Impact of Hybridization on a Threatened Trout of the Southwestern United States

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Abstract: *Trouts native to the American Southwest provide an excellent example of the plight of endangered fishes from this region. The native species, Apache trout and Gila trout (*Oncorhynchus apache *and* O. gilae, *respectively) have faced drastic reduction in habitat and detrimental interactions with introduced species, resulting in a dramatic decrease in numbers and sizes of populations. We used biochemical methods to identify diagnostic markers for the estimation of genetic relatedness and analysis of hybridization among native trouts and introduced cutthroat and rainbow trouts (*O. clarki *and* O. mykiss, *respectively). Restriction endonuclease analysis of mitochondrial DNA (mtDNA) indicated that Apache and Gila trout were very similar to each other, and more similar to rainbow trout than cutthroat. Diagnostic allozyme marker loci indicated that Apache trout hybridized extensively with rainbows in four populations and provided no evidence for reproductive isolation between the forms. Analysis of mtDNA, however, indicated that introduced haplotypes were rare in these same individuals, identifying a bias in the direction of gene exchange between species. The potential reproductive isolation and lack of information concerning population structure necessitate further study of Apache trout to determine the appropriate management strategy for this threatened species. This case demonstrates that extreme care must be exercised when considering elimination of any contaminated population lest the unique genetic identity of the native taxon be lost forever.*

Resumen: *Las truchas nativas del Sudoeste Norteamericano proveen un excelente ejempo de las serias y difíciles condiciones en que se encuentran los peces en peligro de extinción de esta región. Las especies nativas, trucha Apache y trucha "Gila" (*Oncorhynchus apache *y* O. gilae, *respectivamente) han sufrido una drástica reducción en su habitat e interacciones nocivas con especies introducidas, lo que resultó en una dramática disminución en el número y tamaño de sus poblaciones. Métodos bioquímicos fueron usados para identificar marcadores diagnósticos para la estimación de parentesco genético y análisis de hibridación entre truchas nativas y las truchas introducidas "Cutthroat" y Arcoiris (*O. clarki *y* O. mykiss, *respectivamente). Análisis de restricción de endonucleasas de ADN mitocondrial (mtDNA) indicaron que las truchas Apache y "Gila" eran muy similares entre sí, y más similares a la trucha Arcoiris que a la "Cutthroat." Loci marcadores diagnósticos de alocimas indicaron que, en cuatro poblaciones, la trucha Apache se ha hibridizado extensivamente con la arcoiris y no proveyeron de evidencia alguna que indicase aislamiento reproductivo entre especies. Sin embargo, el análisis de mtDNA indicó que los haplotipos encontrados en las especies introducidas eran raros en estos mismos individuos, indicando un sesgo en la dirección del intercambio genético entre las especies. El potencial aislamiento reproductivo y la falta de información sobre la estructura poblacional de la trucha Apache necesita ser estudiada con más detalle para poder determinar la estrategia de manejo apropiada para esta especie en peligro. Estos casos demuestran que se debe tener extremo cuidado cuando se considera la eliminación de cualquier población contaminada para no correr el riesgo de que la identidad genética del taxón nativo se pierda para siempre.*

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Introduction

Many fishes native to the American West are seriously threatened with extinction, largely due to human influence (reviewed in Minckley & Deacon 1991). Water management practices have reduced and extensively modified aquatic habitats and their surrounding landscapes. Non-native species, usually introduced as sport or bait fish, have also seriously affected native species through competition, predation, and hybridization. The impact is so severe that twenty-one taxa have already been eliminated. Of 224 fish taxa from the United States considered threatened, endangered, or candidates for one of these categories, 105 are from west of the continental divide. This is especially significant since only about 170 species are native to the West, compared with approximately 600 in waters east of the Rocky Mountain axis (Minckley & Douglas 1991).

The status of native western trouts provides an excellent example of the impact of human activities. Members of this group are prized for food and sport, resulting in widespread domestication, propagation, and transport (Kincaid & Berry 1986; Allendorf et al. 1987). Introduced trouts (such as brown trout, *Salmo trutta*, and brook trout, *Salvelinus fontinalis*) prey upon or outcompete native species, and transfer of other taxa among western watersheds (rainbow trout, *Oncorhynchus mykiss* [formerly *Salmo gairdneri*]; cutthroat trout, *O.* [formerly *S.*] *clarki*) has resulted in considerable loss of diversity in native taxa due to extensive introgression between native and non-native forms (Rinne & Minckley 1985; Allendorf & Leary 1988).

Two salmonids, Apache trout (*O.* [formerly *S.*] *apache*, Miller, 1972) and Gila trout (*O.* [formerly *S.*] *gilae*, Miller, 1950), are native to the lower Colorado River drainage, Arizona–New Mexico, U.S.A. The Apache trout occupies high-elevation tributaries of the Little Colorado and upper Salt rivers, and a limited part of the San Francisco River in the White Mountains of eastern Arizona (Minckley 1973, Rinne & Minckley 1985). The Gila trout was originally present in tributaries of the Verde River in Arizona and upper Gila River in New Mexico, but it has been extirpated from Arizona (Minckley 1973, Rinne 1980). The former is federally listed as threatened and the latter is endangered (U.S. Fish and Wildlife Service [USFWS] 1989), largely due to interactions with introduced trouts and reductions in habitat (USFWS 1979 and references therein). Previous studies of molecular and morphological characteristics indicate that introduced rainbow trout hybridize extensively with Apache and Gila trouts, producing hybrid swarms and ultimately replacing the natives (Rinne & Minckley 1985; Loudenslager et al. 1986).

We report results of genetic characterization of a number of trouts introduced and native to the lower Colorado River drainage, and further assess the impact of hybridization on Apache trout. Initially, we used restriction endonuclease analysis of mitochondrial DNA (mtDNA) to identify diagnostic markers and estimate genetic similarities. Diagnostic allozymic and mtDNA restriction fragment patterns were then used to determine the extent of introgression into populations of Apache trout. The strict maternal inheritance of mtDNA allows for identification of biases in the direction and extent of introgression.

Materials and Methods

Sampling

To identify genetic markers, we obtained representatives of each species from hatcheries or natural populations (Table 1). Previous studies (Rinne & Minckley 1985; Loudenslager et al. 1986; Rinne 1988) indicated that mostly rainbow trout were involved in hybridization with the natives. Our initial surveys also implicated cutthroat trout, so representative samples of several cutthroat subspecies were included. Each of the introduced species has already been extensively characterized for allozymic and mtDNA variation (Busack et al. 1979; Berg & Ferris 1984; Gyllensten et al. 1985; Ferris & Berg

Table 1. Source and sizes of reference samples.*

Taxon	*Source*	*Sample size*	
		Allozymes	*mtDNA*
Rainbow	Arlee strain (WCNFH)	10	5
	Erwin strain (WCNFH)	10	5
	Fish Lake strain (WCNFH)	10	5
	Shasta strain (WCNFH)	10	5
Cutthroat			
henshawi	Heenan Lake strain (NDWLMH)	5	2
pleuriticus	Figure Eight Lake, Uinta Co., UT	5	0
	Jones Cabin Creek, Wasatch Co., UT	0	1
stomias	S. Platte River strain (BFTC)	4	2
subsp.	SnakeRiver strain(SSSFH)	4	5
virginalis	Indian Creek, Otero Co. NM (MNFH)	4	2
Apache	E. Fork White River (WCNFH)	20	10
Gila	Main Diamond Creek (MNFH)	20	10

* *Hatchery abbreviations are as follows: BFTC, Bozeman Fish Technology Center, MT; MNFH, Mescalero National Fish Hatchery, NM; NDWLMH, Nevada Department of Wildlife Lake Mead Hatchery; SSSFH, Sterling Springs State Fish Hatchery, AZ; WCNFH, Williams Creek National Fish Hatchery, AZ.*

1987; Gyllensten & Wilson 1987; Leary et al. 1987). Allozymic variation of the native Arizona–New Mexico species has also been surveyed (Loudenslager et al. 1986; D. Morizot et al., personal communication), but mtDNA variation has not been reported previously.

Four hatchery strains of rainbow trout commonly stocked in Arizona were analyzed, as were several subspecies of cutthroat (*O. clarki* subsp. [Snake River cutthroat], *O. c. henshawi* [Lahontan cutthroat], *O. c. pleuriticus* [Colorado River cutthroat], *O. c. stomias* [greenback cutthroat], and *O. c. virginalis* [Rio Grande cutthroat]) because of their geographic proximity to Apache and Gila trouts or history of stocking in the region. Yellowstone cutthroat (*Oncorhynchus c. bouveri*) has also been widely stocked; however, this subspecies and the Snake River form are virtually identical genetically (Allendorf & Leary 1988; Williams & Shiozawa 1989), and the latter presumably represented variation in the Yellowstone form. We assumed that variation in the hatchery strains analyzed was characteristic of these taxa, allowing their use as reference specimens.

Reference samples of Apache and Gila trouts were also obtained from hatchery stocks. The Apache stock originated in 1983 from progeny of 20 males and 30 females collected from the East Fork of the White River, Apache County, Arizona. This stock was supplemented in 1984 with progeny of approximately 38 females and 22 males from the same locale (for further description of this stock, see David 1990). The Gila trout stock originated in 1988 from progeny of 10 males and 10 females from Main Diamond Creek, Grant County, New Mexico. In addition to these, allozymes of hatchery-produced rainbow X Apache F_1 hybrids (Williams Creek National Fish Hatchery) were examined to insure proper identification of heterozygotes.

Four Arizona populations suspected to contain hybrid individuals were sampled in 1989: (1) Boggy Creek, Salt River drainage, Apache County (T4N, R27E, Sec. 1)(N = 14); (2) Paddy Creek, Little Colorado River drainage, Apache County (T4S, R30E, Sec. 15)(N = 72); (3) KP Creek, Blue River drainage, Greenlee County (T8S, R30E, Sec. 15)(N = 50); and (4) Chitty Creek, Gila River drainage, Greenlee County (T9S, R28E, Sec. 1)(N = 63). The Apache trout is native to all localities except Chitty Creek, where identity of the original trout inhabitant remains equivocal (Marsh et al. 1991). KP Creek was treated with piscicide in 1969, after which Apache trout from Sterling Springs State Fish Hatchery (from Ord Creek, Apache County; Minckley & Brooks 1985) were restocked. Trouts from three of these creeks, Chitty, Paddy, and KP, were previously studied by Loudenslager et al. (1986).

Protein electrophoresis. Loudenslager et al. (1986) and Morizot (personal communication) identified several presumptive allozyme loci as diagnostic for Apache, Gila, and rainbow trouts. Three of these, alcohol dehydrogenase (*Adh-A*, EC 1.1.1.1), lactate dehydrogenase (*Ldh-C*; EC 1.1.1.27), and a tripeptidase (*Pep-LGG*, EC 3.4.11.4, L-leucyl-glycyl-glycine as substrate), were selected as markers because electromorphs were readily scored and easily interpreted. Enzyme products were resolved with 12% starch gels (Sigma Chemical Co.). *Adh-A* was resolved from liver, using the borate buffer system described in Murphy et al. (1990). *Ldh-C* and *Pep-LGG* were resolved from eye and muscle, respectively, using the Tris-borate-EDTA I buffer system also described in Murphy et al. (1990).

Restriction Endonuclease Analysis of mtDNA

MtDNA was isolated by equilibrium-density ultracentrifugation, and fragments produced by restriction endonuclease digestion were visualized by end-labelling and autoradiography (Dowling et al. 1990). Restriction enzymes used to cleave mtDNAs varied, depending upon the analysis. For preliminary assessment of similarity, mtDNAs from single reference specimens of Apache, Gila, and rainbow trouts were digested with 12 six-base recognizing restriction endonucleases (*Ava*I, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Eco*RI, *Hin*dIII, *Mlu*I, *Nco*I, *Nhe*I, *Puu*II, *Spe*I, and *Xba*I), and fragments were electrophoretically separated on 1.0% agarose and 4.0% polyacrylamide gels. Since this analysis indicated little divergence, mtDNAs from representatives of each species (two individuals of Apache, Gila and each strain of rainbow trout, and a single cutthroat trout) were cleaved with four four-base recognizing restriction endonucleases (*Hinf*I, *Hin*PI, *Mbo*I, *Scr*FI) and fragments were electrophoretically separated on 1.5% agarose and 4.0% polyacrylamide gels. Estimates of genetic relatedness among haplotypes (percent sequence divergence) were calculated from the proportion of shared fragments (Avise et al. 1979). Divergence estimates were clustered by the unweighted pair group method using arithmetic averages (UPGMA; Sneath & Sokal 1973) to identify genetic relationships.

Results and Discussion

Characterization of Reference Specimens

Allozymes. Analysis of reference specimens for *Adh-A*, *Ldh-C*, and *Pep-LGG* (Table 1) supported their use as markers for discriminating introduced from native trouts; the various forms were fixed for alternate alleles at each locus. In addition, *Pep-LGG* discriminated between rainbows and cutthroats, as all forms of the latter exhibited alleles different from those of the former. Some subspecies of cutthroat not analyzed in this study (e.g., *clarki*, *lewisi*) exhibit the same allele as rainbow trout (Leary et al. 1987), but there are no records of these subspecies being stocked in Arizona. These loci

did not allow discrimination of Apache and Gila trout. Loudenslager et al. (1986) reported a peptidase diagnostic for Gila trout (*Pep-A*, glycyl-L-leucine as substrate), but we were unable to replicate their results.

Heterozygous genotypes for these loci were identified in hatchery-reared rainbow × Apache hybrids. For *Adh-A*, heterozygous individuals exhibited the three-banded pattern expected for a dimeric protein. Heterozygotes for *Ldh-C* and *Pep-LGG* were both expressed as intermediate smears due to small differences in migration distances between alleles.

Mitochondrial DNA. Preliminary analysis of representative mtDNAs from Apache, Gila, and rainbow trout with six-base recognizing restriction enzymes indicated limited divergence among species. Only two enzymes (*Ava*I, *Nhe*I) varied, each at a single cleavage site. Sequence divergences (Nei & Tajima 1983) were correspondingly low (0.4–0.9%).

Cleavage of mtDNAs of Apache, Gila, four strains of rainbow, and cutthroat trouts with four-base recognizing enzymes revealed considerably more variation, as demonstrated by estimates of sequence divergence (Table 2). UPGMA supported close relationships among all rainbow strains (Fig. 1). In fact, individuals of some strains possessed mtDNAs identical to those of others, likely due to the practice of using individuals from several geographic regions in production of strains. The mtDNAs of Apache and Gila trouts were more similar to each other than either was to rainbow trout (Fig. 1), and they clustered closely to rainbow trout (approximately 1.5% sequence divergence). The mtDNA of cutthroat trout was the most divergent, differing from all others by approximately 3.8% (Fig. 1). This level of divergence between rainbows and cutthroats is approximately the same as that reported previously (about 4%; Gyllensten & Wilson 1987).

The level of divergence between the Apache-Gila trout cluster and rainbow trout is similar to that between subspecies groups of cutthroats (Williams & Shiozawa 1989). While this comparison may indicate relatively limited mtDNA divergence between these three taxa, it is important to note that all available data

Table 2. Percent shared fragments (above diagonal) and sequence divergence estimates (×100, below diagonal) calculated from comparison of fragment patterns for several strains and species of trouts.*

	1	*2*	*3*	*4*	*5*	*6*	*7*
1	—	90.7	97.3	97.8	86.2	83.5	65.5
2	0.82	—	90.6	89.3	83.9	82.1	65.8
3	0.23	0.83	—	93.3	85.2	83.3	64.3
4	0.19	0.95	0.58	—	84.8	83.0	64.3
5	1.25	1.48	1.35	1.39	—	93.5	63.2
6	1.52	1.67	1.54	1.57	0.57	—	62.3
7	3.62	3.58	3.78	3.58	3.94	4.00	—

* *Haplotypes are as follows: rainbow trout strains from (1) Fish Lake; (2) Erwin, Shasta; (3) Arlee; (4) Arlee, Shasta; (5) Apache trout; (6) Gila trout; (7) cutthroat trout.*

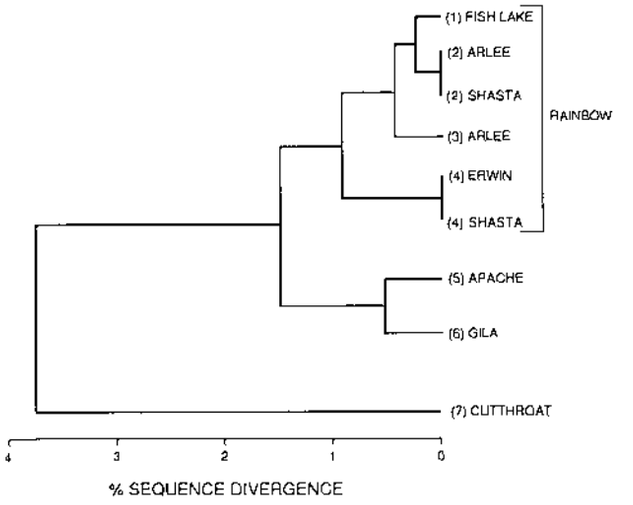

Figure 1. UPGMA representation of the relationships among species studied. Numbers depict haplotypes provided in Table 2.

(from morphology, allozymes, mtDNA) must be considered in assigning taxonomic status. Levels of divergence for molecular characters are not precisely correlated with taxonomic status (Buth 1984); therefore, the lack of genetic divergence does not determine the specific status of the taxa in question. The existence of morphological features diagnostic for Apache and Gila trout (Miller 1950, 1972) is sufficient for their continued recognition as distinct species.

The diagnostic characteristics of two enzymes (*Mbo*I and *Nhe*I) were verified by digestion of mtDNAs from a larger number of individuals representing each taxon (Table 1). For *Nhe*I, Apache and Gila trouts exhibited the same restriction fragment pattern, differing from that of rainbows by at least one site change (Fig. 2A). For *Mbo*I, all three taxa exhibited distinct fragment patterns, with Apache and Gila trout mtDNAs differing by one site (Fig. 2B). The mtDNA of the native species was so different from rainbow trout that it was impossible to infer the number of site changes from fragment patterns. Representatives of the various forms of cutthroat also differed from Apache, Gila, and rainbow trouts by numerous fragment differences in both enzymes.

Dynamics of Hybridization

Allozymes. All individuals collected from the four wild populations were characterized using the three diagnostic allozyme loci. Many individuals from Boggy, KP, and Paddy creeks were of mixed ancestry, indicating extensive gene exchange between native and introduced forms. Such individuals were heterozygous at some or all marker loci or were homozygous for alleles derived from introduced and native forms at different loci. All alleles scored for the *Pep-LGG* locus exhibited mobili-

ties identical to those of Apache and Gila trouts or rainbow reference specimens, making it likely that rainbow trout were the major (or only) contributor of introduced alleles. All individuals from Chitty Creek exhibited only rainbow trout alleles for all three diagnostic loci and, unless specifically noted, will not be considered further. Loudenslager et al. (1986) reported similar results for Chitty Creek fish, finding no native alleles at four diagnostic loci. The Boggy Creek sample was small ($N = 14$), and therefore statistical analyses of it are less likely to be informative.

Observed genotypic distributions were compared to Hardy-Weinberg expectations for the three diagnostic allozyme loci (Table 3). Deviations from Hardy-Weinberg expectations were not detected, providing no evidence for reproductive discontinuity. Significant deficiency of heterozygous individuals for multiple loci would have indicated some barrier to gene exchange between species (such as assortative mating or selection against hybrids).

G tests (Sokal & Rohlf 1981) were performed to test for nonrandom association of alleles from different loci (linkage disequilibrium) as a further test for reproductive isolation. If native and introduced species are mating randomly and hybrids are not selectively inferior, alleles from one locus are likely to be randomly distributed relative to alleles from other loci. If native and introduced forms are distinct, however, specific homozygous genotypes will occur more frequently than expected from random assortment (Bert & Harrison 1988). Of nine possible comparisons for the three localities, none was significant, further indicating random mixture of native and introduced alleles.

A hybrid index score, the number of native (Apache and Gila trouts) alleles, was calculated for each individual using genotypes from each of the three diagnostic loci. "Pure" introduced and native individuals would score 0 and 6 respectively, while hybrids would score from 1 to 5. An F_1 hybrid would score 3, as would some backcross and F_2 hybrids. The distribution of scores was skewed toward intermediate values in Boggy Creek, while at KP and Paddy creeks scores appear normally distributed as expected for randomly mating populations (Fig. 3).

MtDNA. The restriction enzymes *Nhe*I and *Mbo*I (Fig. 2) were used to identify mtDNAs of all individuals from each population except Chitty Creek, where mtDNAs from only 22 of 63 individuals were surveyed. All individuals analyzed from Chitty Creek exhibited mtDNA of rainbow trout, with variation typical of a variety of strains. For the remaining three populations, all individuals possessed mtDNA from Apache trout, with the exception of five fish from Paddy Creek which had cutthroat trout mtDNAs (Fig. 3). Digestion of these last mtDNAs with additional restriction enzymes diagnostic for the different cutthroat subspecies (*Bcl*I, *Eco*RV, and *Pst*I) and comparison with reference mtDNAs and published fragment patterns (Williams & Shiozawa 1989) identified the source as the Colorado River subspecies.

The complete absence of rainbow trout mtDNAs is particularly surprising, given its preferential stocking and the high frequency of rainbow allozyme alleles. Even if the cutthroat mtDNAs had been that of rainbows, statistical analyses of the number of introduced mtDNAs observed relative to expected values clearly show far fewer mtDNAs from introduced species than expected in the hybrid populations (Table 4), indicating a bias in the direction of introgression. Four explanations may be advanced to explain this phenomenon: (1) stochastic elimination of rainbow trout mtDNA, (2) selection favoring Apache trout mtDNA, (3) differential selection among reciprocal crosses, or (4) differential assortative mating.

Population sizes of Apache trout are frequently small (reviewed in Rinne 1978), increasing the likelihood that variants will be lost by genetic drift. Despite increased probability of loss, this is an unlikely explanation for the observed patterns because of the presence of Colorado River cutthroat mtDNAs in Paddy Creek. Stocking records are sketchy, making it difficult to identify stocks used and timing and frequency of introduction. Nonetheless, we know that stocking of this subspecies in northeastern Arizona began in the late 1800s and continued until around 1930, when it was supplanted by

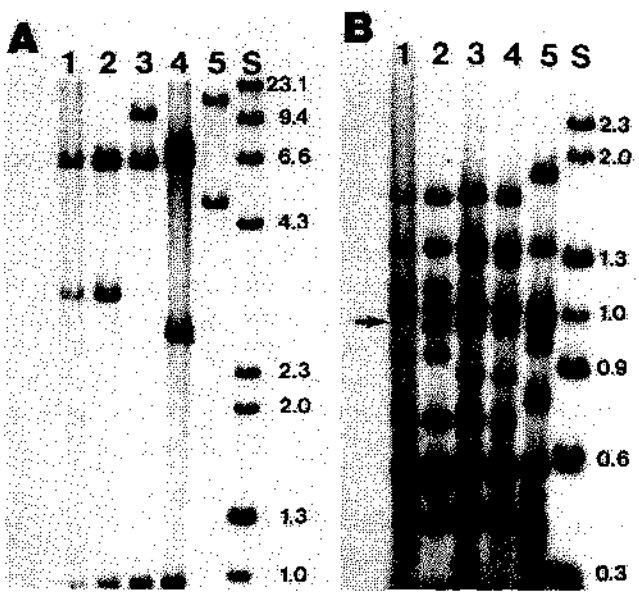

Figure 2. Autoradiographs of trout mtDNA variation revealed by (A) Nhe*I and (B)* Mbo*I (using 1.0% and 1.5% agarose gels, respectively). Sample identification is as follows: (1) Gila trout, (2) Apache trout, (3 and 4) rainbow trout, (5) cutthroat trout, (S)* Hin*dIII digested λ DNA + Hae*III *digested ϕXRF DNA (sizes in kilobases on the margin). The arrow on B identifies the site difference between Gila and Apache trout.*

Table 3. Tests of Hardy-Weinberg equilibrium for diagnostic allozyme loci.*

	Adh-A				*Ldh-C*				*Pep-LGG*			
Locality	*NN*	*IN*	*II*	*F*	*NN*	*IN*	*II*	*F*	*NN*	*IN*	*II*	*F*
Boggy Creek	10	4	0	0.75	9	4	1	1.42	8	4	2	2.02
KP Creek	19	25	6	0.88	29	16	5	1.43	30	17	3	1.11
Paddy Creek	17	43	12	1.48	30	33	9	1.01	19	31	22	1.34

* *F values calculated using procedures outlined by Cockerham (1969, 1973). Genotypes are identified as follows: NN = homozygous for native alleles, II = homozygous for introduced alleles, NI = heterozygous for native and introduced alleles. All tests were insignificant.*

Yellowstone cutthroat (B. Rosenlund personal communication). Since the 1940s, rainbow trout have been predominantly stocked, with cutthroat used only occasionally (Rinne 1988) and not known to include the Colorado River form (J. Novy personal communication). If mtDNAs of rainbow trout were eliminated by drift, then those of Colorado River cutthroat, which should be far rarer, would have likely suffered the same fate. The presence of mtDNAs from the latter therefore indicates that drift has not been a major factor in elimination of mtDNA haplotypes for the last fifty to sixty years.

Selection for the mtDNA of Apache trout over that of rainbow trout requires local adaptation of mtDNA from the native form (or coevolving nuclear genes), favoring it over that of rainbow trout in a background of the combined nuclear genomes of both species. While selection of different haplotypes has been documented (MacRae & Anderson 1988; but see Nigro & Prout 1990), evidence for such an event is limited (reviewed in Avise 1986; Moritz et al. 1987). Coevolution of the nuclear and mitochondrial genomes makes it improbable that a single divergent haplotype would be favored in the variety of hybrid nuclear backgrounds.

Experimental crosses between rainbow and Apache trouts have produced mixed results. Rinne et al. (1985) had difficulty producing F_1 hybrids between them; but R. David (personal communication) has been successful. While this suggests some genetic compatability, hybrid breakdown is frequently detected at the F_2 or backcross stages (Endler 1977, Shaw 1981). We therefore cannot evaluate the effect of selection against specific hybrid combinations in determining the observed pattern.

The pattern could also be produced by differential assortative mating. Stocking practices and unique life history attributes could produce directionality of introgression. Male rainbow trout typically reach maturity at a smaller size than females (Scott & Crossman 1973). Because rainbows are most commonly stocked as fingerlings or juveniles, males may be more likely to survive to reproduce than females, producing biased patterns of mtDNA introgression consistent with those observed here. Given the complete absence of rainbow trout mtDNA in our samples from Boggy, Paddy, and KP creeks, mortality of introduced females must be virtually absolute. Such extensive mortality seems inconsistent with the widespread establishment of rainbow trout in Arizona (Minckley 1973).

Alternatively, differential assortative mating may reflect innate properties of these species. Assortative mating plays a major role in trout reproductive behavior, including male-male and female-female competition (Gross 1984; Van den Berghe & Gross 1989). If female Apache trout mate assortatively more frequently than those of rainbow trout or prefer male rainbows (as described in Ryan & Wagner 1987), the result would be a preponderance of mtDNA from Apache trout in hybrids. A similar pattern is also expected if male rainbows are better at obtaining mates than are male Apache trout (or female Apaches are better than female rainbows).

Strong discordance of results from Chitty Creek and Paddy, Boggy, and KP creeks is perplexing. Complete replacement of the native species by rainbow trout in Chitty Creek could be due to overstocking, although geographic isolation of this stream makes this explanation unlikely (W. L. Minckley personal communication). Alternatively, this pattern could result from genetic differences between the native trout of these locations. Apache trout is native to Boggy, KP, and Paddy creeks, whereas the native species in Chitty Creek may have been Gila trout (Minckley 1973; Marsh et al. 1991). Therefore, the contrasting patterns may reflect differences in response to hybridization between Apache and Gila trout. Unfortunately, it is not possible to identify the trout formerly native to Chitty Creek, making this hypothesis untestable. Studies of hybridization between Gila and rainbow trouts in New Mexico may provide more information for resolving this question.

Management Implications of Hybridization for Apache Trout

In many fishes of western North America, the genetic integrity of a native taxon may be compromised by extensive hybridization from an introduced form (Echelle 1991). Studies of western American trouts have identified their general propensity to hybridize, and the consequences of such interactions may be serious. For example, Gyllensten et al. (1985) found random association of diagnostic allozyme and mtDNA genotypes, indicating admixture of gene pools from Yellowstone

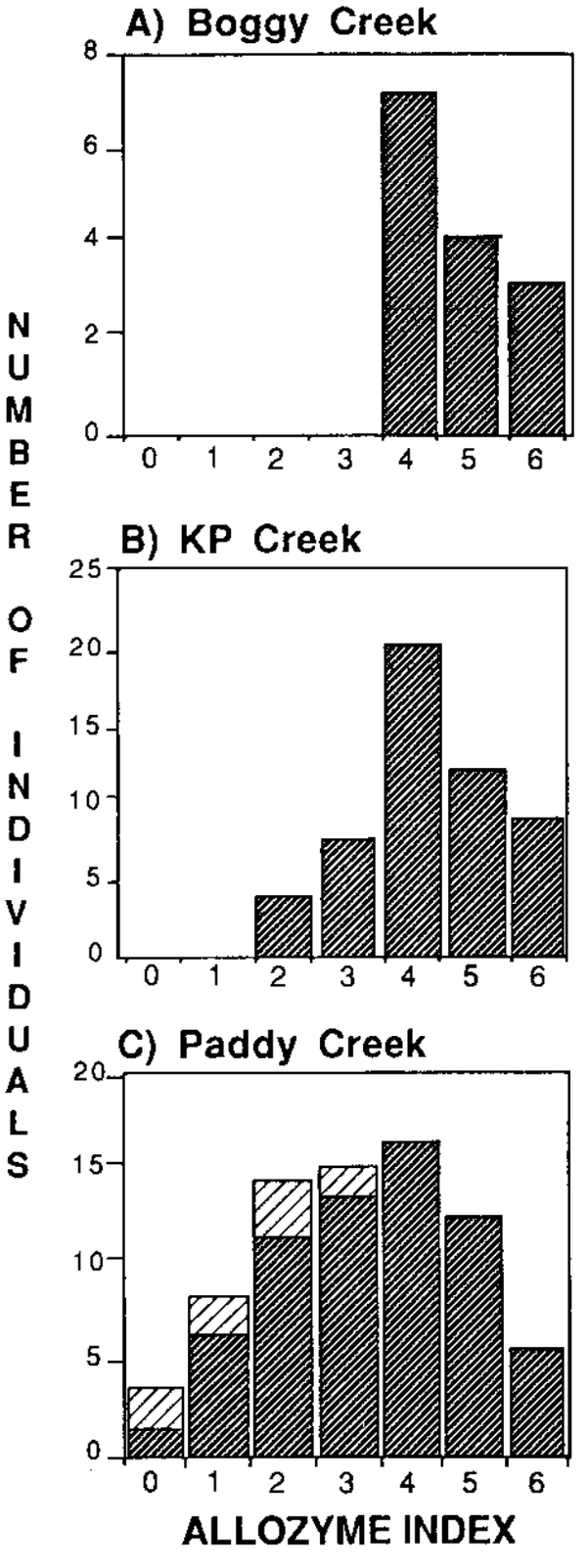

Figure 3. Distribution of allozyme index scores and mtDNA haplotypes from three populations containing hybrid individuals. Allozyme scores of 0 and 6 are typical of "pure" introduced and native individuals. Shading denotes the mtDNA haplotype of individuals with particular allozyme index scores: dark stripes for Apache trout and light stripes for Colorado River cutthroat.

and westslope cutthroats when the two subspecies had been artificially placed in contact through fisheries' management activities. Using similar data, Allendorf & Leary (1988) suggested that the westslope cutthroat is in danger of being contaminated or replaced by the Yellowstone cutthroat or rainbow trout throughout its range. The authors advocated recovery of westlope cutthroat by eradication of all populations where evidence for introgression was found in more than 1% of the individuals, followed by replacement with stocks of similar genetic composition.

The potential for loss of Apache and Gila trouts through hybridization is also significant, with the Chitty Creek population providing an excellent example. Early collections indicated the presence of a native species (either Apache or Gila trout; Marsh et al. 1991). Genetic analysis of recent collections (Loudenslager et al. 1986; this study) failed to detect any evidence of a native species, indicating complete replacement by rainbow trout which were probably transported above a barrier waterfall by fishermen (Rinne & Turner 1991).

The ultimate objective of the Apache trout recovery plan is restoration of the species to nonthreatened status. It will be delisted when thirty self-sustaining, discrete populations are established and maintained throughout its historic range (USFWS 1979). The choice of populations for renovation or maintenance may, however, be critical to its survival, and the use of these allozyme markers for assessing the impact of hybridization may be problematic.

Water and landscape management practices and interactions with nonnative trouts have reduced the historic range of Apache trout by approximately 95% (Rinne 1988). Even prior to these disturbances, the range of Apache trout was naturally fragmented, consisting of many disjunct populations in isolated headwater streams. This pattern makes it almost certain that some stocks have become differentiated and have possibly adapted to local conditions, as frequently occurs in salmonids (reviewed by Behnke 1972). While these allozyme markers identify the existence of introgression, they contain no information concerning the distribution of genetic variation or the impact of hybridization on locally adaptive variation. Genetic differences responsible for local adaptation are most likely encoded at nuclear gene loci, and the allozyme markers used to date are not linked to these selected loci (if such loci exist), as evidenced by their free exchange between species.

Unlike data from allozyme markers, biased introgression of mtDNA provided evidence for a barrier to indiscriminate gene exchange between Apache and rainbow trout. Reproductive isolation could be due to biological features (such as selection against hybrids or assortative mating) or human influence (such as stocking practices). The former would indicate that introgression is biologically limited (Lamb & Avise 1986; Dowling et al.

Table 4. Tests of association for mtDNA and allozyme markers (Zar 1974).*

Locality	*mtDNA*	*OBS*	*EXP*	*G*	*SIG*
Boggy Creek	NATIVE	14	11	1.2	NS
	INTRODUCED	0	3		
KP Creek	NATIVE	50	36	16.6	$P < 0.001$
	INTRODUCED	0	14		
Paddy Creek	NATIVE	67	40	17.3	$P < 0.001$
	INTRODUCED	5	33		

* *Values are observed (OBS) and expected (EXP) numbers of mtDNA haplotypes (NATIVE = Apache trout; INTRODUCED = cutthroat trout), test value (G, using Yates' correction), and significance levels (SIG). The expected values are based on the average frequency of nuclear genes (*Adh-A, Ldh-C, Pep-LGG*) at each locality.*

1989) and that Apache trout may remain partially distinct in spite of hybridization. The latter alternative would indicate that no innate barrier to gene exchange exists and that continual stocking of rainbows will eventually result in complete replacement. Given the difference in impact provided by these alternative explanations, identification of the barriers to gene exchange is essential for management of Apache trout.

In the absence of genetic information, management decisions will be difficult. Elimination of populations based on morphological or allozymic evidence of introgression will reduce the frequency of alleles from introduced taxa but may result in loss of locally adaptive genetic variation as well (Allendorf & Leary 1988). Introgression between Apache and rainbow trouts has been widespread, with 65% of Apache trout populations surveyed containing individuals with alleles contributed by rainbows. In many instances, however, introgression appears limited, with frequency of alleles from Apache trout less than 75% in only four (15%) of twenty-six populations recently studied (D. Morizot et al. personal communication). As a matter of fact, alleles contributed by rainbow trout did not outnumber those of Apache trout in any population surveyed.

Given the limited introgression of rainbow trout alleles into Apache trout populations, one must consider whether widespread eradication of contaminated populations is prudent. Renovation of all streams containing hybrid individuals, while expedient, would eliminate a large proportion of existing populations, possibly causing irreplaceable loss of genetic diversity. The most conservative management alternative is to analyze the geographic distribution of genetic variation in Apache trout to identify patterns of population structure, then devise discrete management strategies for each subpopulation identified. In instances where introgression seems extensive, selective removal of rainbow-like fish could be carried out, while retaining those with a greater proportion of Apache trout features. In this way, conservation of genetic variation should be maximized, while minimizing the loss of local adaptations. Continued monitoring of molecular and morphological features in hybridized populations may allow identification of the characteristics responsible for reproductive isolation and assessment of temporal changes in the extent of introgression.

Use of hatchery-produced Apache trout to achieve recovery goals (USFWS 1979) can be an especially dangerous option where genetic resources are concerned. For example, the present hatchery stock from the East Fork of the White River (David 1990) is one of the more homozygous populations studied to date (Loudenslager et al. 1986; D. Morizot et al. personal communication). Thus, introduction of large numbers of that stock to renovated habitats will do nothing to maintain genetic diversity within the species. Doing so might well compromise the genetic integrity of the native trout through homogenization, just as much or more than has already happened through introgressive hybridization.

A major goal of conservation biology is maintenance of diversity, both in terms of the number of species and of genetic variation within species (reviewed in Schonewald-Cox et al. 1983). Genetic variation is necessary for the organism to adapt to changing environments, making maintenance of genetic diversity the primary objective of genetic management (Templeton 1990). As exemplified in the case of Apache trout, extreme care must be exercised when considering elimination of any contaminated population lest the unique genetic identity of the native taxon be lost forever.

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