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Genetic Structure of Columbia River Redband Trout Populations in the Kootenai River Drainage, Montana, Revealed by Microsatellite and Allozyme Loci

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Abstract.—We describe the genetic divergence among 10 populations of redband trout *Oncorhynchus mykiss gairdneri* from the upper Columbia River drainage. Resident redband trout from two watersheds in the Kootenai River drainage and hatchery stocks of migratory Kamloops redband trout from Kootenay Lake, British Columbia, were analyzed using allele frequency data from microsatellite and allozyme loci. The Kamloops populations have significantly different allele frequencies from those of the Kootenai River drainage. Of the total genetic variation detected in the resident redband trout, 40.7% (microsatellites) and 15.5% (allozymes) were due to differences between populations from the two Kootenai River watersheds. The divergence among populations within each watershed, however, was less than 3.5% with both techniques. Our data indicate that watershed-specific broodstocks of redband trout are needed by fisheries managers for reintroduction or the supplementation of populations at risk of extinction.

The Columbia River redband trout *Oncorhynchus mykiss gairdneri*, a subspecies of rainbow trout *O. mykiss*, is native to the Fraser and Columbia River drainages east of the Cascade Mountain crest up to the barrier falls on the Pend Oreille, Spokane, Snake, and Kootenai rivers (Behnke 1992). In contrast, coastal rainbow trout *O. m. irideus* are native to waters that are mainly west of the Sierra Nevada and Cascade Range crest in the continental United States.

The Kootenai River drainage is the only watershed in Montana where redband trout are native (Holton 1990; Behnke 1992). Using known allele frequency differences between redband and coastal rainbow trout at two allozyme loci (Allendorf and Utter 1979), Allendorf et al. (1980) determined that the range of redband trout in the upper Columbia River basin extends into northwestern Montana to at least the Kootenai River barrier falls (Figure 1). The allele frequencies of 95 populations from throughout the range of the two rainbow trout subspecies are listed in the Appendix. The data indicate that redband trout usually have a frequency of *LDH-B2*76* greater than 0.250 and a frequency of *sSOD-1*152* less than 0.100. In contrast, coastal rainbow trout usually have a frequency of *LDH-B2*76* less than 0.100 and a frequency of *sSOD-1*152* greater than 0.150. Based on these criteria, most samples of rainbow trout from the Kootenai River drainage have redband trout characteristics (Sage et al. 1992). The exceptions appeared to be redband trout populations that have hybridized with westslope cutthroat trout *O. clarki lewisi*, coastal rainbow trout, or both.

Columbia River redband trout exhibit a wide variety of life history strategies. Anadromous

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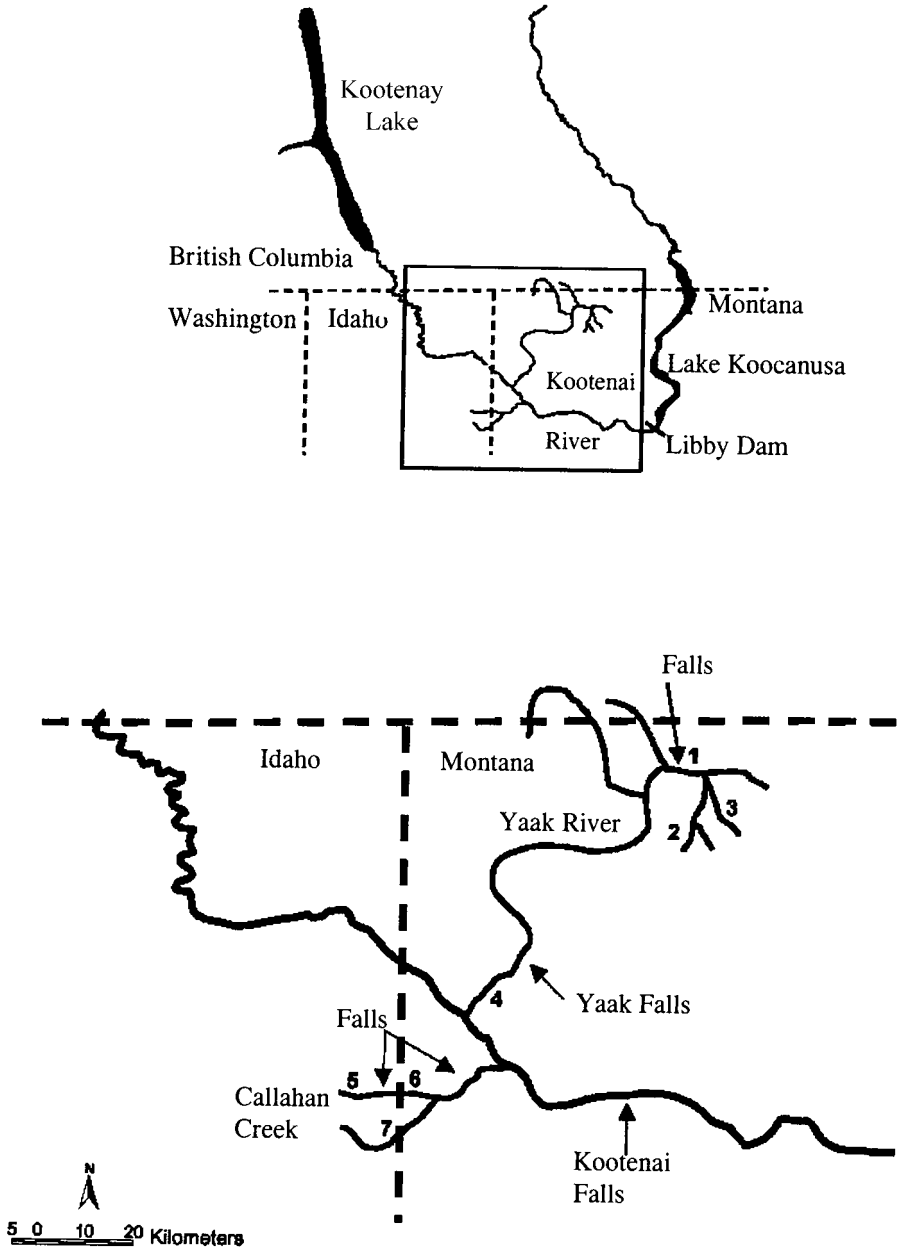

FIGURE 1.—Sampling sites for redband trout populations in the Kootenai River drainage. Site numbers correspond to the locations given in Table 1.

stocks of redband trout (steelhead) historically migrated almost 1,600 km to the middle and upper Columbia River drainage (Behnke 1992). Many of these stocks are now extinct due to dams impeding upstream migration. The Kamloops redband trout of Kootenay Lake, British Columbia, represent an adfluvial form that attains a large body size due to their piscivorous diet and advanced size at sexual maturity. Kamloops redband trout reportedly spawn in Kootenai River tributaries in Montana downstream from Kootenai Falls (Huston 1995). Fluvial stocks occupy large rivers and spawn in smaller tributaries. Resident forms inhabit smaller tributaries and headwater areas for their entire lives. The Kootenai River drainage in Montana definitely supports resident populations (Muhlfeld

et al. 2001b). A migratory fluvial and/or adfluvial component may also exist, but the presence of hybridized populations in the lower portions of the drainage makes their detection problematic.

The different redband trout life history forms are indistinguishable using meristic counts, coloration patterns (Behnke 1992), or allozyme data (e.g., Campton and Johnston 1985; Leary 1997). It is unknown whether there is a strong genetic basis for the various redband trout life history characteristics. Furthermore, it is unknown whether different life history forms exist in the same redband trout population, as they do in some other salmonids, such as migratory and residual sockeye salmon *O. nerka* (Foote et al. 1989).

As with other native salmonids, populations of redband trout have declined due to a complex combination of land and water practices (logging, mining, agriculture, grazing, and dams) and hybridization and competition with nonnative fishes (Williams et al. 1989; Behnke 1992). Based on species distribution patterns and the existence of nonhybridized populations, Sage et al. (1992) concluded that redband trout and native westslope cutthroat trout coexisted in the Kootenai River drainage with little or no hybridization prior to widespread stocking. Numerous introductions of nonnative trout, primarily coastal rainbow trout and eastern brook trout *Salvelinus fontinalis* beginning in the late 1800s (Hanzel 1960), along with more recent introductions of westslope cutthroat trout, have led to intensive competition, species replacement, and hybridization (Allendorf et al. 1980). Consequently, many nonhybridized populations persist only in headwater streams that may serve as refugia until effective conservation and restoration programs are implemented.

Recently, concern has arisen that the redband trout in the Kootenai River basin are at a high risk of extinction (Muhlfeld 1999). In response to population declines, resident forms of redband trout are considered a species of special concern by the U.S. Fish and Wildlife Service, the American Fisheries Society, and all of the states comprising their historic range (Idaho, Oregon, Washington, Nevada, California, and Montana). The U.S. Forest Service and the Bureau of Land Management (Lee et al. 1997) classify them as a sensitive species. The Kootenai River redband trout was petitioned for listing under the Endangered Species Act in 1994 but was not listed due to a lack of information. Montana Fish, Wildlife, and Parks is considering developing a redband trout broodstock for reintroduction into areas from which it has been extirpated as well as the supplementation of populations at risk of extinction in the Kootenai River drainage (Muhlfeld et al. 2001a).

For reintroduction programs to be genetically sound, knowledge of the population genetic structure of the taxon, at least in the region of the introductions, is essential (Leary et al. 1995). Our objectives were (1) to assess the genetic diversity within and among several populations of redband trout in the Kootenai River drainage using microsatellite loci, (2) to compare these data with results obtained from allozyme loci, and (3) to analyze two hatchery stocks of Kamloops redband trout to determine their genetic similarity to the resident redband trout populations and assess their suitability for use in Kootenai River drainage restoration projects.

Methods

Samples.—Wild redband trout of multiple age-classes from seven waters in the Kootenai River drainage in Montana and Idaho were sampled by hook and line during spring and summer 1997 and 1998 for microsatellite analysis (Figure 1; Table 1). In addition, two samples of Kamloops redband trout were obtained. These fish were the direct or indirect descendants of Kootenay Lake, British Columbia, fish. The Gerrard Kamloops redband trout were the progeny of fish collected from Kootenay Lake in 1997 and spawned at the Wardner Hatchery in British Columbia. The Murray Springs State Trout Hatchery fish were the progeny of fish from Spoon Lake, Wyoming, and were presumed to be Duncan Kamloops redband trout because Spoon Lake had been stocked with fish descended from Kamloops redband trout that had been maintained at the Ennis National Fish Hatchery. The Ennis broodstock originated from Duncan Kamloops redband trout spawned at the Wardner Hatchery. A sample of Arlee rainbow trout was taken from the Jocko River State Trout Hatchery in Arlee, Montana, in 1998 as a representative strain of coastal rainbow trout.

Samples for allozyme analysis were collected in 1990 and 1991 from the upper Yaak River drainage and in 1994 from the Callahan Creek drainage (Figure 1; Table 1). In addition, two stocks of Kootenay Lake Kamloops redband trout were obtained. A sample of the Gerrard stock consisting of the 1994 ($N = 10$), 1998 ($N = 25$), and 2000 ($N = 50$) year-classes was collected from the Wardner Hatchery in British Columbia. Duncan Kamloops redband trout were obtained from the Ennis National Fish Hatchery and the Murray Springs State

TABLE 1.—Sample sites and numbers of fish sampled for microsatellite and allozyme analysis. Different individuals were analyzed with the two techniques for all locations except Murray Springs Hatchery. The numbers preceeding the names correspond to the locations shown in Figure 1; NA = sample not analyzed using the indicated technique.

Sample site	Population code	Sample size	
		Microsatellites	Allozymes
Wild populations			
Upper Yaak River			
1. East Fork Yaak River	EFYR	16	5
2. Basin Creek	BASIN	30	28
3. Porcupine Creek	PORC	11	24
Lower Yaak River			
4. Yaak River (below falls)	YRBF	31	NA
Callahan Creek			
5. North Fork Callahan Creek (above falls)	NFCA	32	26
6. North Fork Callahan Creek (below falls)	NFCB	28	NA
7. South Fork Callahan Creek	SFCC	33	25
Hatchery populations			
Kootenay Lake Kamloops			
Duncan (Ennis National Fish Hatchery)	ENNIS	NA	50
Duncan (Murray Springs State Trout Hatchery)	MSPR	30	60
Gerrard (Wardner Hatchery)	GERR	32	85
Arlee coastal rainbow trout (Jocko River State Trout Hatchery)	ARLEE	30	50

Trout Hatchery. The Ennis hatchery fish were the progeny of fish collected from Kootenay Lake. Arlee rainbow trout were sampled in 1994.

Except for the Murray Springs sample, different individuals were analyzed for microsatellites and allozymes. Most of the allozyme data were collected before microsatellite analysis was available, and the samples were not archived. Subsequently, nonlethal samples were taken for microsatellite analysis. Samples for the two techniques were collected in the same areas and at most three generations apart.

Microsatellites.—DNA was extracted from fin clips that had been stored in 95% ethanol or lysis buffer using the Gentra DNA isolation kit (Gentra Systems, Inc.). Microsatellite loci were amplified in an MJ Research PTC-100 thermocycler using fluorescently labeled primers (*Ocl 2*, Condrey and Bentzen 1998; *Omy 0004*, Holm and Brusgaard 1998; *One*μ*11*, Scribner et al. 1996; *Rgt 6*, Sakamoto et al. 1996; *Ssa 197*, O'Reilly et al. 1996). The amplified products were separated by size in 7% denaturing polyacrylamide gels and visualized using a Hitachi FMBIO-100 fluorescent imager. Each 10-μL reaction contained the following components: deoxynucleotide triphosphates, labeled primer, unlabeled primer, $MgCl_2$, 0.8 units *Taq* DNA polymerase with supplied buffer, and 50–100 ng DNA template. Annealing temperatures were 55°C for *Ocl 2* and *Ssa 197*, 56°C for *One*μ*11*, 58°C for *Rgt 6*, and 62°C for *Omy 0004*. Product sizes were determined using MapMarkerLOW size standards (Bio Ventures, Inc.) and Hitachi FMBIO software (MiraiBio, Inc. 1999). Each gel also included previously analyzed individuals to ensure consistent scoring across all populations.

Allozymes.—Horizontal starch gel electrophoresis was used to determine the genetic characteristics of each fish at 38 allozyme loci coding for proteins present in muscle, liver, or eye tissue (Leary 1997). Electrophoresis followed the procedures of Allendorf and Utter (1979) and Leary and Booke (1990). Gel buffers and stains used to reveal the position of particular enzymes in the gels after electrophoresis followed the protocols of Allendorf et al. (1977).

Data analysis.—The three year-classes of the Gerrard stock and the 1997 and 1998 creek samples were initially compared for temporal differences in allele frequencies. Allele frequencies, heterozygosities, deviations from Hardy–Weinberg expectations, exact probabilities of population homogeneity, and fixation statistics were calculated using GENEPOP (Raymond and Rousset 1995).

In rainbow trout, some pairs of allozyme loci produce a protein with identical function and electrophoretic mobility. Such pairs of loci are commonly termed isoloci (Allendorf and Thorgaard 1984). When genetic variation exists at isoloci, it is not possible from the gels to determine at which locus the variant exists. To estimate allele frequencies at the isoloci (*sIDHP-1,2**, *sMDH-A1,2**, and *sMDH-B1,2**), therefore, each pair was considered to be a single gene with four rather than two alleles per individual. Since the genotypes of individual loci could not be determined at isoloci,

those loci could not be used to test for deviations from expected Hardy–Weinberg genotypic distributions.

We used three procedures to investigate the levels of genetic divergence among the sampled populations. Allele frequencies and the Cavalli-Sforza and Edwards (CSE) chord distance option of PHYLIP (Felsenstein 1993) were used to construct a matrix of genetic distance estimates for all pairwise population comparisons. We used the unweighted pair-group method with arithmetic averages (UPGMA) algorithm in PHYLIP to construct a dendrogram of the genetic similarity among populations. Bootstrap values for branch points were calculated using the SEQBOOT algorithm with 100 replicates in PHYLIP. The unrooted tree was visualized using TREEVIEW PCC (Page 1996). We also conducted a principal components analysis using the covariance matrix of allele frequencies (Minitab, Inc. 1996) omitting the largest allele at each microsatellite locus and the common rainbow trout allele at each allozyme locus to account for the nonindependence of allele frequencies within a locus (for a review, see Cavalli-Sforza et al. 1993). Finally, using the hierarchical gene diversity analysis procedure of Chakraborty (1980), we partitioned the total amount of genetic variation, estimated as the average expected heterozygosity, into (1) the proportion due to genetic variation within samples and (2) the proportion due to genetic differences among samples, both within and between drainages. Differentiation of the Callahan and Upper Yaak River drainages, as measured by the index of genetic diversity, G_{ST} (Nei 1973), was also calculated. It is unlikely that additional, unsampled populations exist within these small watersheds, so the estimates of the within- and between-watershed divergence should be unbiased.

Results

Genetic Variation within Populations

All five microsatellite loci analyzed were variable in all samples (Table 2). The number of alleles per locus varied from 2 to 13 among the samples, averaging 8. The average expected heterozygosity in the wild populations ranged from 0.187 in the sample from North Fork Callahan Creek above the falls to 0.533 in that from the Yaak River below the falls.

Microsatellite allele frequencies did not differ significantly between any of the 1997 and 1998 collections. The samples from both years from each creek were therefore pooled for further analysis. After correction for multiple tests (Rice 1989), a statistically significant ($P = 0.00$) deviation from the expected Hardy–Weinberg genotypic proportions was only observed at a single locus in one population; the sample from the Yaak River below the falls had an excess of heterozygotes at *Omy 0004*.

Evidence of genetic variation was detected among the samples at 14 of the 38 allozyme loci screened (Table 3). The number of alleles per variable locus ranged from 2 to 4 among the samples, averaging 2.2. The relative heterozygosity at the 14 variable loci in the wild populations ranged from 0.041 in the Basin Creek sample to 0.077 in the sample from the South Fork of Callahan Creek.

There were no differences among the three year-classes of Gerrard Kamloops redband trout, so they were combined for further analysis. No significant deviations from the expected Hardy–Weinberg genotypic proportions were observed at any allozyme locus in any sample.

Genetic Variation among Populations

Microsatellite allele frequency differences among populations were substantial. Pairwise comparisons of allele frequencies between populations indicated statistically significant ($P < 0.05$) divergence between most pairs of populations at one or more loci (Table 4). The only exceptions were the comparison between the East Fork Yaak River and its tributary, Basin Creek, and that between the South Fork and North Fork (above the falls) of Callahan Creek. All branch points of the UPGMA unrooted tree of overall genetic similarity based on CSE values are strongly supported (Figure 2).

The genetic distance estimates from the microsatellite data indicate some geographic structuring of the redband trout populations in the Kootenai River drainage. Samples within watersheds group together. The upper Yaak River samples, however, are quite different from both the Callahan Creek samples and the Kamloops hatchery stocks. Fish from the lower Yaak River are genetically much more similar to the Arlee coastal rainbow trout than they are to any of the other populations sampled in the Kootenai River drainage, indicating a possible genetic contribution by coastal rainbow trout to this group of fish.

A hierarchical gene diversity analysis (Chakraborty 1980) of the microsatellite data also indicates substantial divergence between the fish from the Callahan Creek watershed and those from

TABLE 2.—Allele frequencies and expected average heterozygosities (H_s) at five microsatellite loci for wild redband trout from seven sites in the Kootenai River drainage and three hatchery populations. Population codes are explained in Table 1.

Locus	Allele size	Wild populations							Hatchery populations		
		EFYR	BASIN	PORC	YRBF	NFCA	NFCB	SFCC	MSPR	GERR	ARLEE
*Ocl 2**	128	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000	0.000	0.021
	132	0.000	0.000	0.000	0.086	0.000	0.000	0.000	0.020	0.000	0.479
	134	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.125
	136	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
	140	0.000	0.000	0.000	0.483	1.000	0.852	0.984	0.800	0.968	0.146
	142	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.020	0.032	0.000
	144	0.292	0.483	0.773	0.000	0.000	0.056	0.000	0.080	0.000	0.000
	146	0.583	0.431	0.182	0.414	0.000	0.074	0.000	0.040	0.000	0.229
	148	0.125	0.086	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*Omy 0004**	130	0.000	0.000	0.000	0.097	0.000	0.000	0.000	0.017	0.000	0.103
	134	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.241
	136	0.000	0.000	0.000	0.161	0.000	0.111	0.000	0.138	0.000	0.241
	138	0.875	0.733	0.818	0.274	0.000	0.037	0.000	0.000	0.017	0.000
	140	0.031	0.067	0.000	0.048	0.000	0.074	0.000	0.052	0.150	0.190
	144	0.000	0.000	0.000	0.290	0.000	0.000	0.000	0.086	0.000	0.052
	146	0.063	0.067	0.182	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	148	0.000	0.000	0.000	0.048	0.000	0.000	0.015	0.000	0.000	0.000
	150	0.031	0.133	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	152	0.000	0.000	0.000	0.032	1.000	0.759	0.985	0.534	0.750	0.000
	156	0.000	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.050	0.086
	158	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.103	0.017	0.086
	160	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.069	0.000	0.000
*Rgt 6-1**	149	0.000	0.000	0.000	0.052	0.000	0.000	0.000	0.033	0.000	0.161
	151	0.031	0.069	0.000	0.000	0.000	0.000	0.000	0.083	0.050	0.000
	153	0.000	0.000	0.000	0.034	0.000	0.000	0.000	0.000	0.000	0.000
	159	0.000	0.000	0.000	0.190	0.000	0.036	0.000	0.000	0.000	0.554
	161	0.063	0.000	0.000	0.000	1.000	0.839	0.984	0.067	0.083	0.000
	163	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000
	167	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.000	0.017	0.000
	169	0.313	0.207	0.182	0.000	0.000	0.018	0.000	0.000	0.233	0.000
	171	0.031	0.000	0.045	0.017	0.000	0.000	0.000	0.533	0.167	0.018
	173	0.406	0.552	0.182	0.707	0.000	0.036	0.016	0.033	0.117	0.196
	175	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.133	0.333	0.054
	179	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.117	0.000	0.018
	181	0.156	0.172	0.591	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*Oneμ11**	142	0.313	0.183	0.273	0.467	0.594	0.625	0.561	0.586	0.667	0.600
	144	0.688	0.817	0.727	0.533	0.406	0.375	0.439	0.362	0.333	0.400
	146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.052	0.000	0.000
*Ssa 197**	111	1.000	0.893	0.850	0.833	0.323	0.407	0.350	0.370	0.328	0.481
	115	0.000	0.107	0.150	0.167	0.677	0.593	0.650	0.630	0.672	0.519
H_s		0.399	0.425	0.404	0.533	0.187	0.386	0.211	0.545	0.436	0.633

the upper Yaak River watershed as well as relatively little divergence within watersheds. Of the total genetic diversity among these samples (H = 0.584), 40.7% is due to allele frequency differences between the two watersheds, 3.3% to allele frequency differences among populations within watersheds, and the remaining 56.0% to genetic variation within populations. This differentiation is reflected in an index of genetic diversity (G_{ST}) among these populations of 0.426.

The pattern of divergence revealed by a comparison of allozyme frequencies is generally concordant with that revealed by the microsatellites. The genetic distance estimates, however, are not as large. The UPGMA unrooted tree based on CSE values produced three major groups of samples (Figure 3); those in the upper Yaak River drainage, the Callahan Creek and Kamloops redband trout samples, and the very divergent coastal Arlee. Interestingly, despite their recent common ancestry, two of the most divergent samples within the Callahan Creek and Kamloops group are the Duncan Kamloops redband trout from Ennis National Fish Hatchery and Murray Springs State Trout Hatchery.

It is important to note that the results of the above analyses are highly influenced by genetic differences among the samples at *LDH-B2**. The

TABLE 3.—Allele frequencies and expected average heterozygosities (H_S) at 14 allozyme loci for wild redband trout from five sites in the Kootenai River basin and four hatchery populations. The frequency of the 100 allele at each locus is not included. Population codes are explained in Table 1.

		Wild populations					Hatchery populations			
Locus	Allele	EFYR	BASIN	PORC	NFCA	SFCC	ENNIS	MSPR	GERR	ARLEE
*CK-A1**	76	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.050
*G3PDH-1**	140	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000
*GPI-B2**	Null	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000
*IDDH**	200	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.000	0.000
*mIDHP-2**	140	0.000	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.140
*sIDHP-1,2**	114	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.045
	71	0.333	0.500	0.469	0.096	0.140	0.320	0.258	0.322	0.055
	40	0.167	0.000	0.031	0.231	0.250	0.087	0.117	0.138	0.110
*LDH-B2**	76	1.000	0.964	0.896	0.635	0.280	0.767	0.492	0.782	0.030
*LDH-C**	95	0.000	0.000	0.000	0.019	0.060	0.000	0.000	0.000	0.070
*sMDH-A1,2**	120	0.000	0.000	0.000	0.010	0.000	0.017	0.000	0.000	0.000
*sMDH-B1,2**	125	0.000	0.000	0.000	0.000	0.000	0.023	0.009	0.000	0.000
	83	0.000	0.000	0.000	0.000	0.010	0.000	0.004	0.165	0.135
*PEPA-1**	115	0.000	0.000	0.000	0.000	0.000	0.000	0.070	0.012	0.000
*PGM-2**	90	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.070
*PGM-1r**	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.990
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010
*sSOD-1**	152	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.220
H_S		0.044	0.041	0.054	0.072	0.077	0.070	0.103	0.087	0.115

frequency of *LDH-B2*76* varies from 1.0 to 0.89 in the samples from the upper Yaak River, then progressively decreases from 0.77 in the Ennis Duncan sample to 0.03 in the Arlee sample.

Hierarchical gene diversity analysis of the allozyme data, like that of the microsatellite data, indicates substantial divergence between the fish from the Callahan Creek and upper Yaak River watersheds but relatively little divergence within watersheds. Of the total genetic variation detected (0.071), 15.5% is due to genetic differences between fish from the two watersheds, only 3.4% to genetic differences between populations within watersheds, and 81.1% to genetic variation within populations.

We combined both microsatellite and allozyme data for the principal components analysis (Figure 4). We eliminated the samples from the Yaak River below the falls, Ennis National Fish Hatchery, and North Fork Callahan Creek (below the falls) from the analysis because we had data from only one technique for these samples. The pattern of divergence among populations is generally concordant with that from the UPGMA analyses (Figures 2, 3). The first principal component, which accounts for 67% of the allele frequency variation, highlights the genetic divergence among the upper Yaak River samples and the Callahan Creek samples while the Kamloops redband trout and the Arlee samples occupy an intermediate position. The second principle component, which accounts for an additional 19% of the allele frequency variation, mainly contrasts the Arlee coastal rainbow trout sample with all other samples.

TABLE 4.—Number of loci at which microsatellite allele frequencies are significantly ($P < 0.05$) different in pairwise comparisons of wild redband trout from seven sites in the Kootenai River drainage and three hatchery populations. The *P*-values were corrected using the Bonferroni test (Rice 1989). Population codes are explained in Table 1.

	Population								
Population	EFYR	BASIN	PORC	YRBF	NFCA	NFCB	SFCC	MSPR	GERR
BASIN	0								
PORC	1	1							
YRBF	3	4	3						
NFCA	4	5	4	4					
NFCB	5	5	5	4	3				
SFCC	4	5	4	4	0	2			
MSPR	5	5	5	4	3	2	3		
GERR	5	5	5	4	2	2	2	3	
ARLEE	4	5	4	4	3	3	3	3	3

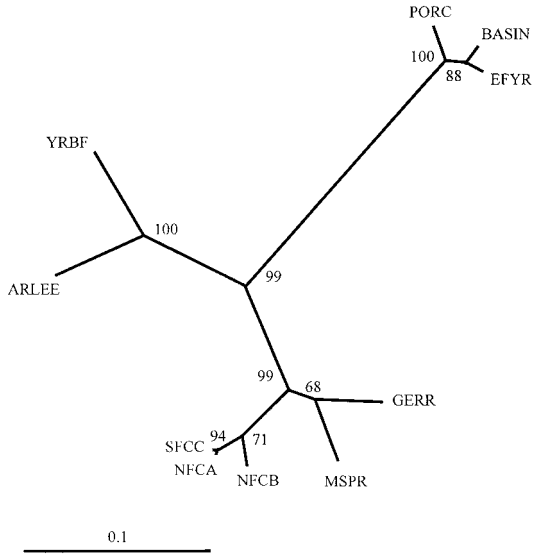

FIGURE 2.—Dendrogram based on Cavalli-Sforza and Edwards chord distance calculated from the microsatellite allele frequencies of samples using the unweighted pair-group method with arithmetic averages. Bootstrap values are shown where branch points had more than 50% support. Population codes are explained in Table 1.

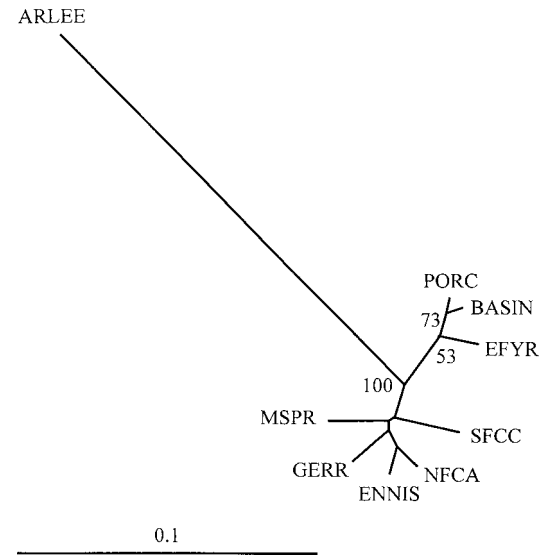

FIGURE 3.—Dendrogram based on Cavalli-Sforza and Edwards chord distance calculated from allozyme allele frequencies using the unweighted pair-group method with arithmetic averages. Bootstrap values are shown where branch points had more than 50% support. Population codes are explained in Table 1.

Hybridization with Coastal Rainbow Trout

The microsatellite data indicate that the fish collected from the Yaak River below the falls and Murray Springs Hatchery have very likely hybridized with coastal rainbow trout. Excluding these two samples, six alleles were unique to coastal rainbow trout (Table 2). Four of these apparent coastal rainbow trout markers were detected in the lower Yaak River, and all six were observed in the Murray Springs sample. Furthermore, the lower Yaak River fish appear to be much more similar to Arlee coastal rainbow trout than they are to any of the other fish sampled (Figure 2). The simplest explanation for these results is hybridization with coastal rainbow trout. This result is not surprising, as other samples of redband trout from the Yaak River have been found to contain coastal rainbow trout genes (Sage et al. 1992).

The allozyme data also strongly indicate that coastal rainbow trout genes occur in fish from Murray Springs. The frequency of *LDH-B2*76* in the Duncan Kamloops redband trout maintained at the Ennis National Fish Hatchery was 0.77 in the 1987 year-class and 0.76 in the 1992 year-class (R. F. Leary, unpublished data). Allele *sSOD-1*152* was detected only in the 1992 sample and at a frequency of only 0.01 (Leary, unpublished data). Thus, these samples strongly indicated that the fish are redband trout (see Appendix). In contrast, the frequency of *LDH-B2*76* had decreased to 0.49 and that of *sSOD-1*152* increased to 0.02 in the Murray Springs sample. Furthermore, variant alleles at seven other loci detected in the Murray Springs sample were not detected in the other two Kamloops redband trout samples (Table 3). The alleles at all these loci are commonly detected in populations of coastal rainbow trout (Leary et al.

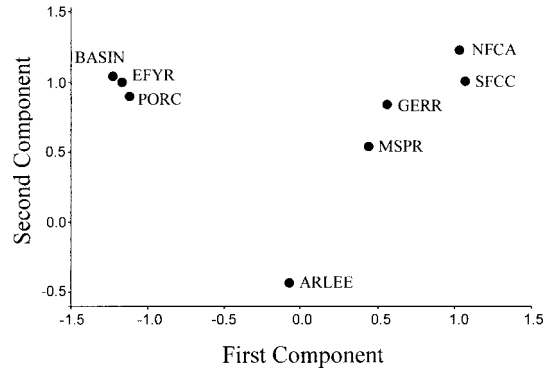

FIGURE 4.—Plot of the first two principle components calculated from the combined microsatellite and allozyme data. The first component accounts for 67% of the allele frequency variation and the second component for 19%. Population codes are explained in Table 1.

1983a). Overall, the simplest explanation for these marked genetic differences between the Murray Springs samples and the Duncan Kamloops redband trout samples from other hatcheries is hybridization with coastal rainbow trout.

Hybridization with coastal rainbow trout in the Murray Springs fish is not readily apparent in the allozyme UPGMA dendrogram (Figure 3). This is a reflection of the fact that the differences in *LDH-B2*76* frequencies account for most of the variation among populations. The Kamloops redband trout and Callahan Creek drainage samples have intermediate *LDH-B2*76* frequencies and thus tend to group together. Hybridization in this analysis is reflected only by the divergence between Murray Springs and the other two Kamloops redband trout samples.

Discussion

Both the microsatellite and allozyme data sets lead to similar conclusions regarding the population genetic structure of redband trout from the Kootenai River drainage. Because the lower Yaak River and Murray Springs fish appear to be hybridized with coastal rainbow trout, they are of no value in redband trout conservation and restoration.

Relatively little genetic divergence occurs among the populations in Callahan Creek and the upper Yaak River and in the Kamloops redband trout populations in Kootenay Lake. Both techniques indicate that approximately 3.5% of the total genetic variation is due to differences among populations within the two Kootenai River drainage watersheds. This suggests that the population structure within these watersheds has remained relatively stable between the collections used to obtain allozyme and microsatellite data.

The pattern of genetic variation revealed by both allozymes and microsatellites indicates geographic structuring among populations of redband trout in the Kootenai River drainage. There are substantial allele frequency differences between the Callahan Creek and upper Yaak River watersheds at several loci. The percent of total genetic variation detected due to differences between fish from the two watersheds was much higher for microsatellites (41%) than for allozymes (16%) but was quite high for both. The value from allozyme data is 1.5−2 times that obtained in a comparison of redband trout populations from the Snake River drainage (8%, Wishard et al. 1984; 7.4%, Leary, unpublished data) and the Columbia River drainage in eastern Washington (10%; Leary 1997) and over 9 times that of coastal rainbow trout sampled from Oregon and Washington (1.7%, Reisenbichler and Phelps 1985).

The high level of genetic divergence indicates that there has been very limited gene flow (genetic exchange) between the fish in Callahan Creek and those in the upper Yaak River for an extended period of time. The existence of three falls limiting upstream migration increases the possibility that adaptive differences exist between fish from the two drainages. With limited gene flow, over time even weak selective differences can result in local adaptations. The possibility of adaptive differences cautions against transferring fish between the drainages. Such transfers could disrupt local adaptations and compromise population productivity and viability.

The Kootenay Lake Kamloops redband trout are also quite divergent from the upper Yaak River redband trout (Figures 2–4). Allele frequencies at all five microsatellite loci are significantly different between the Gerrard stock and the upper Yaak River redband trout population (Table 4). The Kamloops redband trout hatchery stocks are more similar, however, to the Callahan Creek fish (Figures 2–4). Gene flow between Kootenay Lake and Callahan Creek redband trout is possible since migratory Kamloops redband trout have been found in the Kootenai River upstream of the mouth of Callahan Creek and the barriers on Callahan Creek could have been breached by migrating Kamloops redband trout in the past. However, mitochondrial DNA data reveal nearly fixed differences between the Kootenay Lake Kamloops stock and all of the redband trout populations of the Kootenai River. A restriction fragment length polymorphism analysis of the ND2 region using eight restriction enzymes indicates that all but one fish from the Kootenai River tributaries exhibited the C haplotype, while only one had the A haplotype. In contrast, 28 Gerrard Kamloops fish exhibited the A haplotype, 6 the B haplotype, and only 1 the C haplotype (M. Powell, Hagerman, Idaho, personal communication).

Montana Fish, Wildlife, and Parks is considering the development of a redband trout broodstock for reintroduction into areas from which they have been extirpated as well as the supplementation of populations at risk of extinction in the Kootenai River drainage (Muhlfeld et al. 2001a). Considering the genetic differences between the resident redband trout and the migratory Kamloops redband trout it is probably inappropriate to use a

Kootenay Lake stock for reintroduction into any of the tributaries of the Kootenai River.

Transfers of redband trout that were confined within the Callahan Creek or upper Yaak River watershed would probably not cause adverse genetic changes to those populations. Thus, our data suggest that a restoration program would require a separate broodstock for each watershed within the Kootenai River drainage where redband trout exist. In addition, they indicate that separate broodstocks may be required for other watersheds as well.

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References

Allendorf, F. W., D. M. Espeland, D. T. Scow, and S. Phelps. 1980. Coexistence of native and introduced rainbow trout in the Kootenai River drainage. Proceedings of the Montana Academy of Sciences 39: 28–36.

Allendorf, F. W., N. Mitchell, N. Ryman, and G. Stahl. 1977. Isozyme loci in brown trout (*Salmo trutta*): detection and interpretation from population data. Hereditas 86:179–190.

Allendorf, F. W., and G. H. Thorgaard. 1984. Tetraploidy and the evolution of salmonid fishes. Pages 1–53 *in* B. Turner, editor. Evolutionary genetics of fishes. Plenum, New York.

Allendorf, F. W., and F. M. Utter. 1979. Population genetics. Pages 407–454 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, volume 8. Academic Press, New York.

Behnke, R. J. 1992. Native trout of western North America. American Fisheries Society, Monograph 6, Bethesda, Maryland.

Campton, D. E., and J. M. Johnston. 1985. Electrophoretic evidence for a genetic admixture of native and nonnative rainbow trout in the Yakima River, Washington. Transactions of the American Fisheries Society 114:782–793.

Cavalli-Sforza L. L., P. Menozzi, and A. Piazza. 1993. The history and geography of human genes. Princeton University Press, Princeton, New Jersey.

Chakraborty, R. 1980. Gene-diversity analysis in nested subdivided populations. Genetics 96:721–726.

Condrey, M. J., and P. Bentzen. 1998. Characterization of coastal cutthroat trout (*Oncorhynchus clarki clarki*) microsatellites and their conservation in other salmonids. Molecular Ecology 7:787–789.

Currens, K. P., C. B. Schreck, and H. W. Li. 1990. Allozyme and morphological divergence of rainbow trout (*Oncorhynchus mykiss*) above and below waterfalls in the Deschutes River, Oregon. Copeia 1990:730–746.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package), version 3.5c. University of Washington, Department of Genetics, Seattle.

Foote, C. J., C. C. Wood, and R. E. Withler. 1989. Biochemical genetic comparison of sockeye salmon and kokanee, the anadromous and non-anadromous forms of *Oncorhynchus nerka*. Canadian Journal of Fisheries and Aquatic Sciences 46:149–158.

Hanzel, D. A. 1960. The distribution of the cutthroat trout (*Salmo clarki*) in Montana. Proceedings of the Montana Academy of Sciences 19:32–71.

Holm, L.-E., and K. Brusgaard. 1998. Two polymorphic dinucleotide repeats in rainbow trout (*Oncorhynchus mykiss*). Animal Genetics 30:162–163.

Holton, G. D. 1990. A field guide to Montana fishes. Montana Department of Fish, Wildlife and Parks, Helena.

Huston, J. E. 1995. A report on the Kootenai River drainage native species search for the U.S. Fish and Wildlife Service. Montana Fish, Wildlife, and Parks, Kalispell.

Knudsen, K. L., F. W. Allendorf, and R. F. Leary. 1982. Genetic analysis of DeSmet rainbow trout from Harrison Lake and comparison to other rainbow trout stocks. University of Montana, Population Genetics Laboratory Report 82/6, Missoula.

Leary, R. F. 1989. Genetic and meristic variation in Bellaire, Fish Lake, Tasmanian, and Whytheville rainbow trout. University of Montana, Population Genetics Laboratory Report 89/6, Missoula.

Leary, R. F. 1997. Hybridization between introduced and native trout in waters of the Colville National Forest. University of Montana, Wild Trout and Salmon Genetics Laboratory Report 97/3, Missoula.

Leary, R. F. 2001. Allozyme analysis of rainbow trout populations in the lower Snake River drainage. University of Montana, Wild Trout and Salmon Genetics Laboratory Report 01/1, Missoula.

Leary, R. F., and F. W. Allendorf. 1981. Genetic analysis of rainbow trout in the Ivan Hale Hatchery, Idaho. University of Montana, Population Genetics Laboratory Report 81/4, Missoula.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983a. Genetic variation in eight strains of rainbow trout maintained by the USFWS in Montana. University of Montana, Population Genetics Laboratory Report 83/5, Missoula.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1983b. Genetic analysis of four rainbow trout populations from Owyhee County, Idaho. University of Montana, Population Genetics Laboratory Report 83/6, Missoula.

Leary, R. F., F. W. Allendorf, and G. K. Sage. 1995. Hybridization and introgression between introduced

and native fish. Pages 91–101 *in* J. L. Schramm, Jr. and R. G. Piper, editors. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society, Symposium 15, Bethesda, Maryland.

Leary, R. F., and H. E. Booke. 1990. Starch gel electrophoresis and species distinctions. Pages 141–170 *in* C. B. Shreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Leary, R. F., K. L. Knudsen, and F. W. Allendorf. 1981. Genetic variation in rainbow trout, brown trout, and brook trout from the Harriman Trout Company, Montana. University of Montana, Population Genetics Laboratory Report 81/7, Missoula.

Lee, D. C., J. R. Sedell, B. E. Reiman, R. F. Thurow, and J. E. Williams. 1997. Broadscale assessment of aquatic species and habitats. *In* T. M. Quigley and S. J. Arbelbide, editors. An assessment of ecosystem components in the interior Columbia basin and portions of the Klamath and Great basins. U.S. Forest Service General Technical Report PNW-GTR.

Minitab, Inc. 1996. Minitab, release 11.21. Minitab Inc., State College, Pennsylvania.

MiraiBio, Inc. 1999. FMBIO analysis, version 8.0. MiraiBio, Inc., Alameda, California.

Muhlfeld, C. C. 1999. Seasonal habitat use by redband trout (*Oncorhynchus mykiss gairdneri*) in the Kootenai River drainage, Montana. Master's thesis. University of Idaho, Moscow.

Muhlfeld, C. C., D. H. Bennett, and B. Marotz. 2001a. Summer habitat use by Columbia River redband trout in the Kootenai River drainage, Montana. North American Journal of Fisheries Management 21:223–235.

Muhlfeld, C. C., D. H. Bennett, and B. Marotz. 2001b. Fall and winter habitat use and movement by Columbia River redband trout in a small stream in Montana. North American Journal of Fisheries Management 21:170–177.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences of the USA 70:3321–3323.

O'Reilly, P. T., L. C. Hamilton, S. K. McConnell, and J. M. Wright. 1996. Rapid detection of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Canadian Journal of Fisheries and Aquatic Sciences 53:2292–2298.

Page, R. D. M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. Comparative and Applied Bioscience 12:357–358.

Raymond, M., and F. Rousset. 1995. GENEPOP, version 1.2: population genetics software for exact tests and ecumenicism. Journal of Heredity 83:248–249.

Reisenbichler, R. R., and S. R. Phelps. 1985. Genetic structure of steelhead trout (*Salmo gairdneri*) from the north coast of Washington state. U.S. Fish and Wildlife Service, Seattle National Fishery Research Center Report, Seattle.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Sage, G. K., R. F. Leary, and F. W. Allendorf. 1992. Genetic analysis of 45 populations in the Yaak River drainage, Montana. University of Montana, Wild Trout and Salmon Genetics Laboratory Report 92/3, Missoula.

Sakamoto, T., N. Okamoto, and Y. Ikeda. 1996. Application of PCR primer pairs from rainbow trout to detect polymorphisms of CA repeat DNA loci in five confamilial species. Fisheries Science 62:552–555.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:685–693.

Williams, J. E., J. E. Johnson, D. A. Hendrickson, S. Contreras-Balderas, J. D. Williams, M. Avarro-Mendoza, D. E. McAllister, and J. E. Deacon. 1989. Fishes of North America endangered, threatened, or of special concern. Fisheries 14(6):2–20.

Williams, R. N., R. F. Leary, and K. P. Currens. 1997. Localized genetic effects of a long-term hatchery stocking program on resident rainbow trout in the Metolius River, Oregon. North American Journal of Fisheries Management 17:1079–1093.

Williams, R. N., D. K. Shiozawa, J. E. Carter, and R. F. Leary. 1996. Genetic detection of putative hybridization between native and introduced rainbow trout populations in the upper Snake River. Transactions of the American Fisheries Society 125:387–401.

Wishard, L. N., J. E. Seeb, F. M. Utter, and D. Stefan. 1984. A genetic investigation of suspected redband trout populations. Copeia 1984:120–132.

Appendix: Additional Allele Frequencies

TABLE A.1.—Allele frequencies of populations of Columbia River redband trout and coastal rainbow trout for *LDH-B*76* and *sSOD-1*152*.

Population	*LDH-B2*76*	*sSOD-1*152*	Source
Deschutes River, Oregon			
Bakeoven Creek	0.596	0.093	Currens et al. 1990
Barlow Creek	1.000	0.009	Currens et al. 1990
Big Log Creek	0.674	0.021	Currens et al. 1990
Buck Hollow Creek	0.619	0.091	Currens et al. 1990
Deschutes River	0.484	0.075	Currens et al. 1990
East Foley Creek	0.667	0.043	Currens et al. 1990
Gate Creek	0.000	0.025	Currens et al. 1990
Little Badger Creek	0.000	0.000	Currens et al. 1990
Metolius River	0.031	0.000	Williams et al. 1997
Nena Creek	0.606	0.056	Currens et al. 1990
Three Mile Creek	0.020	0.030	Currens et al. 1990
Upper Tygh Creek	0.000	0.000	Currens et al. 1990
Middle Columbia River, Washington			
Umatilla River	0.610	0.080	Campton and Johnston 1985
Wells Dam	0.490	0.090	Campton and Johnston 1985
Upper Columbia River, Washington			
Barnaby Creek	0.560	0.000	Leary 1997
Deadman Creek	0.840	0.000	Leary 1997
Lane Creek	0.820	0.000	Leary 1997
Nancy Creek	0.500	0.000	Leary 1997
North Fork Deadman Creek	0.700	0.000	Leary 1997
North Fork Trout Creek	1.000	0.000	Kanda[a]
South Fork Chewelah Creek	0.791	0.021	Kanda[a]
Tonata Creek	0.860	0.000	Leary 1997
West Fork Trout Creek	1.000	0.000	Kanda[a]
Kootenai River, Idaho and Montana			
Boyd Creek	1.000	0.000	Sage et al. 1992
Callahan Creek	0.300	0.000	Sage[b]
East Fork Basin Creek	0.944	0.000	Sage et al. 1992
Grass Creek	0.480	0.000	Sage[b]
North Fork Yaak River	0.990	0.000	Sage et al. 1992
Saddle Creek	1.000	0.000	Sage[b]
West Fork Basin Creek	0.911	0.000	Sage et al. 1992
Salmon River, Idaho			
Bergamin Creek	0.680	0.010	Allendorf and Leary[c]
Chamberlin Creek	0.760	0.010	Allendorf and Leary[c]
Five Mile Creek	0.354	0.000	Allendorf and Leary[c]
Snake River, Idaho			
Cabin Creek	0.670	0.000	Williams et al. 1996
Columbia Creek	0.680	0.040	Williams et al. 1996
Doby George Creek	0.420	0.070	Williams et al. 1996
Hordan Creek	0.640	0.040	Wishard et al. 1984
King Hill Creek	0.770	0.050	Williams et al. 1996
Sinker Creek	0.683	0.027	Leary et al. 1983b
Middle Fork Brownlee Creek	0.782	0.000	Leary 2001
Dukes Creek	0.500	0.000	Leary 2001
Hells Canyon Reservoir	0.571	0.018	Leary 2001
Indian Creek (lower)	0.600	0.086	Leary 2001
Indian Creek (upper)	0.950	0.050	Leary 2001
Cove Creek	0.593	0.000	Leary 2001
Middle Malad River	0.717	0.050	Leary 2001
Clear Creek	0.793	0.000	Leary 2001
Elk Creek	0.550	0.000	Leary 2001
East Fork Pine Creek	0.705	0.023	Leary 2001
Pine Creek	0.333	0.019	Leary 2001
Powder River	0.516	0.043	Leary 2001
Rock Creek	0.525	0.050	Leary 2001
Sheep Creek	0.766	0.017	Leary 2001
Sturgill Creek	0.942	0.008	Leary 2001
Bear Creek	0.817	0.000	Leary 2001
Crooked River	0.617	0.000	Leary 2001
Lick Creek	0.567	0.000	Leary 2001

Appendix: Additional Allele Frequencies

TABLE A.1.—Continued.

Population	*LDH-B2*76*	*sSOD-1*152*	Source
Columbia River redband hatchery strains			
Oxbow	0.658	0.067	Leary 2001
Pahsimeroi	0.651	0.016	Leary 2001
Dworshak	0.700	0.000	Campton and Johnston 1985
Coastal rainbow trout hatchery strains			
Allison	0.050	0.150	Leary[d]
Arlee	0.030	0.220	Leary[d]
Bellaire	0.000	0.080	Leary 1989
Bow	0.020	0.184	Leary[d]
Cape Cod	0.000	0.240	Williams et al. 1996
DeSmet	0.090	0.130	Knudsen et al. 1982
Eagle	0.010	0.333	Wishard et al. 1984
Eagle Lake	0.000	0.250	Williams et al. 1996
Erwin	0.000	0.440	Williams et al. 1996
Fall	0.030	0.310	Leary[d]
Fish Lake	0.020	0.290	Leary[d]
Goldendale	0.000	0.290	Campton and Johnston 1985
Harrison	0.110	0.290	Leary[d]
Hayspur	0.000	0.380	Williams et al. 1996
Jocko	0.035	0.194	Leary et al. 1981
McConnaughy	0.000	0.100	Williams et al. 1996
Nampa	0.130	0.170	Leary et al. 1983b
Paint Bank	0.050	0.670	Leary[d]
Pekisko	0.033	0.208	Leary[d]
Post Creek	0.000	0.187	Leary et al. 1981
Raven	0.000	0.288	Leary[d]
Shasta	0.000	0.336	Leary et al. 1983a
South Tacoma	0.000	0.350	Campton and Johnston 1985
Sullivan	0.016	0.250	Leary[d]
Tasmanian	0.000	0.240	Leary et al. 1983a
Whytheville	0.000	0.230	Williams et al. 1996
Introduced coastal rainbow trout populations			
Colorado River, Colorado	0.020	0.400	Leary[d]
Depuy's Spring, Montana	0.000	0.179	Leary[d]
Firehole River, Wyoming	0.051	0.276	Leary[d]
Gunnison River, Colorado	0.000	0.531	Leary[d]
Jakey's Fork, Wyoming	0.010	0.315	Leary[d]
Madison River, Montana	0.120	0.400	Leary[d]
Marias River, Montana	0.205	0.192	Leary[d]
South Platte River, Colorado	0.010	0.292	Leary[d]
Tobacco River, Montana	0.083	0.222	Leary and Allendorf 1981

[a] Kanda, N. and R. F. Leary. Unpublished data.
[b] Sage, G. K. Unpublished data.
[c] Allendorf, F. W, and R. F. Leary. Unpublished data.
[d] Leary, R. F. Unpublished data.