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ARTICLE

# Historical Influences of Volcanism and Glaciation in Shaping Mitochondrial DNA Variation and Distribution in Yellowstone Cutthroat Trout across Its Native Range

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*Abstract*

**While Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* are probably the best known and one of the most extensively researched of all the subspecies of cutthroat trout, relatively little is known about their genetic structure and evolutionary history. In this study, we assessed the genetic variability and population structure among 50 populations of Yellowstone cutthroat trout over a large portion of their range in Idaho, Montana, Utah, Wyoming, and Nevada using restriction fragment length polymorphism and sequencing analysis of the mitochondrial ND1 and ND2 gene regions. Among the more than 1,000 samples analyzed, a total of 17 haplotypes were observed. These data indicate significant geographic structuring of the genetic variation between drainages and varying levels of reproductive isolation among populations within drainages. Much of this genetic structuring is clearly the product of long-term historical processes (basaltic volcanism and glaciations) that have isolated populations for substantial periods of time and then, in many cases, allowed secondary contact and subsequent admixture of divergent populations. Comparisons of samples between major basins were consistent with the results of previous allozyme and mtDNA investigations indicating that cutthroat trout in the Bear River basin in Utah have a more recent common ancestor with Yellowstone cutthroat trout than with the populations of Bonneville cutthroat trout *O. c. utah* in the central and southern portions of their range in Utah. The results from this study should assist managers with future conservation and management planning efforts for both subspecies.**

Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* are among the most widely distributed and best known of the 13 described subspecies of cutthroat trout native to the western United States. Their range extends upstream from Shoshone Falls on the Snake River in Idaho, across the continental divide into Wyoming, and into the Yellowstone River drainage in Montana (Figure 1). Like all western trout, they have experienced substantial declines in abundance and distribution throughout their historical range. These declines prompted a series of legal petitions to list the subspecies under the U.S. Endangered Species Act (USFWS 2001, 2005), although listings have been deemed unwarranted (USFWS 2006). Efforts are ongoing to document the current distribution and abundance of Yellowstone cutthroat trout and to develop management strategies to enhance and preserve populations and maintain their genetic diversity (May et al. 2003; Meyer et al. 2003b, 2006; Cegelski et al. 2006).

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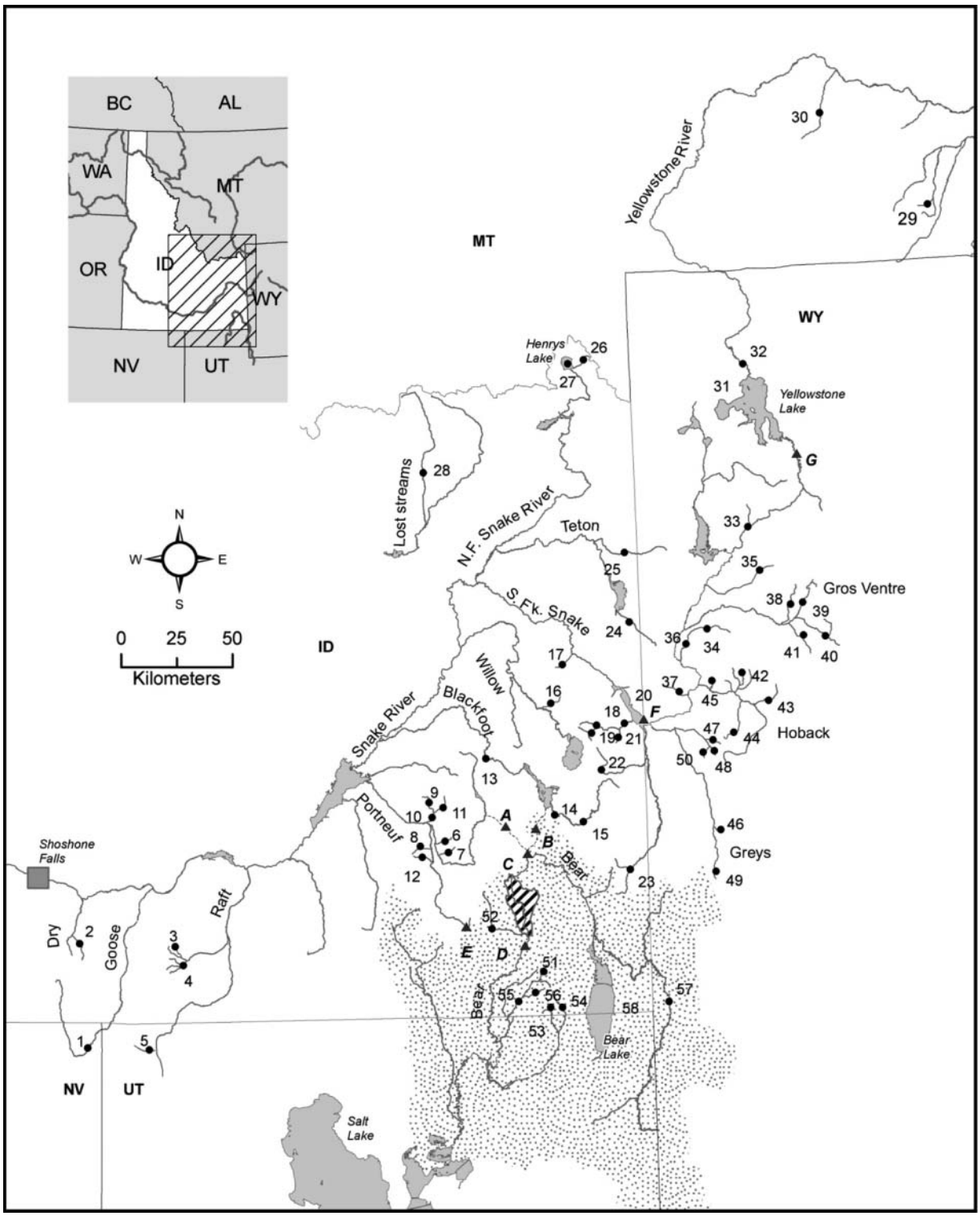


FIGURE 1. Locations of 58 of the sample sites in Nevada, Idaho, Wyoming, and Utah (circles, with numbers corresponding to those in Table 1). Three sampling locations are not shown (Daniel Fish Hatchery, Wyoming [59]; Glenwood Fish Hatchery, southern Utah [60]; and South Fork Johnson Creek, central Utah–Nevada border [61]). The range of Yellowstone cutthroat trout extends from a natural barrier (Shoshone Falls, noted on the map) to the Yellowstone River drainage. The stippled area designates the Bear River basin (Bonneville Basin). Triangles identify landmarks referred to in the text, as follows: A = the former course of the Bear River; B = the alternate course of the Bear River; C = Soda Point; D = Oneida Narrows; E = Red Rocks Pass; F = Palisades Reservoir; and G = Two-Oceans Pass. The area with diagonal lines marks historic Lake Thatcher.

Understanding the influence and relative importance of contemporary and historical processes in shaping the pattern of genetic variation among and within populations of Yellowstone cutthroat trout is crucial to developing and effectively evaluating current and future genetic conservation efforts. Recent genetic examinations of Yellowstone cutthroat trout using microsatellite DNA have revealed that contemporary processes have been instrumental in shaping the observed genetic population structure across the subspecies' range (Cegelski et al. 2006). Droughts, habitat alteration (from dams and diversions), and nonnative fish introductions appear to have influenced genetic diversity and gene flow at various spatial scales. Contemporary processes alone, however, appear insufficient to fully explain the patterns of genetic diversity between and within populations of Yellowstone cutthroat trout.

Yellowstone cutthroat trout evolved in the upper Snake, Yellowstone, and Bear River basins of Idaho, Wyoming, and Utah (Behnke 1992), possibly diverging from an ancestor in the main Bonneville Basin during the early Pleistocene Epoch (Smith et al. 2002). The climatic and geological events in these basins throughout the Pleistocene were particularly complicated and chaotic (Alt and Hyndman 1989). In the southern part of the Yellowstone cutthroat trout's range, massive basaltic eruptions and lava flows blocked and redirected entire river drainages, as exemplified by the history of the Bear River (Bouchard et al. 1998). The river currently flows into the Great Salt Lake in the Bonneville Basin. However, it is believed that historically it flowed north to the Snake River, most likely through the Portneuf River Canyon or possibly through the Blackfoot River drainage (Figure 2; Link et al. 1999). About 500,000 years ago, basaltic volcanism in both the Gem and Blackfoot River valleys produced large lava fields (Scott et al. 1982; Kuntz et al. 1992). It has been hypothesized that these lava flows repeatedly blocked the northward drainage of the Bear River (Scott et al. 1982). These events, along with southern regional tilt (Mabey 1971) probably contributed to the formation of a series of lakes in the Thatcher basin in southern Idaho collectively called Lake Thatcher (Figure 2; Bright 1963; Bouchard et al. 1998). Sedimentological evidence suggests that over the next 500,000 years the Bear River reversed course multiple times as

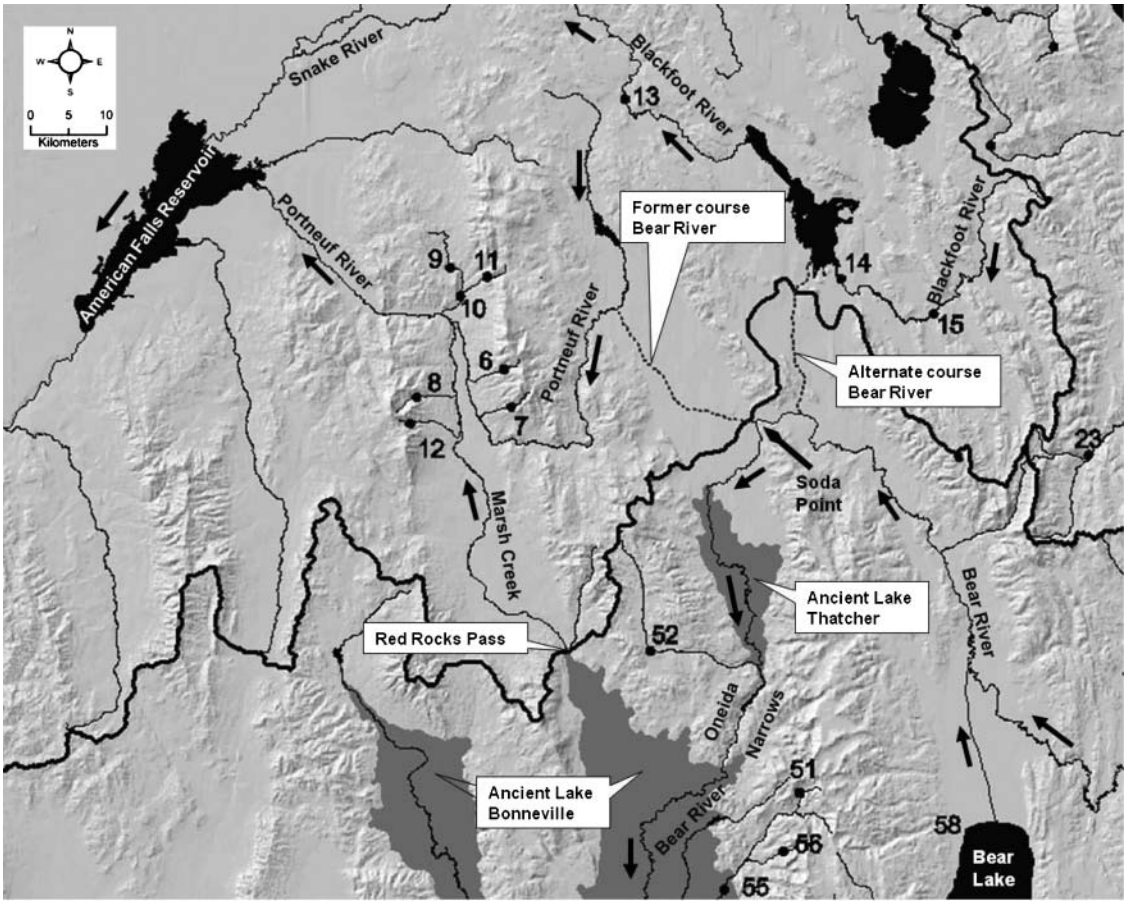


FIGURE 2. Map of the Portneuf, Blackfoot, and Bear River–Lake drainages. The dark black line shows the present-day divide between the Bonneville and Snake River basins. Black arrows indicate the present-day direction of river flow. Major features referenced in the text include Red Rocks Pass, ancient Lake Bonneville, ancient Lake Thatcher, and the alternate courses of the Bear River drainage (dashed lines) during historic diversions.

a result of basalt flows, alternately flowing south into the Lake Thatcher area or north toward the Snake River basin via surface or subsurface flows (Bouchard et al. 1998). Lake Thatcher's connection with the Bonneville Basin is not clear during much of this time period. However, it is known that by about 20,000 years ago Lake Thatcher filled and eventually overflowed into the Bonneville Basin after the evacuation of Oneida Narrows, dramatically increasing the volume of water entering the basin and historic Lake Bonneville (Link et al. 1999). Since that time the Bear River has flowed into the Bonneville Basin except for a very brief period between 14,000 and 11,000 years ago, when a catastrophic flood drained Lake Bonneville into the Snake River basin (Bouchard et al. 1998).

In contrast to the basaltic volcanism in the southern part of the Yellowstone cutthroat trout's range, the historically dominant geological process affecting the upper Snake and Yellowstone River drainages in Wyoming was glaciation, of which there were two periods. The older glaciation (Bull Lake) took place from 160,000 to 130,000 years ago (Love et al. 2003). During this time, ice covered all of the Yellowstone and Jackson Hole area, with glaciers filling the Wind River and Gros Ventre valleys and extending as far south as the mouth of the Hoback River (Love et al. 2003). The more recent Pinedale glaciation lasted from more than 30,000 to about 14,000 years ago; it was not as extensive but still covered all of the Yellowstone Plateau and much of the area north of Jackson Hole, including Jackson Lake and Pacific Creek (Love et al. 2003).

Collectively, these historical processes created a complicated history of hydrological diversion, isolation, and reconnection throughout these basins, providing numerous opportunities for population isolation, divergence, and reconnection. Among-population biological diversity of Yellowstone cutthroat trout has long been recognized and extensively documented (Varley and Gresswell 1988; Behnke 1992; Meyer et al. 2003a, 2006), suggesting that these historical processes did result in periods of isolation and divergence. However, little is currently known about the genetic variation and distribution underlying that diversity. In addition, although previous research has focused on describing the phylogenetic relationships among the major cutthroat trout subspecies (Loudenslager and Gall 1980; Allendorf and Leary 1988; Williams et al. 1994), no studies have examined the phylogeography of Yellowstone cutthroat trout across its present range.

Phylogeography is the study of the geographic distribution of genetic lineages within and among closely related species and the processes that have shaped that structuring (Avise 2000). It is an important field of study for ichthyologists because it provides insight into the processes that have generated the inter- and intraspecific diversification of fish over a wide range of geographical scales (e.g., Bernatchez and Wilson 1998; Johnson 2002; Durand et al. 2003). Phylogeographic studies have also been increasingly used to identify evolutionarily distinct lineages for conservation (e.g., McCusker et al. 2000; Morrison et al. 2006; Robalo et al. 2007; Gum et al. 2009) and to resolve taxonomic uncertainties (e.g., Poulin et al. 2004; Metcalf et al. 2007; Snoj et al. 2010). These issues are relevant to the conservation and management of Yellowstone cutthroat trout. For example, previous studies using allozyme and mitochondrial DNA (mtDNA) analyses over a limited sampling area indicate that the cutthroat trout within the Bear River basin, though formally recognized as a separate subspecies (Bonneville cutthroat trout *Oncorhynchus clarkii utah*), are genetically more closely related to Yellowstone cutthroat trout than to other Bonneville cutthroat trout populations found in the Bonneville Basin (Martin et al. 1985; Toline et al. 1999).

This study focuses on a phylogeographic investigation of Yellowstone cutthroat trout across its historic range, with the goals of (1) providing insight into the role of historical events in shaping current geographic patterns of genetic divergence and distribution and (2) furthering the understanding of the colonization and isolation history of Yellowstone cutthroat trout with respect to cutthroat trout found in the Bonneville Basin in Idaho and Utah.

To address these goals, we examined 1,155 Yellowstone cutthroat trout samples from 50 sampling locations or populations across the subspecies' range in the Snake River basin. We also included a large number of cutthroat trout samples (273 samples from 9 sampling locations) from the Bear River drainage (Bonneville Basin). The samples were examined using mtDNA restriction fragment length polymorphism (RFLP) and sequencing analyses to assess the genetic variation and population structure of the subspecies in a phylogenetic and phylogeographic context. The utility of mtDNA analyses for assessing intra- and interspecific phylogenetic patterns has long been established (Avise et al. 1987, 1994); we combined both RFLP and sequencing analyses to provide maximum efficiency and resolution.

## METHODS

### Sample Collection

From 1999 to 2003, nonlethal fin tissue samples were collected from 50 Yellowstone cutthroat trout populations (primarily by electrofishing) across the species' present range in Idaho, Montana, Utah, Wyoming, and Nevada (Figure 2; Table 1). During the same period, samples were also collected from 11 populations of Bonneville cutthroat trout: nine from the Bear River basin in northern Utah and southern Idaho (including a reference population from Daniel Fish Hatchery, Wyoming); one from Glenwood Fish Hatchery, Utah (founded from fish from the Sevier River drainage in the southern Bonneville Basin), representing the southern portion of their range in Utah; and one from South Fork Johnson Creek in the Deep Creek Mountains along the Utah–Nevada border, representing the western portion of their range. Fin clips were stored in 100% nondenatured ethanol or lysis buffer (0.5M EDTA, 2M tris, 5M NaCl, 20% sodium dodecyl sulfate, and deionized $H_2O$) prior to DNA extraction.

## Genetic Analysis

*DNA extraction*.—Total genomic DNA was extracted using a standard salt–chloroform protocol adapted from Sambrook et al. (1989) and Dowling et al. (1990) and described in detail by Campbell (2000).

*Polymerase chain reaction amplification*.—The combined NADH dehydrogenase 1 and 2 (ND12) gene regions (3,558 base pairs) were amplified following procedures described by Toline et al. (1999). Primers flanking the ND12 region, 5′-GCC TCG CCT GTT TAC CAA AAA CAT-3′ at position number 2,988 within the 16S ribosomal RNA (ND12 L) and 5′-CCG GCT CAG GCA CCA AAT AC-3′ at position number 6,547 within the cytochrome c oxidase I gene (ND12 H), were purchased from Integrated DNA Technology (Coralville, Iowa). The ND12 mtDNA gene region was amplified in a 40-μL reaction consisting of 0.5–3.0 μL DNA extract (unquantified), 4.0 μL 10× buffer (Perkin Elmer), 4.0 μL $MgCl_2$, 3.2 μL bovine serum albumin, 1.0 μL DMSO, 4.0 μL of each primer, 3.2 μL 10.0-mM deoxynucleotide triphosphates (10 mM each of deoxyadenosine triphosphate, deoxycytidine triphosphate, deoxyguanosine triphosphate, and deoxythymidine triphosphate), 0.15 μL *Taq* polymerase (Perkin Elmer), and 13.45–15.95 μL deionized $H_2O$. Polymerase chain reaction conditions consisted of an initial denaturing cycle of 94°C for 3 min, followed by 39 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 1 min, and extension at 72°C for 4 min, with a final extension at 72°C for 5 min.

*Restriction enzyme digestion of amplification products*.—Amplification products were digested with four restriction enzymes (*Dpn*-II, *Hin*f-I, *Msp*-I and *Rsa*-I) that had previously yielded polymorphisms in Yellowstone cutthroat trout and Bonneville cutthroat trout samples (Toline et al. 1999). Digests were electrophoresed on 3% agarose/synergels with tris-acetate-EDTA buffer or 6% acrylamide gels with tris-borate-EDTA and visualized as band patterns (fragments) when stained with ethidium bromide and fluoresced under ultraviolet light. Each unique banding pattern (polymorphism) generated by a specific restriction enzyme was rerun on a 6% acrylamide gel, photographed, and assigned a letter designation. The letter designations for each of the four restriction enzymes were later combined and designated as a composite haplotype.

*DNA sequencing*.—The entire ND12 gene region was sequenced on one to four representatives of each observed haplotype (GenBank accession numbers EU186781–EU186801). Internal primer sequences were designed using the online software program Primer3 (Rozen and Skaletsky 2000; http://frodo.wi.mit.edu/primer3/) using the complete mtDNA sequence for rainbow trout *O. mykiss* (Zardoya et al. 1995) available on GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). The internal primer sequences are as follows:

ND12 reverse, number 1: 5′-CCTGATCCAACATCGAGGT-3′
ND12 forward, number 2: 5′-ACCTCGATGTTGGATCAGG-3′
ND12 reverse, number 2: 5′-GCGTACTCGGCTAGGAAAAA-3′
ND12 forward, number 3: 5′-GGGCAGTGGCACAAACTATT-3′
ND12 reverse, number 3: 5′-GGTATGGGCCCGAAAGCTTA-3′
ND12 forward, number 4: 5′-TAAGCTTTCGGGCCCATACC-3′
ND12 reverse, number 4: 5′-GGGTCGGGGATTTAGTTCAT-3′
ND12 forward, number 5: 5′-ATGAACTAAATCCCCGACCC-3′.

Sequencing reactions were performed with a BigDye Terminator Cycle Sequencing Ready Reaction Kit (version 3.1; Applied Biosystems) using the internal forward and reverse primers as well as the primers used in the initial amplification (ND12 H and L). Sequenced products were cleaned using gel filtration plates (Edge Biosystems, Gaithersburg, Maryland) and run out on a Prism 3730 DNA sequencer (Applied Biosystems). Sequences were edited using Sequencher (version 4.1.2; Gene Codes Corporation, Ann Arbor, Michigan), and the consensus sequences were aligned using the Clustal X program (version 1.81; Thompson et al. 1997) on the San Diego supercomputer center workbench.

## Statistical Analysis

Haplotype frequencies from the RFLP analyses and aligned DNA sequences (3,483 base pairs) from the observed haplotypes were used to prepare input files for the program ARLEQUIN (version 3.5.1.2; Excoffier and Lischer 2010). The program was then used to calculate within-population nucleotide diversity ($\pi$) and haplotype diversity ($h$). ARLEQUIN was also used to perform a hierarchical analysis of population subdivision via an analysis of molecular variance (AMOVA). The AMOVA analyses were run several ways: all 61 sample locations grouped according to the major drainage basin to which they belong (20 groups); Snake River sample locations grouped as to whether they were below (22 locations) or above Palisades Reservoir (28 locations); and Snake River sample locations grouped as to whether they were below or above Palisades Reservoir as well as according to the major drainage to which they belong (10 and 7 groups, respectively). We felt it was appropriate to make the division at Palisades Reservoir since drainages that flow into the Snake River upstream of this area in Wyoming have been affected by extensive glaciation periods that did not affect Snake River drainages below the reservoir in Idaho (Love et al. 2003; USFS 2004).

Version 2.1 of the software program MEGA (Molecular Evolutionary Genetics Analysis; Kumar et al. 2001) was used to obtain basic descriptive statistics (including the average base composition, the transition : transversion ratio, the number of variable and parsimony informative sites) as well as to estimate the pairwise sequence divergence between haplotypes and to construct a bootstrapped (10,000 replicates) neighbor-joining tree (both with the Kimura two-parameter model).

Two additional approaches were used to examine the phylogenetic relationships among haplotypes. First, the relationships among haplotypes were inferred based on Bayesian posterior probabilities implemented estimated in MrBayes version 3.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck

TABLE 1. Haplotype frequencies among the 61 sample locations. Superscripts refer to drainage designations, as follows: 1 = Goose, 2 = Dry, 3 = Raft, 4 = Portneuf, 5 = Blackfoot, 6 = Willow, 7 = South Fork Snake, 8 = McCoy, 9 = Salt, 10 = Teton, 11 = Henrys Lake, 12 = Lost Streams, 13 = Yellowstone, 14 = Upper Snake, Wyoming, 15 = Gros Ventre, 16 = Hoback, 17 = Greys, 18 = Bear, 19 = Sevier, and 20 = Deep Creek. Fish sampled from locations within the Bear, Sevier, and Deep Creek River drainages are formally recognized as *Bonneville cutthroat trout*. The remaining drainages are all in the Snake River basin and fish sampled from these areas are formally designated as *Yellowstone cutthroat trout*.

| | | Haplotype | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sample Location | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Goose Creek[1] | 1 | | | | | | | 31 | | | | | | | | | |
| Dry Creek[2] | 2 | 14 | | | | | | 14 | | | 1 | | | | | | |
| New Canyon Creek[3] | 3 | 1 | | | | | | 26 | | | | | | | | | |
| Green Creek[3] | 4 | 6 | | | | | | 24 | | | | | | | | | |
| Basin Creek[3] | 5 | | | | | | | | | | 1 | | | 27 | | | |
| Robbers Roost Creek[4] | 6 | | | | | | | 16 | | | | | | | 7 | 7 | |
| Harkness Creek[4] | 7 | | | | | | | | | | | | | | 19 | 8 | |
| Bell Marsh Creek[4] | 8 | | | | | | 1 | 2 | | | 1 | | | | 18 | 3 | |
| Upper Rapid Creek[4] | 9 | 1 | | | | | | 2 | | | | | | | 17 | 2 | |
| Main Rapid Creek[4] | 10 | | | | | | 1 | 1 | | | | | | | 10 | 3 | |
| Inman Creek[4] | 11 | 2 | | | | | 17 | 10 | | | | | | | 1 | | |
| Goodenough Creek[4] | 12 | | | | | | | 1 | | | | | | | 2 | 12 | |
| Lower Blackfoot River[5] | 13 | 1 | | | | | | | | | 5 | | | | | | |
| Middle Blackfoot River[5] | 14 | | | | | | 3 | | | 24 | 12 | | | | | | |
| Upper Blackfoot River[5] | 15 | | | | 1 | | 1 | | | 20 | 4 | | | | | | |
| Lava Creek[6] | 16 | 16 | | | | | | | | | | | | | | | |
| Garden Creek[7] | 17 | | | | | | | | | | 13 | | | | | | |
| Barnes Creek[8] | 18 | 15 | | | | | 1 | | | | 9 | 2 | 3 | | | | |
| Clear Creek[8] | 19 | 16 | | | | | 2 | | 1 | | 7 | 2 | 3 | | | | |
| McCoy Creek[8] | 20 | 18 | | | | | | | | | 6 | | | | | | |
| Fish Creek[8] | 21 | 23 | | | | | | 1 | | | 4 | 1 | | | | | |
| Tincup Creek[9] | 22 | 9 | | | | | | | | | 5 | | | | | | |
| Crow Creek[9] | 23 | 26 | | | | | | | | | 4 | | | | | | |
| Mike Harris Creek[10] | 24 | | | | | | 28 | | | | | | | | | | |
| South Fork Badger Creek[10] | 25 | 8 | | | | | | | | | | | | | | | |
| Tyghee Creek[11] | 26 | | | | | | 27 | | | | | | | | | | |
| Henrys Lake[11] | 27 | 6 | | | 9 | | 28 | | | | | | | | | | |
| Middle Dry Creek[12] | 28 | | | | | | 26 | | | | | | | | | | |
| Brushy Fork Creek[13] | 29 | | | | 20 | | | | | | | | | | | | |
| Placer Creek[13] | 30 | | | | 27 | | | | | | | | | | | | |
| Yellowstone Lake[13] | 31 | 30 | | | 31 | | | | | | | | | | | | |
| Lehardy Rapids River[13] | 32 | 7 | 1 | | 15 | 1 | | | | | | | | | | | |
| Pacific Creek[14] | 33 | 6 | | 7 | 2 | | | | | | | | | | | | |
| Jackson National Fish Hatchery[14] | 34 | 29 | | | 3 | | | | | | | | | | | | |
| Ditch Creek[14] | 35 | 6 | | | 4 | | | | | | 5 | | | | | | |
| Flat Creek[14] | 36 | 16 | | | | | | | | | | | | | | | |
| Cabin Creek[14] | 37 | 6 | | | | | | | | | 3 | | | | | | |
| Cottonwood Creek[15] | 38 | 11 | 1 | | | | | | 1 | | 11 | | | | | | |
| North Fork Fish Creek[15] | 39 | 17 | | | 3 | | | | | | 2 | | | | | | |
| Leeds Creek[15] | 40 | 8 | | | | | | | | | 1 | | | | | | |
| Bacon Creek[15] | 41 | 12 | | | | | | | | | 2 | | | | | | |
| Boulder Creek[16] | 42 | 16 | | | 1 | | | | | | 7 | | | | | | |

*(Continued on next page)*

TABLE 1. Continued.

| Sample Location | | Haplotype | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Dell Creek[16] | 43 | 15 | | | 1 | | | | | | 3 | | | | | | |
| Bondurant Creek[16] | 44 | 14 | | | | | | | | | 1 | | | | | | |
| Bull Creek[16] | 45 | | | | | | | | | | 20 | | | | | | |
| Martin Creek[17] | 46 | 8 | | | | | | | | | | | | | | | |
| Steer Creek[17] | 47 | 19 | | | | | | | | | 5 | | | | | | |
| Blind Trail Creek[17] | 48 | 22 | | | | | | | 5 | | 2 | | | | | | 5 |
| Poison Creek[17] | 49 | 5 | | | | | | | 1 | | 1 | | | | | | |
| South Fork Little Greys River[17] | 50 | 12 | | | | | | | 2 | | 1 | | | | | | 2 |
| Maple Creek[18] | 51 | | | | | | | | 21 | | | 1 | 3 | | | | |
| Cottonwood Creek[18] | 52 | | | | | | | | 0 | | | 12 | 10 | | | | |
| Logan R.[18] | 53 | | | | | | | | 14 | | | 3 | 5 | | | | |
| Beaver Creek[18] | 54 | | | | | | | | 9 | | | 1 | 3 | | | | |
| Cub R.[18] | 55 | | | | | | | | 17 | | | 3 | 9 | | | | |
| Sugar Creek[18] | 56 | | | | | | | | 23 | | | 1 | 1 | | | | |
| Peagram Creek[18] | 57 | | | | | | | | 22 | | | 1 | 2 | | | | |
| Bear Lake[18] | 58 | | | | | | | | 46 | | | 13 | 5 | | | | |
| Daniel Fish Hatchery[18] | 59 | | | | | | | | 1 | | | 16 | 31 | | | | |
| Glenwood Fish Hatchery[19] | 60 | | | | | | | | | | | | | 24 | | | |
| South Fork Johnson Creek[20] | 61 | | | | | | | | | | | | | 52 | | | |
| Total | | 449 | 2 | 7 | 117 | 1 | 135 | 128 | 163 | 44 | 136 | 28 | 75 | 103 | 74 | 35 | 7 |
| Percent | | 29.9 | 0.1 | 0.5 | 7.8 | 0.1 | 9.0 | 8.5 | 10.8 | 2.9 | 9.0 | 1.9 | 5.0 | 6.9 | 4.9 | 2.3 | 0.5 |



2003). The software jModelTest was initially used to estimate the model of nucleotide substitution (Posada 2008). The likelihood calculations were carried out with the following parameters: three different model selection strategies (since MrBayes will only allow three schemes), equal or unequal base frequencies, a proportion of invariable sites and rate variation among sites, and a maximum likelihood optimized base tree. Following the likelihood analysis, the Akaike information criterion was used to pick the best model. The specified model (general time reversible, gamma distribution plus a proportion of invariant sites [GTR, I + G]) was then used in MrBayes along with default priors, run for 60,000 generations, and sampled every 10th generation. The average SD of split frequencies was 0.011, indicating convergence. The first 25% of samples were discarded as "burn in" to ensure sampling from a stationary posterior distribution. In the second approach, we constructed a haplotype network based on a statistical parsimony approach (Templeton et al. 1992) using the software program TCS 1.13 and a 95% connection limit criterion (Clement et al. 2000).

## RESULTS

### RFLP and Sequencing Analyses

A total of 1,504 samples from the 61 sample locations (both Yellowstone cutthroat trout and Bonneville cutthroat trout) were analyzed with the four-enzyme RFLP screen, revealing 16 separate haplotypes (Table 1). To obtain accurate estimates of the sequence divergence between haplotypes, one to four representative samples of each haplotype were sequenced (Table 1). We were unable to obtain a complete sequence for haplotype 16. It was present in only two sample locations (South Fork Little Greys River [$n = 2$] and Blind Trail Creek, Wyoming [$n = 5$]) and at a frequency of less than 0.5% in the entire data set. Two additional haplotypes were discovered through sequencing (haplotypes 11b and 13b). All sequences were submitted to GenBank (accession numbers EU186781–EU186801).

Of the 3,483 base pairs successfully sequenced, the average base composition across haplotypes was as follows: 25.4% T, 29.7% C, 26.3% A, and 18.6% G. A total of 94 sites (85 transitions and 9 transversions) were variable among the 17 haplotypes (Table 2) and 70 of these were parsimony informative. Trees produced from both the neighbor-joining and Bayesian analyses showed similar topologies. Only the neighbor-joining tree is shown (Figure 3), with bootstrap values/clade credibility values shown above the branches. Two divergent clades were observed (A and B; 100% bootstrap support), with an average sequence divergence of 1.58% (Table 3). Clade A contained haplotypes 1 through 12. Sequence divergence within clade A ranged from 0.03% to 0.58% (mean, 0.30%). Significant structuring of haplotypes within clade A was

Table 2. Variable sites in 17 haplotypes found in 3,483 bp of th e combined ND 1 and 2 mitochondrial gene regions. Numbers above the sequences correspond to the position of the polymorphic site starting from the 5' end of the ND 1 region. Dots indicate an identical nucleotide at the position relative to Haplotype 1.

| Haplotype | Variable Sites |
|---|---|
| | 1111111111111111111111111111111122222222222222222222222222222222222222222222333333333333 |
| | 2223346777888999000011111111223334556667778911111122222334444444444455566777777778888990000000001 |
| | 0560568156556279678023456791903923756822767001236024568011222445745903023566881488061112223891 |
| | 8070020254490501352208705153136361746113812342109891975439027081870978053439574023172351897354 |
| 1 | AGAAGGAGACGGTACGGAACAGTACGACATAGAGCGTACGCAACAGGCATGATAAGAGTGCCTCAGATGGGGATAATAGCCCTATGGGACTGGG |
| 2 | .....A..... |
| 3 | .....A..... |
| 4 | .....A.....CA.A.C..... |
| 5 | ..G...A.....G.....CA.A..... |
| 6 | .A.....A.....T.....T.....C.....G.....G..A.....G..... |
| 7 | .....A.GG...AT.....AG.....G..A.....GA.....A.. |
| 8 | .....G.....A.G...A.....AG.....G..A.....GA.....A.....A.. |
| 9 | C.....T.....C..A.G...A.....AG.C...G..A.....GA.....A.. |
| 10 | C.....GT.....A.G...A.....AG.C...G..A.....GA.....A.. |
| 11a | ...G..G..A.....G....A.....A.....G..AG...G.G.AA.....GA..... |
| 11b | ...G..G.....G....A.....A.....G..AG...G.G.AA.....GA..... |
| 12 | ...GA.....A.....A.....A....G.....AG...G.G.AA.....GATTT.....A |
| 13a | ..G.....CGTAAG..GACGT...GCG.GA.A.G.AAG.T....G..GCGGA.AC.AT.A.A.C.AT. G.CGCG.....GC.AAGAC.A. |
| 13b | ..G.....ACGTAAG..GACGT...GCG.GA.A.G.AAG.T....G..GCGG..AC.AT.A.A.C.AT. G.CGCG.....GC.AAGAC.A. |
| 14 | ..G.....G...CGTAAG..GACGT...GC..GA.A.G.AAG.T.A..G.AGCGG..AC.AT.A.A.C.AT. G.CGCG....CGC.AAGAC.A. |
| 15 | ..G..A..G...CGTAAG..GACGT...GC..GA.A.GTAAG.T.A..G.AGCGG..AC.AT.A.A.C.AT. G.CGCG.....GC.AAGAC.A. |

observed, with more than 50% bootstrap support for all internodes. Two subclades (with relatively weak bootstrap support) separated haplotypes 1–6 (A1; 54%) from haplotypes 7–12 (A2; 51%). Clade B consisted of the remaining four haplotypes clustered into two groupings: haplotypes 13a and 13b (92%) and haplotypes 14 and 15 (95%). Sequence divergence within clade B ranged from 0.06% to 0.20% (mean, 0.15%).

The large divergence of the two major clades was also clearly demonstrated in the parsimony analyses (Figure 4). Haplotypes 1–12 (clade A) were joined in a single haplotype network with 95% confidence. However, haplotypes 13–15 (clade B) could not be joined with haplotypes from clade A via statistical parsimony with 95% confidence. A high degree of divergence between the haplotypes within the subclades was also observed, with a large number of mutational steps (7–11) separating many haplotypes.

## Haplotype Diversity and Distribution

Across the 61 sampling locations, the average sample size was 25 and the average number of haplotypes observed per location was 2.5. Clear Creek ($n = 31$) had the highest number of haplotypes (6). The most common haplotype observed was haplotype 1 (Table 1); it was observed in more sample locations than any other haplotype ($n = 34$) and was present in most of the Snake River drainages sampled in Idaho and Wyoming. Haplotype 10 was also widely distributed across the sampling area, occurring in 27 sample locations. The majority of the haplotypes, however, were generally limited to specific geographic areas. Haplotype 7 was primarily limited to populations in the Goose, Raft, and Portneuf River drainages in Idaho. Haplotypes 14 and 15 were found only in the Portneuf River drainage. Haplotype 9 was restricted to the upper Blackfoot River drainage. Haplotype 6 was predominant in the Henrys Fork drainage (Henrys Lake and Tyghee Creek). Haplotype 4 was primarily found in the Yellowstone River basin and the upper Snake River basin. Haplotypes 8, 11, and 12 were primarily limited to the Bear River basin. Haplotype 13a was limited to South Fork Johnson Creek and the Glenwood Hatchery, and haplotype 13b was limited to one population in the upper Raft River.

Haplotype and nucleotide diversity varied widely across the 61 sample locations (Table 4.) Haplotype diversity ranged from 0.0000 (14 locations) to $0.7048 \pm 0.0535$ (mean $\pm$ SE) in Ditch Creek. The overall haplotype diversity across all sampling locations was $0.3319 \pm 0.0735$. Nucleotide diversity ranged from 0.0000 to $0.0086 \pm 0.0043$.

Analysis of molecular variance at the 61 sample locations grouped according to drainage indicated that there was significant partitioning at each hierarchical level (Table 5). However, the highest percentage variance was attributed to differences between drainages (52.3%; $P < 0.001$), which is consistent with the restriction of many haplotypes to one or two drainages. When the Snake River sample locations were grouped into those below and those above Palisades Reservoir (i.e., nonglaciated versus glaciated areas), AMOVA analyses showed that below the reservoir mtDNA variation is partitioned predominantly among populations (78.4%) while above the reservoir it is partitioned predominantly within populations (67.5%). A similar pattern was observed when sample locations above and below the reservoir were further grouped by drainage: below the reservoir genetic variation was partitioned predominantly among populations within drainages (51.6%), while above the reservoir it is partitioned predominantly within populations (66.2%).

Haplotype and nucleotide diversity were similar across the 20 drainages (Table 4). The highest values for both were observed in the Portneuf River drainage ($h = 0.4895$, $\pi = 0.0039$). The

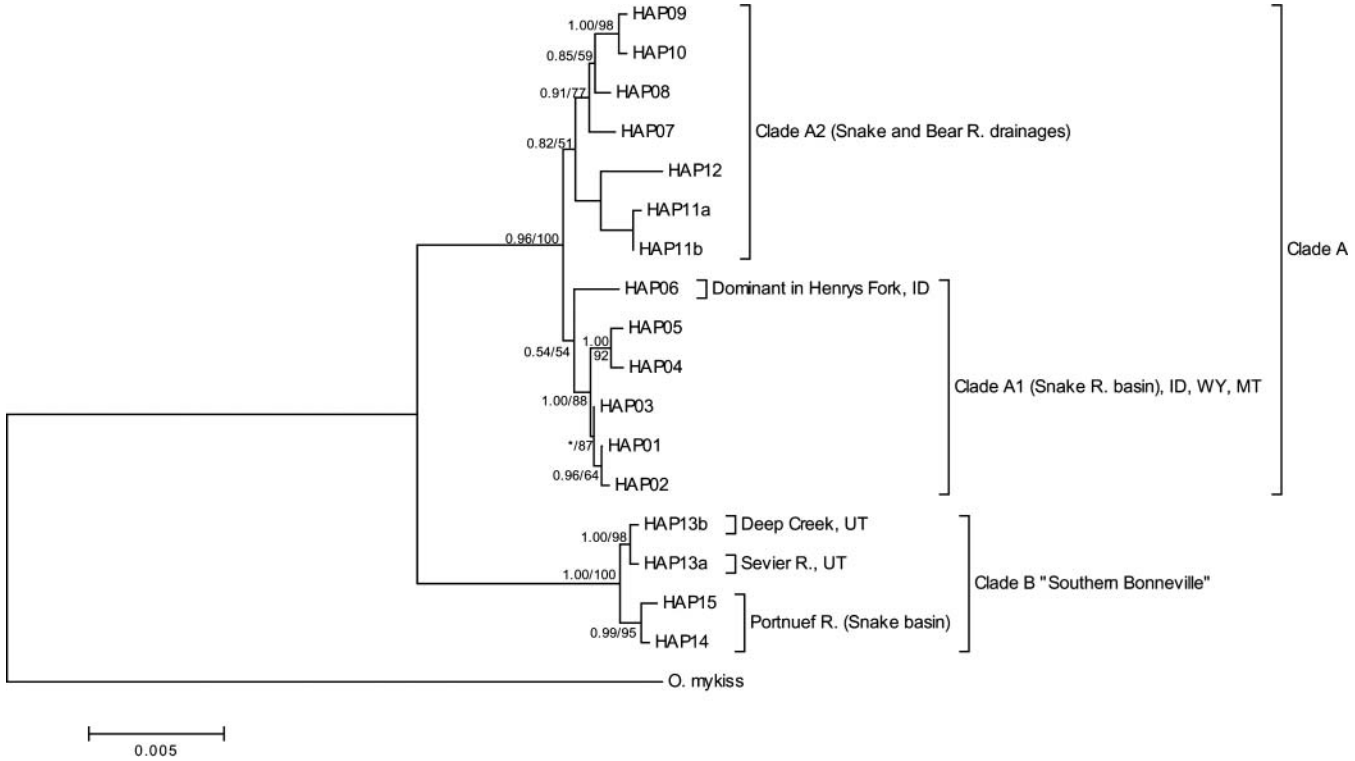


FIGURE 3. Neighbor-joining dendrogram based on the Kimura-2 distance for the 17 haplotypes identified through sequencing. Bootstrap values (all >50) were based on 10,000 replications. Rainbow trout *O. mykiss* was used as an out-group. A tree produced from the Bayesian analyses (not shown) had the same topology except that haplotype 3 (HAP03) was collapsed into clade A1 (noted with an asterisk). Clade credibility values from the Bayesian analyses are shown next to bootstrap values in parentheses. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura two-parameter method and are the number of base substitutions per site.



Portneuf River drainage also had the second highest number of haplotypes observed (6), one less than the McCoy Creek drainage.

The highest pairwise nucleotide divergence among all haplotypes observed within a drainage was 1.67% between haplotypes 6 and 15 in the Portneuf River drainage, whereas the nucleotide divergence was 0.06% between haplotypes 9 and 10 in the Blackfoot River drainage. The nucleotide divergence among the two most common haplotypes in almost every drainage was 0.35% or more. For example, in the Gros Ventre, Hoback, and Greys River drainages in Wyoming, haplotypes 1 (subclade A1) and 10 (subclade A2) are both found in each of these drainages and diverge from one another at 0.40%.

Comparisons of haplotype diversity and divergence across the two basins (Snake River and Bonneville) demonstrate discordance between the genetic relationships among various haplotypes and the basins and taxa in which they are found. Haplotype 13 was fixed in representative samples of Bonneville cutthroat trout from South Fork Johnson Creek and the Glenwood Hatchery but was not observed in any sample locations in the Bear River drainage. All of the haplotypes observed in the Bear River drainage cluster with the haplotypes found in Yellowstone cutthroat trout in the Snake River basin (clade A). However, haplotype 13 is found in high frequency in Basin Creek, a tributary to the Raft River (Snake River basin). In addition, haplotypes 14 and 15 are unique to the Portneuf River drainage (Snake River basin) but cluster with haplotype 13 (clade B).

## DISCUSSION

It is clear from recent microsatellite DNA investigations (Cegelski et al. 2006) that many of the processes controlling the present-day patterns of genetic diversity and gene flow in Yellowstone cutthroat trout across their range in Idaho are contemporary (recent droughts, habitat alteration, water diversions, etc). However, an examination of the distribution, diversity, and divergence of mtDNA lineages clearly demonstrates that the patterns of genetic variation across the subspecies' range have also been strongly shaped by long-term historical processes. Throughout much of the range of Yellowstone cutthroat trout in the Snake River basin in Idaho and Bonneville cutthroat trout in the Bonneville Basin in Idaho and Utah, the dominant process has been basaltic volcanism that influenced the shared hydrological history of the Bear, Portneuf, and Blackfoot River drainages.

The geological–hydrological history shared by these three drainages is evident in the distribution and divergence patterns

TABLE 3. Sequence divergence (below diagonal) and number of base pair differences (above diagonal) among the 17 observed haplotypes. Haplotypes 11b and 13b were only resolved through sequencing. A rainbow trout (RBT) ND12 sequence was included for comparison purposes (Hat Creek, Salmon River, Idaho; GenBank accession number EU186789).

| | Haplotype | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Haplotype | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11a | 11b | 12 | 13a | 13b | 14 | 15 | RBT |
| 1 | | 1 | 1 | 5 | 6 | 9 | 12 | 12 | 14 | 14 | 15 | 14 | 18 | 50 | 50 | 52 | 53 | 156 |
| 2 | 0.03 | | 2 | 6 | 7 | 10 | 13 | 11 | 13 | 13 | 16 | 15 | 19 | 51 | 51 | 51 | 52 | 157 |
| 3 | 0.03 | 0.06 | | 4 | 5 | 8 | 11 | 11 | 13 | 13 | 14 | 13 | 17 | 49 | 49 | 51 | 52 | 155 |
| 4 | 0.14 | 0.17 | 0.12 | | 3 | 12 | 15 | 15 | 17 | 17 | 17 | 16 | 20 | 53 | 53 | 55 | 56 | 157 |
| 5 | 0.17 | 0.20 | 0.14 | 0.09 | | 13 | 14 | 14 | 16 | 16 | 18 | 17 | 21 | 50 | 50 | 52 | 53 | 156 |
| 6 | 0.26 | 0.29 | 0.23 | 0.35 | 0.38 | | 13 | 13 | 15 | 15 | 16 | 15 | 17 | 54 | 54 | 56 | 57 | 158 |
| 7 | 0.35 | 0.38 | 0.32 | 0.43 | 0.40 | 0.38 | | 6 | 8 | 8 | 13 | 12 | 16 | 53 | 53 | 55 | 56 | 155 |
| 8 | 0.35 | 0.32 | 0.32 | 0.43 | 0.40 | 0.38 | 0.17 | | 6 | 6 | 13 | 12 | 16 | 51 | 51 | 51 | 52 | 153 |
| 9 | 0.40 | 0.38 | 0.38 | 0.49 | 0.46 | 0.43 | 0.23 | 0.17 | | 2 | 15 | 14 | 18 | 55 | 55 | 55 | 56 | 155 |
| 10 | 0.40 | 0.38 | 0.38 | 0.49 | 0.46 | 0.43 | 0.23 | 0.17 | 0.06 | | 15 | 14 | 18 | 55 | 55 | 55 | 56 | 155 |
| 11a | 0.43 | 0.46 | 0.40 | 0.49 | 0.52 | 0.46 | 0.38 | 0.38 | 0.43 | 0.43 | | 1 | 13 | 58 | 58 | 60 | 61 | 160 |
| 11b | 0.40 | 0.43 | 0.38 | 0.46 | 0.49 | 0.43 | 0.35 | 0.35 | 0.40 | 0.40 | 0.03 | | 12 | 57 | 57 | 59 | 60 | 159 |
| 12 | 0.52 | 0.55 | 0.49 | 0.58 | 0.61 | 0.49 | 0.46 | 0.46 | 0.52 | 0.52 | 0.38 | 0.35 | | 61 | 61 | 63 | 64 | 161 |
| 13a | 1.46 | 1.49 | 1.43 | 1.55 | 1.46 | 1.58 | 1.55 | 1.49 | 1.60 | 1.60 | 1.69 | 1.66 | 1.78 | | 2 | 6 | 7 | 159 |
| 13b | 1.46 | 1.49 | 1.43 | 1.55 | 1.46 | 1.58 | 1.55 | 1.49 | 1.60 | 1.60 | 1.69 | 1.66 | 1.78 | 0.06 | | 6 | 7 | 159 |
| 14 | 1.52 | 1.49 | 1.49 | 1.60 | 1.52 | 1.63 | 1.61 | 1.49 | 1.60 | 1.60 | 1.75 | 1.72 | 1.84 | 0.17 | 0.17 | | 3 | 160 |
| 15 | 1.55 | 1.52 | 1.52 | 1.63 | 1.55 | 1.66 | 1.63 | 1.52 | 1.63 | 1.63 | 1.78 | 1.75 | 1.87 | 0.20 | 0.20 | 0.09 | | 161 |
| RBT | 4.67 | 4.70 | 4.63 | 4.70 | 4.66 | 4.73 | 4.63 | 4.57 | 4.63 | 4.63 | 4.79 | 4.76 | 4.82 | 4.76 | 4.76 | 4.79 | 4.82 | |



of the dominant haplotypes found in samples from these basins. Haplotypes 7–12 cluster together and are predominantly found within the Portneuf, Blackfoot, and Bear River drainages. Haplotypes 11a, 11b, and 12 strongly cluster together (94% bootstrap support), are significantly diverged from all other "Yellowstone" haplotypes (0.38–0.61%), and are found throughout the Bear River basin. Using a molecular clock estimate for salmonid mtDNA of 1% per million years (Smith 1992), it is possible that these haplotypes represent lineages that were isolated as part of the initial diversion of the Bear River from the Snake River sometime during the last 600,000 years. The absence of these haplotypes in the Portneuf and Blackfoot rivers and their relatively large divergence from all other haplotypes suggest that they originated in Lake Thatcher and that these rivers had to have been isolated from the Bear River basin for a very long time. Haplotypes 11 and 12 were observed in extremely low frequency in one area outside the Bear River basin (Barnes Creek [$n = 2$], Clear Creek [$n = 2$], and Barnes Creek [$n = 2$], all tributaries to McCoy Creek). However, these tributaries were stocked with "unidentified cutthroat" from the Grace Hatchery during the mid-1950s and 1960s that were very likely of Bear River basin origin (Idaho Department of Fish and Game [IDFG], unpublished stocking records, 1913–1966).

Haplotypes 7, 8, 9, and 10 also strongly cluster together (79%). Haplotype 9 is unique to the upper Blackfoot River drainage and exhibits very low divergence (0.06%) from haplotype 10, the other dominant haplotype in the Blackfoot River drainage. These two haplotypes diverge from haplotypes 7 and 8 by 0.17–0.23%. Haplotype 7 is the most common haplotype in the Raft and Goose River drainages. It is also found in high frequency in the Portneuf River drainage. Haplotype 7 diverges from haplotypes 8 and 9–10 by 0.17–0.23%. Haplotype 8 is the dominant haplotype in the Bear River basin and diverges from haplotypes 7 and 9–10 by 0.17%. The fact that haplotypes 7, 8, and 9–10 all differ from one another by about 0.17–0.23% in terms of sequence divergence suggests that these three main lineages diverged from a common lineage at approximately the same time. It is plausible that these lineages represent the isolation of three main drainages as a result of the basaltic volcanism that occurred in the Gem and Blackfoot valleys sometime prior to 140,000 years ago.

The catastrophic Bonneville flood provided the most recent (although very temporary) opportunity for faunal exchange between the Bonneville Basin and the Snake River basin and is undoubtedly the reason for the presence of haplotypes 14 and 15 in the Portneuf River drainage. These haplotypes are widely divergent from other "Yellowstone" haplotypes (~1.6%) and clearly cluster with a "southern" Bonneville cutthroat trout haplotype (haplotype 13a) found fixed within Bonneville cutthroat trout from reference populations in west-central and southern Utah. The lack of distribution of haplotypes 14 and 15 outside the Portneuf River drainage in the Snake River basin suggests

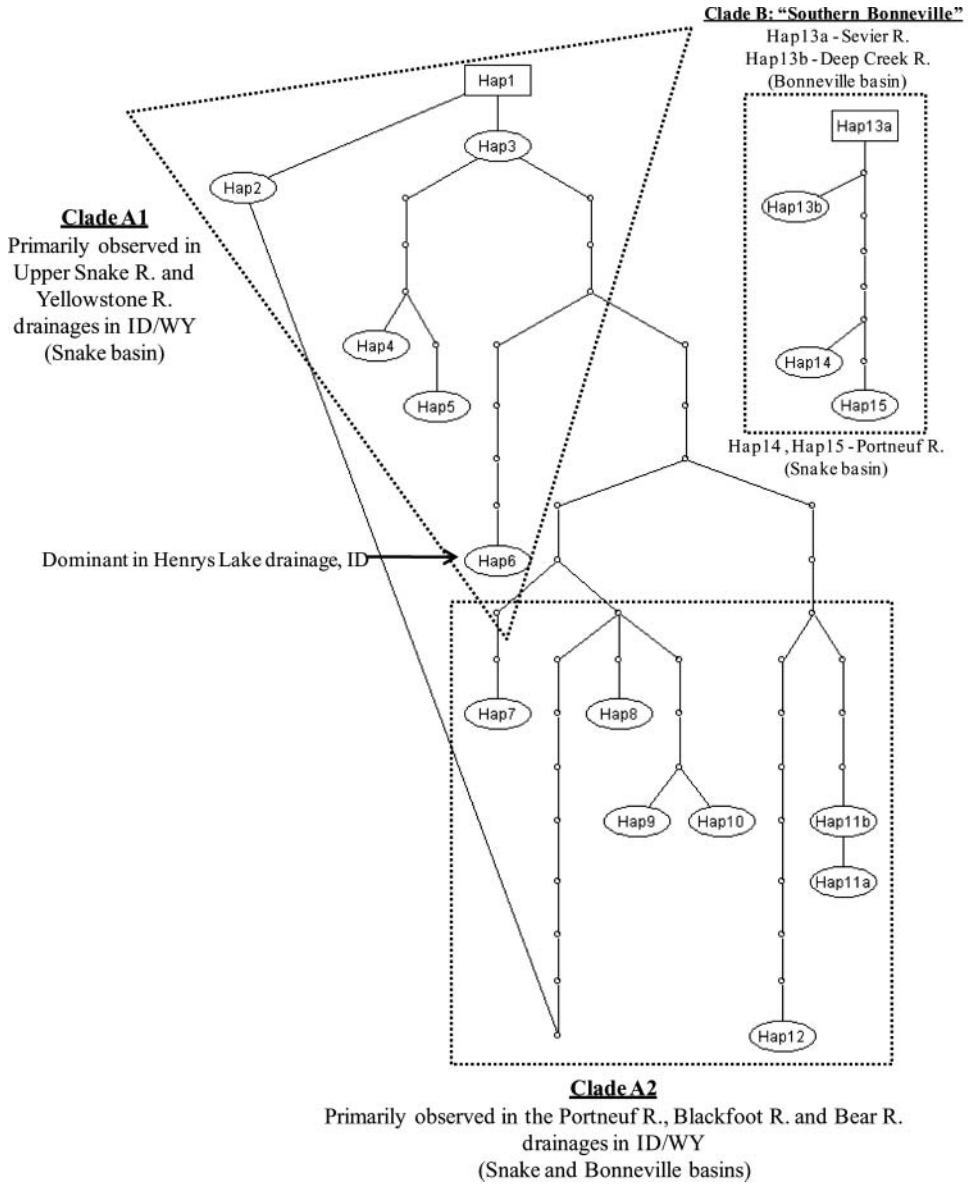


FIGURE 4. Network of 17 mitochondrial ND12 haplotypes. The rectangles represent inferred ancestral haplotypes, the ellipses additional haplotypes, and the small circles missing haplotypes needed to connect the observed ones. Each line represents a one-base-pair difference between haplotypes. To resolve clade A1 and clade A2 into a single network with 95% parsimony probability required 26 steps. An additional 24 steps were required to join clades A and B into a single network (not shown).

that major upstream dispersal barriers have existed since their introduction into the Portneuf River. This is likely, given the large number of current and suspected historical waterfalls present in the main Snake River basin in Idaho. The absence of "southern" Bonneville cutthroat trout haplotypes in the Bear River basin suggests that while the Thatcher and Bonneville basins were undoubtedly connected through the Oneida Narrows (~20,000 years ago; Bouchard et al. 1998), fish movement upstream must have been limited. The amount of sequence divergence between the "Yellowstone" and "southern Bonneville" haplotypes suggests that they have been separated for approximately 1.6 million years. Johnson (2002) proposed an identical estimate for the time since divergence between two clades of Utah chub *Gila atraria* from the Snake River and Bonneville basins. Johnson (2002) also found "southern Bonneville" Utah chub haplotypes in the Portneuf River but not in samples from Bear Lake.

The presence of haplotype 13b in one population in the upper Raft River in Utah is more difficult to explain. The haplotype clearly clusters with "southern Bonneville" haplotypes and may also be the result of the Bonneville flood. Another hypothesis is that there were opportunities for headwater transfer between the Raft River and rivers flowing south into ancient Lake Bonneville.

TABLE 4. Mean haplotype diversity ($h$), number of pairwise differences (PD), and nucleotide diversity ($\pi$) observed in all 61 populations with associated standard errors. Superscripts refer to the drainages into which the population were grouped (see Table 1).

| | $h$ | | PD | | $\pi$ | |
|---|---|---|---|---|---|---|
| Sample location | Mean | SE | Mean | SE | Mean | SE |
| Goose Creek[1] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Dry Creek[2] | 0.5517 | 0.0388 | 6.5517 | 3.1885 | 0.0019 | 0.0010 |
| New Canyon Creek[3] | 0.0741 | 0.0674 | 0.8889 | 0.6406 | 0.0003 | 0.0002 |
| Green Creek[3] | 0.3310 | 0.0890 | 3.9724 | 2.0445 | 0.0011 | 0.0007 |
| Basin Creek[3] | 0.0714 | 0.0652 | 4.0000 | 2.0610 | 0.0011 | 0.0007 |
| Robbers Roost Creek[4] | 0.6276 | 0.0586 | 29.4322 | 13.2356 | 0.0084 | 0.0042 |
| Harkness Creek[4] | 0.4330 | 0.0750 | 1.2991 | 0.8384 | 0.0004 | 0.0003 |
| Bell Marsh Creek[4] | 0.4767 | 0.1154 | 16.5200 | 7.6127 | 0.0047 | 0.0024 |
| Upper Rapid Creek[4] | 0.4026 | 0.1247 | 14.1429 | 6.5945 | 0.0041 | 0.0021 |
| Main Rapid Creek[4] | 0.5429 | 0.1327 | 15.0286 | 7.1241 | 0.0043 | 0.0023 |
| Inman Creek[4] | 0.5816 | 0.0643 | 10.0943 | 4.7449 | 0.0029 | 0.0015 |
| Goodenough Creek[4] | 0.3619 | 0.1448 | 8.2667 | 4.0600 | 0.0024 | 0.0013 |
| Lower Blackfoot[5] | 0.3333 | 0.2152 | 4.6667 | 2.6604 | 0.0013 | 0.0009 |
| Middle Blackfoot[5] | 0.5344 | 0.0602 | 2.9636 | 1.5843 | 0.0009 | 0.0005 |
| Upper Blackfoot River[5] | 0.3969 | 0.1097 | 2.8923 | 1.5696 | 0.0008 | 0.0005 |
| Lava Creek[6] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Garden Creek[7] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Barnes Creek[7] | 0.6667 | 0.0632 | 9.9701 | 4.6903 | 0.0029 | 0.0015 |
| Clear Creek[7] | 0.6860 | 0.0718 | 9.8409 | 4.6291 | 0.0028 | 0.0015 |
| McCoy Creek[7] | 0.3913 | 0.0912 | 5.4783 | 2.7312 | 0.0016 | 0.0009 |
| Fish Creek[7] | 0.3621 | 0.1049 | 4.9606 | 2.4850 | 0.0014 | 0.0008 |
| Tincup Creek[9] | 0.4945 | 0.0876 | 6.9231 | 3.4649 | 0.0020 | 0.0011 |
| Crow Creek[9] | 0.2391 | 0.0917 | 3.3471 | 1.7661 | 0.0010 | 0.0006 |
| Mike Harris Creek[10] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| SF Badger Creek[10] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Tyghee Creek[11] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Henrys Lake[11] | 0.5249 | 0.0698 | 5.3223 | 2.6198 | 0.0015 | 0.0008 |
| Middle Dry Creek[12] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Brushy Fork Creek[13] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Placer Creek[13] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Yellowstone Lake[13] | 0.5082 | 0.0119 | 2.5410 | 1.3850 | 0.0007 | 0.0004 |
| Lehardy Rapids River[13] | 0.5435 | 0.0849 | 2.5942 | 1.4391 | 0.0007 | 0.0005 |
| Pacific Creek[14] | 0.6476 | 0.0716 | 1.5048 | 0.9587 | 0.0004 | 0.0003 |
| Jackson NFH[14] | 0.1754 | 0.0841 | 0.8770 | 0.6317 | 0.0003 | 0.0002 |
| Ditch Creek[14] | 0.7048 | 0.0535 | 8.3810 | 4.1118 | 0.0024 | 0.0013 |
| Flat Creek[14] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Cabin Creek[14] | 0.5000 | 0.1283 | 7.0000 | 3.6324 | 0.0020 | 0.0012 |
| Cottonwood Creek[15] | 0.6014 | 0.0548 | 7.4529 | 3.6087 | 0.0021 | 0.0012 |
| NF Fish Creek[15] | 0.3939 | 0.1186 | 3.6061 | 1.9013 | 0.0010 | 0.0006 |
| Leeds Creek[15] | 0.2222 | 0.1662 | 3.1111 | 1.7793 | 0.0009 | 0.0006 |
| Bacon Creek[15] | 0.2637 | 0.1360 | 3.6923 | 1.9852 | 0.0011 | 0.0006 |
| Boulder Creek[16] | 0.4891 | 0.0843 | 6.4022 | 3.1421 | 0.0018 | 0.0010 |
| Dell Creek[16] | 0.3684 | 0.1254 | 4.4211 | 2.2817 | 0.0013 | 0.0007 |
| Bondurant Creek[16] | 0.1333 | 0.1123 | 1.8667 | 1.1307 | 0.0005 | 0.0004 |
| Bull Creek[16] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Martin Creek[17] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

*(Continued on next page)*

TABLE 4. Continued.

| | $h$ | | PD | | $\pi$ | |
|---|---|---|---|---|---|---|
| Sample location | Mean | SE | Mean | SE | Mean | SE |
| Steer Creek[17] | 0.3442 | 0.0987 | 4.8188 | 2.4374 | 0.0014 | 0.0008 |
| Blind Trail Creek[17] | 0.4039 | 0.0995 | 4.9163 | 2.4654 | 0.0014 | 0.0008 |
| Poison Creek[17] | 0.5238 | 0.2086 | 6.4762 | 3.4888 | 0.0019 | 0.0011 |
| SF Little Greys River[17] | 0.3619 | 0.1448 | 4.4571 | 2.3268 | 0.0013 | 0.0007 |
| Maple Creek[18] | 0.2900 | 0.1095 | 4.4000 | 2.2472 | 0.0013 | 0.0007 |
| Cottonwood Creek[18] | 0.5195 | 0.0379 | 6.7532 | 3.3091 | 0.0019 | 0.0011 |
| Logan R.[18] | 0.5498 | 0.0952 | 8.0563 | 3.8894 | 0.0023 | 0.0012 |
| Beaver Creek[18] | 0.5000 | 0.1364 | 7.5385 | 3.7647 | 0.0022 | 0.0012 |
| Cub R.[18] | 0.5690 | 0.0672 | 8.5271 | 4.0596 | 0.0024 | 0.0013 |
| Sugar Creek[18] | 0.1567 | 0.0957 | 2.2667 | 1.2883 | 0.0007 | 0.0004 |
| Peagram Creek[18] | 0.2267 | 0.1062 | 3.3867 | 1.7940 | 0.0010 | 0.0006 |
| Bear Lake[18] | 0.4430 | 0.0628 | 6.1007 | 2.9415 | 0.0018 | 0.0009 |
| Daniel FH[18] | 0.4814 | 0.0492 | 6.3404 | 3.0591 | 0.0018 | 0.0010 |
| Glenwood FH[19] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| SF Johnson[20] | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

The Blackfoot, Portneuf, and Bear River drainages are not the only area in Idaho in which basalt flows probably helped shape haplotype distribution. Large basalt flows erupted over the floor of the Henrys Fork drainage approximately 200,000 years ago, probably filling the Henrys Fork River (Alt and Hyndman 1989). These flows could have isolated populations, and the subsequent formation of two large waterfalls (upper Mesa Falls [34.75 m high] and lower Mesa Falls [19.81 m]) would have prevented upstream migration. The dominance of haplotype 6 in Henrys Lake and Tyghee Creek just downstream from the lake may reflect these past isolation events. Haplotype 6 appears to be most related to haplotypes 1, 2, and 3;

TABLE 5. Total molecular variance at different hierarchical levels of population structure. The percentage variation, probability ($P$) estimated from permutation tests, and the $F$-statistic are given at each hierarchical level (Excoffier et al. 1992).

| Structure | Source of variation | % Total variance | Fixation indices | $P$ |
|---|---|---|---|---|
| Grouped according to drainage | Among groups | 52 | $F_{ST} = 0.77648$ | $< 0.001$ |
| | Among populations within groups | 26 | $F_{SC} = 0.53179$ | $< 0.001$ |
| | Within populations | 22 | $F_{CT} = 0.52259$ | $< 0.001$ |
| Snake River sample locations below Palisades Reservoir[a] | Among populations | 78 | $F_{ST} = 0.78381$ | $< 0.001$ |
| | Within populations | 22 | | |
| Snake River sample locations above Palisades Reservoir[b] | Among populations | 33 | $F_{ST} = 0.32530$ | $< 0.001$ |
| | Within populations | 67 | | |
| Sample locations below Palisades Reservoir grouped by drainage | Among groups | 27 | $F_{ST} = 0.79092$ | $< 0.001$ |
| | Among populations within groups | 52 | $F_{SC} = 0.71146$ | $< 0.001$ |
| | Within populations | 21 | $F_{CT} = 0.27540$ | $< 0.001$ |
| Sample locations above Palisades Reservoir grouped by drainage | Among groups | 17 | $F_{ST} = 0.33848$ | $< 0.001$ |
| | Among populations within groups | 17 | $F_{SC} = 0.20664$ | $< 0.001$ |
| | Within populations | 66 | $F_{CT} = 0.16618$ | $< 0.001$ |

[a]Locations 1–17 and 24–28 in Figure 1.
[b]Locations 18–23 and 29–50 in Figure 1.

however, sequence divergence estimates are still relatively high (0.23–0.26%), suggesting isolation for quite some time. Haplotype 6 is found outside the Henrys Fork area, but its disjunct distribution in the Teton, Portneuf, and Blackfoot rivers could be from hatchery stocking. All three areas have been stocked with fish from Henrys Lake (IDFG stocking database; http://fishandgame.idaho.gov/fish/stocking/).

The diversity and divergence of mtDNA haplotypes throughout much of the range of Yellowstone cutthroat trout in Idaho appears to be the product of older vicariant events associated with basaltic volcanism. However, the observed pattern of mtDNA haplotype structuring in the Snake and Yellowstone River drainages in Wyoming is reflective of a more recent colonization history following the last glacial periods. From a broad-scale perspective, this is evident from the AMOVA analyses, which indicate that much more of the mtDNA variation in Wyoming is partitioned within populations (67.5%) than is the case in Idaho (21.6%), implying less divergence of populations between drainages. For the most part, the predominant haplotypes throughout the Greys, Hoback, and Gros Ventre drainages are haplotypes 1 and 10. As mentioned previously, these two haplotypes are quite divergent from one another (0.40%) and fall into distinct subclades. Both haplotypes are predominant in the Salt River drainage and the tributaries to Palisades Reservoir. Haplotype 1 is widely distributed throughout Idaho. Haplotype 10 probably originated in the Blackfoot River drainage and was fixed in samples from a single population sampled below Palisades Reservoir in the South Fork of the Snake River. It is likely that both of these haplotypes originated in Idaho and postglacial colonizations distributed them throughout the Snake River drainage in Wyoming.

Certainly the patterns of haplotype distribution and divergence at sample locations from the Yellowstone River, Yellowstone Lake, Pacific Creek, and Jackson National Fish Hatchery are consistent with a pattern of very recent colonization and subsequent population expansion. The two dominant haplotypes in these areas (haplotypes 1 and 4) are probably products of the colonization of populations south of the Pinedale glaciation boundary through Pacific Creek and Two-Ocean Pass, as proposed by Behnke (1992). These two haplotypes are too divergent (0.14%) to have been derived during the last 10,000–14,000 years. Haplotype 4 exhibits a north–south cline in frequency distribution, becoming less frequent as one moves downstream in the Snake River drainage, and is absent in samples from the Greys River drainage in Wyoming. Haplotype 4 is also absent from samples from the Salt River drainage (Idaho and Wyoming) and in fact is only found in two locations in Idaho (Blackfoot River [$n = 1$] and Henrys Lake [$n = 9$]), both of which have been stocked with fish from Yellowstone Lake. The high frequency of haplotype 4 in Pacific Creek and Yellowstone Lake, and the fact that it is fixed within two populations in the Yellowstone River, Montana, downstream of the lake are consistent with founding effects.

While some tributaries to the Yellowstone River in south-central Montana and Wyoming (e.g., Bighorn and Powder rivers) may have been free of ice during the Bull Lake and Pinedale glaciations, it is unlikely that the presence of haplotype 4 in Yellowstone Lake is the product of colonization from a possible northern glacial refugia. Large waterfalls on the Yellowstone River just downstream of the lake (the lower falls has a current height of 93.9 m and the upper falls a current height of 33.2 m) probably formed during deglaciation approximately 10,000 years ago and would certainly have acted as upstream migration barriers (Pierce et al. 2003). It is likely that haplotype 4 originated in the upper Snake River basin, being derived from haplotype 1 during the interglacial period between the Bull Lake and Pinedale glaciations.

The remaining three haplotypes observed in Pacific Creek and the Yellowstone Lake–River drainage show evidence of recent divergence. Haplotype 3, which is unique to samples from Pacific Creek, is probably a recent derivation from haplotype 1 (0.03% divergence). Haplotype 2, which is found in one sample from LeHardy Rapids Hatchery, is probably also recently diverged from haplotype 1 (0.03%). Finally, haplotype 5, which is found in one sample from LeHardy Rapids Hatchery, appears to have been recently derived from haplotype 4 (0.09% divergence).

## Summary and Implications for Conservation and Management

The mtDNA lineage diversity and distribution of Yellowstone cutthroat trout are clearly the products of long-term historical processes that have isolated populations for substantial periods of time and then, in many cases, allowed secondary contact and subsequent admixture of the divergent populations. In Idaho, the dominant historical force responsible for this vicariance is volcanism, which has resulted in strong partitioning of genetic diversity at the drainage level. Subsequently, genetic preservation efforts in Idaho will require at minimum the protection of populations within each of the major drainages. Recent rangewide evaluations of Yellowstone cutthroat trout population distribution, abundance, and hybridization (Meyer et al. 2006), coupled with genetic data (Cegelski et al. 2006; this study), should assist managers with the identification and prioritization of populations in each drainage.

While an emphasis on drainage level conservation will be important, managers should keep in mind that in some instances significant portions of the genetic diversity within drainages is partitioned among populations (e.g., the Portneuf River drainage). In these instances, conservation should be directed at preserving multiple populations at the individual stream level. This will be a challenging task, especially since contemporary influences (drought and habitat degradation and fragmentation) appear to significantly impact gene flow and genetic diversity in some areas (Cegelski et al. 2006).

Patterns of mitochondrial haplotype diversity and divergence contrast between areas that have historically been impacted by basaltic volcanism as opposed to glaciation. Less of the total haplotype diversity in the upper Snake and Yellowstone rivers in Wyoming and Montana is partitioned among the major drainages, with the same two haplotypes being observed in high frequencies in three drainages. The most obvious explanation for this difference is that Yellowstone cutthroat trout have only had access to much of this area in the last 10,000–50,000 years because of glaciation restrictions that have limited opportunities for isolation and divergence. This pattern is exemplified in the Yellowstone Lake and River drainages, where only one major haplotype and several minor, closely related haplotypes are observed. It is important to emphasize however, that while less of the total haplotype diversity is partitioned among the major drainages, there are still differences in haplotype frequency among drainages and populations within drainages, suggesting that the Yellowstone cutthroat trout in these areas do not act as one large panmictic unit (also see Novak et al. 2005). Additional fine-scale analyses are needed in Wyoming to better understand the genetic structure and gene flow within drainages.

The present data are consistent with those from previous allozyme and mtDNA studies demonstrating that the cutthroat trout in the Bear River basin share a more recent common ancestor with Yellowstone cutthroat trout than with the populations of Bonneville cutthroat trout in the central and southern portions of their range in Utah (Smith et al. 2002). The rangewide and Idaho conservation management plans for Bonneville cutthroat trout acknowledge this shared ancestry (Lentsch et al. 2000; Teuscher and Capurso 2007), but both treat the cutthroat trout in the Bear River drainage as *O. c. utah* rather than *O. c. bouvieri*. The Idaho plan suggests that the genetic similarities are due to connections (30,000 years ago) between the Snake and Bear River drainages as a result of the Bonneville flood (Teuscher and Capurso 2007). The rangewide plan assumes that the Bear River was a tributary to the Snake River until 25,000–35,000 years ago, when the Bear River drainage was diverted into the Bonneville Basin (Lentsch et al. 2000). Our results indicate that the cutthroat trout in the Bear and Snake rivers share a much longer, more complicated evolutionary history stretching back nearly half a million years, one that has largely been independent from that of the cutthroat trout in the main Bonneville Basin. Although current conservation plans already stipulate that the cutthroat trout in the Bear River drainage be managed separately from the Bonneville cutthroat trout outside this drainage, it seems appropriate that this distinction be made more formal by changing the former's taxonomic status from *O. c. utah* to *O. c. bouvieri*.

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