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Mitochondrial DNA Variation in Gila Trout, *Oncorhynchus gilae*: Implications for Management of an Endangered Species

BRETT R. RIDDLE, DAVID L. PROPST, AND TERRY L. YATES

Gila trout (*Oncorhynchus gilae*) is an endangered species restricted to relictual populations in the Gila and San Fransisco River drainages of southwestern New Mexico. We employed nucleotide sequences from the mitochondrial DNA (mtDNA) control region and restriction sites from the whole mtDNA genome, to assess population-level variation and phylogenetic status of trout samples from southwestern New Mexico. Our results indicate existence of a diagnosable Gila trout mtDNA lineage in four relictual populations from the Gila River and San Fransisco River drainages. Results support recognition of Gila and Apache trouts as a distinct evolutionarily significant unit. Further, populations in the Gila River drainage are connected historically through metapopulation dynamics and should be treated as a separate management unit relative to the San Fransisco River drainage.

NATIVE fishes of southwestern North America have become vulnerable to extinction over the past century because of numerous factors, including introductions of nonnative species that have led to extreme population fragmentation (Minckley and Deacon, 1991). Genetic studies provide wildlife managers with methods to infer genetic and demographic consequences of historical introductions of nonnative species on native species (Wayne and Jenks, 1991; Dowling and Childs, 1992; Dowling and DeMarais, 1993). Even without problems associated with nonnative introductions, management decisions should be based on genetically grounded evolutionarily significant units (ESUs). Although amount and kinds of data necessary to delineate separate ESUs are debated (Waples, 1991; Moritz et al., 1995), we agree with Moritz et al. (1995) and place emphasis on recognition of phylogeographically structured, reciprocally monophyletic clades that likely result from a history of allopatric divergence among populations. The concept of management units (MUs; Moritz, 1994) differs from ESUs in utilizing interpopulation distribution of gene and allele frequencies to understand ecologically relevant processes such as metapopulation structure and patterns of gene flow. Thus, only statistically significant differences in mitochondrial or nuclear gene frequencies are necessary for recognition of separate MUs (Moritz et al., 1995). ESUs and MUs are complementary and equally important perspectives from which to consider conservation of native North American fishes. ESU delineation could provide a sound basis for identification of populations that should be afforded legal protection under the US Endangered Species Act (ESA), whereas subsequent delineation of MUs could provide an operational basis for implementing management and recovery plans for a specified ESU.

The Gila River drainage of Arizona and New Mexico supports two native species in the genus *Oncorhynchus* (Fig. 1): Apache trout, *O. apache* (Miller, 1972) and Gila trout, *O. gilae* (Miller, 1950). The taxonomic and conservation history of *O. gilae* were reviewed recently (Propst et al., 1992). Widespread introduction of other species of *Oncorhynchus*, especially rainbow trout, *O. mykiss*, and subsequent hybridization among rainbows and natives resulted in large-scale losses of formerly widespread populations of *O. gilae*. As a consequence, *O. gilae* was listed as endangered in 1967 under the Endangered Species Protection Act and afforded protection under the New Mexico Wildlife Conservation Act of 1974. Currently *O. gilae* is restricted to four relictual populations in small first- and second-order streams (Fig. 1) and to six replicated populations (Propst et al., 1992).

Several studies have supported specific distinctiveness of *O. gilae* relative to other North American *Oncorhynchus* (Beamish and Miller, 1977; Loudenslager et al., 1986; Dowling and Childs, 1992). Miller (1950) proposed that *O. gilae* was derived from an *O. mykiss*-type ancestor that had gained access to the lower Colorado River and subsequently to the lower Gila River during the late Pliocene or early Pleistocene. Needham and Gard (1959) hypothesized that *O. gilae* was derived from *O. clarki* (cutthroat trout). Subsequently, Needham and Gard (1964) proposed a hybrid origin (*O. mykiss* × *O. clarki*), with subsequent divergence of *O. gilae*, *O. apache*, *O. chrysogaster* (Mexican golden trout of Pacific slope streams of the Sierra Madre, Mexico), and *O. aquabonita* (golden trout of Kern River basin, California). Loudenslager et

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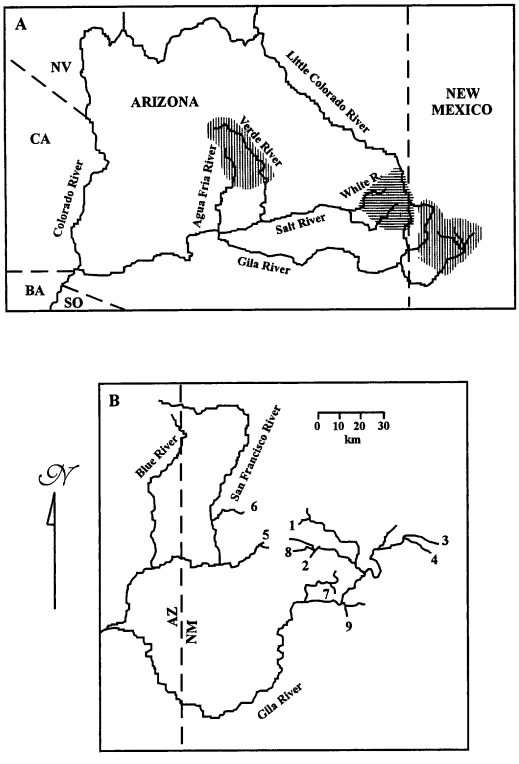

Fig. 1. (A) Historic and current range of Gila (vertical lines) and Apache trout (horizontal lines) in New Mexico and Arizona. (B) Magnification of (A) showing distribution of relictual populations of Gila trout (1–5) and nonnative rainbow trout (6–9) in the Gila and San Fransisco River drainges: 1 = Iron Creek; 2 = McKenna Creek; 3 = Main Diamond Creek; 4 = South Diamond Creek; 5 = Spruce Creek; 6 = Mineral Creek; 7 = Miller Spring Creek; 8 = White Creek; 9 = Corral Canyon Creek.

al. (1986) used nuclear isozyme variation to demonstrate close genetic similarity between *O. gilae* and *O. apache* relative to *O. mykiss*, and an affinity among these three species relative to *O. clarki*. Dowling and Childs (1992) could not replicate the isozyme differences between *O. gilae* and *O. apache* reported by Loudenslager et al. (1986) but did find diagnostic differences by using restriction fragment analysis of mtDNA in *O. apache* and in exemplar specimens of *O. clarki*, *O. gilae*, and *O. mykiss*.

In this study, we used nucleotide sequences in the control region of mtDNA and restriction site analysis of whole-genome mtDNA to further characterize mtDNA of *O. gilae* relative to mtDNA of *O. apache*, *O. mykiss*, and *O. clarki*. We examined mtDNA diversity within and among seven populations of *Oncorhynchus* from the Gila River drainage (four representing relictual populations of *O. gilae*), and two populations from the San Fransisco River drainage (one representing a relictual population of *O. gilae*). We addressed the following questions: (1) are the five relictual populations of *O. gilae* genetically "pure" in mtDNA perspective, or have they been contaminated by past introductions of nonnative species? (2) can a discrete ESU, consistent with the nominate species *O. gilae*, be identified? and (3) at what geographic scale should management of *O. gilae* populations be conducted, i.e., as single or separate MUs?

Materials and Methods

Sample sizes of species and populations examined are in Appendix 1 and Figure 1. DNA was extracted from individuals by using one of three methods. For most individuals, purified-mtDNA was isolated from 0.5–1.5 gm of frozen lateral muscle, liver, kidney, or heart tissue by using CsCl-propidium-iodide gradient centrifugation (Dowling et al., 1996). For other individuals, total genomic DNA was isolated using either a standard phenol-chloroform extraction protocol (Hillis et al., 1996) or A.S.A.P. columns and protocols (Boehringer-Mannheim Biochemicals).

The complete control region of mtDNA was PCR amplified in 100 μL reactions by using double-stranded PCR reaction conditions reported for a previous study of variation in mtDNA of *Oncorhynchus* (Bernatchez et al., 1992). The light-strand primer was located in proline tRNA (primer L19; Bernatchez et al., 1992); the heavy-strand primer was in 12sRNA (primer 12SB-H; Palumbi et al., 1991). About 45 μL of the PCR product was electrophoresed in an agarose gel and visualized using ethidium bromide. A gel slice containing the PCR fragment was purified using Genclean (BIO 101) kits. Eight μL of this product were used in DNA sequencing reactions with either the L19 primer or the S-phe primer (Nielsen et al., 1994) by using a double-stranded "snap-freeze" protocol (Palumbi et al., 1991) with Sequenase version 2.0 (US Biochemical). Sequence data for 45 individuals were generated for up to 377 base pairs (bps) of the 5′ end (positions 1–377 numbered according to *O. mykiss* accession L29771 in GenBank) of the control region. For a representative subset of 23 individuals, an additional 170–196 base pairs of the 3′ end (positions 817–987) of the control region were examined to increase potential number of variable characters available for analyses and also to interpret findings within the framework of known mtDNA control region sequence variation in western North American *O. mykiss* (Nielsen et al.,

1994). Sequences are accessioned in GenBank (AF013468–AF013480). For a representative subset of 14 individuals, restriction endonuclease digestions of whole-genome mtDNA were performed using reaction conditions supplied by the manufacturer. Following digestion, DNA fragments were end-labeled with ^{32}P-dNTPs and electrophoresed through 1.2% agarose and 3.5% polyacrylamide vertical gels using a standard protocol (Dowling et al., 1996).

Sequences were aligned by eye and sites numbered according to GenBank sequence L29771. Sequence divergence among mtDNA haplotypes was calculated by using the proportion of nucleotide sites that differed between two sequences [S. Kumar K. Ramura, and M. Nei, MEGA (Molecular evolutionary genetics analysis) vers. 1.01, 1993, unpubl.]. Restriction-site maps for each restriction enzyme were generated by using MacVector v4.0 (International Biotechnologies, Inc.) to generate a reference map over the complete mtDNA genome of *O. mykiss* (GenBank L29771). These maps were used as a basis to infer restriction-site variation from restriction fragment digests (Appendix 1). For *Msp*I, a restriction enzyme recognizing a four-base sequence, only variation among fragments larger than 350 bps were used to map site changes. Restriction sites were used to estimate sequence divergence (J. C. Miller, RESTSITE vers. 1.2, 1991, unpubl.) among composite restriction-site mtDNA haplotypes. A "total evidence" matrix of all variable control-region sequence and whole-genome restriction-site characters was used to construct maximum parsimony trees (PAUP, vers. 3.1; Swofford, 1993) by using six unordered characters states (A, C, G, T, or gap; missing data were excluded only for taxa where character state was unknown) for sequence data and presence or absence states for restriction-site data. *Oncorhynchus clarki* was designated as outgroup.

TABLE 1. VARIABLE SITES IN 5′- AND 3′-ENDS OF MTDNA CONTROL REGION. Insertion or deletion denoted by "*." Sites numbered according to GenBank L29771 sequence (designated here as haplotypes C5.1 and C3.10).

	Variable sites
	11111111222222233
5′ end	11112345501577899112226647
	80189197459031450689167233 5
	Character number
	111111111222222222
	123456789012345678901234567
C5.1	CGCCACGATATTTCATTCTAGTTATGG
C5.2	----------A----------
C5.3	----------A---T---------C--
C5.4	----------AA
C5.5	----------A-
C5.6	T-TA------AC----------GC--
C5.7	T-TA------A----------GC--
C5.8	TA--TTATCTACCT-AAACTACCGC??
	Variable sites
	1
	888***888888999999999990
3′ end	124***789999001344455666770
	922***563469462903747039381
	Character number
	223333333333444444444455555
	890123456789012345678901234
C3.10	CAA***TGAAGTTCAATATCAAAGTAG
C3.01	----------T-------AG----
C3.02	------C------T-------AG----
C3.04	----------T--C---GAG----
C3.05	----------T--C---G*G----
C3.06	----------CT--C---G*G----
C3.07	----------T--C----AG----
C3.08	----------T--C----*G----
C3.11	----------A--T-------AG----
C3.12	----------T--C----*G----
C3.13	----------T--C---G*G----
C3.03	----------T-------A-----
C3.09	----------T-------AG----
C3.14	---A---------T-------AG--G-
C3.16	AGGGTA-AGGAC-TTGCCCTGAGAA-A

RESULTS

Nucleotide variation across 377 bp at the 5′ end of the control region (n = 10) included 27 variable characters (27 total substitutions, 17 transitions, 10 transversions; Table 1). Nine of the substitutions were phylogenetically informative, resulting in eight different haplotypes (C5.1–C5.8). Variation across 195 sites at the 3′ end (n = 16) included 27 variable characters (24 total substitutions, 20 transitions, 5 transversions, 3 insertion/deletion sites); six substitutions and one insertion were phylogenetically informative, resulting in 16 haplotypes (C3.1–C3.16). An increase of approximately 300 bps at the 3′ end of the control region, identified via restriction-site analysis (below) and via electrophoretic mobility of PCR-amplified fragments, diagnosed all five individuals sampled from the population in Spruce Creek. Thus, only bps 888–987 of the 3′ sequence from the population in Spruce Creek were aligned and analyzed with other sequences in this study. Combined 5′ and 3′ variation assessed across nine individuals resulted in eight different haplotypes. Sequence

TABLE 2. COMPOSITE HAPLOTYPES BASED ON SINGLE DIGESTIONS WITH 14 RESTRICTION ENDONUCLEASES. Restriction site differences and total number of sites compared are given in lower matrix; sequence divergence estimates are given in upper matrix. Divergence estimates for haplotypes R7 and R8 are unavailable because of missing data.

Haplotype number	Composite haplotype[a]	R.1	R.2	R.3	R.4	R.5	R.6	R.7
R.1	EGAACAEAAABAAA	—	0.0023	0.0206	0.0094	0.0159	0.0530	
R.2	EEAACAEAAABAAA	2/49	—	0.0206	0.0120	0.0159	0.0530	
R.3	DDAAAACACABAAB	17/49	17/49	—	0.0127	0.0166	0.0593	
R.4	CAAAAABAAABAAC	9/49	10/49	11/49	—	0.0189	0.0579	
R.5	ACAAAAAAAAAAAD	14/49	14/49	13/49	15/49	—	0.0562	
R.6	FFBBDBDADCCCA?	24/31	24/31	26/31	25/31	25/31	—	
R.7	CGAAA?EA?DB???	4/38	6/38	17/38	9/38	17/38	20/22	—
R.8	CGAAE?EA?DB???	2/38	4/38	15/38	7/38	15/38	18/22	2/38

[a] Letters represent restriction fragment patterns for *Ava*I, *Ava*II, *Bam*HI, *Bcl*I, *Bgl*II, *Bst*EII, *Bst*NI, *Eco*RI, *Hin*dI, *Hin*dIII, *Kpn*I, *Sal*I, *Xba*I, and *Msp*I.

divergence among eight combined 5′ and 3′ haplotypes ranged from 0.18–9.46% (data available: www.nscee.edu:80/unlv/Colleges/College of Science and Math/Biology/Riddle.html).

A total of 122 restriction sites were inferred from digestion of whole-mtDNAs with 14 restriction enzymes. Forty-nine restriction sites varied across 14 individuals (Appendix 2), although about half of these sites varied only among *O. clarki* and the remaining taxa. Eight different composite mtDNA haplotypes (Table 2) differed from one another by 2–26 restriction-site changes. Estimates of sequence divergence among haplotypes ranged from 0.23% (*O. mykiss* haplotypes R.1 and R.2) to an average of 5.59% (*O. clarki* haplotype R.6 and *O. mykiss, O. gilae,* and *O. apache* haplotypes R.1–R.5).

A large fraction of the restriction-site variation was revealed by the enzyme BstNI. A total of 19 *Bst*NI sites are present in the *O. mykiss* L29771 mtDNA genome, compared with an average of 2.7 sites per enzyme for others (except *Msp*I, which recognizes a four-base sequence) used in this study. Although the relatively large number of restriction sites that differentiate *O. gilae* and *O. apache* mtDNA seems surprising given the absence of control region sequence variation between the two species, nearly half of those differences represent variation at the common sequence CC$^{a}_{t}$GG recognized by *Bst*NI.

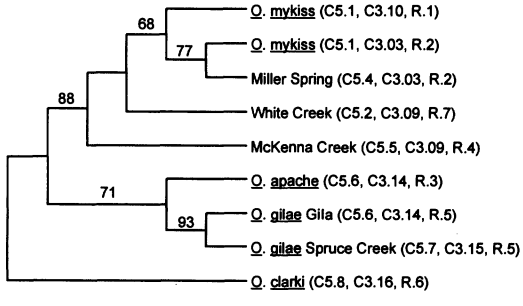

Fig. 2. Phylogenetic analysis of mtDNA haplotypes. Total evidence, branch-and-bound, maximum-parsimony tree from combined sequence and restriction-site haplotypes; numbers on branches are values resulting from 1000 bootstrap replicates.

Length variation was detected in haplotype R.5 from the population in Spruce Creek. R.5 differed from all other haplotypes in this study by a 300 base-pair increase in length that was inferred to occur at the 3′ end of the control region (between bps 618 and 1048) through mapping of fragment size variation generated by enzymes *Ava*I, *Ava*II, *Bgl*II, *Hin*dI, *Hin*dIII, *Sal*I, and *Xba*I. This length mutation is consistent with control region sequence results described above.

Maximum-parsimony analysis of combined control region sequence and restriction-site variation (Fig. 2) generated a tree 42 steps long (CI = 0.714, RI = 0.789). Strong bootstrap support was found for a separate clade of *O. gilae* and *O. apache* haplotypes that is basal to known and putative *O. mykiss* haplotypes.

The 5′ end of the control region was sequenced in a total of 41 individuals representing seven populations in the Gila drainage and two populations in the San Francisco drainage (Appendix 1). Seventeen individuals from populations in Main Diamond, South Diamond, and Iron Creeks had haplotype C5.6, whereas five individuals from the population in Spruce Creek had haplotype C5.7. In the population from McKenna Creek, three individuals had haplotype C5.5 and three had C5.6. The populations from Corral, Mineral, White, and Miller Spring Creeks contained one or more of the haplotypes C5.1, C5.2, C5.3, and C5.4.

DISCUSSION

Evolutionary divergence of Gila trout mtDNA.—Both control-region sequence and whole-mtDNA restriction-site datasets uncovered mtDNA haplotypes that distinguish relictual populations of *O. gilae* and *O. apache* relative to *O. mykiss* and *O. clarki*, whereas restriction-site data distinguished *O. gilae* from *O. apache*. Evidence that *O. gilae* and *O. apache* share a common ancestor relative to *O. mykiss* corroborates results of previous genetic studies (Loudenslager et al., 1986; Dowling and Childs, 1992). Both methods revealed similar levels of interspecific sequence divergence between *O. gilae/O. apache* relative to *O. mykiss* haplotypes. Haplotypes of *O. gilae*, *O. apache*, and *O. mykiss* form a distinct and divergent clade relative to those of *O. clarki*, supporting results of previous studies (Dowling and Childs, 1992).

The generally equivalent numbers of haplotypes and levels of divergence between *O. gilae*, *O. apache*, and *O. mykiss* mtDNA are comparable to intraspecific mtDNA variation revealed by both sequencing and restriction-site approaches in other salmonids (Bernatchez and Danzmann, 1993). For example, control-region sequence divergence ranged from 0.96–1.44% for 640 bp in *Salmo trutta* (Bernatchez et al., 1992). Additionally, restriction-site divergence is similar to values reported by Wilson et al. (1985) among populations of *O. mykiss* from the northwest coast and by Gyllensten and Wilson (1987) between Arlee and Eagle Lake strains of rainbow. Furthermore, control-region sequence and restriction-site values parallel one another in levels of divergence, similar to results reported for *S. trutta* (Bernatchez et al., 1992; Giuffra et al., 1994). Divergence of *O. gilae* and *O. apache* from *O. mykiss* is not appreciably greater than maximum intraspecific divergence values within *O. mykiss*. Regardless, the two species form a clade with multiple apomorphic character states and share a biogeographic history in distinct southwestern North American drainages.

Delineation of ESUs and MUs.—Although the issue of using genetic characters to define species boundaries has been controversial (Dowling et al., 1992; Wayne, 1992), the more important issue from a conservation perspective is whether a population or group of populations share a history of evolutionary divergence relative to other populations (Waples, 1991; Moritz et al., 1995). Under the criteria of phylogeographic structuring of a monophyletic set of haplotypes, our mtDNA data support recognition of *O. gilae* and *O. apache* as an ESU relative to other populations within the *O. mykiss* clade. Notably, the two share a unique haplotype at the 3′ end of the control region distinct from the large sample of natural and hatchery *O. mykiss* sampled by Nielsen et al. (1994). Previous morphological diagnosis of an *O. gilae*-like population (now extinct) as far west as the Verde River in west-central Arizona (Miller, 1950), and extinction of many populations of native trout in the San Francisco and Gila drainages, prevent a realistic assessment of whether extant populations of *O. gilae* and *O. apache* each comprise a separate ESU. Biogeographic proximity and a likely shared biogeographic history would support the more conservative approach of considering both species as a single ESU, pending future analyses of highly variable components of the genome. Additional support for recognition of an *O. gilae/O. apache* ESU comes from congruence across multiple independent datasets that include morphology (Miller, 1950; David, 1976), isozymes (Loudenslager et al. 1986), and mtDNA (this study; Dowling and Childs, 1992).

We found no evidence for presence of a native *O. gilae* or *O. apache* mtDNA in populations from Miller Spring, Mineral, White, or Corral Canyon Creeks. All known haplotypes from these populations are closely related to known haplotypes of *O. mykiss* and likely derived through historic releases of hatchery-raised trout. Several haplotypes are present in Mineral and White Creeks and could indicate stocking from a variety of rainbow strains or of polymorphism within strains (Dowling and Childs, 1992). That no mtDNA polymorphism was discovered among 17 individuals from three putative populations of *O. gilae* in the Gila River drainage is consistent with the hypothesis that these populations were formerly connected. A wildfire in 1989 demonstrated the vulnerability of relictual populations in South Diamond and Main Diamond Creeks to extinction or extreme decreases in population size (Propst et al., 1992). Over time frames of tens to hundreds of years, we might anticipate similarly extreme perturbations to impact any one of the headwater streams where relictual populations currently exist.

Given the absence of mtDNA variation among populations in the Gila drainage, occurrence of two haplotypes among six individuals in McKenna Creek is of interest. One of the haplotypes in McKenna Creek has greater phylogenetic affinities with *O. mykiss* than with *O. gilae/O. apache*. We suggest this haplotype represents a recent introduction of rainbow trout. First, the maternally inherited mtDNA genome is subject to rapid fixation in small populations

through genetic drift. Census surveys indicate that McKenna Creek supports a small number of breeding individuals compared with other relictual populations (Propst et al., 1992). Thus, maintenance of ancestral mtDNA polymorphism in McKenna Creek and fixation of a single haplotype in all others would be surprising from the perspective of population-level lineage sorting. Second, if these two haplotypes coexisted in a former Gila drainage metapopulation, then random genetic drift (under a model of selective neutrality) would dictate that the probability of fixation of the same haplotype in each of three currently isolated relictual populations would be only 0.125. Finally, although we cannot entirely rule against retained ancestral polymorphism to explain a unique haplotype in McKenna Creek, the fact that it is more divergent from the common *O. gilae* form than is the *O. gilae* haplotype in Spruce Creek (San Fransisco drainage) or that in *O. apache* also argues for a recent introduction of *O. mykiss* mtDNA.

The fixation of a unique haplotype with a diagnostic 300-bp length increase in the population from Spruce Creek suggests that the San Fransisco and Gila drainages were isolated historically. Although previous isozyme analyses (Loudenslager et al., 1986) did not indicate divergence in allele frequencies between populations in Spruce Creek and other relictual populations of Gila trout, available evidence weakly supports recognition of Gila and San Fransisco drainages as separate MUs. Investigations of rapidly evolving elements in the nuclear DNA (e.g., microsatellites) may further refine and improve conservation strategies.

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Literature Cited

Beamish, R. J., and R. R. Miller. 1977. Cytotaxonomic study of Gila trout, *Salmo gilae*. J. Fish. Res. Bd. Can. 34:1041–1045.

Bernatchez, L., and R. G. Danzmann. 1993. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of Brook charr (*Salvelinus fontinalis* Mitchill). Mol. Biol. Evol. 10: 1002–1014.

———, R. Guyomard, and F. Bonhomme. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. Mol. Ecol. 1:161–173.

David, R. E. 1976. Taxonomic analysis of Gila and Gila × Rainbow trout in southwestern New Mexico. Unpubl. master's thesis, New Mexico State Univ., Las Cruces.

Dowling, T. E., and M. R. Childs. 1992. Impact of hybridization on a threatened trout of the southwestern United States. Conserv. Biol. 6:355–364.

———, and B. D. Demarais. 1993. Evolutionary significance of introgressive hybridization in cyprinid fishes. Nature 362:444–446.

———, ———, W. L. Minckley, and M. E. Douglas. 1992. Use of genetic characters in conservation biology. Conserv. Biol. 6:7–8.

———, C. Moritz, J. D. Palmer, and L. H. Rieseberg. 1996. Nucleic acids III: analysis of fragments and restriction sites, p. 249–320. *In:* Molecular systematics. D. M. Hills, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Sunderland, MA.

Giuffra, E., L. Bernatchez, and R. Guyomard. 1994. Mitochondrial contral region and protein coding genes sequence variation among phylogenetic forms of brown trout *Salmo trutta* from northern Italy. Mol. Ecol. 3:161–171.

Gyllensten, U., and A. C. Wilson. 1987. Mitochondrial DNA of salmonids: intraspecific variability detected with restriction enzymes, p. 301–317. *In:* The application of population genetics to fisheries management. N. Ryman and F. M. Utter (eds.). Univ. of Washington Press, Seattle.

Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis, and E. A. Zimmer. 1996. Nucleic acids IV: sequencing and cloning, p. 321–381. *In:* Molecular systematics. D. M. Hills, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Sunderland, MA.

Loudenslager, E. J., J. N. Rinne, G. A. E. Gall, and R. E. David. 1986. Biochemical genetic studies of native Arizona and New Mexico trout. Southwest. Nat. 31:221–234.

Miller, R. R. 1950. Notes on the cutthroat and rainbow trouts with the description of a new species from the Gila River, New Mexico. Occ. Pap. Mus. Zool. No. 529. Univ. of Michigan, Ann Arbor.

———. 1972. Classification of the native trouts of Arizona with the description of a new species, *Salmo apache*. Copeia 1972:401–422.

Minckley, W. L., and J. E. Deacon. 1991. Battle against extinction: native fish management in the American West. Univ. of Arizona Press, Tucson.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Mol. Ecol. 3:401–411.

———, S. Lavery, and R. Slade. 1995. Using allele frequency and phylogeny to define units for conservation and management. Am. Fish. Soc. Symp. 17:249–262.

Needham, P. R., and R. Gard. 1959. Rainbow trout in Mexico and California with notes on the cutthroat series. Univ. Calif. Publ. Zool. 67:1–124.

———. 1964. A new trout from central Mexico: *Salmo chrysogaster*, the Mexican golden trout. Copeia 1964:169–173.

Nielsen, J. L., C. A. Gan, and W. K. Thomas. 1994.

Differences in genetic diversity for mitochondrial DNA between hatchery and wild populations of *Oncorhynchus*. Can. J. Fish. Aquat. Sci. 51:290–297.

PALUMBI, S., A. MARTIN, S. ROMANO, W. O. MCMILLAN, L. STICE, AND G. GRABOWSKI. 1991. The simple fool's guide to PCR. Vers. 2.0. Univ. of Hawaii, Honolulu.

PROPST, D. L., J. A. STEFFERUD, AND P. R. TURNER. 1992. Conservation and status of Gila trout, *Oncorhynchus gilae*. Southwest. Nat. 37:117–125.

SWOFFORD, D. L. 1993. PAUP: Phylogenetic analysis using parsimony. Vers. 3.1. Illinois Nat. Hist. Sur., Champaign.

WAPLES, R. S. 1991. Pacific salmon, *Oncorhynchus* spp., and the definition of a "species" under the Endangered Species Act. Mar. Fish. Rev. 53:11–22.

WAYNE, R. K. 1992. On the use of molecular genetic characters to investigate species status. Conserv. Biol. 6:590–592.

———, AND S. M. JENKS. 1991. Mitochondrial DNA analysis implying extensive hybridization of the endangered red wolf *Canis rufus*. Nature 351:565–568.

WILSON, G. M., W. K. THOMAS, AND A. T. BECKENBACH. 1985. Intra- and inter-specific mitochondrial DNA sequence divergence in *Salmo*: rainbow, steelhead, and cutthroat trouts. Can. J. Zool. 63:2088–2094.

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APPENDIX 1. DISTRIBUTION OF MTDNA HAPLOTYPES AMONG INDIVIDUALS. Sample designation was based on morphological identification of taxon. Parentheses indicate individuals assayed with a subset of five enzymes. Asterisk indicates length variation, described in text.

Sample designation		Reference sample or population	Hatchery, reference, or drainage	Haplotypes		
				Control region		Restriction site
				5′	3′	
18228	cutthroat	*O. clarkii*	Indian Creek, NM	C5.8	C3.16	R.6
L29771	rainbow	*O. mykiss*		C5.1	C3.10	R.1
18182	rainbow	*O. mykiss*	Lost Lake, ID	C5.1	C3.03	R.2
18279	rainbow	*O. mykiss*	Symes Creek, CA	C5.1	C3.03	—
ST01	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.01	—
ST02	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.02	—
ST03	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.03	—
ST04	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.04	—
ST05	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.05	—
ST06	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.06	—
ST07	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.07	—
ST08	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.08	—
ST09	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.09	—
ST10	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.10	—
ST11	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.11	—
ST12	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.12	—
ST13	rainbow	*O. mykiss*	Nielsen et al. (1994)	—	C3.13	—
18090	apache	White River	E. Fork White River	C5.6	C3.14	R.3
18307	rainbow	Miller Spring	Gila	C5.1	—	—
18309	rainbow	Miller Spring	Gila	C5.1	C3.03	—
18310	rainbow	Miller Spring	Gila	C5.1	—	(R.2)
18314	rainbow	Miller Spring	Gila	C5.1	—	—
18315	rainbow	Miller Spring	Gila	C5.4	C3.03	(R.2)
18291	rainbow	Mineral	San Francisco	C5.1	C3.03	—
18298	rainbow	Mineral	San Francisco	C5.2	C3.9	—
18300	rainbow	Mineral	San Francisco	C5.3	—	—
18245	rainbow	White	Gila	C5.2	C3.09	R.7
18249	rainbow	White	Gila	C5.4	—	(R.2)
18257	rainbow	White	Gila	C5.1	—	—
18258	rainbow	White	Gila	—	C3.09	R.8

APPENDIX 1. CONTINUED

Sample designation		Reference sample or population	Hatchery, reference, or drainage	Haplotypes		
				Control region		Restriction site
				5′	3′	
18003	gila	McKenna	Gila	C5.5	C3.09	—
18008	gila	McKenna	Gila	C5.6	C3.14	—
18020	gila	McKenna	Gila	C5.5	C3.09	—
18023	gila	McKenna	Gila	C5.6	—	—
18027	gila	McKenna	Gila	C5.5	C3.09	R.4
18028	gila	McKenna	Gila	C5.6	C3.14	—
18031	gila	Spruce	San Francisco	C5.7	C3.15	—
18038	gila	Spruce	San Francisco	C5.7	C3.15	R.5*
18039	gila	Spruce	San Francisco	C5.7	C3.15	—
18044	gila	Spruce	San Francisco	C5.7	—	—
18045	gila	Spruce	San Francisco	C5.7	—	—
11403	rainbow	Corral Canyon	Gila	C5.1	C3.03	—
11404	rainbow	Corral Canyon	Gila	C5.1	C3.03	(R.2)
18066	gila	Iron	Gila	C5.6	—	—
18075	gila	Iron	Gila	C5.6	—	—
18077	gila	Iron	Gila	C5.6	C3.14	R.5
18080	gila	Iron	Gila	C5.6	—	—
18081	gila	Iron	Gila	C5.6	—	—
18082	gila	Iron	Gila	C5.6	—	—
18084	gila	Iron	Gila	C5.6	—	—
18122	gila	South Diamond	Gila	C5.6	—	—
18123	gila	South Diamond	Gila	C5.6	C3.14	R.5
18125	gila	South Diamond	Gila	C5.6	—	—
18135	gila	South Diamond	Gila	C5.6	—	—
18143	gila	South Diamond	Gila	C5.6	—	—
18151	gila	Main Diamond	Gila	C5.6	—	—
18157	gila	Main Diamond	Gila	C5.6	—	—
18159	gila	Main Diamond	Gila	C5.6	C3.14	R.5
18167	gila	Main Diamond	Gila	C5.6	—	—
18170	gila	Main Diamond	Gila	C5.6	—	—

APPENDIX 2. VARIABLE RESTRICTION SITES (SITE POSITION BASED ON GENBANK L29771 SEQUENCE) ACROSS COMPOSITE RESTRICTION-SITE HAPLOTYPES BASED ON 14 RESTRICTION-ENDONUCLEASE ENZYMES.

Site	Position	Restriction enzyme	Haplotype							
			R.1	R.2	R.3	R.4	R.5	R.6	R.7	R.8
1	1231	*Ava*I	0	0	0	0	0	1	0	0
2	5664	*Ava*I	0	0	1	1	0	0	1	1
3	6304	*Ava*I	1	1	1	1	1	0	1	1
4	14434	*Ava*I	1	1	0	1	1	0	1	1
5	15184	*Ava*I	0	0	0	0	1	0	0	0
6	15674	*Ava*I	0	0	0	0	0	1	0	0
7	3647	*Ava*II	1	0	0	1	0	?	1	1
8	4012	*Ava*II	0	0	0	0	1	?	0	0
9	6541	*Ava*II	0	0	1	1	0	?	0	0
10	12591	*Ava*II	1	1	0	1	0	?	1	1
11	14636	*Ava*II	0	1	0	0	0	?	0	0
12	10836	*Bam*HI	0	0	0	0	0	1	0	0
13	12021	*Bam*HI	1	1	1	1	1	0	1	1
14	9529	*Bcl*I	1	1	1	1	1	0	1	1
15	10204	*Bcl*I	0	0	0	0	0	1	0	0
16	10454	*Bcl*I	0	0	0	0	0	1	0	0
17	618	*Bgl*II	1	1	1	1	1	1	0	1
18	6273	*Bgl*II	0	0	0	0	0	1	0	0
19	8773	*Bgl*II	1	1	0	0	0	1	1	1
20	16059	*Bgl*II	1	1	1	1	1	1	0	1
21	577	*Bst*EII	0	0	0	0	0	1	?	?
22	8134	*Bst*EII	0	0	0	0	0	1	?	?
23	13134	*Bst*EII	0	0	0	0	0	1	?	?
24	1419	*Bst*NI	1	1	0	0	1	?	1	1
25	1554	*Bst*NI	1	1	0	1	1	?	1	1
26	5626	*Bst*NI	0	0	1	1	1	?	0	0
27	7071	*Bst*NI	1	1	0	1	0	?	1	1
28	7213	*Bst*NI	1	1	0	1	0	?	1	1
29	8569	*Bst*NI	1	1	0	0	0	?	1	1
30	10196	*Bst*NI	0	0	0	0	1	?	0	0
31	11637	*Bst*NI	1	1	0	1	0	?	1	1
32	15094	*Bst*NI	0	0	0	0	1	?	0	0
33	15714	*Bst*NI	1	1	0	0	1	?	1	1
34	16669	*Bst*NI	0	0	1	0	0	?	0	0
35	1411	*Hin*dI	0	0	0	0	0	1	?	?
36	5418	*Hin*dI	1	1	1	1	1	0	?	?
37	6995	*Hin*dI	0	0	0	0	0	1	?	?
38	15888	*Hin*dI	1	1	0	1	1	1	?	?
39	4631	*Hin*dIII	0	0	0	0	0	0	1	1
40	5335	*Hin*dIII	0	0	0	0	0	1	0	0
41	6823	*Kpn*I	1	1	1	1	0	0	1	1
42	8823	*Kpn*I	1	1	1	1	1	0	1	1
43	9972	*Kpn*I	1	1	1	1	1	0	1	1
44	10163	*Kpn*I	1	1	1	1	1	0	1	1
45	11782	*Kpn*I	1	1	1	1	1	0	1	1
46	5416	*Sal*I	1	1	1	1	1	0	?	?
47	15433	*Sal*I	1	1	1	1	1	0	?	?
48	15069	*Msp*I	1	1	1	0	1	?	?	?
49	16484	*Msp*I	1	1	0	1	0	?	?	?

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