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Localized Genetic Effects of a Long-Term Hatchery Stocking Program on Resident Rainbow Trout in the Metolius River, Oregon

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Abstract.—Hatchery rainbow trout *Oncorhynchus mykiss* have been stocked in the Metolius River in central Oregon since 1938, and legal-sized (≥160 g) yearling trout were stocked annually from 1947 until 1995. In 1996, management objectives shifted to emphasize wild trout, and hatchery stocking ceased. We examined allozyme and mitochondrial DNA (mtDNA) variation among three naturally occurring populations of rainbow trout in the Metolius River to investigate possible hybridization with hatchery-produced rainbow trout. We also examined two commonly used hatchery strains of rainbow trout, one of which has supplied nearly all of the catchable hatchery trout in the Metolius. Both allozyme and mtDNA data showed the two hatchery samples to have genetic characteristics typical of hatchery populations derived from coastal rainbow trout *O. mykiss irideus*. Rainbow trout sampled from the lower Metolius River, approximately 30 km downstream of the headwaters, had allozyme and mtDNA characteristics typical of interior rainbow trout *O. m. gairdneri*. The two samples from the upper Metolius River, where stocking activities occurred, had allozyme profiles intermediate between interior and coastal types and mtDNA haplotypes characteristic of both interior and coastal populations. We attributed the upper-river results to hybridization between indigenous rainbow trout and the hatchery trout that had been stocked there for nearly 60 years. We attribute the lack of hybridization in the lower Metolius River to ecological isolation: the upper river meanders through park-like habitat, whereas the lower river has greatly increased water flows and velocities and a steep gradient, creating a habitat that may be inhospitable to hatchery-reared rainbow trout. Stocked hatchery trout that drift or migrate downstream into the lower river likely perish or are carried farther downstream into Lake Billy Chinook, where they are subject to lethal infection by the myxosporean parasite *Ceratomyxa shasta* and where a robust population of bull trout *Salvelinus confluentus* exists. If some fish from the genetically pure interior rainbow trout population in the lower Metolius River were to migrate to the upper river and spawn there, the hybridized upriver population would receive a steady infusion of genes from native fish. Future monitoring of life history and genetic attributes of the upper and lower rainbow trout populations could reveal whether such an infusion occurs.

Fisheries managers and scientists have recently questioned the effects of hatchery programs on aquatic ecosystems and attempted to define new roles for hatcheries and hatchery-reared fish (Busack and Currens 1995; Schramm and Piper 1995; White et al. 1995). The potential for adverse effects of hatchery fish on wild fish has long been recognized (Rich 1939; Schuck 1943), and mounting empirical evidence indicates that such effects often are realized (Reisenbichler and McIntyre 1977, 1986; Vincent 1984, 1987; Evans and Willox 1991; Hindar et al. 1991; Waples 1991; Eriksson and Eriksson 1993; Flagg et al. 1995).

As the role of hatcheries in mitigation programs has come into question (Philipp et al. 1993; Utter et al. 1993; Washington and Koziol 1993; Utter 1994), managers have focused their attentions on identifying and preserving the genetic diversity found in native trout populations (Trotter 1987; Gresswell 1988; Krueger and May 1991; Behnke 1992; Angermeier and Williams 1994; Moyle and

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Yoshiyama 1994). Native populations are thought to have a higher probability of long-term persistence in a habitat, due to evolutionary adaptation to local environmental conditions, than hatchery-derived fish (Allendorf et al. 1987; Allendorf and Leary 1988). Additionally, recent studies and reviews (Carl and Healey 1984; Taylor 1991; Levings 1995, Currens et al. 1997, this volume) have demonstrated differences in local adaptation and fitness among stocks within relatively small geographic areas, suggesting that local adaptation may occur at smaller geographic scales than previously recognized. With the recognition that local adaptation can occur at the level of individual drainages, or even tributaries within drainages, managers and researchers increasingly urge that remnant native fish populations be identified and managed as unique and irreplaceable resources. Consequently, identification of genetically pure native trout populations is now a goal of many management programs.

Previous studies have demonstrated the utility of genetic analyses for assessment of hybridization between fish populations and for identification of remnant native salmonid populations (Campton 1987). Genetic analysis has been useful in detecting hybridization between various subspecies of cutthroat trout *Oncorhynchus clarki* (Busack and Gall 1981; Gyllensten et al. 1985; Leary et al. 1987), between cutthroat and rainbow trout *O. mykiss* (Leary et al. 1984; Campton and Johnston 1985), and among populations of rainbow trout (Wishard et al. 1984; Campton and Johnston 1985; Currens et al. 1990; Williams et al. 1996). Much of this analysis has relied on detectable differences among alleles of protein-coding gene loci (i.e., allozymes). Because many fish species also show geographical differentiation in the distribution of mitochondrial DNA (mtDNA) haplotypes (Birt et al. 1986; Avise et al. 1987; Avise and Vrijenhock 1987; Billington and Hebert 1991), analysis of mtDNA variation also has proven useful for stock discrimination (Wilson et al. 1987; Ward et al. 1989) and, in conjunction with nuclear DNA information, for analysis of hybridization between native and introduced fish (Bermingham and Avise 1986; Williams et al. 1996).

Rainbow trout native to both the Columbia and Fraser river drainages can be separated into two major groups, coastal and interior, depending upon where they spawn. The crest of the Cascade Mountains separates the two groups. Behnke (1992) has suggested that coastal rainbow trout *Oncorhynchus mykiss irideus* and interior Columbia basin rainbow trout (redband trout in his terminology) *O. m. gairdnerii* differ at the subspecies level. The two subspecies differ in morphology (Behnke 1992), allozyme frequencies (Allendorf and Utter 1979), and mtDNA haplotypes (Wilson et al. 1985; Williams et al. 1996). Native rainbow trout in the Metolius system are interior rainbow trout, because the Deschutes River basin, which includes the Metolius River, is the first major tributary of the Columbia River east of the Cascade Mountain crest.

Hatchery rainbow trout have been stocked throughout western North America, and numerous examples of introgressive hybridization between native and hatchery trout have been described (Busack et al. 1979; Allendorf et al. 1980; Campton and Johnston 1985; Campton and Utter 1985; Allendorf 1988; Hindar et al. 1991; Schramm and Piper 1995; Williams et al. 1996). Most hatchery rainbow trout, including the Cape Cod and Arlee strains sampled in this study, were derived from wild coastal rainbow trout originally collected from the lower McCloud River (Needham and Behnke 1962; Busack et al. 1979; Crawford 1979; Busack and Gall 1980), and they retain genetic characteristics or markers of their coastal origin. Nevertheless, hybridization between interior and coastal rainbow trout has sometimes been difficult to detect conclusively with allozymes both because the compared populations lacked fixed or nearly fixed allele frequency differences and because the comingled populations had not been genetically analyzed beforehand (Wishard et al. 1984; Campton and Johnston 1985; Leary et al. 1987; Williams et al. 1996).

Williams et al. (1996) examined allele frequencies at the lactate dehydrogenase and superoxide dismutase loci (*LDH-B2** and *sSOD-1**) in 27 natural and 7 hatchery populations of rainbow trout. Hatchery populations, founded primarily from coastal rainbow trout as previously observed (Needham and Behnke 1962; Kinunen and Moring 1978; Busack et al. 1979; Busack and Gall 1980), generally had high frequencies (>0.90) of the *LDH-B2*100* allele and lower frequencies (≤0.90) of the *sSOD-1*100* allele. In contrast, interior rainbow trout populations tended to have frequencies of *sSOD-1*100* exceeding 0.90 but highly variable frequencies of *LDH-B2*100* (range, 0.02–1.00).

The Metolius River in central Oregon has been managed by the Oregon Department of Fish and Wildlife (ODFW) as a premier rainbow trout fishery with both wild and hatchery components. Hatchery rainbow trout were first introduced to the river in 1938, and annual stocking continued

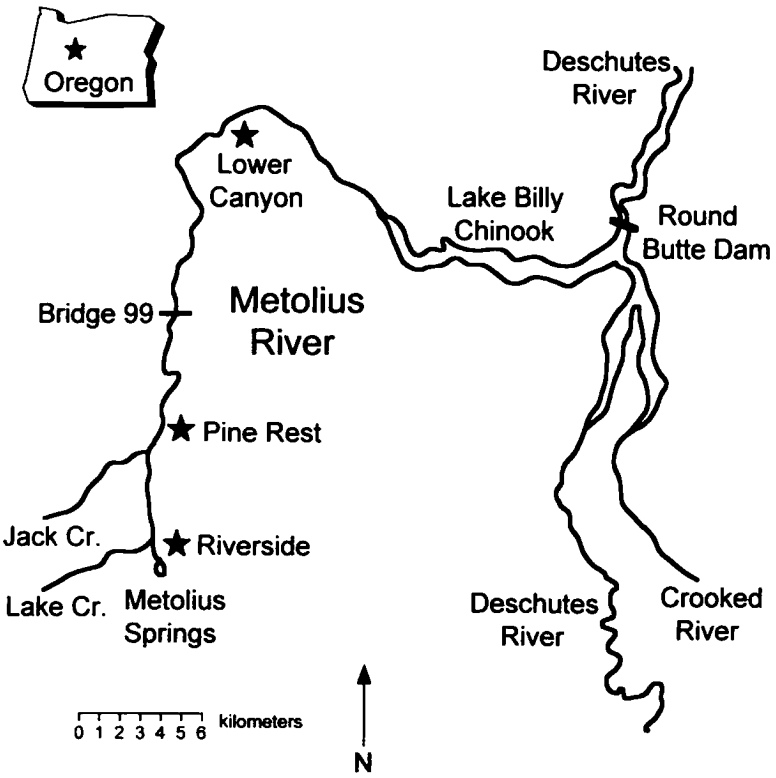

FIGURE 1.—Sampled populations of naturally occurring rainbow trout in the Metolius River in central Oregon.

through 1995. Currens (1987) and Currens et al. (1990) compared one Metolius River rainbow trout population to rainbow trout populations in the lower Deschutes basin and noted significant introgression with hatchery rainbow trout in the Metolius River sample that was absent from populations in the lower Deschutes River basin. Here, we expand on the work of Currens (1987) and Currens et al. (1990, 1997) by using both allozyme and mtDNA data to determine if introgression has occurred from hatchery rainbow trout into resident rainbow trout populations throughout the Metolius River system as a result of nearly 60 years of stocking. Our goal was to determine the overall extent of hybridization in the river and to identify any populations that may not be hybridized.

Study Area

The Metolius River is one of the major headwater tributaries of the Deschutes River in central Oregon (Figure 1). From its origin in a series of headwater springs on the east slope of the Cascade Mountains, it flows north and east approximately 44 km before reaching Lake Billy Chinook. Prior to the construction of Round Butte Dam, which forms Lake Billy Chinook, the Metolius River flowed 68 km before joining directly into the Deschutes River.

The Metolius River changes character dramatically over its present length. Near its headwaters, it flows at approximately 3 m^3/s through meadows flanked with ponderosa pine *Pinus ponderosa*. After gaining volume from various springs and tributaries, particularly in its middle and lower reaches, it attains flows of approximately 40 m^3/s in the remote canyon section above Lake Billy Chinook. Over its length, the river drops an average of 6.3 m/km, but most of the drop occurs below Bridge 99 (Figure 1), which lies near the midpoint of the river's course. The lower reaches of the river are nearly continuous whitewater.

Mitigation and Stocking History

Anadromous fish passage into the upper Deschutes River basin, including the Metolius River (Figure 1), has been blocked since the early 1960s, when fish passage facilities at Round Butte and Pelton Dams failed. The blockages eliminated runs

of native spring chinook salmon *Oncorhynchus tshawytscha* and sockeye salmon *O. nerka* from the upper basin. Kokanee (residual sockeye salmon) presently migrate upstream from Lake Billy Chinook to spawn in the headwater gravel areas such as Lake Creek (Fies and Robart 1988). There are no confirmed records of steelhead (anadromous rainbow trout) using the Metolius (Fies and Robart 1988); however, anecdotal accounts suggest that steelhead may have been there as they were in other upper Deschutes tributaries (Nehlsen 1995). Regardless, rainbow trout are native to the Metolius drainage (Fies and Robart 1988). Bull trout *Salvelinus confluentus* are native to the Metolius and upper Deschutes system. Presently, the Metolius River and Lake Billy Chinook support a large and robust population of bull trout (Ratliff et al. 1996). The river also contains introduced brown trout *Salmo trutta* (moderate numbers) and brook trout *Salvelinus fontinalis* (rare).

Rainbow trout fingerlings were first stocked in the Metolius in 1938, and plantings of yearling trout (catchable size; approximately 160 g) began in 1947. The Wizard Falls Hatchery, 13 km below the headwater springs, was built primarily to raise rainbow trout for release into the Metolius River following complaints of poor fishing in the late 1930s and early 1940s (Fies and Robart 1988). Plants of catchable size rainbow trout for the last three decades averaged approximately 175,000 fish annually (Currens et al. 1997, see their Figure 2). In 1995, stocking of hatchery fish was discontinued altogether, and the Metolius River is presently managed as a catch-and-release wild trout fishery.

Methods

At the time of our sampling, rainbow trout had been stocked in the Metolius River between Lake Creek and Bridge 99 (Figure 1) since 1938. We collected specimens above, within, and below the stocking, so each sample represents a stocking "treatment" in this natural experiment. Fish were obtained by electrofishing or angling, and only fish larger than 10 cm total length were retained for analysis. The Riverside sample (Figure 1; 17 fish) came from above Lake Creek and hence above the zone of stocking. The Pine Rest sample (N = 18) was taken approximately midway in the stocking zone. The Lower Canyon sample (N = 16) was obtained 25–33 km below the headwaters, well below the stocking zone. We also collected 24 fish of the Cape Cod hatchery strain maintained by ODFW. The Cape Cod strain was the primary hatchery strain stocked in the Metolius, particularly over the last two decades (Currens et al. 1997).

Samples were placed on dry ice and transported to Oregon State University, where they were maintained at −80°C. Tissues from the three natural populations were subsequently shipped to the University of Montana for allozyme analysis and to Boise State University for mtDNA analysis. Samples of the Cape Cod hatchery strain were analyzed for both allozyme and mtDNA variation at Boise State University. All specimens in the study were examined for both allozyme and mtDNA variation. Because we had previously used the Arlee hatchery strain of rainbow trout maintained by the Montana Department of Fish, Wildlife and Parks as a reference population for allozyme analysis (Leary et al. 1987; Williams et al. 1996), we also included data from that population in our analysis.

Allozyme analysis.—Horizontal starch gel electrophoresis (Utter et al. 1974; Aebersold et al. 1987; Leary and Booke 1990) was used to assay genetic variation at 19 presumptive loci (including 3 isoloci) encoding for proteins in muscle, liver, or eye tissue of all specimens (Table 1). Nomenclature of loci and alleles followed Shaklee et al. (1990). Allelic mobilities are relative to that of the most common allele (designated **100*) at each locus in the Arlee hatchery strain of rainbow trout.

Allele frequencies at isoloci (*sAAT-3,4**, *sIDHP-1,2**, and *sMDH-B1,2**) were estimated by treating each pair as a single gene with four rather than two copies per individual. We estimated the amount of electrophoretic differentiation among populations with Nei's (1978) measure of standard genetic distance. Calculation of genetic distances was based on all the loci. Genetic distances were calculated with the BIOSYS-1 program (Swofford and Selander 1981). We used GENEPOP (Raymond and Rousset 1995b) and Fisher's exact test to examine differences among populations (Raymond and Rousset 1995a). This testing procedure is robust even for small sample sizes, such as ours, that might otherwise limit the ability to detect hybridization events and draw inferences (Weir 1990). We examined differences among all possible combinations of populations through iterative runs whereby the most genetically divergent population was eliminated from the next iteration. The final iteration included only the two natural populations (Pine Rest and Riverside) that were the most similar to one another.

Mitochondrial DNA analysis.—Mitochondrial DNA was isolated with phenol–chloroform sepa-

TABLE 1.—Enzymes, Enzyme Commission numbers (EC; IUBMBNC 1992), and loci examined in rainbow trout samples.

Enzyme	EC number	Locus	Tissue[a]	Buffer[b]
Aconitate hydratase	4.2.1.3	*sAH-2**	L	AC
Aspartate aminotransferase	2.6.1.1	*sAAT-1**, *sAAT-2**	L	AC, RW
		*sAAT-3,4**	M	AC, RW
Creatine kinase	2.7.3.2	*CK-A1**	M	RW
Cytosol nonspecific dipeptidase	3.4.13.18	*PEPA-1**, *PEPA-2**	E	SR
Isocitrate dehydrogenase (NADP$^+$)	1.1.1.42	*sIDHP-1,2**	L	AC
L-Lactate dehydrogenase	1.1.1.27	*LDH-B1**, *LDH-B2**	E	SR
		*LDH-C**	E	SR
Malate dehydrogenase	1.1.1.37	*sMDH-B1,2**	M	AC
beta-*N*-Acetylhexosaminidase	3.2.1.52	*bHA**	L	RW
Phosphoglucomutase	5.4.2.2	*PGM-2**	M	AC+, RW
Superoxide dismustase	1.15.1.1	*sSOD-1**	L	RW
Tripeptide aminopeptidase	3.4.11.4	*PEPB**	E	SR

[a] E = eye, L = liver, and M = muscle.

[b] Buffer systems were as follows. AC was the *N*-(3-aminopropyl)-morpholine and citric acid buffer of Clayton and Tretiak (1972); pH = 6.7 for liver and 6.5 for muscle. AC+ was the same as AC except two drops of 2-mercaptoethanol and 15 mg of beta-nicotinamide adenine dinucleotide were added for every 225 mL of gel buffer; pH = 6.3 for liver, 6.3 for eye, and 6.9 for muscle. RW was the tris–citric acid buffer of Ridgway et al. (1970). SR was the tris–citric acid buffer of Gall and Bentley (1981).

ration and ethanol precipitation (Lansman et al. 1981; Sambrook et al. 1989; Dowling et al. 1990). Frozen liver and heart were the primary tissue sources, and skeletal muscle was used if volumes of liver and heart tissues were insufficient. The isolated mtDNAs were digested with 10 type II hexanucleotide restriction enzymes (*Bam*H I, *Bcl* I, *Bgl* II, *Bst*E II, *Eco*R I, *Eco*R V, *Hind* III, *Nhe* II, *Pst* I, and *Pvu* II) and 2 type II pentanucleotide restriction enzymes (*Ava* I and *Hinc* II) according to the buffer system and incubation temperatures recommended by the manufacturer (Boehringer-Mannheim and Promega Corp.). We have previously used these restriction enzymes to examine mtDNA variation among numerous salmonid populations and taxa, and several (particularly *Ava* I and *Nhe* II) were informative in separating coastal and interior rainbow trout mtDNA haplotypes (Williams et al. 1996). Fragments of DNA were separated by electrophoresis in agarose gels (0.8%), transferred to nylon membranes via capillary transfer, and probed (Southern 1975; Dowling et al. 1990) with digoxigenin-labeled rainbow trout mtDNA.

Fragment patterns were visualized with an alkaline phosphatase conjugate colorimetric procedure (Boehringer-Mannheim *Genius*® kit) and compared to a 1-kilobase molecular weight marker (Bethesda Research Laboratories). Fragments of questionable identity were rerun in adjacent lanes to minimize the chance of scoring two nonhomologous fragments as identical. All unique restriction fragment patterns (= haplotypes) for each enzyme were designated by a letter and a composite mtDNA haplotype, noting haplotypes for all 12 restriction enzymes, was produced for each specimen. Fragment patterns observed for all specimens are listed by restriction enzyme and haplotype in the appendix.

Composite mitochondrial DNA haplotypes (henceforth haplotypes) were analyzed with REAP (Restriction Enzyme Analysis Package) (McElroy et al. 1991) which estimates the number of nucleotide substitutions per restriction site (p) via the Nei (1987) method for each enzyme class (Dowling et al. 1990). Estimates from each enzyme class were combined, and an overall p was calculated (Nei and Miller 1990). The analysis produced a diagonal matrix of distances (p-values) between all pairs of mtDNA haplotypes. From the matrix, a distance network was produced as a way of estimating evolutionary relationships among mtDNA haplotypes. We used the least-squares method of Fitch and Margoliash (1967) from the PHYLIP 3.5 package (Felsenstein 1993), which assumes additivity, independence, and equal rates of evolutionary divergence among lineages (KITSCH program).

Results

Allozyme Analysis

Allozyme analysis revealed 12 polymorphic loci among the three natural and two hatchery populations of rainbow trout (Table 2). Eight of these loci exhibited allele frequencies that were

TABLE 2.—Allele frequencies at the polymorphic loci for three natural populations of rainbow trout from the Metolius River in central Oregon and two hatchery rainbow trout populations. All other loci in Table 1 were monomorphic for the same allele in each population.

		Natural populations			Hatchery populations		
Locus	Allele	Lower Canyon	Pine Rest	Riverside	Cape Cod strain	Arlee strain	χ^2 (df)[a]
*sAAT-1**	*100	1.000	1.000	1.000	0.917	1.000	26.92 (4)***
	*200				0.083		
*sAAT-3,4**	*100	1.000	1.000	0.981	1.000	1.000	
	*90			0.019			
*sAH-2**	*100	0.438	1.000	0.684	1.000	1.000	145.68 (4)***
	*80	0.562		0.316			
*CK-A1**	*100	1.000	1.000	1.000	0.875	0.955	13.44 (4)**
	*76				0.125	0.045	
*bHA**	*100	1.000	0.906	0.917	0.587	0.440	74.60 (4)***
	*72		0.094	0.083	0.413	0.560	
*sIDHP-1,2**	*100	0.688	0.695	0.712	0.648	0.750	36.87 (12)***
	*114			0.038		0.058	
	*71	0.156	0.194	0.212	0.093	0.032	
	*40	0.156	0.111	0.038	0.259	0.160	
*LDH-B2**	*100	0.719	0.833	0.842	1.000	0.985	51.95 (8)***
	*112	0.250	0.167	0.158		0.015	
	*76	0.031					
*LDH-C**	*100	0.969	1.000	1.000	1.000	0.950	
	*95	0.031				0.050	
*sMDH-B1,2**	*100	1.000	0.833	0.842	0.861	0.873	
	*83		0.167	0.158	0.139	0.127	
*PEPA-1**	*100	0.750	0.889	0.947	1.000	1.000	53.76 (8)***
	*115	0.031					
	*97	0.219	0.111	0.053			
*PGM-2**	*100	0.969	0.944	0.921	0.939	0.945	
	*90	0.031	0.056	0.079	0.061	0.055	
*sSOD-1**	*100	1.000	0.906	0.974	0.792	0.775	19.25 (4)***
	*152		0.094	0.026	0.208	0.225	

[a] Contingency table chi-square statistic for homogeneity of allele frequencies among samples. Asterisks denote $P < 0.01$** or $P < 0.001$***.

statistically heterogeneous among samples, indicating that they came from genetically divergent populations (Table 2). Much of the divergence was due to differences between the hatchery samples (Cape Cod and Arlee) and the Lower Canyon natural population. The hatchery populations possessed the *CK-A1*76*, *bHA*72*, *sMDH-B1,2*83*, and *sSOD-1*152* alleles at appreciable frequencies, but these alleles were either absent or scarce (frequency, <0.10) in the Lower Canyon population. The Lower Canyon and Riverside natural populations also possessed the *sAH*80* allele that was absent from the hatchery populations. Similarly, all three natural populations possessed the *PEPA-1*97* allele, which was absent from the hatchery populations.

Coastal rainbow trout typically have high frequencies (>0.90) of *LDH-B2*100* and usually possess *sSOD-1*152* at frequencies greater than 0.10 (Allendorf and Utter 1979; Campton and Johnston 1985; Williams et al. 1996). In contrast, interior populations usually have much lower *LDH-B2*100* and *sSOD-1*152* allele frequencies. The two hatchery samples had allele frequencies at *LDH-B2** and *sSOD-1** typical of coastal rainbow trout populations (Table 2; Figure 2), whereas, the Lower Canyon sample had allele frequencies more typical of interior rainbow trout populations (Table 2; Figure 2). The Riverside and Pine Rest samples exhibited allele frequencies at these loci that were intermediate between the coastal profile of the Cape Cod and Arlee hatchery strains and the profile of the Lower Canyon sample (Figure 2). These samples

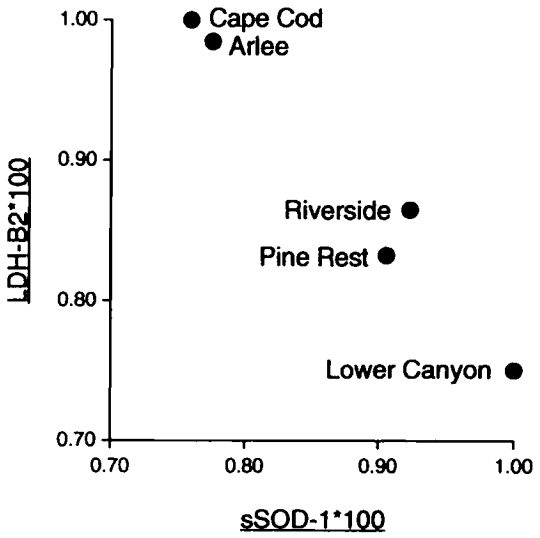

FIGURE 2.—Allele frequencies for *sSOD-1*100* and *LDH-B2*100* in three natural rainbow trout populations from the Metolius River and two hatchery populations.

also had intermediate allele frequencies at the *bHA**, *sMDH-B1,2**, and *PEPA-1** loci (Table 2). Such results are expected of populations where hybridization has occurred between coastal rainbow trout and interior rainbow trout.

Estimated genetic distances between populations support the relationships depicted in Figure 2. Genetic distances were greatest between the Lower Canyon population and the two hatchery populations (0.061 for Cape Cod and 0.064 for Arlee), whereas the two hatchery populations differed little from one another (0.003). The Pine Rest sample, from the stocked section of the Metolius River, was essentially equidistant from the Lower Canyon (0.027), Cape Cod (0.020), and Arlee (0.023) samples. The Riverside sample was slightly closer to the Lower Canyon (0.014) and Pine Rest (0.013) samples than to the Cape Cod (0.026) and Arlee (0.027) samples.

We used Fisher's exact test (Raymond and Rousset 1995a, 1995b) to examine differences among Metolius River populations and the Cape Cod hatchery strain. For these four populations, 7 of 12 loci differed significantly ($P < 0.05$; Table 2), and the populations differed highly significantly from one another ($P < 0.001$). The main differences were between the hatchery and natural populations. Only 2 of 12 loci differed significantly among the river samples ($P < 0.05$). The river populations remained different overall ($P < 0.001$), but the two upper-river samples (Pine Rest and Riverside) did not differ significantly from one another in spite of having highly significant differences in allele frequencies at *sAH-2**.

Mitochondrial DNA Analysis

Analysis of mitochondrial DNA variation among the three natural populations and the Cape Cod hatchery sample revealed 15 mtDNA haplotypes, labeled A–O (Tables 3, 4). Haplotype A, which occurred in 15 of 16 specimens from the Lower Canyon sample, was also the predominant haplotype in the Pine Rest and Riverside samples (Table 4). The predominant occurrence of haplotype A in these three samples and its absence from the Cape Cod (and Arlee) hatchery sample suggests it is the predominant indigenous haplotype of trout native to the Metolius River.

Allozyme analysis of lower Deschutes River rainbow trout by Currens et al. (1990) showed little evidence of hybridization with hatchery strains derived from coastal rainbow trout; the lower Deschutes fish exhibited allozyme profiles typical of interior rainbow trout. Consequently, we examined mtDNA variation in seven specimens from Bake Oven Creek in the lower Deschutes system. Of these, five specimens had haplotype A and two had haplotype B—the same two haplotypes found in the Lower Canyon sample. Thus, it is likely that A is the predominant haplotype for native rainbow trout in the Deschutes system and that B represents an indigenous but less common haplotype. Haplotype A was also the predominant one observed by Williams et al. (1996) in rainbow trout populations of the Owyhee River in northern Nevada and of Snake River tributaries in southwestern Idaho.

The predominant mtDNA haplotype observed in the Cape Cod hatchery sample (haplotype O, Table 3) was also the predominant haplotype observed in the Arlee Hatchery strain of rainbow trout (33 of 40 specimens analyzed: S. H. Forbes, University of Montana, personal communication), and in the Hayspur Hatchery strain used by Idaho Fish and Game in southern Idaho (Williams et al. 1996). This haplotype, therefore, appears to be characteristic of hatchery strains derived from coastal rainbow trout.

Haplotype diversity differed among the sampled populations. The Lower Canyon sample exhibited two mtDNA haplotypes, whereas the Pine Rest, Riverside, and Cape Cod samples had seven, six and four haplotypes, respectively. Within each population, haplotypes differed from one another

TABLE 3.—List of 15 composite mtDNA haplotypes from 75 Metolius River and Cape Cod strain rainbow trout. Haplotypes are denoted by uppercase letters and represent individual fragment patterns (denoted by lowercase letters) detected with the restriction enzymes *Ava* I, *Bam*H I, *Bcl* I, *Bgl* II, *Bst*E II, *Eco*R I, *Eco*R V, *Hinc* II, *Hind* III, *Nhe* II, *Pst* I, and *Pvu* II, respectively. Fragment patterns are listed in the appendix.

Sample	Haplotype designation	Composite mtDNA haplotype	Number of fish
Metolius River			
Lower Canyon	A	a a a a a a a a a a a a	15
	B	a a a a a a a a a b a a	1
Pine Rest	A	a a a a a a a a a a a a	7
	D	a a a c a a a a a d a a	1
	E	a a a a a a a a a a a a	6
	F	a a a a a a a a a c a a	1
	G	a a a a a a a a a a a a	1
	H	a a a c a a a a a a a a	1
	I	a a a c a a a c a a a a	1
Riverside	A	a a a a a a a a a a a a	8
	C	a a a a a a a a a c a a	1
	D	a a a c a a a a a a a a	1
	I	a a a c a a a c a a a a	5
	J	a a a c a a a c a a a a	1
	K	a a a a a a a a a a a a	1
Cape Cod hatchery strain	L	a a a c a a a a b a a a a	1
	M	a a a a a a a a b a a a a	6
	N	a a a a a a a a b a a a a	2
	O	a a a c a a a a b a a a a	15

over a range of values that was low in the Lower Canyon sample (0.17% sequence divergence), intermediate in the Cape Cod sample (0.28–0.80% divergence), and high in the Pine Rest (0.13–1.35%) and Riverside (0.17–1.59%) samples. The low divergence between haplotypes in the Lower Canyon sample is typical of that observed in nonhybridized populations (Billington and Hebert 1991). In contrast, the high sequence divergence among mtDNA haplotypes within the other three samples is typical of that observed in populations formed by intraspecific hybridization (Wilson et al. 1985; Billington and Hebert 1991; Williams et al. 1996).

A Fitch–Margoliash (1967) unrooted network of the 15 observed mtDNA haplotypes (Table 5; Figure 3) divided the haplotypes into two major groupings. The principal division was primarily related to variation at restriction sites produced by *Ava* I, *Bgl* II, *Hinc* II, and *Nhe* I (Table 3; see appendix). The two groupings of haplotypes are denoted "interior" or "coastal" in Figure 3 based on the presence of haplotype A or haplotype O, respectively. Specimens from all three natural populations and several in the Cape Cod sample exhibited haplotypes that were genetically similar to haplotype A and, therefore, clustered in the "interior" grouping. Haplotypes in the "coastal" cluster came from the Pine Rest, Riverside, and Cape Cod samples. Within each of the two clusters, haplotype sequences diverged by approximately 0.50–0.80%; the divergence between the two clusters was approximately 1.35%.

Discussion

Genetic Status and Detection of Hybridization

Allozyme and mtDNA analysis showed the Pine Rest and Riverside samples to be genetic mixtures (i.e., hybrid swarms) of the interior and coastal forms of rainbow trout. These samples had allozyme profiles intermediate between coastal and interior profiles and mtDNA haplotypes that appeared to originate from both interior and coastal rainbow trout (Figure 3). These results likely stem from hybridization between hatchery rainbow trout of coastal origin and the indigenous interior rainbow trout of the Metolius River system during nearly 60 years of stocking in the upper river.

In contrast, the Lower Canyon sample had an allozyme profile generally typical of interior rainbow trout and possessed only mtDNA typical of interior rainbow trout (Figure 3; Table 4). Taken together, the allozyme and mtDNA data strongly suggest that the Lower Canyon population is a genetically pure interior rainbow trout population.

TABLE 4.—Frequencies of the 15 observed mtDNA haplotypes from the Metolius River and Cape Cod hatchery rainbow trout samples. Haplotype designations are from Table 3.

Haplotype	Sample				Total
	Lower Canyon	Pine Rest	River-side	Cape Cod	
A	15	7	8	0	30
B	1	0	0	0	1
C	0	0	1	0	1
D	0	1	1	0	2
E	0	6	0	0	6
F	0	1	0	0	1
G	0	1	0	0	1
H	0	1	0	0	1
I	0	1	5	0	6
J	0	0	1	0	1
K	0	0	1	0	1
L	0	0	0	1	1
M	0	0	0	6	6
N	0	0	0	2	2
O	0	0	0	15	15

However, the frequency of *LDH-B2*100* in the Lower Canyon sample (0.75), while lower than that in the Pine Rest (0.83) and Riverside (0.87) samples, is higher than Currens et al. (1990) observed among fish from Bake Oven (0.40) and Big Log (0.33) creeks in the Deschutes River downstream of the Metolius.

Williams et al. (1996) reviewed geographic variation at the *LDH-B2** and *sSOD-1** loci among 27 natural and 7 hatchery strains of rainbow trout and found that natural variation in some populations would suggest hybridization based on the commonly accepted criteria described above. Interior rainbow trout tended to have high frequencies (> 0.90) of *sSOD-1*100* but highly variable frequencies of *LDH-B2*100*. Williams et al. (1996) concluded that allele frequencies at these two loci, particularly at *LDH-B2**, are reliable indicators of hybridization only when allozyme diversity in a region has been well documented. Currens et al. (1990) examined allozyme variation among rainbow trout from 22 natural populations in the lower Deschutes basin along with 1 sample from the Metolius River and 5 hatchery rainbow trout strains. That study and Currens et al. (1997) form the background and larger geographical context within which our results can be interpreted. Thus, the *LDH-B2*100* frequency of the Lower Canyon population, which is higher than that observed in populations from the lower Deschutes (Currens et al. 1990), could be due either to natural variation or to slight introgression with hatchery rainbow trout over the long stocking history of the Metolius River. Analysis of mtDNA variation showed no evidence for introgression; therefore, if introgression has occurred in the Lower Canyon population, it is very slight and impossible to definitively detect given our methods and samples sizes.

Mitochondrial DNA haplotype diversity within the Lower Canyon sample (15 specimens with haplotype A and one specimen with haplotype B) was typical of nonhybridized interior salmonid populations (Billington and Hebert 1991; Shiozawa and Evans 1995; Williams et al. 1996; Williams et al. 1997). Most such populations exhibit one or a few mtDNA haplotypes, one being predominant and the others being minor variants of the predominant one. Percent sequence divergence among these haplotypes is typically less than 0.60%. In con-

TABLE 5.—Percentage sequence divergences ($100 \cdot p$) among the 15 observed mtDNA haplotypes from the Metolius River and Cape Cod hatchery samples of rainbow trout. Haplotype designations are from Table 3.

Haplo-type	Haplotype													
	A	B	C	D	E	F	G	H	I	J	K	L	M	N
B	0.173													
C	0.170	0.379												
D	0.183	0.396	0.250											
E	0.296	0.476	0.469	0.477										
F	0.458	0.722	0.332	0.583	0.126									
G	0.670	0.919	0.898	0.510	0.386	0.574								
H	0.961	1.160	0.998	0.370	1.350	1.435	0.980							
I	1.352	1.608	1.435	0.799	0.919	1.020	0.565	0.303						
J	1.181	1.413	1.383	0.993	0.790	0.963	0.395	0.476	0.171					
K	0.382	0.587	0.575	0.574	0.496	0.661	0.842	0.921	1.005	0.821				
L	0.563	0.772	0.755	0.566	0.675	0.842	0.647	0.723	0.804	0.630	0.175			
M	0.198	0.388	0.358	0.272	0.479	0.637	0.729	0.570	0.835	0.706	0.149	0.275		
N	0.536	0.771	0.755	0.756	0.198	0.376	0.563	1.096	0.742	0.552	0.255	0.431	0.235	
O	1.314	1.594	1.406	0.736	0.933	1.041	0.551	0.703	0.353	0.540	1.018	0.801	0.778	0.783

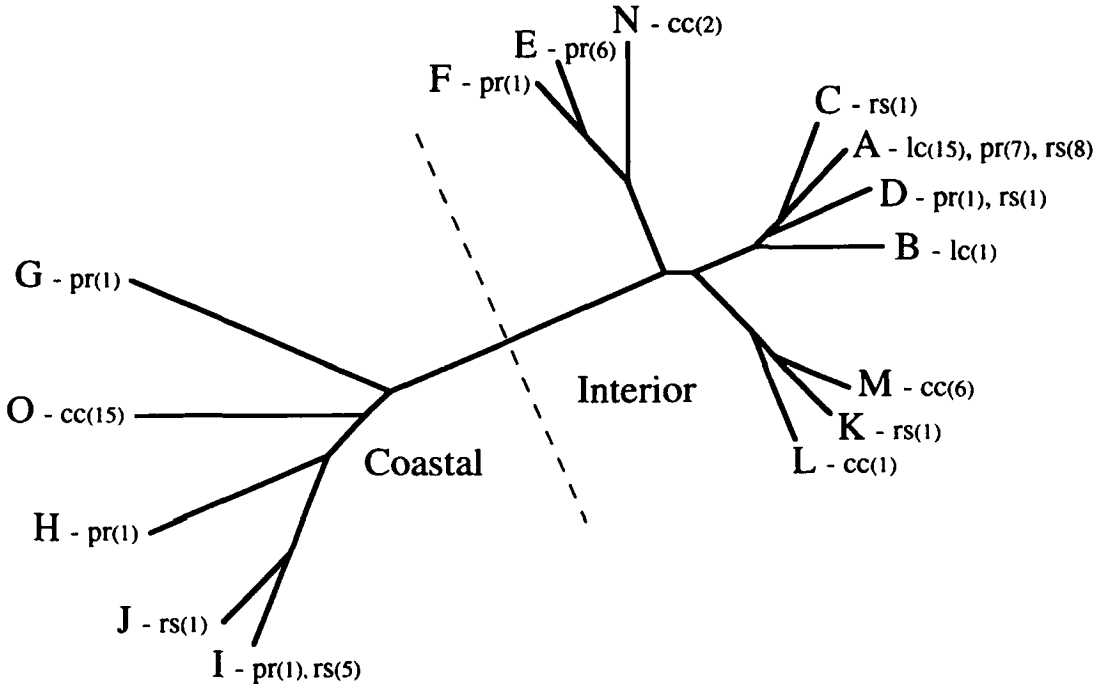

FIGURE 3.—Unrooted Fitch–Margoliash (1967) network showing inferred evolutionary relationships among 15 mtDNA haplotypes found in three natural rainbow trout populations from the Metolius River and one hatchery population. Haplotype letter designators (from Table 3) are followed by lowercase abbreviations denoting the populations in which the haplotype was observed (Tables 3, 4; cc = Cape Cod hatchery strain, lc = Lower Canyon, pr = Pine Rest, and rs = Riverside). Haplotype frequencies (numbers of individuals) within each population are shown parenthetically.

trast, the occurrence of multiple (≥ 4) and divergent (>80%) haplotypes within a single population can be inferred to represent hybridization and introgression (Billington and Hebert 1991; Williams et al. 1996). The Pine Rest and Riverside samples had six or more haplotypes that diverged from one another by 1.35% or more, reflecting the hybridized nature of these populations.

The Cape Cod hatchery sample had an allozyme profile typical of coastal rainbow trout, and had mtDNA haplotypes typical of interior and coastal rainbow trout origin. Williams et al. (1996) observed this same genetic pattern in the Hayspur hatchery strain of rainbow trout commonly used for stocking southern Idaho streams.

Genetic and Conservation Implications

Because the entire Metolius River system is relatively small, coursing only 44 km from its headwaters to its confluence with Lake Billy Chinook, we did not expect to find such marked differences in genetic attributes among the three sample populations. Currens (1987) had documented hybridization between native and hatchery rainbow trout in the Metolius River, although his sample was confined to the Pine Rest area (approximately 10 km downstream from the headwater springs), where stocking had occurred since 1938. Our Riverside sample, collected 2–3 km downstream from the headwater springs in an area that was not stocked with hatchery trout, exhibited substantial allozyme and mtDNA introgression, as did our sample from the Pine Rest area. Although the two samples did not differ significantly from one another based on allozyme analysis, they each exhibited a number of unique mtDNA haplotypes. There are no barriers to fish movement throughout the entire Metolius River system. Our analysis shows that fish movement and gene flow between hatchery and native rainbow trout has occurred between the Pine Rest area and the Riverside area.

In contrast to the two upper-river samples, the Lower Canyon sample, collected 25–33 km downstream of the headwaters, showed no evidence of hybridization with hatchery rainbow trout. We be-

lieve that ecological factors are responsible for the genetic isolation of the lower river rainbow trout from the hybridized upper-river population. Whereas the upper 15 km of the Metolius River has numerous large, deep pools, gravel runs, a moderate elevational gradient, and small flow volume (3 m^3/s near headwaters), the lower river lies within a constrained canyon reach and has a steep elevational gradient with few pools and heavy flows (40 m^3/s near the confluence with Lake Billy Chinook). Rearing in a protected hatchery environment does not prepare fish for survival in conditions like those in the lower Metolius River. Local anglers report that few hatchery rainbow trout (all are fin-clipped) are ever caught in the lower river (J. Judy, G. Kish, M. Leitheiser, and R. Robinson, personal communications). Hatchery rainbow trout are also susceptible to predation by a robust bull trout population in the lower Metolius River and Lake Billy Chinook. Additionally, hatchery rainbow trout are susceptible to the lethal effects of the myxosporean parasite *Ceratomyxa shasta*, which is present in the Deschutes system, including the Metolius River and Lake Billy Chinook. Fishes native to the drainage appear to be genetically resistant to the parasite (Ratliff 1981, 1983). Water temperatures in the Metolius River (9–10°C) appear to be below the temperature threshold for ceratomyxosis infection (> 10°C), so hatchery rainbow trout in the Metolius River are not affected by the disease unless they migrate downstream into the warmer waters of Lake Billy Chinook (see Currens et al. 1997).

With the recent decision to curtail the hatchery stocking program and to direct management actions toward wild fish objectives in the Metolius system, the Lower Canyon population may have a valuable role to play in the restoration and rebuilding of native Metolius rainbow trout populations. If some of the genetically pure interior rainbow trout in the lower river migrate upstream to spawn, they could sustain an infusion of genes from native fish back into the hybridized populations, shifting the genetic attributes of the upriver population toward those of Lower Canyon fish. Uncertainties about the extent of fluvial spawning migrations and rates of gene flow, should be resolved by population monitoring. Monitoring of both life history and genetic attributes of the upper and lower river rainbow trout populations could allow estimation of the time required to achieve natural restoration.

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Appendix: Mitochondrial DNA Fragment Patterns of Metolius River and Hatchery Rainbow Trout

TABLE A.1.—Mitochondrial DNA fragment patterns (haplotypes a–d) of rainbow trout from the Metolius River and the Cape Cod and Arlee hatchery strains for each of 12 restriction enzymes (bold subheads). Data are numbers of base pairs in fragments; the bottom entry in each enzyme column is the total number of base pairs for the haplotype. Inferred gains or losses of restriction sites among haplotypes (assumed to have resulted from single nucleotide substitutions) can be discerned by matching up values with the same letter across columns.

Haplotype			
a	b	c	d
	***Ava* I**		
6,100	6,100	6,100	
2,800	2,800 y		
2,180	2,180	2,180	
1,820	1,820	1,820	
		y 1,780	
1,240	1,240	1,240	
1,200	1,200	1,200	
	z 1,150	1,150	
		y 1,020	
620 z			
530 z			
270	270	270	
16,760	16,760	16,760	
	***Bam*H I**		
12,400			
4,300			
16,700			
	***Bcl* I**		
12,000			
4,700			
16,700			
	***Bgl* II**		
11,950	11,950 y		
		y 7,350	
4,750 z			
		y 4,600	
	z 3,500	3,500	
	z 1,250	1,250	
16,700	16,700	16,700	
	***Bst*E II**		
16,140			
560			
16,700			
	***Eco*R I**		
7,800			
4,150			
4,050			
730			
16,730			
	***Eco*R V**		
8,400			
7,000			
820			
330			
16,550			

TABLE A.1.—Continued.

Haplotype			
a	b	c	d
	***Hinc* II**		
5,600	5,600 y		
4,000 z			
3,550	3,550	3,500	
		y 3,300	
	z 3,280	3,280	
2,750	2,750	2,750	
		y 2,300	
	z 820	820	
445	445	445	
225	225	225	
220	220	220	
16,790	16,890	16,890	
	***Hind* III**		
6,600	6,600		
	z 3,760		
3,480 z			
2,200	2,200		
1,780	1,780		
1,200	1,200		
1,100	1,100		
276 z			
16,636	16,916		
	***Nhe* I**		
9,600 z	9,600 x		
		x 8,100	
	y 7,100 v		
			z 6,900
6,000 y		v 6,000	6,000
			z 2,700
		x 1,500	
1,100 y		v 1,100	1,100
16,700	16,700	16,700	16,700
	***Pst* I**		
10,950			
4,100			
1,550			
280			
16,880			
	***Pvu* II**		
6,950			
4,800			
2,850			
2,350			
16,680			