

## Evidence of Sexually Dimorphic Introgression in Pinaleno Mountain Apache Trout

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**Abstract.**—The high-elevation headwater streams of the Pinaleno Mountains support small populations of threatened Apache trout *Oncorhynchus apache* that were stocked following the chemical removal of nonnative salmonids in the 1960s. A fisheries survey to assess population composition, growth, and size structure confirmed angler reports of infrequent occurrences of *Oncorhynchus* spp. exhibiting the external morphological characteristics of both Apache trout and rainbow trout *O. mykiss*. Nonlethal tissue samples were collected from 50 individuals in the headwaters of each stream. Mitochondrial DNA (mtDNA) sequencing and amplification of nuclear microsatellite loci were used to determine the levels of genetic introgression by rainbow trout in Apache trout populations at these locations. Sexually dimorphic introgression from the spawning of male rainbow trout with female Apache trout was detected using mtDNA and microsatellites. Estimates of the degree of hybridization based on three microsatellite loci were 10–88%. The use of nonlethal DNA genetic analyses can supplement information obtained from standard survey methods and be useful in assessing the relative importance of small and sensitive populations with a history of nonnative introductions.

Interest in applying genetic information to management of fisheries resources is increasing (Allendorf and Ferguson 1990; Crandall et al. 2000; Ingvarsson 2001). This is due in part to the continuing conflict between the ecological and political interests surrounding the preservation of threatened and endangered species and their critical habitat (Bisson 1995; Avise and Walker 2000; Hendry et al. 2000). Biologists are aware of the need to manage imperiled species and biological diversity on an ecosystem scale and are becoming increasingly familiar with the principles of evolutionary and population genetics required to make appropriate management decisions (Behnke 1995; Waples et al. 2001).

In the 1960s, the Arizona Game and Fish Department (AGFD) began a recovery program for native Gila trout *Oncorhynchus gilae*. The urgency of preserving the declining populations of these trout and the limited amount of suitable habitat led to the renovation and stocking of isolated high-elevation streams outside the historical range of

this species (Rinne 1990; Rinne and Turner 1991), including several streams in the Pinaleno Mountains of Graham County (Taylor and Hayes 1991; Porath et al. 1998). Trout were taken from Ord Creek in the White Mountains of Arizona and stocked into the Pinaleno Mountain streams from 1965 to 1971. Subsequently, the Ord Creek stock was taxonomically defined as Apache trout *O. apache* and described by Miller (1972).

Recovery plans included removing all fish from these streams prior to stocking with Apache trout. According to historical stocking records maintained by the AGFD, these streams were first stocked with rainbow trout *O. mykiss* in 1934. Records indicate that brook trout *Salvelinus fontinalis* and “native trouts” (described as black-spotted natives, spotted mountain trout, Wyoming natives, natives, and cutthroat trout *O. clarki* spp.) were stocked for several years beginning in 1936. Brown trout *Salmo trutta* were also stocked in 1938 to provide sportfishing opportunities. To remove existing fish from the treatment areas, liquid rotenone was applied in high concentrations in the headwaters and allowed to flow downstream. These treatments proved difficult because of limited vehicle access and rugged terrain (W. Silvey, AGFD, personal communication). Removal efficiency decreased as distance from the application

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site increased and the chemical became more dilute. Treatments were conducted on Grant Creek in 1960, Grant and Ash creeks in 1965, and Marijilda Creek in 1968. The remaining perennial streams were treated in 1969.

The Apache trout was downlisted from endangered to threatened under the Endangered Species Act in 1975 to permit sportfishing for this species (Behnke 1992). Populations in the Pinaleno Mountain streams were then managed with bag limit restrictions. The terrain and a U.S. Forest Service area closure from November through May limited angler access. Although AGFD biologists documented Apache trout in earlier surveys of the headwater streams (Porath et al. 1998), anglers reported catching an occasional rainbow trout, brook trout, or brown trout, indicating that the treatments were not completely successful. There were also several reports of anglers catching putative hybrids of rainbow trout and Apache trout. Using isozyme locus polymorphisms, Carmichael et al. (1993, 1995) found that 19 of 31 populations investigated in the White Mountains of Arizona were hybridized and identified introgression with rainbow trout as one of the primary threats to Apache trout.

Hybrid individuals, by definition, carry a mixture of parental genomes from divergent species (Campton 1987). One theoretical, negative effect of the mixing of divergent genomes is the potential loss of local adaptation in the native stock. Locally adaptive traits influenced by hybridization include changes in energy storage or stamina, growth and maturation rates, early development rate, migration timing, and morphological and physiological characteristics (reviewed in Arnold 1997). Reductions in fitness occur when F2 or backcross populations from fertile hybrids contain postzygotic reproductive isolating mechanisms leading to hybrid breakdown.

Motivation to investigate the Pinaleno Mountain streams increased following the Clark Peak fire in April–May 1996. Coronado National Forest officials wanted to investigate the fire's effects on the fish populations in several streams. The state was interested in the ability of the streams to support either "high quality" or "native wildfish" populations as an expansion of the sportfishing component of its strategic plan. The U.S. Fish and Wildlife Service (USFWS) wanted to know the genetic status of the Pinaleno Mountain streams because the stock in Ord Creek had been found to be extensively hybridized with rainbow trout (Carmichael et al. 1993).

Allozyme analysis by the USFWS of tissue sam-

ples from 30 fish collected from Grant Creek in 1997 was inconclusive in determining hybridization levels (L. Ruiz, USFWS, personal communication). Because these headwater streams only support small numbers of individuals, the removal of additional samples was considered undesirable. A nonlethal method was therefore sought to determine the level of Apache trout introgression with rainbow trout in each of these streams.

### Methods

*Study site.*—The physical geography of the Pinaleno Mountains is typical of isolated mountain ranges throughout the desert southwestern United States. Perennial streams are rare and frequently located only at high elevations where they are supported by annual snowpack. The eight perennial streams of the Pinaleno Mountains are relatively small (mean wetted width, <2 m). Headwaters typically originate in low-gradient alpine forests and meadows near the summit and descend rapidly through a series of plunge pools. Stream flows become ephemeral or subsurface as they approach the mountain base, with the exception of Grant and Ash creeks, which are diverted for human use in their lower reaches. An unimproved road provides recreational access to the summit area and is closed during the winter.

An electrofishing survey of these streams conducted during the summer of 1997 (Porath et al. 1998) found that only four of the eight (Ash, Big, Grant, and Marijilda creeks) supported fish populations. Rainbow trout, brook trout, and brown trout were found at the lower reaches (1,950 m) of Grant Creek, while only putative Apache trout were observed in the headwater reaches of these streams in the summit area. Because high morphological and meristic variation has been found in Apache trout populations (Rinne 1985; Rinne and Minckley 1985), field examination of specimens during this survey was unsatisfactory in identifying individuals potentially hybridized with rainbow trout.

We conducted our study on these four streams at the highest elevations (2,500–2,850 m) that supported fish populations. The beginning of perennial flows and the presence of fish defined the upper reach of the sampling area on each stream. An impassable migration barrier identified in the 1997 survey and characterized by a significant drop in elevation determined the lower reach. Each of these reaches is accessible from the summit area and was the location of the original Apache trout stockings. These areas also have the highest probability of containing intact Apache trout popula-

TABLE 1.—Allelic structure for 11 microsatellite loci amplified in western trout species. Size ranges are given in base pairs (bp); asterisks indicate that there was no significant amplification product at tested PCR conditions. Allelic size ranges were resolved on a LI-COR Long Reader 4200 automatic sequencer and include the length of the amplified primer (see Nielsen and Sage 2001).

Locus	Coastal rainbow trout ( <i>N</i> = 1,518)		Gila trout (New Mexico; <i>N</i> = 33)		Apache trout (Arizona; <i>N</i> = 219)		Mexican golden trout (Mexico; <i>N</i> = 28)		Rio Yaqui trout (Mexico; <i>N</i> = 74)		Lahontan cutthroat trout (Nevada; <i>N</i> = 349)	
	Size	No. of alleles	Size	No. of alleles	Size	No. of alleles	Size	No. of alleles	Size	No. of alleles	Size	No. of alleles
<i>Omy2</i> <sup>a</sup>	107–177	26	*	*	*	*	93–149	9	121–149	14	104	1
<i>Omy77</i> <sup>b</sup>	93–155	29	101–133	10	151–171	9	135–139	3	115–119	3	98–148	23
<i>Omy207</i> <sup>c</sup>	97–161	24	121–155	7	121–155	8	114–190	7	106–136	9	96–146	7
<i>Omy325</i> <sup>c</sup>	97–149	27	135	1	113–137	3	105–153	8	95–129	7	101–133	6
<i>Onep.2</i> <sup>d</sup>	182–290	47	202–206	2	202	1	230–242	5	244–322	20	194–286	22
<i>Onep.8</i> <sup>d</sup>	152–190	18	170–178	2	162–170	2	154–160	4	162–168	4	153–195	17
<i>Ots1</i> <sup>e</sup>	151–249	33	159–163	2	161–167	2	159–233	4	221–229	4	159–289	36
<i>Sfo8</i> <sup>f</sup>	171–287	15	173	1	265–283	8	219–255	3	233–241	3	176–286	24
<i>Ssa14</i> <sup>g</sup>	120–168	21	152	1	152	1	154–198	13	132–144	5	105–145	13
<i>Ssa85</i> <sup>h</sup>	101–169	34	105–155	3	105–115	2	107–183	11	135–171	11	85–153	22
<i>Ssa289</i> <sup>g</sup>	104–130	14	100–124	4	96–122	5	110–124	5	108	1	108–122	8

<sup>a</sup> Heath et al. 2001.

<sup>b</sup> Morris et al. 1996.

<sup>c</sup> O'Connell et al. 1997.

<sup>d</sup> Scribner et al. 1996.

<sup>e</sup> Banks et al. 1999.

<sup>f</sup> Angers et al. 1995.

<sup>g</sup> McConnell et al. 1995.

<sup>h</sup> O'Reilly et al. 1996.

tions because of the removal methodology and the barriers to upstream migration. The distances from the barrier to the upper reach were measured by hip-chain and were approximately 2.8 km on Grant Creek, 2.5 km on Ash Creek, 1.6 km on Marijilda Creek, and 1.2 km on Big Creek. Barrier heights ranged from 5 to 7.5 m for Big, Grant, and Marijilda creeks but only 2 m on Ash Creek. Physical habitat was similar at the four reaches, with stream classification categories ranging from A4 to C4 (Rosgen 1985) and gradient from 4% to 14%.

**Fish collections.**—During 12–18 August 1998, streams were sampled using a pulsed-DC backpack electrofisher with a single anode ring and trailing cathode cable. All individuals were collected from low-gradient and uninterrupted flow areas, while stratified random sampling was applied in areas that contained a series of separated plunge pools. Samples taken from any of these pools were limited to three individuals. If more than three fish were captured while electrofishing a single pool, they were divided into the length frequency categories identified in the 1997 survey data, and only one individual per length-group was selected to avoid sibling swamping of genetic information. A total of 50 fish were collected from each of the four streams. Each fish was measured for total

length (mm) and weight (g). A small portion of the lower caudal fin (2 × 2 mm) was removed, placed on folded filter paper, and inserted into a labeled envelope. Tissue samples were thoroughly dried at room temperature and then shipped to the laboratory for genetic analysis.

**Genetic analysis.**—Total genomic DNA was extracted from 50 trout per population according to the methods in Nielsen et al. (1994b). Amplification and visualization of the mitochondrial DNA (mtDNA) D-loop sequence followed the methods given in Nielsen et al. (1994a). Amplification of 11 microsatellite loci used to screen for diagnostic alleles followed the methods given in Nielsen and Fountain (1999). Allelic size ranges and the number of alleles for these 11 microsatellite loci were compared with published data from several southwestern trout species from the USA and Mexico (Table 1). Rainbow trout show the most diversity in allelic size range (93–290 base pairs [bp]) and number of alleles (mean number of alleles per locus = 23). Apache trout allelic size ranges are more limited (96–283 bp), with a significantly ( $P < 0.0001$ ) lower mean number of alleles per locus (3.5). Only Gila trout have a smaller mean number of alleles per locus (2.4). We selected three loci, *Omy77* (Morris et al. 1996), *Omy2* (Heath et al. 2001), and *Sfo8* (Angers et al. 1995), to test rainbow

trout introgression in Apache trout based on the degree of resolution found between the two species for these loci.

Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard (P-E Biosystems, Foster City, California), DNA samples of known size that were rerun on each gel, and a double-stranded reference marker developed in our laboratory showing the most common alleles available for each locus in rainbow trout. The GENESCAN 1.1 and GENOTYPER 2.1 (P-E Biosystems) DNA fragment analysis software packages were used to determine allelic (and genotypic) designations. Comparisons of species-specific allelic diversity (i.e., rainbow versus Apache trout) were based on previously published amplification of microsatellite loci for many trout populations (Nielsen 1996, 1999; Olsen et al. 1996; Nielsen et al. 1997a, 1997b, 1998, 1999a, 1999b, 2000). Nonamplification of the *Omy2* locus in Apache trout using the same polymerase chain reaction (PCR) protocols that successfully amplified this locus in all other rainbow trout populations was used as our third diagnostic trait. We were conservative in judging hybridization, using only those loci with repeatable diagnostic allelic structure (differences in allelic size [*Sfo8* and *Omy77*] or nonamplification [*Omy2*]) to screen for hybridization in Apache trout populations. Individuals with a genotype containing one or more of these markers assigned to rainbow trout were considered hybrids.

Baseline genetic data on Apache trout previously analyzed in our laboratory included those from 30 samples from the Williams Creek National Fish Hatchery. These fish were from production lot 5WC-1 and represented age-1 fish. They were several generations removed from the wild and had descended from fertilized eggs originally collected from the East Fork White River in 1983 and 1984. The East Fork White River is the type locality for the Apache trout (Miller 1972).

Reference collections for hatchery rainbow trout ( $N = 586$ ) came from 12 common hatchery stocks used for supplementation throughout the western United States since the early 1900s (see lists in Nielsen 1996; Nielsen et al. 1994a, 1997a). Our rainbow trout reference data for mtDNA and microsatellites contained analyses of 932 individuals from populations from Alaska, California, Idaho, Missouri, Nevada, Oregon, Washington, and Canada (Nielsen et al. 1998, 1999a, 1999b, 2000; Nielsen 1999), including the putative ancestral coastal

rainbow trout *O. m. irideus* type locality from Sheephaven Creek, California, identified by Behnke (1992). Genetic data for reference trout from Mexico were previously published in Nielsen et al. (1998) and Nielsen and Sage (2001).

Analyses of heterozygosity and  $F_{ST}$  (a function of the probability of the identity of genes within and between units) were performed using ARLEQUIN (Schneider et al. 1997). Genotypic disequilibrium among locus pairs was tested using GENEPOP (Raymond and Rousset 1997). Population independence between paired comparisons of allelic frequencies was tested using Fisher's exact tests based on a Markov chain adaptation of row-by-column contingency tables using GENEPOP. Statistical significance levels for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989). A measure of the number of migrants ( $N_m$ ) was calculated as a surrogate for gene flow using the private allele method (Slatkin 1985; Barton and Slatkin 1986).

The program ARLEQUIN was used to generate a genetic distance matrix among Apache trout populations based on linearized  $F_{ST}$  values. The NEIGHBOR and CONSENSE applications from PHYLIP (Felsenstein 1995) were used to generate a consensus neighbor-joining tree from the  $F_{ST}$  genetic distance values.

## Results

Mitochondrial DNA sequence data showed only one haplotype in all Apache trout analyzed for this marker. This sequence was identical to a previously published sequence for Gila and Apache trout in Nielsen et al. (1998). Strict maternal inheritance and the lack of variation found in 188 bp of the D-loop sequence for all four Apache trout populations does not eliminate the possibility of sexually dimorphic introgression by other species, such as would occur when rainbow trout males spawn with Apache trout females without equivalent mating between rainbow trout females and Apache trout males. This type of mating bias would retain the maternally inherited mtDNA of the Apache trout in successive generations, with or without nuclear gene flow between the species. Therefore, our results based on the mtDNA D-loop sequence gave little resolution in our analyses of rainbow trout introgression in Apache trout except to suggest the possibility of sexually biased gene flow between these species.

Microsatellite allelic structure varied by the size ranges amplified in rainbow and Apache trout for locus *Omy77*, with alleles falling below the 151-bp

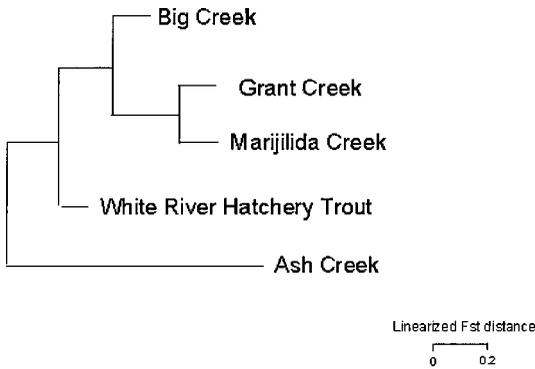


FIGURE 1.—Neighbor-joining tree based on the pairwise linearized  $F_{ST}$  genetic distances reported in Table 2.

threshold found to be unique to rainbow trout and thus possibly indicating introgression in Apache trout. Despite overlap in the upper allelic size ranges between these species for locus *Sfo8*, alleles unique to rainbow trout were found in the smaller size range (<265 bp). No *Sfo8* alleles smaller than 265-bp were found in the hatchery Apache trout derived from the original White River stock. All putative Apache trout with amplification of *Omy2* alleles ( $n = 32$ ) also carried rainbow trout alleles at the *Omy77* locus. We tested for genotypic disequilibrium across all Apache trout populations between these two loci and found no significant disequilibrium ( $\chi^2 = 0.011$ ,  $P = 0.999$ ). Rainbow trout alleles detected at one or more loci were highest in Ash Creek (88%). Lower levels of introgression with rainbow trout were found in Big Creek (16%), Grant Creek (10%), and Marijilda Creek (14%). Only 15 fish from Ash Creek showed evidence of rainbow trout introgression for all three loci. Forty-nine fish carried rainbow trout alleles at one or two loci, indicating substantial backcrossing of hybrids within these populations.

We calculated highly significant genic differentiation among the three putative diagnostic loci ( $P < 0.00013$ ). Average heterozygosity for all loci equaled 0.63. The mean  $F_{ST}$  for all loci combined was 0.18 ( $Omy77 = 0.22$ ;  $Omy2 = 0.27$ ;  $Sfo8 = 0.07$ ), with most genetic diversity occurring at the level of individuals within a population (99%). The analysis of population independence showed significant ( $P < 0.01$ ) differences for all paired comparisons, suggesting differences in population allelic structure for each stream locality. The estimated number of migrants (used as a surrogate for gene flow) among Apache trout populations in this study was 1.6. Population pairwise  $F_{ST}$  values ranged from 0.003 between Grant and Marijilda

TABLE 2.—Population pairwise linearized  $F_{ST}$  genetic distances based on the number of different alleles calculated using ARLEQUIN. The White River population is the federal Apache trout hatchery population from Alchey-Williams Creek Fish Hatchery.

Population	Population			
	Ash Creek	Big Creek	Grant Creek	Marijilda Creek
Big Creek	0.2713			
Grant Creek	0.2855	0.1379		
Marijilda Creek	0.3089	0.1126	0.0033	
White River	0.2195	0.0795	0.1022	0.1201

creeks to 0.31 between Ash and Marijilda creeks (Figure 1; Table 2).

### Discussion

Stocking of rainbow trout in streams and rivers throughout the southwestern United States has caused concern about potential introgressive hybridization with native trout (Dowling and Childs 1992; Carmichael et al. 1993; Utter 2000). The detection of hybridization can be difficult, but improved molecular techniques developed over the last two decades have facilitated such findings (Avisé 2000; Allendorf et al. 2001). Analyses of hybridization and introgression are only as good as the baseline data available for each species (Arnold 1997; Rosenfield et al. 2000; Scribner et al. 2000). Microsatellite loci have been used in many case studies to examine the effects of hybridization (Beaumont and Bruford 1999; Poteaux et al. 2000). After extensive analyses at 11 microsatellite loci, 3 loci were used in this study to test rainbow trout introgression in Apache trout populations. The genetic data used in these analyses included those from an extensive survey of rainbow trout throughout its range as well as those from the Shasta Hatchery and Arlee Hatchery strains that were commonly stocked throughout the range of this species since before the turn of the last century (U.S. Commission of Fish and Fisheries 1874–1901; Busack and Gall 1980; Krueger and May 1987).

These microsatellite markers indicated limited ( $\leq 16\%$ ) levels of introgression in the populations of Apache trout in Grant, Marijilda, and Big creeks, whereas only 12% of the population in Ash Creek was found to be free of putative rainbow trout alleles. Although cross-species amplification of microsatellite loci has been well documented (Estoup and Cornuet 1999), it is important to remember that these loci represent only a small part of the total trout genome and that they were developed for studies of genetic diversity in species

other than Apache trout (the *Omy* series of microsatellites for rainbow trout and *Sfo8* for brook trout). The evidence of introgression that they present should be considered as only part of the potential genomic evidence for introgression. There may be additional informative characters based on other molecular systems or loci not screened in this study that show different or confounding levels of introgression in these same populations.

In this study, we used differences in allelic size range and PCR amplification constraints to screen potential introgressive hybridization between rainbow trout and Apache trout. The conservation of microsatellite loci over long periods of time has been demonstrated (Hamada et al. 1982; Fitz-Simmons et al. 1995). Conservation of microsatellite loci among salmonid species allows the use of heterologous PCR primer pairs in closely related species (Angers and Bernatchez 1996; Jarne and Lagoda 1996; Estoup and Cornuet 1999). However, significant allele size variability among species or subspecies for a single locus may result from variation in mutation rates (Rubinsztein et al. 1995), allele size constraints (Chakraborty and Kimmel 1999), different selective pressures in different environments (Boyce et al. 1996), or variation in the complex evolution of the individual loci between groups (Angers and Bernatchez 1997).

Changes in the microsatellite flanking region sequence have been shown to be a potential source of information on genealogical relationships among species or subspecies (Angers and Bernatchez 1997; Orti et al. 1997; Zhu et al. 2000). Microsatellite flanking region polymorphisms may also create constraints on PCR amplification of loci or individual alleles (i.e., null alleles) among closely related species (Jarne and Lagoda 1996; Lehmann et al. 1996; Chakraborty and Kimmel 1999). Therefore, the degree and types of molecular variation (i.e., nonamplification) that we found between rainbow trout and Apache trout using the microsatellite locus *Omy2* were not unusual or unexpected. The nonamplification of *Omy2* in Apache trout is significant considering the sample sizes of rainbow trout that we have amplified using this locus ( $N > 3,500$ ). It is also significant that we have demonstrated that there is a diversity of trout species and subspecies in which this locus amplifies a product of similar allelic size range (93–181 bp) using standard PCR protocols: coastal rainbow trout throughout its range, California golden trout *O. m. aquabonita*, McCloud River red-band trout *O. m. stonei*, Kern River rainbow trout *O. m. gilberti*, Little Kern River rainbow trout *O.*

*m. whitei*, Baja California Mexican trout *O. m. nelsoni*, Mexican golden trout *O. chrysogaster*, Rio Yaqui trout *O. mykiss* ssp., Lahontan cutthroat trout *O. clarki henshawi*, coastal cutthroat trout *O. c. clarki*, and Paiute cutthroat trout *O. c. seleniris* (see Nielsen et al. 1997b; Nielsen and Sage 2001, 2002). Introgressive relationships that appear congruent at two or more loci for most Apache trout containing putative rainbow trout *Omy2* alleles add support to our conclusions; however, sequencing the flanking region of this locus in Apache trout will be required to confirm that the mutation(s) in the flanking region for *Omy2* lead(s) to nonamplification of this locus in Apache trout.

As in a previously published study of hybridization in Apache trout by Dowling and Childs (1992), we found the direction of rainbow trout introgression to be sexually biased. The absence of rainbow trout mtDNA in any Apache trout shown to carry putative rainbow trout microsatellite alleles supports directionality in gene exchange and differential assortative mating between these species. As proposed by Dowling and Childs (1992), juvenile rainbow trout males may be more likely to survive to reproduction than females because of their tendency to mature at a smaller size. Apache trout females, on the other hand, may mate more frequently than rainbow trout females or prefer male rainbow trout. Alternatively, male rainbow trout may compete more successfully than male Apache trout for female Apache trout. In any case, the result is that mtDNA gives different results on introgression from nuclear data based on allozymes (Dowling and Childs 1992) and microsatellites (this study).

Microsatellite and mtDNA genetic analyses of Apache trout populations indicated that both hatchery and wild populations contained limited genetic diversity in comparison with rainbow trout. These differences could have resulted from the reproductive isolation of stream populations over long periods of time, population bottlenecks, founder effects, or very low effective population size within individual streams. Pairwise comparisons of allelic frequencies among the Apache trout populations, however, showed genetic independence between all possible population pairs, suggesting that each stream population has suffered unique bottleneck events. The estimated number of migrants ( $N_m$ ) and  $F_{ST}$  neighbor-joining analyses also supported limited gene exchange among the Apache trout populations surveyed for this study.

Low population size in isolated habitats has probably contributed to a loss of genetic diversity

in Apache trout over recent history. Introgression from stocked rainbow trout has clearly changed the scale of genetic diversity found within Apache trout, but species-specific allelic structure remains in these populations despite the obvious effects of hybridization. The limited genetic diversity and low heterozygosities found in these trout populations suggest small effective population size (number of breeders) and significant backcrossing by hybrids. Elimination of populations or individuals thought to be hybrids or backcrosses based on molecular genetic analyses will clearly reduce the frequency of putative rainbow trout alleles found in any population. But such activity may also result in the loss of locally adaptive genetic variation not found in any other population of Apache trout (Allendorf and Leary 1988; Dowling and Childs 1992). The limited genetic diversity found in Apache trout makes it important to balance the degree of introgression evident within a population with the diversity found in "pure" Apache trout characteristics before the elimination of hybrids is implemented as a conservation measure.

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