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Population Genetic Structure in Lahontan Cutthroat Trout

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Abstract.—We used 10 microsatellite loci to examine the genetic population structure of cutthroat trout *Oncorhynchus clarki* within the Lahontan Basin complex. Genetic diversity was analyzed for trout from Nevada, California, and Utah representing three putative subspecies: Lahontan *O. c. henshawi*, Paiute *O. c. seleniris*, and Humboldt (an unnamed subspecies) cutthroat trout. We found significant differences in microsatellite diversity among the three putative subspecies found in this area. Analysis of molecular variance partitioned microsatellite variation as 9.8% among subspecies, 27.7% among populations, and 62.5% within populations of Lahontan Basin cutthroat trout. Genetic distance analyses (Cavalli-Sforza–Edwards and F_{st}) supported unique population structure in cutthroat trout from the Humboldt and Pilot Peak drainages. Pairwise F_{st} values for Lahontan cutthroat trout were not significantly correlated with geographic distance between population pairs ($r^2 = 0.008$; $P < 0.0001$), suggesting that they are extremely isolated populations with small effective sizes that are vulnerable to extinction. Two extant hatchery strains of Lahontan cutthroat trout showed genetic associations with different geographic source populations. The Pyramid Lake hatchery strain was most closely associated genetically with fish from Summit Lake. The Pilot Peak hatchery strain was associated genetically with Pilot Peak wild trout (Utah) and Macklin Creek trout (California). The phylogeographic diversity depicted in this study supports unique population structure and suggests important evolutionary relationships needed to evaluate transplanted populations and hatchery supplementation within the basin.

Lahontan cutthroat trout *Oncorhynchus clarki henshawi* are thought to have evolved in a large late Pleistocene lake, Lake Lahontan, which covered approximately 22,000 km^2 about 14,000 years ago (La Rivers 1962). Lahontan cutthroat trout persisted in streams and lakes throughout the Lahontan basin in Nevada, Oregon, and California after the desiccation of Lake Lahontan about 8,000 years ago (Hickman and Behnke 1979). Before this century, this subspecies occupied over 135,000 hectares of lakes and 5,796 km of stream (Gerstung 1986). At the time of European settlement, Lahontan cutthroat trout were native to the Truckee, Carson, Walker, and Quinn rivers. Only a few headwater and lake populations of Lahontan cutthroat trout persisted into the 20th century. Native trout are thought to be extinct in Tahoe, Pyramid, Walker, and Donner lakes (Xu 1982). More recently, Lahontan cutthroat trout have been documented in several Lahontan Basin lakes and in small locally isolated fluvial populations in the Humboldt, Truckee, Carson, and Walker river drainages (Figure 1). Despite recent enhancement efforts and broodstock development, self-sustaining Lahontan cutthroat trout occupy less than 10% of their historic range, and they remain on the federal list of threatened species (Coffin and Cowan 1995). The evolutionary relationship among contemporary geographic distributions of Lahontan cutthroat trout remains controversial.

During the postglacial desiccation of Lake Lahontan, Pyramid Lake retained large numbers of Lahontan trout. The fish in this lake had a long history of coevolution with numerous fish prey, demonstrated early piscivory, and possessed genetic attributes necessary to achieve larger size at age (375–450 mm) than any other stock of this subspecies (Behnke 1992). The extinction of the unique Pyramid Lake Lahontan cutthroat trout began at the start of the 20th century with the construction of Derby Dam, which blocked their spawning grounds in the Truckee River (Knack and Stewart 1984). An extensive sport fishery in the lake accelerated further declines in the Pyramid Lake genotype (Hickman and Duff 1978). Pyramid Lake trout were artificially propagated by various means from 1885 to 1930 by several fisheries agencies. Pyramid Lake hatchery fish were introduced into many remote waters in the Great Basin throughout the first half of the 20th century. An unusual run of Lahontan cutthroat trout found in the Pilot Peak drainage in 1977 was thought to represent the original gene pool derived from eggs

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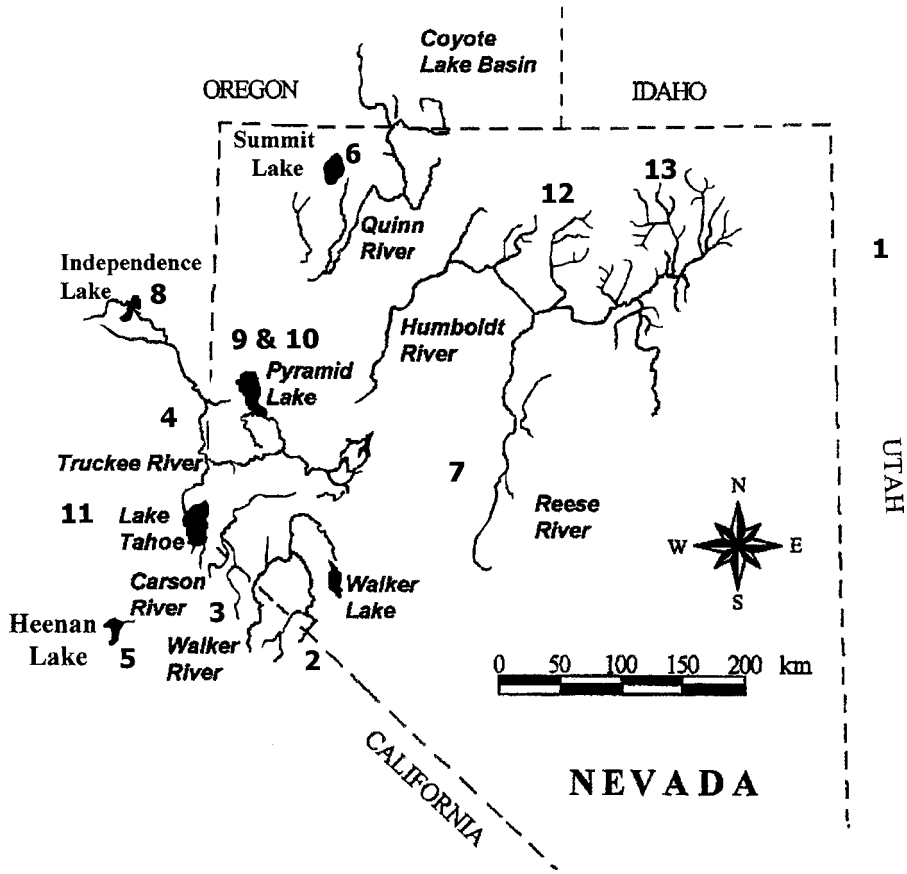

FIGURE 1.—Major river drainages of the Lahontan Basin within which Lahontan cutthroat trout were distributed at the beginning of the 20th century. Numbers correspond to sample numbers presented in Table 1.

stocked from Pyramid Lake Lahontan cutthroat trout (Hickman and Behnke 1979).

The evolutionary history of cutthroat trout in the Lahontan Basin is complex. Lahontan cutthroat trout share the basin with Paiute cutthroat trout *O. c. seleniris*, and Humboldt cutthroat trout (an unnamed subspecies). The Paiute cutthroat trout is differentiated solely by the absence of spots on its body (Behnke 1992). According to Behnke (1992), the fluviate (resident stream form) Humboldt cutthroat trout are similar to other lacustrine (lake predator form) Lahontan cutthroat trout, except that they have fewer gill rakers and fewer scales in the lateral series and above the lateral line.

Early references suggested that there were two forms of cutthroat trout in all of the lakes, a black and a silver form (Behnke 1992), but the evolutionary status of these forms was unknown. Significant controversy dating back to the last century exists over whether or not two forms of Lahontan cutthroat trout existed in the Truckee River basin. Jordan initially recognized the ''silver trout of Lake Tahoe'' as *Salmo henshawi* but later renamed this group *S. clarki tahoensis* (Jordan and Evermann 1898). Jordan and Evermann (1896) named the second group, ''the black Tahoe trout,'' *S. mykiss henshawi*. Snyder (1917) described two distinct spawning runs of cutthroat trout from Pyramid Lake that ascended the Truckee River. Until 1931, the California Department of Fish and Game (CDFG) operated separate hatcheries for ''henshawi'' and ''tahoensis'' trout. Behnke (1992), however, found no evidence for two lineages in the Truckee Basin and suggested that only one subspecies (i.e., *O. c. henshawi*) is native to Lake Tahoe, Pyramid Lake, and the Carson, Walker and Truckee rivers.

The historical record indicates that over the last 120 years significant translocations of trout have occurred throughout the Lahontan Basin. There

has also been a persistent and pervasive effort to introduce nonnative trout, especially hatchery rainbow trout of coastal origins for recreational fishing. Documented hatchery introductions into the basin began as early as 1877. These early introductions included McCloud River rainbow trout *O. mykiss* from California, ''Mackinaw trout'' from the U.S. Fish Commission Fish Hatchery in Missouri, steelhead *O. mykiss* from California, lake trout *Salvelinus namaychus* from Michigan, eastern brook trout *Salvelinus fontinalis* from Virginia, landlocked Atlantic salmon *Salmo salar* from Maine, and various hybrid trout stocks such as rainbow trout × lake trout (listed as *Salmo mykiss henshawi* by State of Nevada Fish and Game Commission Reports 1877–1914).

Two stocks of Lahontan cutthroat trout are currently in artificial propagation at the Lahontan National Fish Hatchery. One stock, Pyramid Lake hatchery trout, is derived from a mixture of several cutthroat trout stocks from Heenan, Walker, Independence, and Summit lakes (Coleman and Johnson 1988; Coffin and Cowan 1995). The second stock, Pilot Peak hatchery trout, originated from transplanted Pyramid Lake cutthroat trout from Morrison Creek in the Pilot Range of eastern Utah. These fish were introduced into the Lahontan National Fish Hatchery for captive propagation in 1996 (Dunham et al. 1998).

Over the last decade, the use of molecular genetics has increased significantly in the study of conservation and population ecology in fishes (Utter 1994). Patterns of genetic variation have a strong historical component (Slatkin 1985, 1993; Avise 2000). The degree of genetic variation found among and between existing subspecies and populations can provide information concerning past patterns of demographic and ecological events (Weir 1996). Genetic structure showing trends in isolation, gene flow, and evolutionary history can provide insight into historic population dynamics not otherwise available (Avise 1994; Dunham et al. 1999) and can give significant inference to management and conservation activities directed at contemporary populations (Nielsen 1995, 1999; Beaumont and Bruford 1999).

Evolutionary divergence is a dynamic process involving markers acting across many different temporal and spatial patterns (Avise 1994; Boyce et al. 1996; Nielsen et al. 1997). Different molecular systems (e.g., allozymes, DNA sequence, restriction fragment length polymorphisms [RFLPs], and microsatellites) present different scales for measuring genetic diversity and may or may not provide congruent information (Scribner et al. 1994; Boyce et al. 1996; Allendorf and Seeb 2000; Crandall et al. 2000). The debate over the efficiency and scale of information available from different molecular markers continues (reviewed in Sunnucks 2000). Therefore, the evaluation of genetic differences that represent biologically meaningful information must be made carefully (Hedrick 1999). It is commonly accepted, however, that the basic properties of the most commonly used molecular systems contain pertinent characteristics important to population genetic analyses (Smith and Wayne 1996; Kocher and Stepien 1997).

Earlier genetic studies of Lahontan cutthroat trout using electrophoretic variation in allozymes (Loudenslager and Gall 1980; Busack and Gall 1981; Leary et al. 1987; Bartley et al. 1987; Bartley and Gall 1993; W. Cowan, Humboldt State University, unpublished data) demonstrated significant genetic divergence between coastal and interior cutthroat trout and among five major cutthroat trout subspecies of the interior Great Basin: Lahontan, Bonneville *O. c. utah*, Colorado *O. c. pleuriticus*, Yellowstone *O. c. bouvieri*, and westslope *O. c. lewisi*. They also provided inference on stocks of hybrid origins (i.e., rainbow trout × cutthroat trout crosses). These markers, however, were not sufficiently polymorphic for clear differentiation among subspecies or populations of cutthroat trout in the Lahontan Basin.

High rates of sequence divergence in mitochondrial DNA (mtDNA) can show a degree of variation among populations on broad geographic scales not found in more slowly evolving proteins and enzymes (Brown et al. 1979; Moritz et al. 1987). Contemporary mtDNA analyses of Lahontan cutthroat trout (Williams et al. 1992, 1998; Shiozawa and Evans 1995, 1998) demonstrated recent evolutionary divergence between the Lahontan and Humboldt lines of cutthroat trout, despite little haplotype divergence among the Lahontan Basin cutthroat trout populations. Furthermore, there was no evidence of introgression from nonindigenous cutthroat trout and hatchery rainbow trout in the Lahontan basin despite many years of stocking throughout northern Nevada streams and rivers (Williams et al. 1998).

Microsatellite DNA represents a class of highly polymorphic, simple sequence, tandem repeat loci. Changes recorded in microsatellite loci are frequently used to determine genetic variation in studies of closely related or endangered species (Ashley and Dow 1994; Forbes et al. 1995; Boyce

et al. 1996; May et al. 1997; Beacham et al. 1999). Additionally, microsatellites can be amplified from nondestructive tissue (fin-clips, scales, hair, feces, mucus, etc.), allowing access to rare or difficult to reach populations. Recently, microsatellites have been used to identify population structure and interspecific hybridization in coastal cutthroat trout (Wenburg et al. 1996, 1998), but until now have not been used in comparisons of trout from the Lahontan Basin.

Comparisons of molecular diversity using different genetic markers can provide insight into directed patterns of evolution and anthropomorphic impacts in natural populations (Taylor et al. 1994; Estoup et al. 1995, 1998; Tessier et al. 1995; Brunner et al. 1998; Nielsen et al. 1999). In an effort to shed light on the evolutionary and phylogeographic relationships among contemporary populations of Lahontan Basin trout, this study presents microsatellite allelic diversity found within and among 10 Lahontan cutthroat trout populations and genetic comparisons among three putative subspecies of cutthroat trout found in the Lahontan Basin (i.e., Lahontan, Humboldt, and Paiute cutthroat trout). Despite small sample sizes in some categories, these analyses provide inference and information important to management activities within the Lahontan Basin. Using genetic data we discuss (1) the evolutionary legacy of trout in the Lahontan Basin in light of significant anthropogenic manipulations of habitat and populations; (2) contemporary population genetic structure in relation to population viability and conservation measures; and (3) choices and concerns for future enhancement activity for cutthroat trout within the basin.

Methods

Sample collection.—Trout tissue samples thought to represent pure Lahontan cutthroat trout came to these analyses from several researchers and resource agencies (Table 1). Many of these are small isolated populations found in fragmented habitats, and thus, sample sizes were limited by the availability of fish. The type–locality samples of Lahontan cutthroat trout from Edwards Creek ($N = 7$) came from the collection of Paul Evans, Brigham Young University. The Nevada Department of Wildlife and the U.S. Fish and Wildlife Service provided samples from Lahontan cutthroat trout including a collection of fish from the Pilot Peak drainage where trout from the original Pyramid Lake Lahontan cutthroat trout population where introduced around the turn of the 19th century (Hickman and Behnke 1979; Behnke 1992).

TABLE 1.—Cutthroat trout populations by subspecies and sources used in this study of genetic structure. Population numbers for cutthroat correspond to sample locations in Figure 1.

Population	Sample number (number amplified)	Transfer/ collector[a]
Lahontan cutthroat trout		
1. Pilot Peak wild–Utah	18	USFWS
2. Slinkard Creek	51	CDFG
3. East Carson River	13	UNR
4. Macklin Creek	44	UNR
5. Heenan Creek	28	CDFG
6. Summit Lake	14	NDW
7. Edwards Creek	40	BYU & UNR
8. Independence Lake	46	USGS/BRD
9. Pyramid Lake LFH[b]	49	USFWS
10. Pilot Peak LFH[b]	39	USFWS
Paiute cutthroat trout		
11. Four Mile Canyon Cr.	16	CDFG
Humboldt cutthroat		
12. Frazer Creek	13	UNR
13. West Mary's River	9	UNR

[a] USFWS = U.S. Fish and Wildlife Service, Reno, Nevada; CDFG = California Department of Fish and Game; UNR = University of Nevada, Reno (Jason Dunham); NDW = Nevada Department of Wildlife; BYU = Brigham Young University (Paul Evans); USGS/BRD = U.S. Geological Survey–Biological Resources Division.
[b] Hatchery strain.

California Department of Fish and Game provided fin clips from Paiute cutthroat trout from Four Mile Canyon Creek, Lahontan cutthroat trout from Heenan Creek, and an unresolved population from Slinkard Creek, Mono County, California. The Paiute cutthroat trout samples were from the lower meadow area of Four Mile Canyon Creek, an area known to support Paiute cutthroat trout (Bartley and Gall 1993). The Slinkard Creek fish are descended from several hundred wild fish transplanted from ByDay Creek in the late 1980s and represent the only endemic cutthroat trout stock remaining in the Walker Lake basin (S. Parmenter, CDFG, personal communication). The Heenan Creek fish, captured directly above Heenan Lake, represent Lahontan cutthroat trout from tributaries of the Carson River (W. L. Somer, CDFG, personal communication).

Scientists at the University of Nevada, Reno, provided additional samples from wild populations of Lahontan and Humboldt cutthroat trout from throughout the current range of the species. The U. S. Fish and Wildlife Service's Lahontan National Fish Hatchery (LNFH) provided hatchery collections from their Pilot Peak and Pyramid Lake strains of cutthroat trout.

TABLE 2.—List of microsatellites and their source publications tested for amplification of DNA from cutthroat trout. Allelic size ranges depict visualized product, including amplified primer. Loci in bold were used in these analyses.

Location	Source	Allele number	Allelic size range (bp)
Omy2	M. O'Connell [a]	1	104
Omy27	M. O'Connell [a]	0	[b]
Omy77	Morris et al. 1996	23	98–148
Omy78	M. O'Connell [a]	1	60
Omy87	M. O'Connell [a]	2	103–105
Omy207	O'Connell et al. 1997	7	96–146
Omy325	O'Connell et al. 1997	6	101–133
Oneμ2	Scribner et al. 1996	22	194–286
Oneμ8	Scribner et al. 1996	17	153–195
Oneμ11	Scribner et al. 1996	9	138–154
Oneμ14	Scribner et al. 1996	0	[b]
Ots1	Banks et al. 1999	36	159–289
Ots2	Banks et al. 1999	0	[b]
Ots4	Banks et al. 1999	1	149
Sfo8	Angers et al. 1995	24	176–286
Ssa4	McConnell et al. 1995	0	[b]
Ssa14	McConnell et al. 1995	13	105–145
Ssa85	O'Reilly et al. 1996	22	85–153
Ssa289	McConnell et al. 1995	8	108–122
Ssos1439	Slettan et al. 1995	24	102–150

[a] Personal communication, Marine Gene Probe Laboratory, Dalhousie University, Halifax, Nova Scotia.

[b] No significant amplification product at tested PCR conditions (available from J.L.N. on request).

TABLE 3.—Microsatellite electrophoretic multiplex system developed for amplification of multiple loci in a single lane of sequencing gel on an ABI-373 automatic sequencer. Loci were stratified by expected size ranges for each locus and mixed after PCR for covisualization on the gel. Primer concentrations are given in parentheses. Two multiplex systems are given (A & B), covering 10 loci.

System	Anneal temperature (°C/cycles)	Locus (dye) (6Fam blue)	(Tet green)	(Hex yellow)
Cutthroat A	54/30	*Omy77* (0.5)	*Ssa85* (0.15)	*Ssos1439* (0.45)
		Oneμ2 (0.4)	*Ots1* (0.4)	*Sfo8* (0.5)
Cutthroat B	50/28	*Ssa14* (1.0)		*Ssa289* (0.5)
				Oneμ8 (0.5)
	52/28		*Oneμ11* (1.0)	

Genetic analyses.—We extracted total genomic DNA from 380 trout fin tissues using Chelex-100 resin (Bio-Rad, Richmond, California). Amplification of microsatellite loci followed methods given in Nielsen et al. (1997). Microsatellite loci taken from the published literature or through verbal agreement with their developers were selected for analysis based on documented variability in *O. clarki*, ease of amplification in polymerase chain reaction (PCR), and allele scoring rigor (Table 2). Electrophoretic multiplex conditions were developed for amplification of cutthroat trout allelic size structure for 10 loci (Table 3). Loci were stratified by expected size ranges and mixed after PCR for covisualization in a single lane on a sequencing gel.

Microsatellite allele sizes (including the amplified primer) were determined in relation to the Genescan-500 internal size standard (P-E Biosystems, Foster City, California), cutthroat trout 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 for this species. The 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 cutthroat trout DNA run on an ABI-373 automatic sequencer. Approximately 5.4% of all samples were run on a second gel and scored independently to verify allelic size.

Analyses of heterozygosity, Fisher's exact tests for Hardy–Weinberg equilibrium, and analysis of molecular variance were calculated using ARLEQUIN (Schneider et al. 1997). Partitioning of microsatellite allelic variance was performed by ARLEQUIN based on population pairwise F_{st} values. Global tests for linkage disequilibrium were performed between all possible pairs of microsatellite loci using ARLEQUIN. Population independence between paired comparisons of allelic frequencies were tested with Fisher's exact tests, based on a Markov chain adaptation of row-by-column contingency tables, using GENEPOP version 3.1a (Raymond and Rousset 1995, 1997). Statistical inference levels for Fisher's exact analyses were set using sequential Bonferroni tests (Rice 1989).

A comparison of regional F_{st}-values with geographic distance was performed to evaluate the relative historical influence of gene flow and drift. Pairwise genetic distance matrices were calculated using Cavalli-Sforza and Edwards (1967) chord distance. Using ARLEQUIN (Nei 1972), we calculated a second set of genetic distance metrics based on F_{st} for all possible pairs of cutthroat trout. Genetic distance data based on chord distance were used to generate an unrooted consensus neighbor-joining tree (NJ; Saitou and Nei 1987) using

NEIGHBOR and CONSENSE applications from PHYLIP version 3.5c (Felsenstein 1999). We used 2,000 random bootstrap replications to assess the reproducibility of branching patterns in our consensus tree (Felsenstein 1985).

Results

Genetic Population Structure

The 10 microsatellite loci amplified from cutthroat trout for this study had allele counts ranging from 8 (*Ssa289*) to 36 (*Ots1*). The average allele count per locus was 19.8. Allele sizes (bp) ranged from 85 to 289 (see Table 2). Hardy–Weinberg probability tests showed no significant deviation from equilibrium expectations for these ten loci in all Lahontan cutthroat trout used for these analyses. Pairwise tests (ARLEQUIN) for linkage disequilibrium between microsatellite loci, based on methods from Slatkin and Excoffier (1996), showed no significant genetic linkage between any paired loci, except for *Omy77* and *Sfo8* (χ^2 = 41.79, df = 22, P = 0.007). However, low allelic diversity was found in Lahontan cutthroat trout for *Sfo8*, one dominant allele (*Sfo8–210*) occurring at a high frequency in all populations. Removal of the *Sfo8* locus had no significant effect on our phylogenetic or genetic distance analyses within Lahontan cutthroat trout. Average F_{st} for all 10 loci combined for all Lahontan cutthroat trout populations (calculated via GENEPOP) was F_{st} = 0.43. Heterozygosity ranged from 0.05 (*One*μ*11*) to 0.54 (*Ots1*); average heterozygosity for all 10 loci was 0.41.

Fisher's exact comparisons of allelic frequency distributions for all 10 loci combined showed population independence between all Lahontan cutthroat trout sample localities (initial α = 0.025; Fisher's adjusted $P < 0.001$ in all cases). We found no significant genetic difference between the type–locality samples for Lahontan cutthroat trout from Edwards Creek sent to our laboratory from Brigham Young University and samples taken more recently from the same drainage, so these samples were combined in all analyses.

Pairwise F_{st}-values calculated for all pairs of putative cutthroat trout subspecies are given in Table 4. Paiute cutthroat trout had no unique alleles at any of the 10 loci compared with Lahontan cutthroat trout. Paiute trout appeared bottlenecked with only one or two alleles per locus. The mean number of alleles per locus in Paiute trout was only 1.4. For three loci (*Omy77*, *One*μ*2*, and *Ots1*), Paiute trout were bottlenecked for the same allele that predominated the Edwards Creek fish. Paiute trout were fixed for alleles *Sfo8–212*. Other than in Paiute trout, this allele was only found at low frequencies (<5%) in Summit and Independence lake samples. Humboldt cutthroat trout allelic diversity contained five unique, rare alleles at three loci (*Ots1*, *Sfo8*, and *Ssa85*), compared with all other Lahontan Basin cutthroat trout.

TABLE 4.—Lahontan Basin cutthroat trout subspecies pairwise F_{st} values calculated from 10 microsatellite loci.

Subspecies pair	F_{st}
Lahontan and Humboldt	0.496
Lahontan and Paiute	0.667
Paiute and Humboldt	0.619

No significant correlation was found between regional F_{st} and geographic distance calculated in kilometers between sample locations (r^2 = 0.008; $P < 0.0001$). This analysis excluded all hatchery stocks, and the transferred Pilot Peak cutthroat trout from Utah were used as a surrogate for Pyramid Lake trout. In the ARLEQUIN's analysis of molecular variance, partitioned Lahontan cutthroat trout genetic variation was as follows: diversity found among putative subspecies (i.e., Paiute, Lahontan, and Humboldt cutthroat trout) = 9.8%; proportion of genetic diversity found among populations = 27.7%; proportion of genetic diversity found within populations = 62.5%.

Population pairwise distance measures based on F_{st} (number of different alleles) calculated for Lahontan Basin cutthroat trout populations ranged from F_{st} = 0.02 (Pilot Peak hatchery and Pilot Peak wild trout) to F_{st} = 0.70 (Slinkard Creek and Paiute cutthroat trout; Table 5). A consensus neighbor-joining tree derived from Cavelli-Sforza and Edwards chord genetic distance analysis is presented in Figure 2. Humboldt River cutthroat trout were separated from the rest of the Lahontan cutthroat trout populations in 81% of the replicate trees. A close genetic association between Pilot Peak wild and the Lahontan National Fish Hatchery's Pilot Peak cutthroat trout strain was supported by 100% of the 2,000 bootstrap trees in these analyses. Fish from the Pilot Peak drainage and Macklin Creek showed similar allelic frequency distributions, based on these analyses, and fish collected from Independence Lake and Heenan Creek proved to be the most genetically distant groups.

Discussion

Higher mutation rates and increased levels of polymorphism associated with nuclear microsat-

TABLE 5.—Pairwise estimates of genetic distance for F_{st} (above diagonal) and the matrix of significant F_{st} probabilities (below diagonal; + = significant) between all possible pairs of Lahontan Basin cutthroat trout populations.

Population by number	Population number												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1 East Carson River		0.453	0.235	0.207	0.211	0.349	0.450	0.278	0.218	0.121	0.431	0.157	0.151
2 Edwards Creek	+		0.339	0.385	0.401	0.470	0.661	0.454	0.458	0.319	0.624	0.374	0.326
3 Frazer Creek		+		0.047	0.079	0.183	0.541	0.041	0.196	0.089	0.335	0.106	0.105
4 Heenan Creek	+	+			0.207	0.352	0.423	0.318	0.233	0.246	0.451	0.089	0.111
5 Independence Lake	+	+	+	+		0.243	0.488	0.216	0.133	0.144	0.467	0.160	0.135
6 Macklin Creek	+	+	+	+	+		0.550	0.343	0.259	0.310	0.586	0.336	0.260
7 Paiute cutthroat trout	+	+	+	+	+	+		0.494	0.575	0.402	0.695	0.522	0.561
8 Pilot Peak Hatchery	+	+		+	+	+	+		0.022	0.276	0.498	0.238	0.184
9 Pilot Peak wild	+	+	+	+	+	+	+			0.186	0.484	0.263	0.170
10 Pyramid Lake Hatchery	+	+		+	+	+	+	+	+		0.461	0.051	0.070
11 Slinkard Creek	+	+	+	+	+	+	+	+	+	+		0.426	0.415
12 Summit Lake	+	+		+	+	+	+	+	+	+	+		0.115
13 West Marys River	+	+		+	+	+	+	+	+	+	+	+	

ellite loci make allele frequency data gathered from these loci useful for studying evolutionary relationships in closely related populations (Goldstein et al. 1995; Takezaki and Nei 1996). For a given population, microsatellite data can potentially represent a different temporal or biogeographic scale than allozyme or mtDNA data (Boyce et al. 1996; Nielsen et al. 1997). Our interspecific phylogenetic analyses of Lahontan cutthroat trout, however, did not disagree with previously published results based on mtDNA (Gyllensten and Wilson 1987; Williams et al. 1992).

Similar to mtDNA analyses, microsatellite data supported significant intraspecific genetic population structure among putative subgroups of Lahontan cutthroat trout. In this study, Humboldt cutthroat trout (i.e., fish from the Humboldt River drainage: West Marys River and Frazer Creek

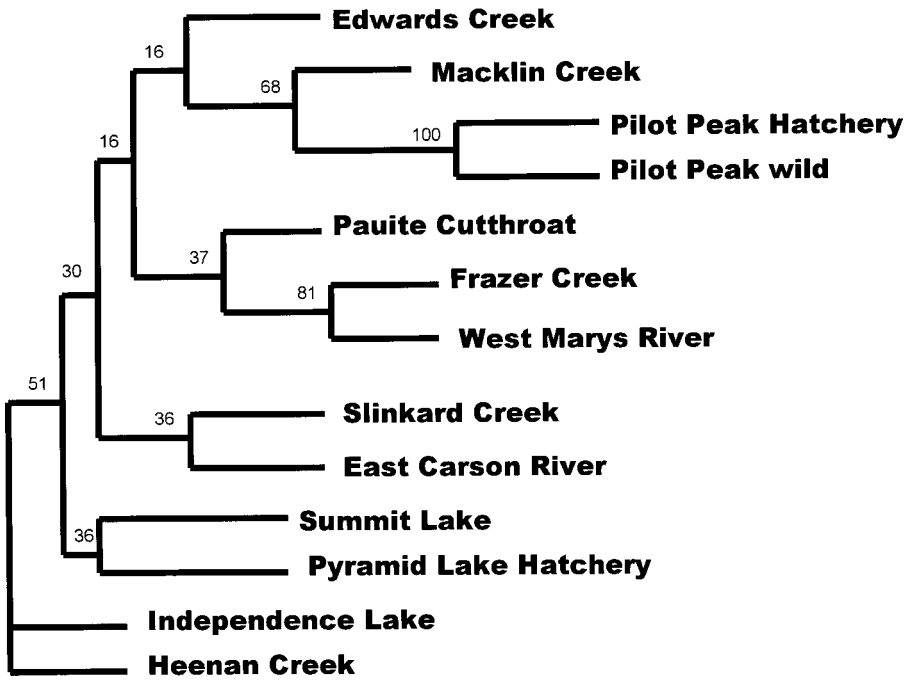

FIGURE 2.—Consensus neighbor-joining tree, based on chord genetic distances (Cavalli-Sforza and Edwards 1967), that was estimated among populations of cutthroat trout. Bootstrap values (%) calculated from 2,000 replicate trees are given at branch points.

trout) separated genetically from western-basin populations (i.e., Carson, Walker, and Truckee rivers) with a high degree of resolution (bootstrap 81% and $F_{st} = 0.496$). These values are comparable to those previously calculated between distinct subspecies of coastal and interior cutthroat trout.

Microsatellite allelic frequency data, however, showed considerable genetic differentiation for the Paiute cutthroat trout subspecies, which was not previously available in earlier genetic studies. The F_{st}-values calculated between Paiute and Lahontan cutthroat trout (0.667) and between Paiute and Humboldt cutthroat trout (0.619) indicate a significant genetic distinction for Paiute cutthroat trout. Much of the genetic structure in Paiute cutthroat trout, however, resulted from genetic bottleneck effects, based on the diminished number of alleles per locus found in the Four Mile Canyon Creek population. The comparisons of Paiute trout with Lahontan Basin cutthroat trout (all $F_{st} > 0.5$) suggest a single colonization event for Paiute trout (Hutchinson and Templeton 1999; Pannell and Charlesworth 1999) that probably occurred before Lahontan and Humboldt trout were isolated from each other during ancient fluctuation in water levels in Lake Lahontan (Benson and Thompson 1987; Behnke 1992).

From microsatellite analyses, trout collected from Heenan Creek appeared to be the most divergent population in relation to Lahontan Basin cutthroat trout. Heenan Lake trout are known to have a history of hybridization with rainbow trout (Behnke and Zarn 1976; Gall and Loudenslager 1981). Residual genetic structure from rainbow trout could confound genetic signatures and suggest false levels of divergence if Heenan Creek fish have mixed with fish in the lake. Introgression may explain the position of this population in our genetic distance analyses. Indeed, microsatellite allelic results from one locus, *Ots1*, suggested residual rainbow trout genes in some fish from this population (J. L. Nielsen, unpublished data).

Within the Truckee River Basin, the Pyramid Lake Hatchery stock from the Lahontan National Fish Hatchery, a stock derived primarily from western-basin lake populations, was most closely related genetically to Summit Lake cutthroat trout. Summit Lake trout contributed significantly to the broodstock development for this hatchery strain (Dunham et al. 1998). A subgroup including trout from Macklin Creek and Pilot Peak (Morrison Creek trout) was closely associated with the Pilot Peak hatchery stock propagated at the Lahontan National Fish Hatchery. The founding gene pool used to establish the federal Pilot Peak strain was drawn from Morrison Creek, the source of the Pilot Peak wild trout population used in this study. Therefore, artificial propagation within the hatchery since 1996 has not significantly changed the genetic signature of this stock.

A high bootstrap value (68%) supported genetic similarity between trout in Macklin Creek, Pilot Peak wild trout, and the Pilot Peak hatchery strain. Macklin Creek trout are thought to be a transferred stock from the Truckee River Basin, where Pyramid Lake fish used to spawn. It is therefore not surprising that these populations group together in these analyses. Macklin Creek fish are currently being used for supplementation of Lahontan cutthroat trout in the Lake Tahoe drainage in California (E. Gerstung, CDFG, personal communication).

Edwards Creek fish are thought to represent a transplanted population from the original Pyramid Lake Lahontan cutthroat trout that also spawned in the Truckee River. Others have suggested that these fish have links to cutthroat trout stocked from Lake Tahoe (Gerstung 1985; Peacock et al. 2001). Our analyses supported a weak genetic association between Edwards Creek fish and transferred Lahontan cutthroat trout populations in Macklin Creek and the Pilot Peak drainage. This diversity in putative transferred stocks from Pyramid Lake may have resulted from different lake genotypes contributing differentially to the original transfers or subsequent genetic bottlenecks at the transfer locations.

We emphasize that small sample sizes were used for some populations and single populations were used to represent entire basins or subspecies in this study (e.g., our use of Four Mile Canyon Creek as representative of Paiute trout and Slinkard Creek to represent the Walker River basin). Additional studies using more loci and larger sample sizes are needed to reinforce the inference gained in this study. We emphasize, however, that all genetic data sets analyzed to date suggest similar large-scale patterns of genetic relatedness throughout the range of Lahontan cutthroat trout.

Additional inference on the molecular genetic architecture of the subdivided groups of Lahontan cutthroat trout can be gained using surrogates for gene flow and drift based on F_{st}, N_m, and Wright's (1931) equation relating the two [$F_{st} \cong 1/(4N_m + 1)$]. Regional equilibrium required for this type of analysis (i.e., analysis based on the island model) is rarely the case in subdivided populations

(McCauley 1993). However, a comparison of regional F_{st}-values with geographic distance calculated between the same population pairs permits evaluation of the historical influences of gene flow and drift in relation to different states of expected genetic structure found along the range of values between equilibrium and disequilibrium (Hutchison and Templeton 1999). The correlation between geographic distance and F_{st} for Lahontan cutthroat trout in this study showed a wide range of scatter. This pattern is similar to the ''case III'' class of populations presented in Hutchison and Templeton (1999). This pattern is consistent with populations in extreme isolation and a very small effective size, where allele frequencies in each population have drifted independently without relation to the geographic distances separating them. As stressed by Templeton et al. (1990) and Sexton et al. (1992), populations in this class are especially vulnerable to extinction.

Whether groups of genetically divergent Lahontan cutthroat trout populations represent unique genetic stocks originally derived from Pyramid Lake or endemic residual groups dating back to the late Pleistocene desiccation of Lake Lahontan is an important evolutionary consideration with significant management implications. It is important to remember that the unique movement and forage patterns that appeared in Lahontan cutthroat trout at the beginning of the last century suggest a diversity of lacustrine and fluviate life histories in the Truckee River basin and in Pyramid Lake. One important management consideration requiring some degree of resolution from genetic studies involves the restoration of the Truckee River basin and Pyramid Lake with their historically endemic cutthroat trout (L. Heki, U. S. Fish and Wildlife Service, personal communication). This will require knowledge of both the endemic genetic structure and different life history adaptations. Unfortunately, there is no way to know if the out-of-basin transfers from Pyramid Lake (i.e., Macklin, Morrison and Edwards Creek trout) have maintained the ability to adapt to a lacustrine life history with the same characteristics as their progenitors.

One controversial issue in this restoration effort is the recovery of fish ''of extraordinary size'' in the Pyramid Lake population (Behnke 1992). Most of the fish put into Pyramid Lake by the hatchery remain small compared to historical records of Pyramid Lake cutthroat trout. Many studies of the genetic architecture of subdivided populations of laboratory organisms have shown that the among-population divergence of some quantitative traits (most notable body size) is driven by local adaptation over time to different environments (Lynch et al. 1999). A truly comprehensive understanding of the phenotypic evolution of large size in the trout of Pyramid Lake will require significant information on the evolutionary forces operating within Lahontan cutthroat trout for size at age and the effects on phenotypic divergence from relative change in the ecology, habitat, and population structure within this lake. The investigation of phenotypic expression of genes influencing life history variability and adaptive traits is new to the genetics community and has had limited application in fishes (see Leroi 2001). Future research along these lines is recommended.

It is clearly an important goal in conservation to protect and provide for the capacity of the evolutionary process so that natural diversity, in all of its aspects, is restored and maintained, as opposed to maximizing current patterns of genetic diversity under artificial conditions (Kark and Blackburn 2000). Working with the best genetic information available for the restoration of Lahontan cutthroat trout, conservation geneticists also need to allow and accept the limitations of this approach in its current form and the possibility that the future of diversity in Lahontan cutthroat trout is tightly linked to ongoing evolutionary processes beyond active human control.

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