. Pp. 69–128 in J. Antonovics and D. Futuyma, eds. *Oxford surveys in evolutionary biology*. Vol. 7. Oxford Univ. Press, Oxford, U.K.
- Heiser, C. B., Jr. 1973. Introgression re-examined. *Bot. Rev.* 39:347–366.
- Howard, D. J. 1986. A zone of overlap and hybridization between two ground cricket species. *Evolution* 40:34–43.
- Hunt, W. G., and R. K. Selander. 1973. Biochemical genetics of hybridisation in European house mice. *Heredity* 31:11–33.
- Keim, P., K. N. Paige, T. G. Whitham, and K. G. Lark. 1989. Genetic analysis of an interspecific hybrid swarm of *Populus*: occurrence of unidirectional introgression. *Genetics* 123:557–565.
- Keim, P., W. Beavis, J. Schupp, and R. Freestone. 1992. Evaluation of soybean RFLP marker diversity in adapted germ plasm. *Theor. Appl. Genet.* 85:205–212.
- Key, K. H. L. 1968. The concept of stasipatric speciation. *Syst. Zool.* 17:14–22.
- Levin, D. A., J. Francisco-Ortega, and R. K. Jansen. 1995. Hybridization and the extinction of rare plant species. *Conserv. Biol.* 10:10–16.
- Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a source of variation for adaptation to new environments. *Evolution* 20:315–336.
- Lindholm, J., and P. Gustafsson. 1991. The chloroplast genome of the gymnosperm *Pinus contorta*: a physical map and a complete collection of overlapping clones. *Curr. Genet.* 20:161–166.
- Marchant, A. D., M. L. Arnold, and P. Willinson. 1988. Gene flow across a chromosomal tension zone I. Relicts of ancient hybridization. *Heredity* 61:321–328.
- Martinsen, G. D., and T. G. Whitham. 1994. More birds nest in hybrid cottonwood trees. *Wilson Bull.* 106:474–481.
- O'Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* 251:1187–1188.
- Paige, K. N., and W. C. Capman. 1993. 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 RFLP's. *Evolution* 47:36–45.
- Paige, K. N., W. C. Capman, and P. Jennetten. 1991. Mitochondrial inheritance patterns across a cottonwood hybrid zone: cytonuclear disequilibria and hybrid zone dynamics. *Evolution* 45:1360–1369.

- Potts, B. M., and J. B. Reid. 1988. Hybridization as a dispersal mechanism. *Evolution* 42:1245–1255.
- Rieseberg, L. H., and S. J. Brunsfeld. 1992. Molecular evidence and plant introgression. Pp. 151–178 in P. S. Soltis, D. E. Soltis, and J. J. Doyle, eds. *Molecular systematics of plants*. Chapman and Hall, New York.
- Rieseberg, L. H., and N. C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization? *Crit. Rev. Plant Sci.* 12:213–241.
- Rieseberg, L. H., and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evol. Trends Plants* 5:65–84.
- Rieseberg, L. H., and J. F. Wendel. 1993. Introgression and Its Consequences in Plants. Pp. 70–109 in R. G. Harrison, ed. *Hybrid zones and the evolutionary process*. Oxford Univ. Press, Oxford, U.K.
- Rieseberg, L. H., S. Beckstrom-Sternberg, and K. Doan. 1990. *Helianthus annuus* ssp. *texanus* has chloroplast DNA and nuclear ribosomal DNA genes of *Helianthus debilis* ssp. *cucumerifolius*. *Proc. Natl. Acad. Sci.* 87:593–597.
- Rieseberg, L. H., H. C. Choi, and D. Ham. 1991. Differential cytoplasmic versus nuclear introgression in *Helianthus*. *J. Hered.* 82:489–493.
- Rieseberg, L. H., D. M. Arias, M. C. Ungerer, C. R. Linder, and B. Sinervo. 1996. The effects of mating design on introgression between chromosomally divergent sunflower species. *Theor. Appl. Genet.* 93:633–644.
- Rieseberg, L. H., J. Whitton, and K. Gardner. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. *Genetics* 152:713–727.
- Rydberg, P. A. 1893. On the American black cottonwood. *Bull. Torr. Bot. Club* 20:46–50.
- Shaw, D. D., A. D. Marchant, N. Contreras, M. L. Arnold, F. Groeters, and B. C. Kohlman. 1993. Genomic and environmental determinants of a narrow hybrid zone: cause or coincidence? Pp. 165–195 in R. G. Harrison, ed. *Hybrid zones and the Evolutionary Process*. Oxford University Press, Oxford, U.K.
- Smith, R. L., and K. J. Sytsma. 1990. Evolution of *Populus nigra* (Sect. Aigeiros): Introgressive hybridization and the chloroplast contribution of *Populus alba* (Sect. *Populus*). *Am. J. Bot.* 77:1176–1187.
- Stace, C. A. 1987. Hybridization and the plant species. Pp. 115–127 in K. M. Urbanska, ed. *Differentiation patterns in higher plants*. Academic Press, New York.
- Stebbins, G. L. 1959. The role of hybridization in evolution. *Proc. Am. Philos. Soc.* 103:231–251.
- Ungerer, M. C., S. J. E. Baird, J. Pan, and L. H. Rieseberg. 1998. Rapid hybrid speciation in hybrid sunflowers. *Proc. Natl. Acad. Sci.* 95:11757–11762.
- Whitham, T. G. 1989. Plant hybrid zones as sinks for pests. *Science* 244:1490–1493.
- Whitham, T. G., and J. Maschinski. 1996. Current hybrid policy and the importance of hybrid plants in conservation. Pages 103–112 in J. Maschinski, D. H. Hammond, and L. Holter, tech. eds. *Southwestern rare and endangered plants: proceedings of the second conference; 1995 September 11–14; Flagstaff, Arizona* USDA For. Ser. RM-GTR-283, Rocky Mountain Forestry and Range Experimental Station, Ft. Collins, CO.
- Whitham, T. G., P. A. Morrow, and B. M. Potts. 1991. Conservation of hybrid plants. *Science* 254:779–780.

Corresponding Editor: S. Tonsor