

Microsatellite diversity and conservation of a relic trout population: McCloud River redband trout

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Abstract

Rainbow trout native to the McCloud River, California, USA (*Oncorhynchus mykiss stonei*) are thought to represent a relic, nonanadromous trout adapted to harsh, fragmented environments. These fish, commonly named McCloud River 'redband' trout, survive in their most primitive form in a small, spring-fed stream, Sheepheaven Creek, in the upper McCloud River drainage. Turn-of-the-century fisheries records document both coastal anadromous steelhead and freshwater resident trout within the McCloud River drainage. The phylogenetic position of the McCloud River redband trout within *O. mykiss* has been debated for over 50 years. Based on phenotypic evidence, these fish were first reported as 'southern Sierra golden trout' by Wales in 1939. Behnke (1970) considered them a relic subspecies of nonanadromous, fine-scaled trout. Allozyme and mitochondrial DNA evidence suggested a coastal lineage. In this study, we examined within- and among-basin genetic associations for Sheepheaven Creek redband trout using 11 microsatellite loci. Within-basin analyses supported unique genetic characteristics in Sheepheaven Creek's trout in comparisons with other McCloud River rainbow trout. Microsatellite data supported significant independence between Sheepheaven Creek fish and hatchery rainbow trout. Inter-basin genetic distance analyses positioned Sheepheaven Creek fish with samples collected from Lassen Creek, a geographically proximate stream containing inland redband trout. California's redband trout shared a close genetic association with Little Kern River golden trout (*O.m. whitei*) and isolated rainbow trout from Rio Santo Domingo, Baja, Mexico (*O.m. nelsoni*), suggesting a vicariant distribution of microsatellite diversity throughout the southern range of this species.

Keywords: McCloud River, microsatellites, molecular systematics, *Oncorhynchus mykiss*, redband trout

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Introduction

Trout populations in North America (*Oncorhynchus mykiss* ssp.) are thought to be polyphyletic containing both coastal anadromous (steelhead) and interior freshwater resident (redband) lineages, with both groups occurring sympatrically in several large river basins. Extensive hybridization between interior and coastal trout are thought to have occurred during and after the last glacial

period with only areas isolated by barriers retaining pure ancestral trout populations (Behnke 1992). Relic interior trout populations with significant morphological and/or genetic differentiation from coastal rainbow trout have been documented in headwater areas of the Kern River (California golden trout), Columbia River (redband trout), and Sacramento River (McCloud River redband trout).

The McCloud River (Fig. 1), once part of the larger Sacramento River drainage, has been isolated from anadromous fish migrations for the last 50 years by the Shasta Dam. Rainbow trout populations found in the McCloud River, however, are thought to originally derive from both resident and anadromous forms of *O. mykiss* (Berg 1987). Tributaries entering the upper McCloud River from the north flow from the base of a dormant volcano, Mount

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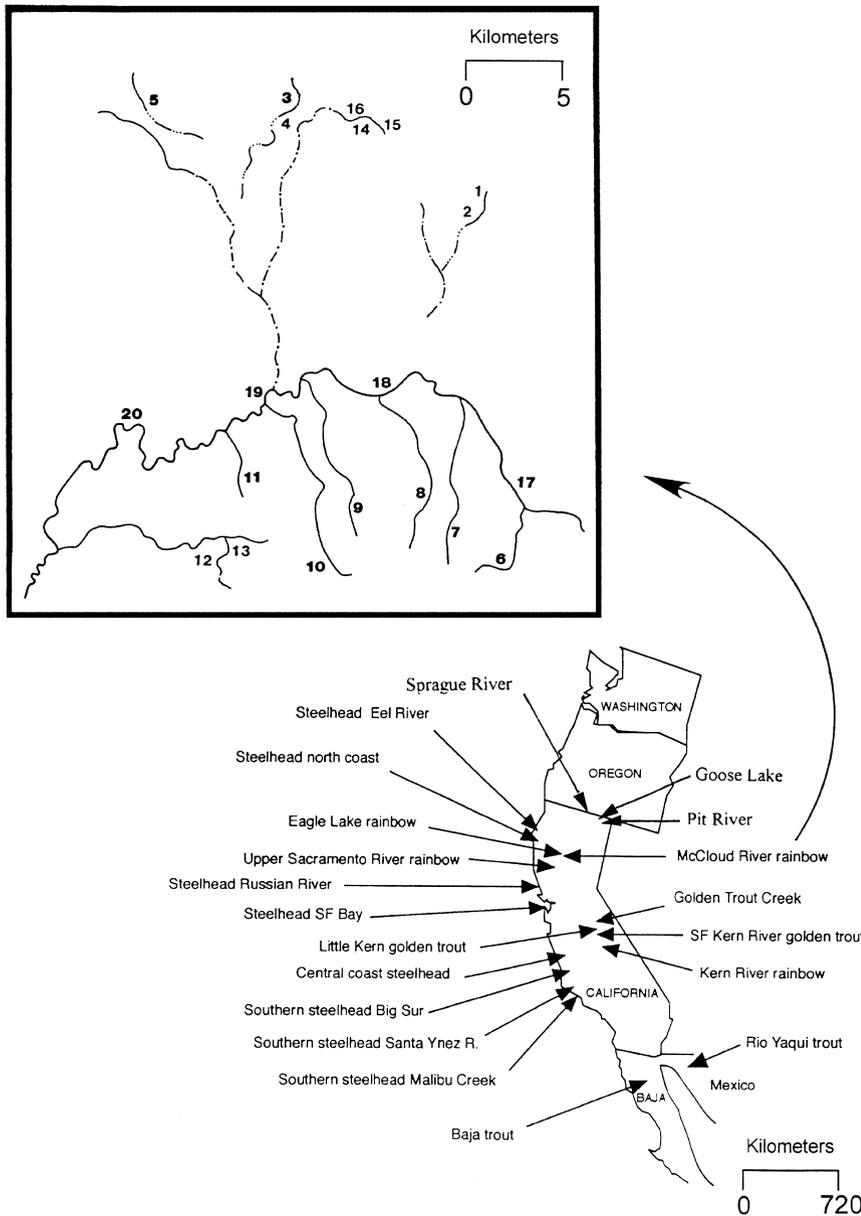


Fig. 1 Map depicting sample collection localities in Oregon, Mexico, California, and throughout the McCloud River drainage (inset). The numbers in the McCloud River drainage represent: (1) Sheepheaven Creek above putative barrier; (2) Sheepheaven Creek below barrier; (3) Trout Creek below water point; (4) Trout Creek above culvert; (5) Swamp Creek; (6) Moosehead Creek; (7) Dry Creek; (8) Bull Creek; (9) Shady Gulch Creek; (10) Tate Creek; (11) Blue Heron Creek; (12) Steep Hollow Creek above culvert; (13) Steep Hollow Creek below culvert; (14) Edson Creek enclosure (upstream); (15) upper Edson Creek; (16) lower Edson Creek; (17) McCloud River above lower railroad crossing; (18) McCloud River above Algoma Bridge; (19) McCloud River below Tate Creek; (20) McCloud River below Upper Falls.

Shasta. These streams have been heavily influenced by volcanism with residual lava formations in this area causing significant underground percolation of freshwater flows and isolated spring-fed trout habitats.

In general, flows to the upper McCloud River from its northern tributaries are subsurface through many miles of dry channel during most of the year. Small populations of rainbow trout found in fragmented stream habitats in this area are thought to represent an undescribed relic subspecies of nonanadromous, fine-scaled trout native to desiccating basins from southern Oregon to the Pit and Sacramento Rivers in northern California (Behnke 1970). These interior trout received the common name 'redband'

trout (*O.m. stonei*, after Behnke 1992) due to a brick-red lateral stripe that distinguishes this group along with other primitive taxonomic structures, such as the presence of vestigial basibranchial teeth in some specimens (Behnke 1992). Today these fish survive in their most primitive form in a small, spring-fed stream, Sheepheaven Creek, in the upper McCloud River drainage.

McCloud River redband trout have been reported to be closely related to the California golden trout complex of the Kern River basin (*O.m. aquabonita* and *O.m. gilberti*) based on early morphological studies (Schreck & Behnke 1971; Hoopagh 1974; Gold 1977). Both the Kern River and Mount Shasta areas are thought to have served as

refugia during the Pleistocene glaciation (Behnke 1992). An alternative hypothesis was posed by Miller (1972) with many of the morphological traits common to both the McCloud River redband and the Kern River golden trout considered to be primitive characters derived independently from more than one ancestral form, that do not necessarily depict true phyletic associations.

Early genetic studies using protein electrophoresis showed significant genetic separation between coastal rainbow trout and interior redband trout of the Columbia River drainage (Allendorf 1975; Utter & Allendorf 1977). However, Berg (1987) demonstrated that, unlike the redband trout of the Columbia basin, the McCloud River redband trout were dominated by the lactate dehydrogenase allele (*LDH-B2*100*) considered diagnostic for coastal trout. In a subsequent report, Berg presented further molecular analyses of McCloud River trout collected in 1989, and showed distinct differences in allelic frequencies for three electrophoretic loci (*MDH-3A*, *ICDH-3A*, and *SOD*) in fish from Sheepheaven, Moosehead, Edson, and Swamp Creeks, when compared to coastal rainbow trout from three hatchery populations (Berg 1994).

Due to habitat loss, drought, and declining populations, early conservation efforts (1970s) to conserve McCloud redband trout included the transfer of fish from Sheepheaven Creek to two putative 'fishless' tributaries in the McCloud River drainage, Swamp Creek and Trout Creek. Recent drought desiccation (1990–94) in Sheepheaven Creek has further reduced the 'type-locality' population of McCloud River redband trout to less than 200 individuals (D. Weidlein, California Department of Fish and Game (CDFG) personal communication). Due to declining population size the McCloud River redband trout was recently assigned 'candidate' status for listing under the U.S. Endangered Species Act of 1973 (USDI 1996). Their status and distribution is still under review by the Department of the Interior.

In genetic studies of rare or threatened populations non-invasive DNA sampling (fin-clips or scales) has provided a distinct advantage over protein analyses including studies of *O. mykiss* populations in the southwestern United States (Nielsen *et al.* 1994a; Nielsen 1996, 1999). Mitochondrial DNA (mtDNA) studies of California's coastal *O. mykiss* populations demonstrated unprecedented levels of genetic diversity in populations at the southern extent of this species' geographical range (Nielsen *et al.* 1994a; Nielsen 1996). *O. mykiss* mtDNA sequence data showed consistent year-to-year frequencies within sampling localities in California and significant haplotype frequency differences among reproductive phenotypes of trout and salmon within basins (Nielsen *et al.* 1994c; Nielsen 1996, 1999; Quinn *et al.* 1996). The dominant mtDNA haplotype found in trout from the McCloud River drainage, including all haplotypes sequenced from Sheepheaven Creek fish,

were identical to the most common coastal steelhead haplotype (MYS1) from northern California (Nielsen 1997). This haplotype also dominated California's hatchery rainbow trout strains (Nielsen *et al.* 1997b).

Fish from the Sacramento River drainage served as broodstock for the development of California's hatchery rainbow trout strains. The U.S. Fisheries Commission started the Mount Shasta Hatchery strain of rainbow trout at Baird Station on the McCloud River in 1897 (U.S. Fisheries Commission 1872–1901). The Mount Shasta Hatchery strain is thought to have had its origins in both coastal anadromous and resident rainbow trout from the Sacramento River (Busack & Gall 1980). Mount Shasta Hatchery rainbow trout have been transplanted all over the world creating self-sustaining populations of *O. mykiss* on all continents, with the notable exception of Antarctica (Gall & Crandell 1992). Concern for the possible hybridization of native McCloud River redband trout and hatchery fish was generated by the annual stocking of yearling hatchery rainbow trout throughout the summer months in the mainstem McCloud River by CDFG from 1954 to 1994. Berg (1994) demonstrated hatchery introgression at several protein loci in many areas of the McCloud River and hatchery supplementation stopped in 1994.

Tributaries flowing into the upper McCloud River from the south were thought to have a high probability of introgression from hatchery rainbow trout due to seasonal connectivity to areas frequently stocked with hatchery fish (i.e. mainstem habitats). Tributaries flowing into the McCloud River from the north (Sheepheaven, Swamp, Trout, and Edson Creeks), however, are generally not connected by surface flows to the mainstem McCloud River. Only during rare high flow events, which last for very short periods, are they connected to the main river by sufficient flows to allow fish migration into the tributaries. Sheepheaven Creek has no documentation of surface flows reaching the mainstem river.

Many microsatellite studies have investigated cryptic genetic structure in aquatic organisms (FitzSimmons *et al.* 1995; Nielsen 1996; Olsen *et al.* 1996; Nielsen *et al.* 1997a). Due to accelerated mutation rates and a high degree of polymorphism, microsatellite loci have been used to investigate population structure when protein-coding loci and/or mtDNA lack sufficient resolution to reveal fine-scale genetic differentiation (Hughes & Queller 1993; Forbes *et al.* 1995; Tessier *et al.* 1995). Microsatellites have provided a powerful tool for the study of gene flow in various fish species including *O. mykiss* (McConnell *et al.* 1995; Colbourne *et al.* 1996; Wenburg *et al.* 1996; Nielsen & Fountain 1999). Due to our lack of knowledge concerning mutational mechanisms the use of microsatellite loci to depict phyletic relationships, however, remains controversial (Bowcock *et al.* 1994; Di Rienzo *et al.* 1994; Goldstein *et al.* 1995a; Takezaki & Nei 1996; Nielsen *et al.* 1997a).

Location	N	Year	Collector
<u>McCloud River trout</u>			
McCloud River above RR crossing	38	1994	CDFG
McCloud above Algoma Bridge	33	1994	CDFG
McCloud below Tate Creek	39	1994	CDFG
McCloud River below Upper Falls	27	1994	CDFG
Sheepheaven Creek	59	1994	CDFG
Swamp Creek	71	1994	CDFG
Dry Creek	33	1994	CDFG
Tate Creek	57	1994–97	CDFG
Bull Creek	33	1994	CDFG
Shady Gulch Creek	13	1994	CDFG
Trout Creek	26	1994	CDFG
Edson Creek	53	1994	CDFG
Steep Hollow Creek	7	1994	CDFG
Moosehead Creek	33	1994	CDFG
Blue Heron Creek	12	1994	CDFG
<u>Redband Trout</u>			
Couch Creek—Pit River	40	1997	CDFG
Deming Creek—Sprague River, Oregon	37	1997	CDFG
Lassen Creek—Goose Lake	43	1997	CDFG
<u>California Hatchery Rainbow Trout</u>			
Mount Shasta Hatchery strain	36	1994–96	CDFG/JLN
Whitney Hatchery strain	14	1994	CDFG
Hot Creek strain	20	1994	CDFG
Crystal Lake Hatchery Pit/Shasta strain	20	1994	CDFG
Coleman rainbow trout strain	14	1994	CDFG
Eagle Lake strain	14	1994	CDFG
<u>Out-of-Basin Collections</u>			
Northern CA coastal steelhead/trout	72	1992–94	JLN
Southern CA coastal steelhead/trout	85	1994–96	JLN
Kern River rainbow trout	11	1994–95	CDFG
Little Kern golden trout	29	1994	CDFG
Golden Trout Creek golden trout	20	1994	CDFG
South Fork Kern golden trout	33	1994	CDFG
Rio Santo Domingo (Baja) rainbow trout	20	1994–95	GRC
Rio Yaqui rainbow trout	11	1995	BLJ
Total	1023		

CDFG, California Department of Fish and Game; GRC, G. Ruiz-Campos (Facultad de Ciencias, Universidad Autónoma de Baja California); BLJ, B. L. Jensen, U.S. Fish and Wildlife Service, Whiteriver, Arizona.

In this study microsatellite allelic diversity and genetic distance measures were used to draw inference on genetic associations among the McCloud River redband trout and other endemic groups of *O. mykiss* from California, Oregon, and Mexico. We examined the genetic effects of recent conservation efforts directed at the McCloud River redband trout. To study the effects of hatchery supplementation on relic trout stocks in the McCloud River drainage, we compare microsatellite allelic diversity found in six California hatchery rainbow trout strains with self-reproducing trout from 15 localities in the McCloud River drainage, including Sheepheaven Creek.

Materials and methods

Trout caudal fin tissues (2 mm²) were collected noninvasively from hatchery and wild trout populations located throughout California, southern Oregon, and Mexico according to methods given in Nielsen *et al.* (1997a). Dried *Oncorhynchus mykiss* fin tissues were collected from 935 wild and 118 hatchery trout (Table 1). Genetic diversity within the McCloud River drainage was analysed for trout populations above and below a barrier falls on the upper mainstem river, seven tributaries in the southern basin, and four isolated tributaries in the northern

Table 1 Sampling localities and number of fish analysed for 11 microsatellite loci

Locus	Source	No. of alleles	Allelic size range (bp)
Omy2	M. O'Connell (personal communication)*	26	107–177
Omy27	M. O'Connell (personal communication)*	7	99–117
Omy77	Morris <i>et al.</i> (1996)	18	97–155
Omy207	M. O'Connell (personal communication)*	24	96–160
Omy325	M. O'Connell (personal communication)*	18	87–143
Omy2	Scribner <i>et al.</i> (1996)	21	110–290
Omy8	Scribner <i>et al.</i> (1996)	14	144–188
Ots1	M. Banks (personal communication)†	14	151–245
Sfo8	Angers <i>et al.</i> (1995)	15	177–287
Ssa14	McConnell <i>et al.</i> 1995	19	120–168
Ssa289	McConnell <i>et al.</i> 1995	9	108–124

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drainage, including the type-locality for McCloud River redband trout in Sheepeaven Creek. Microsatellite alleles were amplified from 11 loci for 534 McCloud River trout from 15 collection sites (Fig. 1). Intraspecific comparisons between Sheepeaven Creek trout and known redband populations were made by screening microsatellite allelic variation in 40 North Fork Pit River redband trout from Couch Creek, 37 Sprague River red-band from Deming Creek in southern Oregon, and 43 redband trout from Lassen Creek in the Goose Lake drainage.

Genetic diversity in Sheepeaven Creek trout was compared to 281 samples of steelhead/trout collected from basins throughout California and Mexico. Northern California coastal steelhead samples were taken from 11 drainages from the Eel River to the central California coast (Pajora River). Southern California coastal steelhead/trout were collected from three drainages: Big Sur (Carmel River), Santa Ynez River, and Malibu Creek. Kern River rainbow trout were collected from Freeman Creek, Tyndal Creek, and the mainstem Kern River above Milestone Creek. Little Kern River golden trout came from upper Bullfrog Lake, Sheep Creek, and Willow Creek. Golden trout were collected from Johnson Creek and the mainstem Golden Trout Creek. South Fork Kern River golden trout were collected from Taylor, Fay, and Manter Creeks, and the mainstem South Fork Kern River at Ramshaw Meadows. We used 11 fish from the Rio Yaqui drainage, Sonora, Mexico and 20 trout from the Rio Santo Domingo drainage, Baja California Norte, Mexico for comparisons with trout found at the southern extent of the range of *O. mykiss*.

We extracted total genomic DNA from trout fin tissues using Chelex-100 resin (Bio-Rad, Richmond, CA, USA) following methods given in Nielsen *et al.* (1994b). Eleven highly polymorphic microsatellite loci known to amplify dinucleotide repeat units in rainbow trout were amplified

Table 2 Microsatellite primer sources, number of alleles and allelic size ranges for the 11 microsatellite loci found in *Oncorhynchus mykiss*

Table 3 PCR conditions and fluorescent dye used for amplification of 11 microsatellite loci in rainbow trout populations from California, southern Oregon, and Mexico. Primer concentrations for all reactions remained 1 μ M throughout. Multiplex systems for Mykiss A and Mykiss B were run separately in different PCR reactions

	Anneal (°C)	Locus (dye)		
		(6Fam)	(Tet)	(Hex)
Mykiss A	52 30 cycles	Omy27	Ots1	Sfo8 Ssa289
Mykiss B	52 32 cycles	Omy77 Omy207	Ssa14 Omy2	Omy325 Omy8

(Table 2). Microsatellite alleles were visualized manually (32%) or on an ABI 373 automatic sequencer (68%) according to methods given in Nielsen *et al.* (1997a) and Nielsen & Fountain (1999). PCR and multiplex conditions adapted from Nielsen & Fountain (1999) are given in Table 3. Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard (P-E Biosystems, Foster City, CA, USA), *O. mykiss* DNA samples of known size that were rerun on each gel, and a double-stranded reference marker developed in our laboratory showing the most common alleles available for each locus. GENESCAN (version 1.1) and GENOTYPER version 2.1 (P-E Biosystems) DNA fragment analysis software packages were used to score, bin, and output allelic (and genotypic) designations for fish run on the automatic sequencer. Allele scoring consistency between systems (manual and automatic sequencing gels) was insured by rerunning 19.4% of all samples on gels under different systems and scored independently.

Analyses of heterozygosity and Fisher's exact tests for Hardy-Weinberg equilibrium (HW) were performed using ARLEQUIN (Schneider *et al.* 1997). Global tests for linkage disequilibrium were performed between all possible pairs of microsatellite loci using GENEPOP (version 3.1a; Raymond & Rousset 1997). Population independence between paired comparisons of allelic frequencies were tested using Fisher's exact tests, based on a Markov chain adaptation of row-by-column contingency tables (GENEPOP). Statistical significance levels for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989). A measure of the number of migrants (N_m) was calculated as a surrogate for gene flow using the private allele method (Slatkin 1985; Barton & Slatkin 1986). Slatkin (1985) suggests that a single individual exchanged between subdivided populations every other generation is sufficient to prevent differentiation based on genetic drift alone. However, Mills & Allendorf (1996), in their consideration of both genetic and nongenetic factors that influence connectivity among subpopulations, suggest that a minimum of one and a maximum of 10 migrants per generation may be necessary to minimize the loss of polymorphism and heterozygosity within subpopulations while allowing for divergence in allele frequencies among subpopulations. Partitioning of microsatellite allelic variance was performed using analysis of molecular variance (AMOVA) based on F_{ST} from ARLEQUIN.

Pairwise genetic distance matrices were calculated with MICROSAT 1.4 (Dr E. Minch, Department of Genetics, Stanford University, USA, [HTTP://lotke.stanford.edu/distance.html](http://lotke.stanford.edu/distance.html)) using R_{ST} (Slatkin 1995) and D (Nei 1987). Nei's D was developed as an analysis of variance based on the assumptions of the infinite allele model with relatively slow mutation rates. Slatkin's R_{ST} is based on the high-rate stepwise mutation model (Slatkin 1991), but does not require a strict, single-step mutation event (i.e. + one repeat unit) in allelic size for the analysis of genetic distance (Garza *et al.* 1995). Permutation tests were used to assess the probability of obtaining the observed level of differentiation by chance, i.e. determine whether observed estimates of R_{ST} and D were significantly different from zero (Goodman 1997). R_{ST} distance data were used to generate a consensus neighbour-joining tree (NJ; Saitou & Nei 1987) using NEIGHBOUR and CONSENSE applications from PHYLIP version 3.572 (Felsenstein 1995). R_{ST} genetic distance relationships depicted in the consensus NJ tree were tested using random bootstrap replications (1000) to assess the reproducibility of branching patterns (Felsenstein 1985).

Results

Eleven polymorphic microsatellite loci were amplified from *Oncorhynchus mykiss* populations from southern

Oregon, California, and Mexico (Table 2). The number of alleles amplified per locus ranged from 7 to 26 (mean = 17), and the allelic size ranged from 87 to 290 bp. The average standard deviation for allelic size estimates based on GENOTYPER analyses was 0.21 bases allowing tight binning of dinucleotide allelic structure without overlap for all 11 loci. The GENEPOP analyses of pairwise linkage disequilibrium gave P -values ranging from 0.0001 to 0.89. Significant disequilibrium ($P < 0.005$) was found between the following pairs of loci: Omy77 and Omy325 ($P = 0.0001$); Omy77 and Oneμ8 ($P = 0.0003$); Omy77 and Ots1 ($P = 0.0003$); Omy325 and Ots1 ($P = 0.0001$); Oneμ2 and Ssa14 ($P = 0.0001$); Ssa14 and Ssa289 ($P = 0.0003$).

ARLEQUIN showed no significant departures from Hardy-Weinberg equilibrium for the 11 microsatellite loci in tests with all populations combined. Sheepheaven Creek redband trout displayed a significant reduction in the number of alleles when compared to the total allelic distribution amplified for each locus across the total extent of the geographical range surveyed in this species (16–73% of the average allelic diversity found at each locus). Sheepheaven Creek trout did, however, fit HW expectations in tests with all polymorphic microsatellite loci combined. Average heterozygosity for the 11 loci was 0.68. Mean F_{ST} for all 11 loci combined equaled 0.02.

Fisher's exact analyses of independence between Sheepheaven Creek trout and fish collected at 14 locations on the McCloud River (Table 1) showed significant differences (initial $\alpha = 0.025$) in all paired comparisons. Tests of allelic frequency differentiation made between Sheepheaven Creek fish and six strains of hatchery rainbow trout used in California (Table 1) also showed significant independence in all paired comparisons ($\chi^2 = \text{infinity}$; $P < 0.0001$). Interior redband trout populations from the Pit River, Sprague River, and Goose Lake drainages were significantly independent in microsatellite allelic frequencies from California's northern and southern coastal populations, trout found in the mainstem McCloud River, Swamp Creek, and all tributaries in the southern McCloud River drainage, with the exception of Tate Creek (Fisher's $P < 0.001$ in all tests).

Thirty-one rare alleles (found in less than 5% of the population) from nine loci were found to be unique to California coastal stocks. Twenty-eight rare alleles from 10 loci occurred only in interior redband trout populations. Twenty-four Sheepheaven Creek trout (41%) carried one or more exclusive redband allele (locus Omy207 alleles 122 and 128); of these, 11 fish (19%) were homozygous for allele 128. Paired comparisons of fish samples above and below a putative recent barrier (water point dam) on Sheepheaven Creek demonstrated no significant differences in allelic frequencies between subpopulations ($N_m = 11.46$).

Location	Population	D	R_{ST}
McCloud River, California	Trout Creek	0.042	0.002
	Edson Creek	0.069*	0.031
	Tate Creek	0.083*	0.048
	Swamp Creek	0.129*	0.153*
	Moosehead Creek	0.302*	0.219*
	Dry Creek	1.579*	0.525*
	Bull Creek	0.226*	0.271*
	Shady Gulch Creek	0.997*	0.498*
	Blue Heron Creek	0.725*	0.437*
	Steep Hollow Creek	0.244*	0.155*
	McCloud mainstem above RR crossing	0.182*	0.103*
	McCloud at Algoma Bridge	1.804*	0.607*
	McCloud below Tate Creek	0.145*	0.133*
	McCloud below upper falls	0.318*	0.164*
Sprague River, Oregon	Deming Creek	0.097*	0.073*
Pit River, California	Couch Creek	0.061*	0.007
Goose Lake, California	Lassen Creek	0.028	0.004
Northern California	coastal drainages	0.951*	0.488*
Southern California	coastal drainages	0.351*	0.356*
Kern River, California	rainbow trout	0.339*	0.267*
	South Fork Kern golden trout	0.437*	0.302*
	Golden Trout Creek golden trout	1.002*	0.499*
	Little Kern golden trout	0.039*	0.008*
Baja California, Mexico	Rio Santo Domingo	0.051*	0.015
Sierra Madre, Mexico	Rio Yaqui	1.705*	0.624*
California	All hatchery trout combined	1.970*	0.748*

Table 4 Nei's D and R_{ST} (Slatkin 1995) and distance measures calculated between Sheepheaven Creek trout and within and out of basin collections of rainbow trout from California, Oregon, and Mexico

*Indicates genetic distance values significantly different from zero ($P < 0.05$).

AMOVA showed that only 1.7% of the overall microsatellite allelic variance was attributable to geographical classifications of *O. mykiss*, i.e. coastal steelhead, interior redband, California golden, Little Kern golden, and Mexican trout. Variation among populations within geographical groups equalled 15.6% of the total variance. The remaining 82.7% of the variance was found among individuals within populations. Partitioning of molecular variance within the McCloud River drainage showed that less than 1% of the overall variance was found in Sheepheaven Creek fish.

R_{ST} and D genetic distance measures calculated between Sheepheaven Creek trout and other trout populations surveyed in this study are given in Table 4. R_{ST} distance measures calculated from paired comparisons of mainstem and tributary trout populations within the McCloud River drainage ranged from 0.00 (identity) to 1.83. Trout populations within the McCloud River drainage showing close genetic affinity to Sheepheaven Creek fish were those in Trout Creek ($R_{ST} = 0.002$; $D = 0.042$), Edson Creek ($R_{ST} = 0.031$; $D = 0.069$), and Tate Creek ($R_{ST} = 0.048$; $D = 0.083$). In several cases, out-of-basin populations were more similar to Sheepheaven Creek fish than were fish from the rest of the McCloud River drain-

age, i.e. Lassen Creek redband ($R_{ST} = 0.004$; $D = 0.028$), Couch Creek redband ($R_{ST} = 0.007$; $D = 0.061$), and Little Kern golden trout ($R_{ST} = 0.008$; $D = 0.039$). Isolated populations of trout from Baja lacked significant R_{ST} distance from Sheepheaven Creek fish ($R_{ST} = 0.015$), but Nei's D was significant ($D = 0.051$). A Wilcoxon signed-rank test of R_{ST} and D estimates using Sheepheaven Creek trout showed no significant difference in rank between the two measures. An unrooted, consensus NJ tree based on R_{ST} is given in Fig. 2. NJ analysis of D gave a similar consensus tree.

McCloud River tributary locations receiving Sheepheaven Creek trout during the 1970s live-fish transfers had mixed associations to their putative parental stock. Trout Creek samples showed the closest genetic association to the Sheepheaven Creek trout samples ($R_{ST} = 0.002$). The number of migrants (N_m) estimated between Sheepheaven Creek trout and fish in Trout Creek was $N_m = 0.82$. Swamp Creek trout showed a limited genetic relationship to the Sheepheaven Creek redband trout ($R_{ST} = 0.153$; $N_m = 0.37$). Swamp Creek trout shared their closest genetic affinity with trout in the upper McCloud River mainstem ($R_{ST} = 0.04$; $N_m = 0.89$).

Comparisons of genetic distances showed some degree

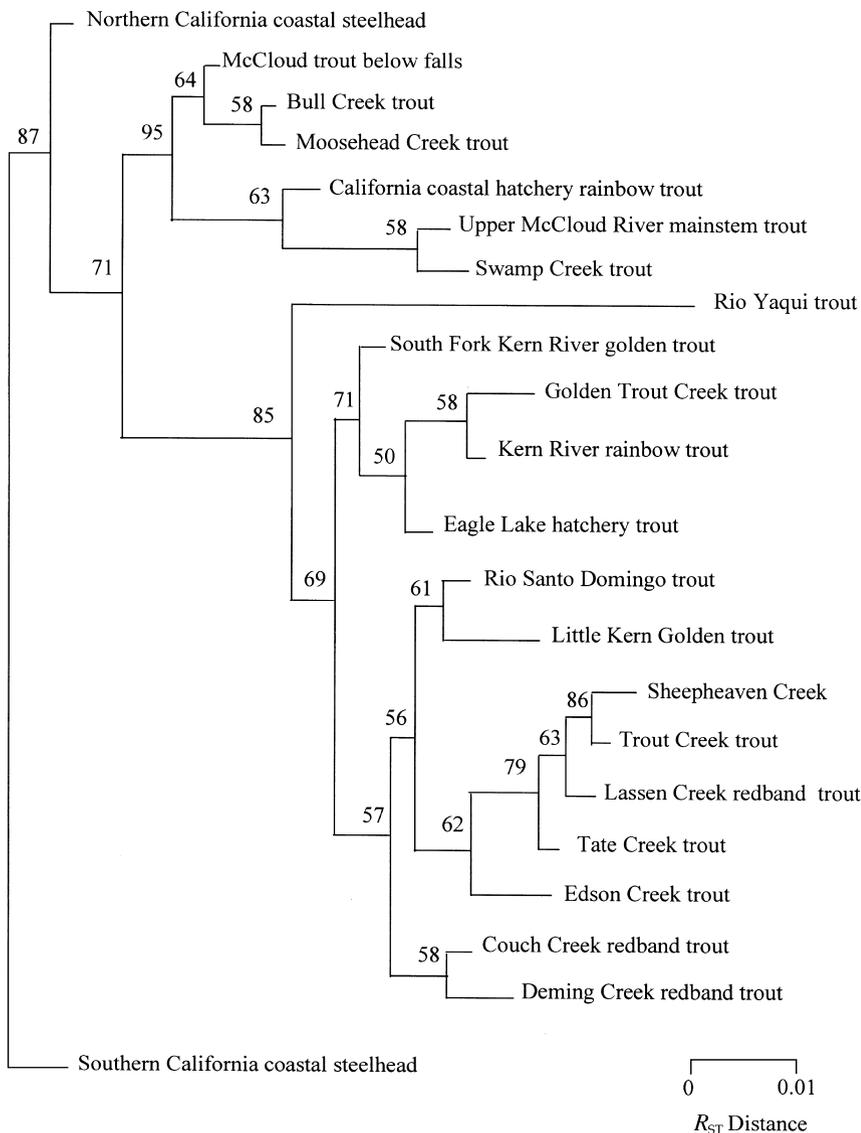


Fig. 2 Unrooted consensus neighbour-joining tree for R_{ST} microsatellite distance measures for McCloud River trout collections, southern Oregon redband, California redband trout, California's coastal stocks of *Oncorhynchus mykiss*, and Mexican trout from the San Pedro Martir basin, Baja and the Rio Yaqui, Sonora Mexico. Bootstrap values (%) calculated from 1000 replicate trees are given at branch points. Trout populations differentiated with bootstrap values < 50% were not included in the tree.

of genetic differentiation among the six hatchery strains sampled (average $R_{ST} = 0.24$). Gene flow estimates also supported isolation of California hatchery stocks with $N_m = 0.64 - 1.72$ in paired comparisons between hatchery strains. Only Eagle Lake hatchery fish showed any significant genetic associations with Sheepheaven Creek trout ($R_{ST} = 0.03$), and Trout Creek fish ($R_{ST} = 0.01$). The Pit-Shasta strain showed the highest levels of genetic associations with wild trout populations in the upper McCloud River (R_{ST} range = 0.02 - 0.06). Trout collected in the McCloud River below Upper Falls (open to Lake Shasta where most hatchery strains have been stocked historically) showed a high degree of similarity with hatchery fish: Crystal Lake Hatchery ($R_{ST} = 0.009$), Mount Shasta Hatchery ($R_{ST} = 0.004$), Coleman Hatchery ($R_{ST} = 0.01$), and Whitney Hatchery ($R_{ST} = 0.02$).

Discussion

Previous analyses showed that trout in the upper McCloud River drainage, including Sheepheaven Creek fish, were monomorphic for the mtDNA haplotype MYS1 common to coastal steelhead throughout California, suggesting an anadromous origin for this group (Nielsen *et al.* 1999). The frequency of occurrence of the lactate dehydrogenase allele (*LDH-B2*100*) also suggested a coastal source for McCloud River redband (Berg 1987). However, DNA extracted from Sheepheaven Creek trout showed unique microsatellite diversity that was not shared with coastal stocks. Significant genetic associations were found between Sheepheaven Creek fish and redband trout from interior drainages in southern Oregon and northern California using microsatellite loci. These

findings support Behnke's position on the unique status of this group within *Oncorhynchus mykiss* (Behnke 1992). Different genetic markers provide varying degrees of resolution and are subject to different rates of mutation (Avice 1994). The lack of congruence we found among nuclear DNA markers suggesting diverse ancestry for Sheepheaven Creek trout may reflect past selective events that are not necessarily consistent with current population structure (Boyce *et al.* 1996).

Behnke speculated on the ancestral nature of Sheepheaven Creek fish in his 1992 monograph. His associations were based on colouration and taxonomic characteristics he considered ancestral to the *O. mykiss* complex, such as the red-banded lateral stripe and basibranchial teeth. The significance of red colouration and occasional occurrence of basibranchial teeth was also noted by Behnke in Little Kern golden trout. It is possible that allozyme and mtDNA 'coastal' genotypes described in other studies reflect ancestral molecular states that have remained fixed in California steelhead and trout populations for long periods of time. A continuous biogeographic cline of haplotype MYS1 in *O. mykiss* from Mexico to Alaska supports a deep lineage for this marker (Nielsen *et al.* 1998). Microsatellite diversity can reflect population-scale changes resulting from genetic drift or migrations in more recent history (Bowcock *et al.* 1994).

Organisms living at the edge of their range often persist in small isolated populations (Soulé 1986; Stacey & Taper 1992). Under the vicariance dispersal model in evolution, a more or less continuous distribution of a species across the landscape has been split by large-scale geological or hydrologic events such as uplifting, continental break-up, or glaciation (Avice 1994). Previous studies supported a Gulf of California Pleistocene refugium for *O. mykiss* (Behnke 1992; Nielsen 1996). Unprecedented levels of genetic diversity for allozyme, mtDNA, and microsatellite loci have been documented at the southern edge of this species range, suggesting a southern source population (Nielsen 1999). The persistence of small, isolated populations of trout at the southern extent of their range carrying ancestral characteristics could have resulted from vicariate dispersal from this refugium with isolation throughout recent history. Volcanism in the upper McCloud River drainage below Mount Shasta (inactive for the last 200–300 years) created many isolated spring-fed streams where natural migrations between populations are impossible. Isolated habitats such as these could support ancestral molecular traits in McCloud River redband trout.

The significance of genetic distance measures based on highly variable loci must be interpreted carefully (Ruzzante 1998; Hedrick 1999). The level of differentiation and genetic distance between groups is influenced to a high degree by differences in heterozygosity. Heterozygosity was

high (average = 68%) for most loci used in this study. Significant disequilibrium between six pairs of loci suggests that selection may have impacted these fish populations or that alleles have drifted as a result of small population sizes. Homoplasy and null alleles frequently found in microsatellite loci can influence the accuracy of genetic distance measures (Estoup *et al.* 1995; Paetkau & Strobeck 1995; Goodman 1997). Several microsatellite loci used in this study have been tested for Mendelian inheritance through controlled mating in *O. mykiss* (Ardren *et al.* 1999). Loci found to have potential problems in *O. mykiss* were dropped from our study. However, the degree of resolution gained from future studies of microsatellite loci may affect the interpretation of our results.

We used two genetic distance measures in our analyses of Sheepheaven Creek redband trout (R_{ST} and D). These measures are based on different assumptions and have different expectations and potential biases based on sample size, allelic distribution, and linearity over time (Goldstein *et al.* 1995b; Takezaki & Nei 1996; Ruzzante 1998). In most cases both genetic distance measures gave similar results, although on different relative scales. Nei's D , however, appears more conservative in analyses of redband populations in the McCloud River where only Trout Creek fish lacked genetic distance values significantly different from zero in comparison with Sheepheaven trout. The connection between statistical significance and actual biological relationships, however, may not be direct or complete. Conclusions drawn from genetic inference should be coupled with other types of ecological or genetic data.

The closest out-of-basin genetic distance relationships for Sheepheaven Creek trout were found with redband trout from southern Oregon and northern California. The Goose Lake drainage was occasionally connected to the headwaters of the Pit River in the upper Sacramento River basin during historic times (Hubbs & Miller 1948). Behnke (1992) considered the Goose Lake basin to be a semidisrupted part of the upper Sacramento River. Microsatellite data supported the ancestral connection between Sheepheaven Creek and Lassen Creek redband trout. Behnke (1992) used gill raker counts to suggest that Lassen Creek trout have been influenced by hybridization with hatchery rainbow trout. Microsatellite data and gene flow estimates among Lassen Creek, Sheepheaven Creek, and contemporary hatchery rainbow trout strains did not support introgression by hatchery trout in either population.

Hatchery rainbow trout in California are maintained in independent phenotypic 'strains' despite considerable stock mixing in past hatchery management practices (Busack & Gall 1980). These strains are used for specific stocking strategies due to perceived or real adaptive differences among the stocks. In contrast to comparisons made

using allozymes by Gall (1993), we found considerable differences in microsatellite allelic distributions among the rainbow trout hatchery strains and wild stocks. As most hatchery rainbow trout were originally derived from fish taken around the turn of the century from the upper Sacramento River (U.S. Fish Communication Reports: 1872–1901), they probably share significant common ancestry with contemporary McCloud River stocks. The Pit/Shasta hatchery strain's association with upper McCloud River rainbow trout could be due to ancestral alleles at several microsatellite loci that were common to the Pit and McCloud Rivers before separation of the basins. The lack of similar allelic distributions in contemporary Pit River trout from Couch Creek, however, argues against this conclusion.

Williams *et al.* (1996) use mtDNA and allozymes to examine introgression between native and introduced rainbow trout in the upper Snake River. Their data supported the hypothetical separation of rainbow trout into two major groups, coastal and interior, separated by the Cascade Mountains (Allendorf 1975; Allendorf & Utter 1979; Berg 1987; Currens *et al.* 1990; Behnke 1992). The assumption used in all of these studies is that hatchery strains of rainbow trout are primarily derived from coastal stocks, and therefore, can be separated genetically from native interior strains. There were numerous diagnostic microsatellite alleles found to differentiate coastal trout from Sheepheaven Creek redband trout. The limited nature and small geographical range we have studied in redband trout, however, suggest caution in the application of these markers to other interior drainages.

Out-of-basin comparisons between Sheepheaven Creek trout and *O. mykiss* populations showed close genetic associations among McCloud River redband, Baja trout, and Little Kern River golden trout. Little Kern Golden trout are listed as an endangered species by the U.S. Federal Government and the State of California. The trout of Baja are listed as a rare subspecies by the Secretaría de Desarrollo Social of the Mexican government and as a species of special concern by Williams *et al.* (1989). Sheepheaven Creek and Baja trout share a lack of molecular diversity at several microsatellite loci, suggesting bottlenecked populations with low effective population size. The alleles bottlenecked in both of these populations occur at similar frequencies in the Little Kern River golden trout. All three populations carried alleles found within unique size ranges for four loci (Omy207, Omy325, Ots1, and Sfo8) that were not found in coastal trout.

The Little Kern golden trout, however, retain a higher level of genetic diversity, with numerous alleles shared with the South Fork Kern River golden trout, suggesting the possibility of a mixed evolutionary lineage for fish in the S.F. Kern River. Shared karyotypes ($2n = 58$) between golden and redband trout raised questions about the evolution-

ary genetic relationships among these groups (Thorgaard 1983). Behnke (1981) suggested that the McCloud River redband trout represent a distinct phylogenetic lineage from the Columbia and Fraser River redband trout. He further suggested that the Kern River system reflects successive invasions by redband trout from the Sacramento River drainage via Lake Tulare in ancient times. Sheepheaven Creek redband trout, according to this hypothesis, represent a remnant primitive form of the Sacramento River basin redband that migrated into the Kern River. The molecular data presented here suggest that outside the Little Kern River golden trout, the Kern River golden trout complex is significantly differentiated from Sheepheaven redband trout.

Genetic differences in stocks with a common ancestor can result from interbreeding and introgression from stocked hatchery trout in the California golden trout complex (Gall 1995). Alternatively, the founding invasion of ancestral trout that started the Kern and Little Kern River populations could have been different, with a more recently shared ancestor between Sheepheaven Creek redband and the Little Kern golden trout. It is also possible that the microsatellite loci used in this study provide insufficient information on the true ancestral relationships between Sheepheaven Creek redband and South Fork Kern River golden trout.

Comparisons between Sheepheaven Creek trout and Mexican trout were not congruent. The genetic associations found between Sheepheaven Creek trout and Baja trout populations did not carry over to Rio Yaqui fish. Previous studies have shown that Rio Yaqui trout carry numerous unique mtDNA haplotypes and microsatellite alleles that have not been found in *O. mykiss* populations in the U.S. (Nielsen *et al.* 1997a, 1998). Although significantly different in their genetic makeup, both Mexican trout populations probably reflect early Pleistocene ancestral divergence of *O. mykiss* from a Gulf of California refugium and deserve special status within the species (Nielsen 1996, 1998; Nielsen *et al.* 1998). Based on these analyses the trout of Baja represent the southern most relic population of *O. mykiss* retaining ancestral trout alleles similar to those in Sheepheaven Creek redband and Little Kern golden trout.

The conservation of a type-locality of a rare species requires an understanding of the history of genetic relationships on a finer, within-basin scale. Wales (1939) reported golden trout (generally believed to be a reference to McCloud River redband trout) in the headwaters of several short, spring-fed creeks in the McCloud River drainage (probably Tate, Edson, and Sheepheaven Creeks). Based on a survey by CDFG, the U.S. Forest Service & Sierra Pacific Industries (1978–95), streams containing putative McCloud River redband trout included Trout, Swamp, Edson, Sheepheaven, Blue Heron, Tate, Moosehead

& Dry Creeks. Berg (1994) showed allozyme frequency associations between Sheepheaven Creek trout and fish from Moosehead, Edson, and Swamp Creeks. Our DNA analyses provide conflicting findings. Swamp and Moosehead Creek trout shared significant allelic diversity with the Sheepheaven Creek redband at no more than one microsatellite locus each. Microsatellite identity with Sheepheaven Creek trout was found only in fish collected from the headwaters of Trout, Edson, and Tate Creek (R_{ST} values not significantly different from zero).

Many native trout populations, both coastal and interior, have been extirpated by habitat decline, introgression by introduced groups, and competitive exclusion (Vincent 1987; Fausch 1988; Hindar *et al.* 1991). Redband population reductions within the McCloud River drainage may have resulted from habitat loss due to recent droughts and/or hybridization with introduced hatchery trout. Introduced hatchery trout may have migrated up tributaries or have been intentionally placed above natural barriers (i.e. Swamp Creek). Much of the mainstem McCloud River and tributaries in the southern drainage have introduced populations of brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) which appear to have displaced McCloud redband populations (D. Maria, CDFG, personal communication).

In an effort to preserve the type-locality of McCloud River redband trout, Swamp Creek, an isolated, 'fishless' tributary in the upper McCloud drainage, received a direct transfer of 28 Sheepheaven Creek trout in 1973 with only one fish surviving high winter flows to 1974 (Hoopaugh 1974). According to unpublished CDFG records a second transfer of 36 Sheepheaven Creek trout into Swamp Creek was made in 1974 (D. Maria, CDFG, personal communication). In 1977, 102 Sheepheaven Creek trout were moved to the Crystal Lake Hatchery and artificially spawned at that site. Of the 102 fish taken as broodstock, 49 survived the first few months of captivity. Due to high levels of mortality in the wild broodstock and their fry (due primarily to disease), the remaining adults and 'several dozen' fry were transferred to stream habitats on Trout Creek (CDFG unpublished report). These habitats had been chemically treated by CDFG prior to the introduction of Sheepheaven Creek fish to eliminate competitive exclusion by non-native stocks.

Microsatellite analyses gave conflicting results on the success of these transfers. It is possible that Swamp Creek and/or Trout Creek were, in fact, not fishless prior to the introduction of Sheepheaven Creek fish, and introduced redband trout suffered from competition or introgression from resident fish. Undocumented introductions of hatchery trout may have occurred since the rescue transfers. The small number of Sheepheaven Creek fish originally transplanted may have experienced low effective population size. Alternatively, modification of the genetic

composition of the source population may have occurred due to founder effects after the transfers. Low population size is thought to produce detrimental genetic effects such as increased inbreeding and loss of heterozygosity and allelic diversity (Lande & Barrowclough 1987; Hedrick & Miller 1992; Hedrick *et al.* 1994). Austere habitats, low effective population size, and limited gene flow are probably equal factors contributing over time to changes in allelic frequency in Sheepheaven Creek redband trout.

Ecological isolation in small populations can lead to changes in genetic diversity very quickly, over a matter of a few dozen generations (Gavrilets & Hastings 1996). Genetic drift accompanying a founder event has been shown to result in the loss of genetic variability at one or several loci within one generation (Templeton 1981). These factors may explain the apparent lack of genetic identity between source and transferred populations of McCloud redband trout. Many animals with highly specialized habitat requirements naturally occur in small populations (Stacey & Taper 1992). The greatest danger of a shift in genetic diversity between the founder and refugium populations would be if substantial fitness effects resulted in changes in the transferred stock. Such changes may make it unable to adapt back into the founder habitats should disaster eliminate the original source population (Hedrick & Miller 1992). Measures linking genetic diversity and actual changes in fitness in natural populations, however, are not available from studies of neutral genetic markers, i.e. microsatellites (Eanes 1987).

The degree of genetic isolation in McCloud River redband trout demonstrates a need to understand the magnitude and nature of dispersal throughout a drainage in the development of successful management and conservation strategies. Unique genetic structure will respond differently in different environments. It is important to know the scale at which the organism uses its environment and the degree of connectivity between environments that has been available in the recent past for successful conservation efforts. Within-basin genetic studies should be important components in any effort to preserve genetic biodiversity.

Ancestral characteristics of *O. mykiss* remain viable and active on an evolutionary scale within the McCloud River refugium. McCloud redband trout are vulnerable to extinction due to low numbers and potential volcanic activity. Microsatellite analyses showed that Sheepheaven Creek fish remain the type-locality for McCloud redband trout. Fish in Trout, Edson, and Tate Creek had similar, albeit not identical, allelic diversity. Their closest out-of-basin genetic relationship was found in redband trout from Lassen Creek in the Goose Lake drainage. Conservation efforts involving live fish transfers and/or artificial propagation programmes designed to capture this unique genetic component in an effort to promote recovery of

McCloud River redband trout must take into consideration the genetic effects to the refugium and the new populations.

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