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MITOCHONDRIAL INHERITANCE PATTERNS ACROSS A COTTONWOOD HYBRID ZONE: CYTONUCLEAR DISEQUILIBRIA AND HYBRID ZONE DYNAMICS

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Abstract.—In this study we examine the cytoplasmic inheritance patterns of an interspecific hybridizing population of Fremont and narrowleaf cottonwoods, using mitochondrial DNA. Three mitochondrial probes showing polymorphisms were used to distinguish between trees of known nuclear inheritance. Every tree screened had only one cytoplasmic genotype, either Fremont or narrowleaf. Thus, these results demonstrate that mitochondria are uniparentally inherited in these trees. Previous studies of the nuclear inheritance of this interspecific hybridizing population of cottonwood trees indicated an asymmetry in the frequency of parental genes. Using mitochondrial markers we tested one hypothesis potentially responsible for this asymmetric distribution (i.e., trees of mixed genotypes will be sterile or will not survive if their cytoplasm is derived from one or the other parent). Our results, however, show that both Fremont and narrowleaf mitochondrial markers are found in trees with mixed nuclear genotypes. Thus, nuclear-cytoplasmic incompatibilities do not appear to account for the asymmetric distribution of nuclear genotypes within the hybrid swarm. An alternative explanation for the observed asymmetric distribution of nuclear genotypes is advanced. Although nuclear-cytoplasmic incompatibilities do not appear to explain the asymmetric distribution of nuclear alleles within the hybrid zone, nonrandom associations between nuclear and cytoplasmic genotypes do exist. For example, all F_1 hybrids had Fremont mitochondrial genotypes. Furthermore, backcrosses between F_1 hybrid and narrowleaf trees have a higher than expected proportion of heterozygous loci and a higher than expected proportion of Fremont mitochondria. We propose that seeds, seedlings, or trees with high proportions of heterozygous loci are at a disadvantage unless they also have the Fremont mitochondrial genotype. While it is generally difficult to infer dynamic processes from static patterns, studies such as ours enable one to gain new insights to the dynamics of plant hybrid zones. 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 suggest that the narrowleaf population may be spreading down the canyon and the Fremont population receding. The eventual fate of the hybrid zone, in relation to these processes, is discussed.

Key words.—Cytonuclear disequilibria, Fremont cottonwood, hybrid zone dynamics, hybridization, mitochondrial inheritance, narrowleaf cottonwood, nuclear inheritance, *Populus angustifolia, Populus fremontii,* restriction fragment.

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With the advent of recombinant DNA technology and its use for the identification of restriction fragment length polymorphisms (RFLPs) we now have the capability of addressing several questions that have proven intractable using conventional methods of investigation (e.g., genetic studies of natural populations of long lived plants that are difficult to analyze by classical segregation approaches; see Keim et al., 1989; Paige et al., 1990). The current surge in the use of molecular genetic techniques has, for example, provided new approaches to the study of hybrid zones, with recent attention focusing on the dynamics of hybrid zones and the processes that have contributed to their present distributions and maintenance (e.g., see Ferris et al., 1983; Millar, 1983; Sage et al., 1986; Baker et al., 1989; Keim et al., 1989; Rand and Harrison, 1989). Most of these studies have focused on animals (Hewitt, 1988). Plant hybrid zones, however, have been much neglected and studies examining both cytoplasmic and nuclear markers are even rarer.

Recently, we conducted a study (Keim et al., 1989) of nuclear inheritance in an interspecific hybridizing population of cot-

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tonwood trees using RFLP markers. Here, we expand our efforts to incorporate studies of cytoplasmic inheritance.

Study Site, Organisms, and Observations

Genetic studies of the interaction of two species of cottonwood trees, Fremont cottonwood, *Populus fremontii,* and narrowleaf cottonwood, *P. angustifolia,* were conducted in the Weber River drainage north of Salt Lake City, Utah. One parental population, *P. fremontii* (Fremont), is located at lower elevations (below 1,310 meters), the other, *P. angustifolia* (narrowleaf), is found at higher elevations (above 1,490 meters). These individuals grow along the banks and in the bottomlands adjacent to the Weber River. 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. The two parental populations are connected by a 13 km overlap zone composed mostly of intermediate phenotypes (with intermediate leaf morphologies) and a few parentals of both types (i.e., a hybrid swarm). It is, however, extremely difficult to distinguish between a pure narrowleaf and a complex backcross (BC_2s, BC_3s, etc.) to the narrowleaf side based on morphology alone (Paige et al., 1990). RFLPs allow one to circumvent this problem, yielding more precise genotypic information. Both tree species are dioecious and wind pollinated.

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., 1990). In individuals making up the hybrid swarm, loci were homozygous for narrowleaf alleles or were heterozygous (both narrowleaf and Fremont fragments were observed). In genetically mixed individuals, however, no homozygous Fremont alleles were found. Thus trees in the hybrid swarm appeared to be either F_1 hybrids or backcrosses between hybrids and narrowleaf parents. No progeny could be attributed to hybrid-hybrid crosses or backcrosses to Fremont.

There are several possible explanations for the asymmetric distribution of Fremont and narrowleaf nuclear genes within the hybrid population. Asymmetry, for example, could result from assortative mating due to phenological differences in flowering, a bias in the population sizes of the two parental species, selection for particular genetic combinations, nuclear incompatibilities inhibiting reproductive success during some stage of development, or nuclear-cytoplasmic incompatibilities (leading to only one type of cytoplasmic inheritance (e.g., narrowleaf) in hybrid trees; Asmussen et al., 1987).

By determining the cytoplasmic inheritance of trees in the hybrid swarm with known nuclear inheritance we have been able to: (1) establish that mitochondrial inheritance is uniparental in cottonwood; (2) test one mechanism potentially responsible for the observed distribution of genotypes within the hybrid swarm (i.e., nuclear-cytoplasmic incompatibilities); (3) gain further insight into the directionality of reproduction within the hybrid swarm and; (4) gain insight into the dynamics of this plant hybrid zone. To accomplish this, we have used RFLP markers to determine the cytoplasmic inheritance of individual trees, using mitochondrial DNA.

Methods and Materials

Mitochondrial inheritance was determined for 40 trees of known nuclear inheritance. The genetic constitution of individual trees (whether a pure parental type, an F_1 hybrid, or a backcross) was determined by the use of nuclear recombinant DNA probes (see Keim et al., 1989 and Paige et al., 1990 for details). A large portion of the nuclear data presented in this paper was taken from Keim et al. (1989). We have since added additional nuclear data, including several new trees and several new markers on previously scored trees.

Mitochondrial DNA Extraction

DNA for the preparation of a mitochondrial DNA library (for detecting polymorphic loci) was extracted from a narrowleaf cottonwood cell suspension tissue culture (prepared by Jill Roth). Eighty ml of cells were homogenized in 10 ml of extraction buffer containing 25 mM Tris (pH = 8.0), 0.3 M Mannitol, 1% PVP 40, 0.1% BSA,

1mM beta-mercaptoethanol and 3 mM EDTA. Homogenization was carried out using a tight-fitting, motor driven Teflon insert in a glass homogenizer.

Following homogenization, debris was removed by centrifugation at 1,000× g for 10 minutes [chromosomal and plastid contamination was significantly reduced by carefully decanting the supernatant and repeating the centrifugation (Lansman et al., 1981; Schmitz, 1988)]. Mitochondria were pelleted from this second low speed supernatant by centrifugation for 20 minutes at 15,000× g. Next, the mitochondrial pellet was resuspended in 2 ml of buffer (25 mM Tris (pH = 8.0), 0.3 M Mannitol, 10 mM $MgCl_2$). A camel hair paintbrush was used to facilitate resuspension of the mitochondrial pellet.

After resuspending the mitochondrial pellet, DNAse I (100 μg/ml) was added and the mixture was incubated at 37°C for 1 hour to remove nuclear DNA. The reaction was stopped by adding EDTA to a final concentration of 100 mM. The temperature of the mixture was elevated to 65°C, to inactivate the DNAse, and the mitochondria were lysed by the addition of sodium dodecyl sulfate (final concentration, 1% SDS) and digested for 1 hour with proteinase K (20 μg/ml) to inactivate nucleases. To each 1.2 g/ml of lysate in TSE [10mM Tris.Cl (pH 8.0), 100mM Nacl, 1mM EDTA (pH 8.0)], 200 μg of Hoechst II dye was added together with 8.7 g of solid cesium chloride. This mixture was centrifuged for 40 hours at 20°C at 36,000 rpm in polyallomer tubes. The gradient was visualized under UV light and the GC rich, mitochondrial DNA band was removed. After dialysis in a Tris-EDTA buffer (two times in 10mM Tris, 0.5mM EDTA, then once in 10mM Tris, 0.05mM EDTA) to remove the cesium chloride, this DNA was used to prepare the mitochondrial DNA library.

Library Construction and Screening

Mitochondrial DNA was digested with HindIII, ligated into the plasmid pBS+ (Strategene Inc.) and transformed into competent cells, (*E. coli* DH5alpha, Bethesda Research Laboratories). Cells were then selected for growth on ampicillin on indicator plates containing X-gal and IPTG (Isopropylthio-B-D-Galactoside). Bacterial colonies containing plasmids with inserts were selected and grown overnight in LB broth (tryptone, yeast-extract, NaCl). Bacteria were lysed and the plasmids were isolated. Inserts were cut out from the vector, using HinDIII, and isolated by gel electrophoresis in low melting agarose (Sea Plaque), diluted in 5X sterile water (volume to weight) and boiled to denature the double-stranded DNA fragment (Langridge et al., 1980; Keim and Shoemaker, 1988). Radioactively labeled probes were then prepared from these inserts by hybridizing a random primer ($pd(N)_6$, Pharmacia) to the denatured strand of DNA and synthesizing DNA containing ^{32}P labeled cytosine.

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 labeled probe, after which the membrane was analyzed by autoradiography (see Keim et al., 1989 for details). 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.

RESULTS

Probes

Plasmid clones prepared from the cottonwood mitochondrial DNA library were screened for polymorphisms by hybridization with southern transfers of DNA restriction digests of narrowleaf and Fremont cottonwood. All 30 probes identified multicopy DNA (most autoradiograms could be developed after an hour whereas the nuclear probes revealed fragments from the same digests only after many days (7–10) of exposure). Three probes were found to be polymorphic and identified fragments of different lengths in the narrowleaf and Fre-

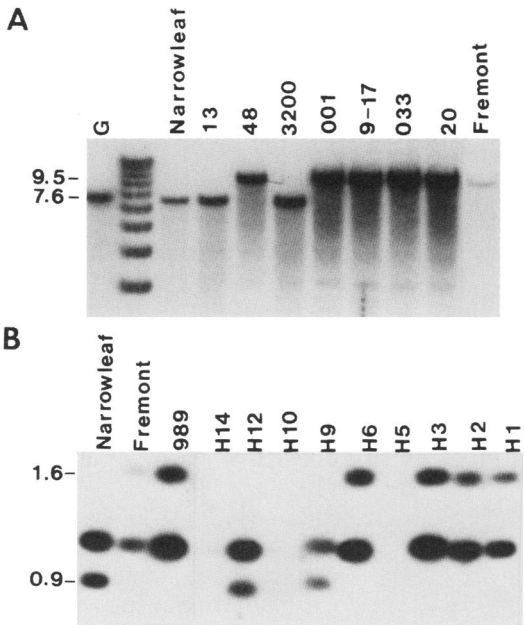

FIG. 1. Two mitochondrial DNA probes used in determining the maternal inheritance of individual cottonwood trees. Probes M30 (A) and M10 (B) were hybridized with fragments of total DNA digested with Bg1II (A) and EcoRI (B). "Fremont" DNA was from tree F; "narrowleaf" DNA was from tree S (Table 1). Remaining tree samples were obtained from all three zones; the pure narrowleaf and Fremont zones (A) and the hybrid zone (B).

mont DNA preparations (Fig. 1A and B). In each case, the polymorphism was observed when DNA was digested by one particular enzyme; other enzymes failed to reveal a difference. This was in striking contrast to our previous experience with nuclear RFLP markers in which most polymorphisms appeared to be the result of rearrangement, resulting in changes in fragment lengths that were observed using several different restriction enzymes (Keim et al., 1989). Data in Figure 1 show that all polymorphic mitochondrial probes identified the DNA of trees as either Fremont or narrowleaf. As expected for cytoplasmic inheritance, no cases occurred in which both types of markers were found in the same tree. Trees 989 and HM-1, for example, had been shown to be nuclear hybrids, heterozygous for all nuclear markers examined [26 for 989, 17 for HM-1; Fig. 2B and D (Keim et al., 1989)], but both trees had only Fremont mitochondrial markers (Fig. 2A and C).

Cytoplasmic Inheritance

Results of examining all of the trees for which a pattern of nuclear inheritance has

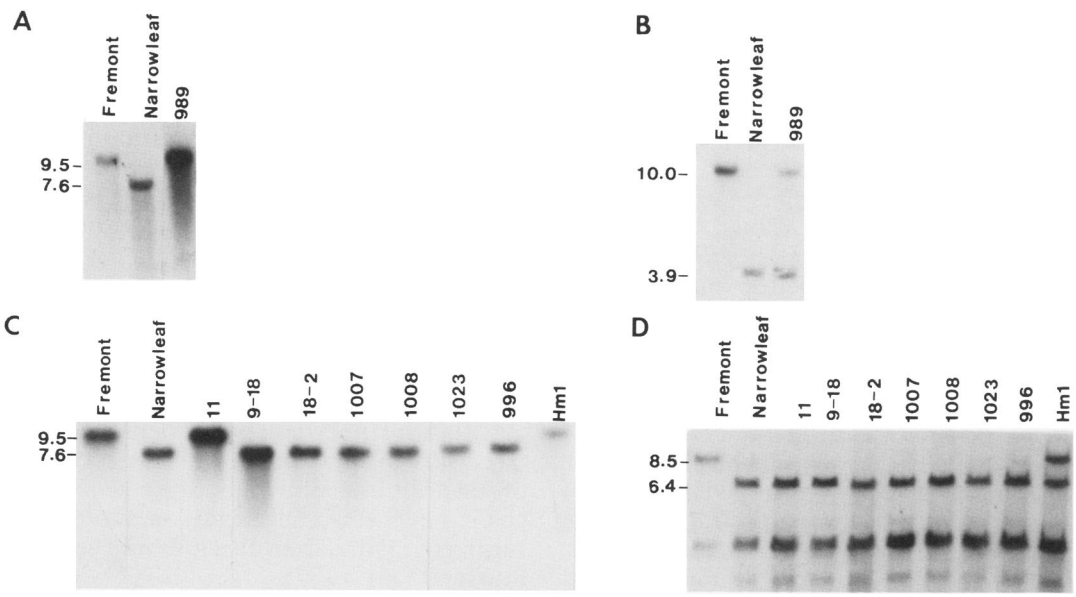

FIG. 2. A comparison of mitochondrial (A and C) and nuclear markers (B and D). Trees 989 and HM-1, for example, are nuclear hybrids, heterozygous for all nuclear markers examined (B and D). Both trees, however, have only Fremont mitochondrial markers (A and C). As expected for cytoplasmic inheritance, no cases occurred in which both types of markers were found in the same tree.

TABLE 1. 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, respectively, F_1 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).

Tree	Nuclear genotype (F:H:N)	Mitochondrial genotype (N or F)	Cross	Tree	Nuclear genotype (F:H:N)	Mitochondrial genotype (N or F)	Cross
F	37:00:00	F	F	11	00:07:19	F	BC_2
20	16:00:00	F	F	18	00:07:13	N	BC_2
H14	05:00:00	F	F	H9	00:03:04	N	BC_2
001	16:00:00	F	F	H10	00:04:07	N	BC_2
012	16:00:00	F	F	1007	00:04:11	N	BC_2
033	16:00:00	F	F	1992	00:01:03	N	BC_2
9-17	18:00:00	F	F	999	00:02;09	F	BC_3
HM-1	00:17:00	F	F_1	1008	00:02:13	N	BC_3
989	00:26:00	F	F_1	1025	00:01:04	N	BC_3
1994	00:05:00	F	F_1	1019	00:01:10	N	BC_4
1997	00:05:00	F	F_1	G	00:00:23	N	N
1979	00:03:01	F	BC_1	S	00:00:29	N	N
1981	00:13:02	F	BC_1	13	00:00:16	N	N
1934	00:03:01	N	BC_1	48	00:00:16	F	N*
1935	00:03:01	F	BC_1	T15	00:00:16	N	N
H1	00:11:02	F	BC_1	996	00:00:19	N	N
H2	00:10:03	F	BC_1	1011	00:00:07	N	N
H6	00:12:04	F	BC_1	1012	00:00:05	N	N
H12	00:06:04	N	BC_1	1023	00:00:12	N	N
H3	00:05:08	F	BC_2	3200	00:00:16	N	N

* Complex backcross.

been determined are summarized in Table 1; the cytoplasmic inheritance is presented as either F (Fremont) or N (narrowleaf). It is immediately apparent that the mitochondrial inheritance of backcross trees in the hybrid swarm can be either Fremont or narrowleaf, ruling out one hypothesis which contends that nuclear-cytoplasmic incompatibilities lead to backcross trees of only one type of cytoplasmic inheritance (e.g., narrowleaf) and an asymmetry in the distribution of nuclear genes.

Nonetheless, our data do suggest an interdependence between nuclear and cytoplasmic inheritance. All of the F_1 hybrid trees (i.e., 50% F/50% N nuclear DNA) that we have looked at so far, for example, have inherited Fremont mitochondria (Table 1). This includes the well established hybrids 989 and HM-1 as well as 1994 and 1997 (five markers each). Furthermore, among trees that are the result of a single backcross between an F_1 hybrid and a pure narrowleaf tree (i.e., BC_1s), those with Fremont mitochondrial DNA have a higher than expected proportion of heterozygous loci (80% instead of 50%; $\chi^2 = 23.40$, $df = 1$, $P < 0.0001$). These include 4 trees each of which has been analyzed for more than 10 nuclear markers and 2 trees analyzed for only 4 nuclear markers each. Two other putative backcross 1 trees have narrowleaf mitochondria, but for only one of these, H12, has the nuclear inheritance been analyzed extensively (interestingly, H12 does not contain as high a proportion of heterozygous nuclear markers as do the majority of backcross 1s that have been analyzed extensively). The other one, 1934, has been analyzed for only four nuclear markers. In addition, a higher than expected proportion of these randomly selected F_1 and BC_1 trees have inherited Fremont mitochondria (82% instead of 50%; $\chi^2 = 4.46$, $df = 1$, $P < 0.025$). This pattern further supports the idea that nuclear and cytoplasmic inheritance are interdependent (e.g., varied interactions between products of nuclear and mitochondrial genotypes could have epistatic effects on fitness resulting in cytonuclear disequilibria; see Asmussen et al., 1987).

We have also found that as the proportion of homozygous nuclear inheritance increases (in BC_2s, BC_3s, and BC_4s) the frequency of trees with narrowleaf cytoplasmic inheritance also increases (8 of 11); such a result

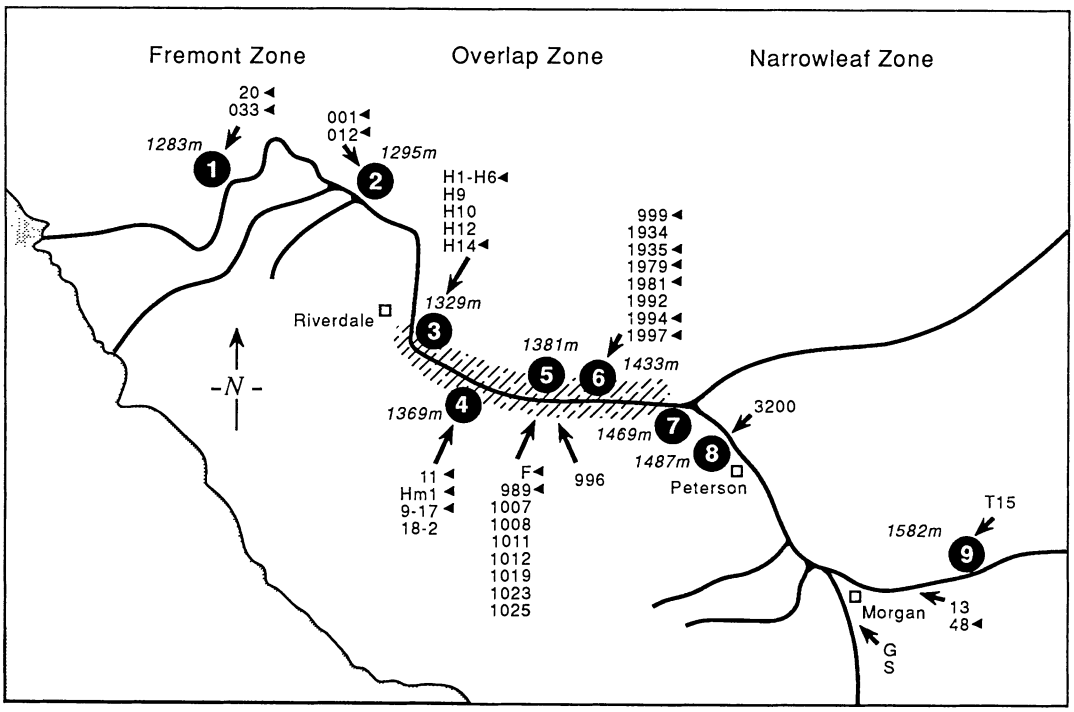

FIG. 3. The geographical distribution and cytoplasmic inheritance patterns of individual cottonwood trees along the Weber River north of Salt Lake City, Utah. Sites of trees are indicated by arrows. Mitochondrial inheritance is indicated by the presence (Fremont) or absence (narrowleaf) of a triangle. The hybrid (or overlap) zone is represented by the shaded area between Riverdale and Peterson (sites 3 through 7), covering a distance of approximately 13 km.

is not significantly different from the expected distribution (73% narrowleaf versus 80%; $\chi^2 = 0.375$, $df = 1$, $P > 0.50$).

Furthermore, nuclear-cytoplasmic disequilibrium values ($D = 0.0463$, $D_1 = 0.093$, $D_2 = -0.093$, $D_3 = 0$; Pearson χ^2 Goodness-of-Fit test = 86.72, $df = 1$, $P < 0.0001$) calculated from the statistics of Asmussen et al. (1987) are consistent with these results, indicating a nonrandom association among nuclear and cytoplasmic genotypes.

Overall, both cytoplasmic genotypes were found throughout the overlap zone (Fig. 3, Table 1). Clearly, cytoplasmically inherited markers from each parental population have successfully penetrated the hybrid swarm, reaching to the boundaries of the overlap zone (trees H10, H12, 999, 1994) or beyond (tree 48).

Tree 48, in particular, represents an interesting anomaly (Fig. 1) in that it has Fremont mitochondria despite its nuclear inheritance (all 16 nuclear markers analyzed have been narrowleaf; (see Table 1). Moreover, it is geographically situated in what we believed to be a population of pure narrowleaf trees (Fig. 3). The fact that Fremont mitochondria can be found in this narrowleaf population suggests that some of these may in fact be complex backcrosses.

DISCUSSION

Mitochondrial Markers

We have isolated probes from a preparation of DNA purified from organelles treated with DNAse. Probes from this G-C rich organelle DNA identify only multicopy DNA. Three polymorphisms (restriction fragment length polymorphisms) were found that distinguished between trees of known Fremont and narrowleaf nuclear inheritance (for this screen we used DNA from trees whose nuclear genotypes have been tested with between 25 and 30 markers). These three probes were used to screen a hybrid swarm of cottonwood trees located between genetically pure populations of Fremont and narrowleaf cottonwood along the Weber River in northern Utah. Every

tree that we have screened has had only one cytoplasmic genotype, either Fremont or narrowleaf. As with most other plant species that have been looked at so far (e.g., see Conde et al., 1979; Schmitz, 1988), no evidence of heterozygous cytoplasmic inheritance was found. Thus, aside from acquiring a unique tool for testing hypotheses, these results are of particular importance in that we have demonstrated that mitochondrial inheritance is uniparental in cottonwood. To date, mitochondrial inheritance has been established for only a limited number of plant species (Schmitz, 1988). Furthermore, since mitochondria are maternally inherited in most angiosperms (e.g., see Schmitz 1988; Stine et al., 1989), it is likely that mitochondria are also maternally inherited in cottonwood (although, as yet, untested).

Unidirectional Introgression and Nuclear-Cytoplasmic Incompatibility

In a previous study it was found that nuclear genotypes of individual trees within the hybrid swarm were consistent with a unidirectional introgression of nuclear genes from the Fremont population into the narrowleaf population (Keim et al., 1989). There are several possible explanations for the observed distribution. Differences in flowering phenology, for example, could account for the observed distribution of genotypes in the hybrid swarm. This hypothesis, however, appears unlikely because significant overlap in phenologies has been observed (Keim et al., 1989). Assortative mating due to a bias in population size (Barton and Hewitt, 1985) is also an unlikely explanation for the observed asymmetric distribution of genotypes in the hybrid swarm. When trees were selected from a region of the hybrid zone immediately adjacent to the Fremont population (where backcrossing to Fremont should be likely) no homozygous (FF) alleles were found in hybrid individuals, suggesting that hybrid by hybrid crosses or backcrosses between hybrid and Fremont trees failed to produce viable offspring (Keim et al., 1989).

Thus, perhaps the most parsimonious explanation for unidirectional gene flow is genetic incompatibility. For example, asymmetry in crosses may be due to an incompatibility between the cytoplasmic genome of one parental type and the nuclear genome of the other (i.e., trees of mixed genotypes will be sterile or will not survive if their cytoplasm is derived from one or the other parent; Asmussen et al., 1987). Examples of such a mechanism might include hybrid dysgenesis (as observed in *Drosophila* [Kidwell et al., 1977; Bingham et al., 1982; Engels 1983; Anxolabehere et al., 1988)] or regulatory system differences governing transcription and translation (Whitt et al., 1977). Our results, however, show that both Fremont and narrowleaf mitochondrial markers are found in trees with mixed nuclear genotypes (in particular, in trees that are products of backcrossing one or more times to narrowleaf parents). Thus, nuclear-cytoplasmic incompatibilities do not appear to account for the asymmetric distribution of nuclear genotypes within the hybrid swarm.

Alternatively, there may be incompatibilities among nuclear genes that could account for the asymmetry in the distribution of nuclear genes within the hybrid swarm (Barton and hewitt, 1985). Incompatibilities, for example, might arise when homozygous Fremont alleles are introduced into a genetic background containing narrowleaf alleles. This idea is consistent with hand pollination experiments showing that backcrosses to Fremont can produce some viable seed, but that seedings are developmentally abnormal, ultimately dying at an early stage. In contrast, backcrosses to narrowleaf resulted in the production of typical, healthy offspring (Keim et al., 1989).

Cytonuclear Disequilibria

Although, nuclear-cytoplasmic incompatibilities do not appear to explain the asymmetric distribution of nuclear alleles within the hybrid zone, nonrandom associations between nuclear and cytoplasmic genotypes were found to exist (using the disequilibrium statistics of Asmussen et al., 1987). The relative proportion of narrowleaf and Fremont nuclear markers found within individual trees in the hybrid swarm suggests that among trees with a high proportion of heterozygous (NF) loci, nuclear inheritance may depend upon cytoplasmic inheritance. For example, all four randomly selected F_1 hybrid trees were found to have Fremont mitochondrial genotypes (HM-1,

989, 1994, 1997; the probability of selecting four F_1 hybrids all with Fremont cytoplasm by chance alone is only 1 out of 16, a relatively rare event). More striking is a higher than expected frequency of heterozygous loci in those progeny of backcrosses between F_1 hybrid and narrowleaf trees that have Fremont mitochondrial genotypes (80% instead of 50%; $\chi^2 = 23.40$, $df = 1$, $P = 0.0001$). Two explanations can be proposed for the excess of heterozygotic nuclear loci found in trees with the Fremont mitochondrial genotype: It is possible that homozygous narrowleaf nuclear loci are incompatible with Fremont cytoplasmic DNA. This explanation—involving the incompatibility of nuclear and cytoplasmic genes—appears unlikely, because Fremont mitochondrial markers are found in trees with extremely high proportions of homozygous narrowleaf nuclear loci (e.g., trees 999 and 48). Moreover, these trees do not appear to have unique combinations of nuclear alleles when compared to other narrowleaf trees (see, for example, Fig. 4 in Keim et al., 1989).

An alternative explanation suggests that seeds, seedlings or trees with high proportions of heterozygous loci are at a disadvantage unless they also have Fremont mitochondria. This explanation is consistent with the observed distribution of nuclear and cytoplasmic alleles in F_1 or first backcross progeny. Trees with an equal proportion of heterozygous to homozygous loci (predicted for a first backcross), for example, are rare. Furthermore, highly heterozygous trees with Fremont cytoplasm (hybrid F_1 trees, or backcross trees such as H1 or 1981) are extremely robust and relatively pest free (see Whitham, 1989; Paige et al., 1990). This suggests that heterosis might be expressed when heterozygous nuclear loci are found in conjunction with Fremont mitochondria. If that were the case, progeny of a single backcross with a preponderance of heterozygous loci in Fremont cytoplasm might have a selective advantage over either parental type within the hybrid zone.

These results are also consistent with the nuclear-cytoplasmic disequilibrium statistics of Asmussen et al. (1987) that support the hypothesis of a strong directionality to interspecific matings (Fremont females crossing with narrowleaf males; assuming mitochondria are maternally inherited in cottonwood) and hybrids backcrossing to only one of the two parental types (to narrowleaf trees, which is also consistent with the results of Keim et al., 1989).

Hybrid Zone Dynamics: Evolutionary Implications

As with nuclear genes, Fremont maternal genes are introgressing into the narrowleaf population. Previous studies have suggested that the acquisition of Fremont nuclear genes by unidirectional introgression into the narrowleaf population may allow these genotypes to become better adapted to lower elevations, the hybrid swarm representing the vanguard of advancing narrowleaf genotypes (Keim et al., 1989). The acquisition of Fremont cytoplasmic genes may also allow these genotypes to compete more effectively with Fremont on its home ground, especially if Fremont mitochondrial genes promote hybrid vigor. The observed linkage disequilibrium between cytoplasmic and nuclear alleles would also allow the persistence of Fremont nuclear and mitochondrial alleles in the hybrid swarm. Thus, the Fremont contribution to the hybrid population would not be diluted as rapidly and could therefore be of selective advantage when encroaching Fremont territory.

While it is generally difficult to infer dynamic processes from static patterns (Endler, 1977, 1982, 1983; Rand and Harrison, 1989) studies such as ours also enable one to gain new insights to the dynamics of plant hybrid zones. Our results, for example, suggest that the narrowleaf population may be 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 would be consistent with such a pattern. This appears to be the case; complex backcrosses, BC_3s and BC_4s, for example, are found at high elevation sites within the hybrid swarm and primarily BC_1s at low elevation sites nearest pure Fremont ($SC = 0.56$, $P < 0.0001$, $N = 40$). Thus, the distribution of backcrosses suggests that the Fremont population may have been prevalent at higher altitudes in the past. As the narrowleaf population spreads and descends (perhaps as the result of the introgression of Fremont genes) the zone of overlap and the hybrid swarm also

descends. Such a pattern would also be consistent with a residue of Fremont cytoplasmic DNA within the narrowleaf population, as we have found. An examination of several randomly chosen trees within the narrowleaf population resulted in the discovery of a tree with Fremont mitochondria despite a nuclear inheritance pattern of narrowleaf (i.e., tree 48; Figs. 2, 3). We are presently seeking further substantiating evidence by examining trees within the narrowleaf population for Fremont cytoplasmic inheritance and by searching for additional independent evidence of the previous existence of Fremont at higher elevations (e.g., a pollen profile).

Hybrid Zone Dynamics: Eventual Fate

Once formed, hybrid zones are either stable or transient (Harrison, 1986). Our previous discussion suggests that the cottonwood hybrid zone is in a state of transition due to directional gene flow and restricted hybridization (i.e., hybrids only backcross with pure narrowleaf; no hybrid by hybrid or hybrid by Fremont crosses occur; see Keim et al., 1989). Therefore, unless some barrier to movement is encountered (Barton, 1979) the hybrid zone will continue to advance, eventually leading to the local extinction of the Fremont parental form (Harrison, 1986) and in time the hybrid zone itself (through repeated backcrossing to narrowleaf). Barriers to movement, for example, might include environmental factors and/or severe reductions in density due to decreased fitness along the cline (Barton, 1979; Barton and Hewitt, 1985). Both of these factors could restrict the movement of the cottonwood hybrid zone and have an overall stabilizing influence. As one proceeds from the pure narrowleaf population to the pure Fremont population (Fig. 3) there is a continual decrease in elevation. Thus, there are likely associated climatic and edaphic factors that could act to inhibit further successful invasion of narrowleaf genotypes. Experimental studies (e.g., seedling transplants) will be necessary to establish the most likely outcome.

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