THESIS

WHOLE GENOME SEQUENCING TO CHARACTERIZE CUTTHROAT TROUT POPULATIONS ACROSS THE CONTINENTAL DIVIDE

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ABSTRACT

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Cutthroat Trout (*Oncorhynchus spp.*) are a biological icon of western North America with the largest native range and species diversity of all North American trout. Currently, there are six recognized lineages believed to be native to the Southern Rocky Mountains. Despite almost a decade of genetic and morphological studies, clarity on the phylogenetic history and evolutionary differences between all lineages of Colorado's Cutthroat Trout remains to be resolved and has implications for future management of populations in this species group throughout the Southern Rocky Mountains. Previous molecular studies have used genetic markers representing only a small portion of the genome, such as with ND2 mitochondrial DNA haplotypes, microsatellites, and amplified fragment length polymorphisms (AFLPs), and have not included a comprehensive nuclear DNA assessment from all extant lineages of Cutthroat Trout found in Colorado. To address this knowledge gap, we used whole genome sequencing to analyze DNA samples from all extant lineages of Southern Rocky Mountain Cutthroat Trout, as well as from five other Cutthroat Trout lineages across North America. Using whole genomes, we have confirmed the nuclear distinctiveness of the "blue" and "green" lineages of former Colorado River Cutthroat Trout, as well the form from the San Juan basin. We additionally identified the persistence of ancestral and unique spatial genetic diversity despite widespread historic stocking. These findings, along with an analysis of long-term effective population sizes, provide key insights into possible natural and anthropogenic drivers of population migration, separation, and speciation. Taken together, our results inform conservation management of Cutthroat Trout lineages across their native range in the Southern Rocky Mountains.

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CHAPTER 1

WHOLE GENOME SEQUENCING TO CHARACTERIZE CUTTHROAT TROUT POPULATIONS ACROSS THE CONTINENTAL DIVIDE

Introduction

Biodiversity exists on a continuum; however, conservation management often recognizes and attempts to maintain diversity at discrete levels, including at the ecosystem, species or subspecies, and genomic levels. In the United States Endangered Species Act (United States 117th Congress 2022), a "species" is defined as any "distinct population segment" of any vertebrate species, however the metrics on which to evaluate "distinctness" are surprisingly vague. Approaches to biodiversity conservation have focused on species and subspecies as the common measure of biodiversity for science and management (Mace 2004; Wilson 2017), with certain legal protections assigned to them under the Endangered Species Act (USFWS 2003; Campton and Kaeding 2005; Rogers et al. 2018). Therefore, conservation actions are more effective when we have correct taxonomic information (Mace 2004; Vogel Ely et al. 2017).

Historically, phenotypic characteristics formed the basis for taxonomic classifications, with the spatial extent of genealogical lineages providing additional insight for determining taxonomic units (Vences et al. 2024). Global geographic and climatic conditions that generated repeated glacial cycles shaped the historical patterns of species expansions and radiations that are linked to contemporary species distributions (Bi et al. 2020; Vilaça et al. 2022). However, anthropogenic impacts to native species during the Holocene have clouded our understanding of the evolutionary history of many species and complicated traditional phenotypic and biogeographical approaches to taxonomic classification. Examples of anthropogenic impacts include widespread stocking of non-native species (Metcalf et al. 2012; Corsi et al. 2013; Hand et al. 2015; Muhlfeld et al. 2016; Kovach et al. 2022; Galland et al. 2024) and intense harvesting of many taxa (Brüniche-Olsen et al. 2021; Wenne 2023; Ovchinnikov and McCann 2023). These anthropogenic influences, particularly widespread movement of species around the landscape, undermine reliable categorization and identification of management units based on geography, and therefore pose major challenges to effective conservation management (Funk et al. 2012).

Next generation sequencing has provided new methods for taxonomic classification and analyses of genetic distinctiveness, and has drastically improved the genetic study of non-model and wild organisms (Allendorf et al. 2010; Whiteley et al. 2010; Behjati and Tarpey 2013; Leliaert et al. 2014; Weckworth et al. 2015; Hernández-Alonso et al. 2023). Advancements in the application of genomic tools in the last decade alone, including reduced costs and increased efficiency of sequencing, have simultaneously uncovered the magnitude of diversity present within many species groups and complicated our understanding of their evolutionary histories and taxonomic relationships (Morin et al. 2004; Allendorf et al. 2010; Metcalf et al. 2012; Nadachowska-Brzyska et al. 2016; Saglam et al. 2017; Rogers et al. 2018; Bestgen et al. 2019; Patton et al. 2019; Segelbacher et al. 2022; Vilaça et al. 2023). Demographic reconstruction is once such method that infers population sizes over evolutionary time, and can provide historical context for contemporary relationships among species (Nadachowska-Brzyska et al. 2016; Cahill et al. 2016; Beichman et al. 2017; Patton et al. 2019; Lucena-Perez et al. 2020; Brüniche-Olsen et al. 2021; Vilaça et al. 2023; Ovchinnikov and McCann 2023). Additionally, genomic information can identify taxa that were previously misclassified based on mitochondrial haplotypes alone, as populations with admixture or incomplete lineage sorting can exhibit mito-nuclear discord (Després 2019; Quattrini et al. 2023; DeRaad et al. 2023). While admixture and genetic diversity analyses have delivered setbacks to conservation programs by revealing that some native populations are deeply inbred or introgressed with non-native species (Metcalf et al. 2007; Muhlfeld et al. 2016; Escalante et al. 2020; Quattrini et al. 2023), they have succeeded in identifying the persistence of ancestral diversity despite stocking (Clemento et al. 2009; Metcalf et al. 2012), as well as unique spatial genetic diversity (Whiteley et al. 2010) and even unique lineages (Saglam et al. 2017).

Cutthroat trout (*Oncorhynchus spp.*) are a biological icon of Western North America as both prized game fish and the only native trout of the Rocky Mountains. With the largest native range and species diversity of all North American trout (Rogers et al. 2018), their level of phenotypic variation has been likened to Galapagos finches (Fausch et al. 2009). Members of this species group currently occupy a range extending from Pacific coastal streams inland to the Rocky Mountains, and from Southern Alaska to New Mexico (Trotter et al. 2018). Cutthroat Trout fossils collected in modern-day western Nevada suggest a split from Rainbow trout (*Oncorhynchus mykiss*) occurred over 10.2 Ma (Stearley and Smith 2016; Smith and Stearley 2018), and gave rise to four major evolutionary groups in North America - Coastal Cutthroat Trout (*O. clarkii*), Westslope Cutthroat Trout (*O. lewisi*), Lahontan Cutthroat Trout (*O. henshawi*), and Yellowstone Cutthroat Trout (*O. virginialis*) (Trotter et al. 2018). Like other freshwater fish, they display high levels of endemism due to the biogeographic constraints intrinsic to riverine ecosystems (Fausch et al. 2002); however, extensive stocking of nonnative salmonids during the last century has led to precipitous declines in both the abundance and distribution of many lineages of Cutthroat Trout (Young et al. 1996; Peterson et al. 2004; Benjamin and Baxter 2012; Al-Chokhachy and Sepulveda 2019; Nordberg et al. 2021; Rosenthal et al. 2022). These anthropogenic extirpations and introductions have presented challenges to more traditional applications of biogeography for taxonomic classification (Olden et al. 2010). Management approaches to address the range of anthropogenic impacts include reestablishing connectivity among isolated populations, translocating individuals, or stocking from broodstock sources to keep population numbers and genetic diversity high (Letcher et al. 2007; Whiteley et al. 2010; Tsuboi et al. 2022; Stack et al. 2024). An important focus then becomes understanding relationships among source and target populations, and balancing inbreeding versus outbreeding risk to ensure that management actions do not further imperil the populations they aim to conserve. Studies leveraging new genomic techniques to investigate relationships amongst lineages of Cutthroat Trout exist across this species' range and have revealed the distinctiveness of some lineages (Pritchard et al. 2009; Saglam et al. 2017), unexpected relationships within other lineages (Shiozawa et al. 2018; Kokkonen et al. 2024), and previously uncategorized diversity (Peacock et al. 2017; Eaton et al. 2018). Despite their conservation significance as well as decades of genetic and morphological studies, the phylogenetic history of Southern Rocky Mountain Cutthroat Trout remains unclear. Gaining a better understanding of the distinctiveness of Southern Rocky Mountain Cutthroat Trout clades as well as genetic relationships between them is important for guiding conservation management across their range (Metcalf et al. 2012; Rogers et al. 2018; Shiozawa et al. 2018; Bestgen et al. 2019; Kokkonen et al. 2024).

Cutthroat Trout in the Southern Rocky Mountains

The Yellowstone Cutthroat Trout group (*O. virginialis*) includes nine unique lineages, six of which appeared in the Southern Rocky Mountains of Colorado roughly 1.3-1.9 Ma. based on fossil-calibrated genomic evidence (Shiozawa et al. 2018) (Fig. 1). Three of these lineages are believed native East of the Continental Divide (Fig. 1 purple, yellow, and orange, respectively): Greenback Cutthroat Trout in the South Platte River basin (GBCT, *O. v. spp*), Yellowfin Cutthroat Trout in the Arkansas River basin (YFCT, *O. v. macdonaldi*) now considered extinct, and Rio Grande Cutthroat Trout in the Rio Grande River basin, including the headwaters of the Pecos and Canadian Rivers (RGCT, *O. v. virginalis*). Three lineages are found West of the Continental Divide, all originally classified under the umbrella of Colorado River Cutthroat Trout (*O. v. pleuriticus*) due to shared connections with the mainstem Colorado River. Recent analysis of full mitogenomes (Van Orden et al., *in prep*) and mitochondrial ND2 haplotypes (Metcalf et al. 2012; Rogers et al. 2018; Shiozawa et al. 2018; Bestgen et al. 2019) affirmed that the Green River Cutthroat Trout (GRCT) and Uncompahgre Cutthroat Trout (UPCT) lineages are as distinct from one another as other lineages in the Southern Rocky Mountains with subspecies designations, so we will refer to both lineages using their new common names recently adopted by the Colorado River Cutthroat Trout Conservation Team: Green River Cutthroat Trout for the former “Blue lineage” of Colorado River Cutthroat Trout, and Uncompahgre Cutthroat Trout for the former “Green lineage” of Colorado River Cutthroat Trout (Rogers et al. 2025). The San Juan Cutthroat Trout (SJCT; Fig. 1) is believed native to its namesake basin and was considered extinct until it was recently rediscovered in multiple San Juan basin headwater streams in 2018 by Colorado Parks and Wildlife (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019), the Green River Cutthroat Trout (GRCT; Fig. 1) is considered native to the Green, White, and Yampa river basins, while the Uncompahgre Cutthroat Trout (UPCT; Fig. 1) is considered native to the headwaters of the Colorado, Gunnison, and Dolores river basins (Metcalf et al. 2012; Rogers et al. 2018).

Colorado carries a long history of native and nonnative trout stocking by both private and public individuals within the state. While private stocking undoubtedly occurred using multiple lineages of

Cutthroat Trout, populations of Cutthroat Trout from the Eastern Slope (GBCT, YFCT, RGCT) were less frequently used in favor of West slope GRCT and UPCT populations for large-scale production and stocking (Metcalf et al. 2012). Notably, West slope GRCT and UPCT hatchery fish were often stocked into originally fishless habitats that occurred above waterfalls and other natural barriers to upstream movement of fish in high alpine lakes and streams, which when combined with local extirpations of native populations throughout the region may have erased the natural patterns of phylogeography (Fausch et al. 2009; Metcalf et al. 2012). While GRCT genetic diversity from populations occurring outside of their putative native range can be traced back to a major source (i.e. the dominant state brood source at Trappers Lake in the headwaters of the White River) by mitochondrial haplotypes, that is not the case for putative UPCT found east of the Continental Divide. UPCT currently residing east of the Continental Divide differ from their West slope counterparts in mitochondrial haplotypes and several key morphological traits (Metcalf et al. 2012; Bestgen et al. 2013, 2019; Rogers et al. 2018). Whether UPCT became established in the Arkansas and South Platte River basins during recent time (within 150 years) with anthropogenic influence or on their own long ago, was the primary motivation for this study. Understanding the genetic and evolutionary history of these unique populations is critical, not only for unraveling the relationships among Cutthroat Trout lineages of the Southern Rocky Mountains, but for determining the appropriate conservation value of these populations.

Objectives

In this study, we used whole genome sequences to better inform conservation management of the Southern Rocky Mountain Cutthroat Trout generally, and to determine the native status of UPCT east of the Continental Divide specifically. Genomic information allowed us to, (1) estimate levels of individual heterozygosity, (2) assess population structure within and among lineages, (3) elucidate the evolutionary relationships among populations, including whether nuclear relationships match those found using mitochondrial DNA, and (4) determine if there are lineage-specific differences in effective population size in the past.

Methods

Study System and Outgroups

Based on mitogenome analysis and geological history from Shiozawa et al. (2018), our current understanding is that the Yellowstone complex contains two monophyletic sister clades: the Yellowstone (*O. v. bouvieri*) / Bear River Bonneville (*O. v. ssp.*) clade, and the Bonneville complex containing Bonneville Cutthroat Trout (*O. c. utah*) and the remaining six lineages of the Southern Rocky Mountains mentioned above. The separation of Yellowstone/Bear River Bonneville and the Bonneville complex likely occurred via an interbasin transfer between the northern Bonneville basin and the southern edge of the upper Snake River Plain. During the middle of the Pleistocene, the Bonneville complex likely underwent a rapid dispersal event around 1.7 Ma with the invasion of the Southern Rocky Mountains from the Bonneville basin.

The subsequent expansion of Cutthroat Trout into the six drainage basins of the Southern Rocky Mountains from the Bonneville basin are less well understood, and possibly occurred via multiple pathways. Evidence from mitochondrial DNA suggests that the GRCT forms a distinct monophyletic group that is a sister clade to the monophyletic group containing the RGCT, GBCT, and UPCT lineages (Shiozawa et al. 2018). Additionally, mitogenome evidence suggests that RGCT were the next to diverge and represent an offshoot of the ancestral lineage that gave rise to the UPCT and GBCT around 1.4 Ma based on fossil-calibrated molecular clock data (Shiozawa et al. 2018). Using new genomic methods for resolving the colonization of the Southern Rocky Mountains is complicated by stocking and anthropogenic influences that have greatly reduced the number and diversity of native populations. For example, the GBCT currently exists in a single stream in the Arkansas River drainage (Bear Creek) outside of its native range that was established via stocking (Metcalf et al. 2012); the Yellowfin Cutthroat Trout has been considered extinct since 1906 (Juday 1906) with only degraded archival samples available; and the recent rediscovery of San Juan lineage has precluded them from previous genomic studies (though they are present here).

DNA Extraction and Whole Genome Sequencing

Whole genomic DNA was collected for 2 individuals each from 10 UPCT populations, 6 GRCT populations, and one population each of the GBCT, RGCT, SJCT, Bonneville, Yellowstone, Lahontan, Westslope, and Coastal Cutthroat Trout for a total of 48 individuals (Fig. 2). DNA from each of the 48 whole genomes was extracted using the manufacturer's standard DNeasy Blood and Tissue Kit protocol (Qiagen, Hilden, Germany) at Pisces Molecular (Boulder, Colorado). After DNA extraction, Novogene Corporation Inc. (Sacramento, CA) sequenced all samples in paired-end 150bp mode on an Illumina HiSeq X platform to a target of 18x average coverage.

Alignment, Variant Calling, and Filtering

The bioinformatic pipeline that we created for processing these data was adapted from Eric Anderson's "mega-non-model-wgs-snakeflow" GitHub repository, found online at: https://github.com/eriqande/mega-non-model-wgs-snakeflow. We trimmed raw reads in paired end mode to remove adapters and low quality reads using fastp (v0.23.4; Chen et al. 2018) with a window size of 4 and minimum mean quality of 20 (options: --cut_right_window_size 4 --cut_right_mean_quality 20). We mapped all cleaned reads of the same read group to the rainbow trout (O. mykiss) reference genome (GCA_013265735.3; Gao et al. 2021) using BWA-MEM2 mem (v2.2.1; Vasimuddin et al. 2019) with default options. We then merged resulting BAM files of a common sample before removing duplicate reads using GATK (v4.2.6.1; Auwera and O'Connor 2020) MarkDuplicates (options: --REMOVE_DUPLICATES true).

Variants from all 48 BAM files were called jointly using the GATK joint calling best practices workflow (Poplin et al. 2017). GATK HaplotypeCaller was used in GVCF mode to generate GVCF files for each sample with minimum base quality of 33 and minimum mapping quality of 20 (options: --min-base-quality-score 33 --minimum-mapping-quality 20). Each sample GVCF was merged using GATK GenomicsDBImport before genotypes were called using GATK GenotypeGVCFs with default flags. Bcftools (v1.9; Li 2011) +setGT and +fill-tags were used to mark any sites with a read depth of 0 or a genotype likelihood of "0,0,0" as missing. GATK SelectVariants and VariantFiltration were used to hard filter SNPs and indels separately (indel options: -filter "QD < 2.0" -filter "QUAL < 30.0" -filter "FS > 200.0" -filter "ReadPosRankSum < -20.0"; snp options: -filter "QD < 2.0" -filter "QUAL < 30.0" -filter "SOR > 3.0" -filter "FS > 60.0" -filter "MQ < 40.0" -filter "MQRankSum < -12.5" -filter

"ReadPosRankSum < -8.0") before merging with GATK MergeVcfs and converting to BCF using Bcftools (v1.9) view. This resulted in a single BCF file for all samples containing 103,596,561 raw variants, with variants that failed any filtering option flagged.

Bcftools (v1.9) view was used to filter this BCF to include only biallelic SNPs that passed all GATK recommended hard filtering options (options: -v snps -m 2 -M 2 -i FILTER=\"PASS\") and mapped to the autosomes (option: -R {region}), and removed any SNP within 5 bp of an indel using filter (options: --Snpgap 5). The resulting BCF file included 62,688,056 biallelic SNPs.

Genetic Diversity

We generated a genotype count report for each individual using the PLINK 2.0 (Chang et al. 2015) --geno-counts flag, and used a custom awk script to pull the number of heterozygous sites from the genotype report. We divided the number of heterozygous sites for each individual by the total reference genome size (2.2Gb) to estimate autosomal heterozygosity. We visualized these data in R 4.3.2 (R Core Team, 2023) using ggplot2 (Wickham 2016). Upon preliminary analysis, one of the Coastal Cutthroat Trout samples (C132065) was identified as an outlier - either due to DNA contamination or admixture - and was removed from all downstream analyses.

Population Structure

Using PLINK 2.0, we applied a 10% missingness filter to remove all variants that were missing in more than 5 of the 48 samples (options: --geno 0.1) which resulted in 55,972,542 SNPs. We then filtered based on minor allele count (MAC) thresholds of 1, 3, and 5 (options: --mac 2, --mac 4, --mac 6) to remove singletons (MAC>1) or population private alleles (MAC>3 and MAC>5). This resulted in approximately the following number of SNPs for each filtering threshold: 40 million for MAC>1, 17 million for MAC>3, and 8.5 million SNPs for MAC>5. The MAC>1 threshold removes singleton sites, while the MAC>3 threshold removes private alleles from the Coastal Cutthroat Trout population after removing the outlier individual, but preserves other population private alleles. The MAC>5 threshold removes any variants that are private to a single population, but preserves those that are private to the GRCT and UPCT. The following population structure analyses were conducted on all three variant sets;

results in the main text were generated with the MAC>1 threshold, while those using MAC>3 and MAC>5 are in the appendix.

We generated eigenvalue and eigenvector files for the 47 samples combined using the PLINK 2.0 --pca flag, and plotted the first, second. We additionally subset to only those variants present in the Rocky Mountain group (Yellowstone, Bonneville, SJCT, RGCT, GBCT, UPCT, GRCT) populations and re-filtered based on 10% missingness and MAC>1. We then re-generated eigenvalue and eigenvector files for the resulting 42 samples combined using the PLINK 2.0 --pca flag. We plotted the first, second, and third principal-component axes for each variant set in R 4.3.2 (R Core Team, 2023) using ggplot2 (Wickham 2016).

To generate a phylogenetic tree, we used the PLINK 2.0 --export phylip used-sites option to convert from BCF to PHYLIP format. Using IQ-TREE 2 (Minh et al. 2020) and the PHYLIP file as input, we generated an alignment file containing only variant sites; this variant-sites only file was used as input for IQ-TREE 2 to generate a phylogenetic tree for each MAC filtering option using the general time reversible (Donnelly and Tavaré 1986) invariable site plus discrete Gamma substitution model (Gu et al. 1995) with an ascertainment bias correction that is recommended for SNP-only data (option: -m GTR+I+G+ASC). We generated branch support files by using the ultrafast bootstrap approximation to run 1000 bootstrap replicates (option: -bb 1000; Hoang et al. 2018; B. Q. Minh, Nguyen, and Von Haeseler 2013). Trees were visualized using the Interactive Tree of Life online program (iToL v6; Letunic and Bork 2024).

We further subset the whole dataset in three ways before analyzing ancestry proportions: first, we filtered to only those variants present in the Rocky Mountain group (Yellowstone, Bonneville, SJCT, RGCT, GBCT, UPCT, GRCT) populations and re-filtered based on 10% missingness and MAC>1; second and third, we filtered to only a single population per Rocky Mountain group lineage (Williamson Lakes for GRCT, and Abrams Creek or Kelso Creek for UPCT) and retained all three a priori UPCT lineage populations currently found on the East slope: Severy Creek, Como Creek, and South Hayden Creek. We used the --make-bed flag in PLINK 2.0 to create the input files needed for running the program ADMIXTURE (v1.3.0; Alexander, Novembre, and Lange 2009) in order to generate a model-based estimation of ancestry in our samples. We used the coefficient of variation for each K to determine the best fit to the data. For each of the three filtering subsets, we tested values of K between 2-21 for the first subset, and values of K between 2-11 for the second and third subsets.

Demographic Reconstruction

We returned to the merged and de-duplicated BAM files from the first step of the filtering process as the starting point for demographic reconstruction analysis. We used BamUtil (v1.0.15; Jun et al. 2015) clipOverlap and the SAMtools (v1.19.2; Li et al. 2009; Danecek et al. 2021) view (option: -L {regions}) to generate cleaned BAM files for each sample with reads that only mapped to autosomal regions. The pairwise sequentially markovian coalescent, or PSMC (Li and Durbin 2011), analysis for demographic reconstruction requires a consensus genome sequence in .fastq format that can be filtered to account for coverage and sequencing errors. We used the 'mpileup', 'call', and 'vcfutils.pl vcf2fq' commands in BCFtools to generate this consensus sequence for each individual sample (command line: bcftools mpileup --full-BAQ -C50 -Ou -f {input.ref} {input.bam} | bcftools call -c - | vcfutils.pl vcf2fq -d 10 -D 36 | gzip > {output}). The BCFtools 'mpileup' command has replaced the deprecated SAMtools 'mpileup' command that has been used in many previous PSMC-based studies. This BCFtools calling pipeline uses single individuals for SNP calling, so variant calling is not based on population allele frequencies and does not assume Hardy–Weinberg equilibrium. We filtered resulting VCF files to a minimum read depth per site of 10 (Nadachowska-Brzyska et al. 2016), and a maximum read depth per site of 36, which is twice the average read depth across all samples as recommended for high coverage genomes in the PSMC documentation (https://github.com/lh3/psmc).

We converted each of these individual consensus sequence files to .psmcfa files using PSMC fq2psmcfa.c with a minimum quality filter of 20 (option: -q 20). We manually chose the PSMC analysis settings (-p and -t options) after many rounds of parameter testing according to suggestions given by Li and Durbin 2011 (https://github.com/lh3/psmc). We set the number of iterations to 25, the upper limit for the time to the most recent common ancestor (TMRCA) to 10, and the initial θ/ρ value to 5 (options: -N25 -t10 -r5). We set the atomic time interval pattern to '-p 10+6*2+18*1+8*2+8*1', which results in 64 atomic time intervals, with 41 free interval parameters. This means that the first population-size estimate spans the first 10 atomic time intervals, the next six estimates span two intervals each, the next 18 estimates span one interval each, the following eight estimates span two intervals each, and the last eight estimates span one interval each. To check for variance in effective population size estimates, we performed 100 bootstrap replicates by randomly sampling with replacement (option: psmc -b) 5-Mb sequence segments obtained from the .psmcfa file after splitting it with the 'splitfa' command. We applied a generation time of 3 years (Downs et al. 1997; Young 2008; Rougemont et al. 2021; Rondeau et al. 2023), and a mutation rate of $8.0 \times 10{-}9$ bp/generation (Rougemont et al. 2021, 2023; Rondeau et al. 2023) to generate the resulting PSMC plots.

Results

Genetic Diversity

The proportion of autosomal heterozygosity ranged from 0.277 to 0.949 sites per 1Kb, with an average of 0.477 sites (Fig. 3). The outlier individual (C132065) had a heterozygosity proportion of 2.061, which was ~2.17x the heterozygosity proportion of the other Coastal Cutthroat Trout individual (C132064, het=0.949) that had the next highest heterozygosity proportion (Fig. A1). An average of 58,621,783 SNPs (range: 55,793,033 - 59,261,355) per individual (ignoring the outlier) were used in the analysis of heterozygosity. A handful of individuals from the GRCT and UPCT had elevated heterozygosity estimates: one from the Navajo River and one from Lake Nanita for GRCT, and both individuals from the Roan Creek, West Antelope Creek, and Como Creek populations from the UPCT. Notably, these are the same populations that showed distance from the cluster of their respective lineage, minus South Hayden Creek. Higher heterozygosity estimates may suggest contemporary admixture has occurred in these populations.

Population Structure

A total of 40,438,798 SNPs were analyzed in the main MAC > 1 filtering set to produce a principal component analysis, where the first principal component axis explained 46.38% and the second axis explained 14.36% of the variation (Fig. 4). The samples appear to cluster into four distinct groups that align with the major evolutionary groups of Cutthroat Trout; however, the unequal and very low sample sizes for the outgroup populations obstruct any reliable estimate of within species variation compared to between species variation. A total of 11,430,255 SNPs were analyzed in the Rocky Mountain group PCA, where the first principal component axis explained 10.99% and the second axis explained 7.45% of the variation (Fig. 5). The first principal component axis separates based on variation in SNPs between the Yellowstone Cutthroat Trout and those populations from the Southern Rocky Mountains, including Bonneville Cutthroat Trout. The second principal component axis separates based on variation between the Southern Rocky Mountain lineages, with UPCT and GRCT / Bonneville Cutthroat Trout at opposite ends of the axes. Only two lineages, GRCT and UPCT, are represented by more than one population and the majority of these populations form lineage-specific clusters. The handful of populations that separate from these clusters are labeled in Fig. 5; for the GRCT these include West Fork Boulder Creek (located in Utah) and 1 individual from Lake Nanita, while for UPCT these include West Antelope Creek, Roan Creek, Como Creek, and South Hayden Creek. Como and South Hayden Creek are located on the East slope.

Using the subset of 11,430,255 variants from the Rocky Mountain group populations, K=4 was identified as the best K based on the lowest coefficient of variation value (Fig. 6); however, K=9 had a CV value that was marginally higher and is presented in the main text as well (Fig. 7). K=4 identifies the Yellowstone Cutthroat Trout as a unique ancestry group in yellow. It also identifies shared ancestry in the GRCT populations in blue, and UPCT populations in green. Notably, South Hayden Creek - an *a priori* UPCT population located on the East slope - shares more ancestry with the other lineages of the Southern Rocky Mountains than with the other UPCT populations. After further filtering to only a single population per Rocky Mountain group lineage (Williamson Lakes for GRCT, and Abrams Creek in the Colorado basin or Kelso Creek in the Gunnison basin for UPCT) and retaining all three *a priori* UPCT lineage populations currently found on the East slope (Severy Creek, Como Creek, and South Hayden Creek), we used 9,151,723 (Abrams for UPCT) or 9,141,840 (Kelso for UPCT) variants to generate ancestry proportion plots (Fig. 8).Different values of K were identified as the first and second best depending on the population used as the representative of the UPCT. Como Creek showed evidence of admixture between GRCT and UPCT in all scenarios except the second best K (K=10) with Abrams Creek for the UPCT. South Hayden Creek was represented as having its own ancestry in all scenarios except the best K (K=7) with Kelso Creek for the UPCT where it grouped with RGCT. Interestingly, at the best K value for the two scenarios, Severy and Como Creek showed shared ancestry with Abrams Creek (which is in the Colorado basin), but not with Kelso Creek (which is in the Gunnison basin); this is different from the finding when all sampled populations of the UPCT were included in the ancestry analysis (Fig. 7).

Maximum Likelihood Tree

The topology of both the maximum likelihood and consensus phylogenetic trees were similar across each of the three MAC filtering sets for relationships within the Rocky Mountain group, with minor differences in the placement of Coastal, Westslope, and Lahontan Cutthroat Trout. The MAC>1 tree was generated with 16,302,152 parsimony-informative sites. The total sum of tree branch lengths was 0.1353 and the sum of internal branch lengths is 45.63% of tree length. The maximum likelihood and consensus trees had the same topology, and all branches had 100% bootstrap support (Fig. 9). Populations generally aligned with their putative lineage, with monophyletic clades highlighted in associated colors; however, South Hayden Creek, an *a priori* UPCT population located on the East slope, does not group with other UPCT populations.

Demographic Reconstruction

Using the pairwise sequentially Markovian coalescent (PSMC) analysis for demographic reconstruction, all lineages besides Coastal Cutthroat Trout had estimated effective population sizes of less than ~30,000 individuals over the last ~30,000 years, with estimated effective population sizes below ~50,000 over the last 100,000 years (Fig. A6). We did not identify any lineage-specific trends in effective population size changes for the Southern Rocky Mountain Cutthroat Trout (Fig. 10). The Como Creek, South Hayden Creek, Greenback Cutthroat Trout, West Antelope Creek, Navajo River (Fig. 10) and Roan

Creek (Fig. 11) populations showed evidence of population expansions and contractions at different points in the past.

Discussion

This is the first study to investigate the relationships of Cutthroat Trout in the Southern Rocky Mountains using whole genome sequence data, and the first nuclear genomic analysis to include all extant lineages of Southern Rocky Mountain Cutthroat Trout. Our results confirm that genetically distinct lineages of Cutthroat Trout exist across the Southern Rocky Mountains, and their current genetic relationships are likely the product of a mosaic of evolution via biogeography as well as contemporary human influence through stocking. Notably, our population structure analyses are able to distinguish between the five putative lineages of Southern Rocky Mountain Cutthroat Trout and additionally identify South Hayden Creek as distinct in its nuclear genome from the rest of the UPCT, including other East slope UPCT populations.

Genetic Diversity

Our heterozygosity estimates are very low compared to SNP-based Rainbow Trout estimates of 15.6 heterozygous sites per 1Kb (Gao et al. 2021). We expected heterozygosity to be low for the majority of populations from the Southern Rocky Mountains because non-admixed Cutthroat Trout populations have been isolated to small headwater streams. This isolation is the result of both natural and anthropogenic fragmentation by physical barriers (Horan et al. 2000; Pritchard et al. 2007; Fausch et al. 2009; Whiteley et al. 2010; Stack et al. 2024) and the loss of 80-95% of their historically occupied range to the invasion of nonnative trout (Peterson et al. 2004; Fausch et al. 2009; Campbell et al. 2012; Kovach et al. 2018). In addition, populations that displayed elevated heterozygosity estimates compared to the average were also distanced from their putative lineage-specific clusters and may suggest evidence of admixture with other Cutthroat Trout lineages. This is confirmed by their admixture plots and intermediate placements in the PCA; specifically, Como Creek, Roan Creek, and one individual each from Lake Nanita and the Navajo River populations. On the other hand, West Antelope Creek also displays higher than average heterozygosity proportions but no evidence of admixture. This population experienced a recent bottleneck event in 2020 due to intense drought; our DNA samples were collected in 2022, so these findings may reflect pre-bottleneck diversity as the impacts of bottleneck events on individual heterozygosity are delayed. Therefore, additional genetic monitoring of West Antelope Creek may reveal reduced diversity in the future and will be important for informing continued management.

At the lower end, observed heterozygosity identifies native populations of greatest concern including many of the UPCT populations, South Hayden Creek, and our representatives from the SJCT and GBCT lineages. It is not necessarily surprising that SJCT and GBCT populations in particular have lower heterozygosity, as SJCT was considered extinct until a few isolated headwater populations were rediscovered in the 2010s (Rogers et al. 2018), and GBCT are represented by a single population that was stocked into Bear Creek outside of its native range in the lower Arkansas basin (Metcalf et al. 2012) GBCT in particular are the focus of a large recovery effort in Colorado; however, hatchery propagation has been complicated by poor survival of offspring with strong evidence of high inbreeding load (Rogers et al. 2022). Outcrosses with UPCT individuals showed pronounced hybrid vigor, suggesting that genetic rescue via outcrossing may be a viable strategy for improving broodstock fitness, yet this raises concerns for extinction via introgression (Rogers et al. 2022). Notably, several UPCT populations showed heterozygosity below that of GBCT; low heterozygosity estimates in these populations are concerning as they likely represent reduced evolutionary adaptation potential in a changing climate. However, recent work shows evidence for adaptive genomic variation in populations of trout that have been small for a long time (Jeon et al. 2025). These findings necessitate further investigation to understand whether these wild populations are already suffering negative fitness consequences, and in need of future management action.

Population Structure

Broadly, the principal component analysis supports the classification of Cutthroat Trout into four major groups, and further differentiates the Southern Rocky Mountain lineages from each other. Additionally, the PCA reveals that many of the GRCT and UPCT populations form tight lineage-specific clusters while other populations or individuals are distanced from these clusters. This distance indicates genetic distinctiveness that may be a product of drift from isolation and resulting genetic bottlenecks, or of admixture between distinct lineages.

All but one of the GRCT populations in our study can be traced back to stocking of progeny derived from wild spawn operations at Trappers Lake a century ago, some with well established one-time stocking events (Lake Nanita, Williamson Lakes). The two GRCT individuals that do not cluster tightly in the PCA with the remaining GRCT samples include one individual each from Lake Nanita and the Navajo River which display admixture and elevated heterozygosity estimates relative to the other GRCT populations. The West Fork Boulder Creek is the only native GRCT population in our study with no known stocking history. Located in Eastern Utah, its distance on the PCA relative to the other GRCT populations is likely the product of native diversity. Among the putative UPCT populations, Roan Creek, Como Creek, West Antelope Creek, and South Hayden Creek are distanced from the main UPCT cluster as well. Roan Creek displays possible admixture with YSCT which may explain why these points are pulled towards the YSCT points on the PCA, while Como Creek displays admixture with GRCT that is reflected in its intermediate position between the GRCT and UPCT. West Antelope Creek is likely similar to West Fork Boulder Creek in that its distance from the main UPCT cluster is likely a product of native diversity, or could be a result of previous drought-related bottleneck. South Hayden Creek lies intermediate to the centralized UPCT cluster and the other SRM lineages likely because it experienced admixture with UPCT individuals, but its nuclear genome remained more distinct. Further discussion of the origin of South Hayden Creek is presented below.

The nuclear phylogenetic tree confirms what previous mitochondrial studies have suggested: the GRCT and UPCT are genetically distinct from one another and deserve taxonomic redescription. Additionally, some lineages within the Southern Rocky Mountains are currently designated as subspecies while others are categorized as lineages, yet these distinctions do not clearly map onto the nuclear phylogeny. This is an area where managers working on the ground with these populations across multiple states may benefit from collaborative taxonomic decision making, as species and subspecies designations influence the possibility of management actions available to imperiled populations, especially those with evidence of introgression (USFWS 2003; Campton and Kaeding 2005).

In general, our nuclear DNA results align with previous mitochondrial DNA results with two notable deviations. The first of these is that the SJCT groups more closely with the UPCT in mitochondrial studies whereas here they align more with GRCT based on the phylogenetic tree. Secondly, when only mitochondrial DNA is analyzed, the South Hayden Creek population is categorized as a member of the UPCT; however, using nuclear DNA this population groups more closely with the Rio Grande Cutthroat Trout. The other East slope UPCT populations - Como and Severy Creek - are genetically similar to one another even though they occur in different basins, and they group mostly closely with the Gunnison basin UPCT populations. This strongly suggests that Como Creek was stocked, as the most likely source for either human-mediated interbasin transfer or natural colonization into the headwaters of South Platte basin where Como Creek is located would be from the upper Colorado River basin, not the upper Gunnison River basin. Additionally, our results as well as prior mitochondrial studies reveal that Como Creek is closely related to Severy Creek, which is located in the lower Arkansas River basin. Given their geographic distance and the finding that these populations share the same mitochondrial haplotype (Rogers et al. 2018; Bestgen et al. 2019) and very similar nuclear diversity, it is unlikely that natural invasion of fish from Severy Creek into the headwaters of the South Platte occurred. Whether both of these UPCT populations were founded on the East slope via stocking from the widely distributed Gunnison basin Grand Mesa hatchery source and related diversity was lost from stocked populations on the West slope (Rogers et al. 2018; Bestgen et al. 2019), or whether a natural invasion occurred between the upper Gunnison and Arkansas basins across the Continental Divide to establish Severy Creek before these fish were used to stock Como Creek remains unclear. However, the distinctiveness of South Hayden Creek, a population within the upper Arkansas River basin near Twin Lakes, provides some insight.

South Hayden Creek

The exact origin of South Hayden Creek trout remains a mystery; however, our results suggest they are not a unique population of UPCT as they are currently managed. South Hayden Creek hosts a UPCT mitochondrial haplotype (Bestgen et al. 2019), yet a nuclear genome that is more similar to RGCT than any of the other lineages included in this study. This similarity to RGCT was also found in nuclear amplified-fragment-length-polymorphism (AFLP) data (Bestgen et al. 2013). Inconsistent ancestry between mitochondrial and nuclear DNA is known as mito-nuclear discord and has been documented in other genomic studies as an outcome of incomplete lineage sorting (DeRaad et al. 2023) or mitochondrial capture that can occur when stocking leads to introgression between native and stocked individuals (Després 2019; Quattrini et al. 2023). Several scenarios that produce mito-nuclear discord in South Hayden Creek fish are possible.

One possibility is that contemporary South Hayden Creek fish are the product of admixture between UPCT fish and Yellowfin Cutthroat Trout - the native fish of the Arkansas River headwaters - that were present on the East slope before Uncompahgre fish were introduced there. This introduction of UPCT could have occurred via human hands, such as direct stocking or an interbasin water transfer, via natural invasion across the Continental Divide through stream capture, or a combination of the two (Metcalf et al. 2012; Rogers et al. 2018; Bestgen et al. 2019). Regardless of their origin, admixture between the ancestral Yellowfin Cutthroat Trout and UPCT individuals may have led to mitochondrial capture of UPCT mitogenomes with maintenance of Yellowfin ancestral nuclear DNA, which would explain the discordance in the position of South Hayden Creek on previous mitochondrial gene trees (Metcalf et al. 2012; Rogers et al. 2018; Shiozawa et al. 2018) or the mitogenome tree presented in (Van Orden et al., *in prep*), and the nuclear tree presented here. This is supported by museum specimens collected from Twin Lakes in the headwaters of the Arkansas River in 1889 that were categorized as Yellowfin Cutthroat Trout based on phenotypic characteristics, but which had a unique mitochondrial haplotype that matches contemporary South Hayden Creek fish (Metcalf et al. 2012; Rogers et al. 2018). Nuclear analysis of pure Yellowfin Cutthroat Trout has not occurred as there are no known extant populations, and nuclear DNA has not been successfully isolated from preserved museum specimens collected from Twin Lakes. However, based on mitochondrial analysis, Yellowfin Cutthroat Trout split from a common ancestor with RGCT (Van Orden et al., *in prep*), much like South Hayden Creek appears on our nuclear tree.

An alternative explanation is that South Hayden Creek fish do not harbor native ancestral nuclear diversity and are the product of admixture with mitochondrial capture between stocked or native UPCT fish and stocked RGCT. This is a less parsimonious explanation as large-scale hatchery production and stocking of Rio Grande Cutthroat Trout in Colorado began well after specimens with the unique South Hayden Creek mitochondrial haplotypes and Yellowfin phenotypes were collected from Twin Lakes in 1889. However, by 1889 other nonnative trout including Rainbow Trout (*O. mykiss*), Lake Trout (*Salvelinus namaycush*), and Atlantic Salmon (*Salmo salar*) had been introduced into Twin Lakes. This explanation would imply that admixture occurred first between UPCT and Yellowfin Cutthroat Trout at which point museum collections occurred, and then additional admixture occurred between stocked RBCT and either the original UPCT with the same South Hayden Creek mitochondrial haplotype or the UPCT-Yellowfin Cutthroat Trout hybrids. Due to the nature of widespread and often poorly documented stocking, this explanation cannot be entirely ruled out.

Further advancements in genomic techniques may allow us to parse between these and other possible scenarios. Although the Yellowfin Cutthroat Trout has been considered extinct since 1906 and are only represented by a handful of museum specimens harboring degraded DNA, advancements in targeted capture techniques improve the possibility of sequencing loci from historical samples (Carpenter et al. 2013; Jiménez-Mena et al. 2022). Additional investigation into the nuclear relationships between populations of RGCT from across their range in the Rio Grande, Pecos, and Canadian River drainages and South Hayden Creek may also provide clarity on the level of distinctiveness of South Hayden Creek today.

Demographic Reconstruction

By visualizing effective population size trends over a long history via demographic reconstruction, we did not observe any lineage specific patterns of population expansions and contractions that would allow us to determine the timing of diversification between lineages. There are a few notable caveats to interpreting PSMC estimates, including that overlapping curves could be due to shared environmental conditions like glacial cycles or regional drought instead of shared demography. Additionally, migration among populations within a species can also look like increases in effective population size (Freedman et al. 2014), while population structuring within a species can present similarly to population declines, and this method does not represent rapid changes in effective population size due to the smoothing effect of the estimation method (Mather et al. 2020). Finally, PSMC is known to estimate the end of panmixia, but not the end of gene flow between two groups. Some work using simulations showed the effect of ancient gene flow on the shape of PSMC curves, including artifactual peaks at the time of simulated migration between two groups (Freedman et al. 2014). Further work with simulations and other methods for estimating effective population size, divergence time, and gene flow among our samples could be useful.

It is notable that all populations appear to have experienced relatively low effective population sizes (<25,000 individuals) over a long period of time (10-30,000 ybp), which may be protective against negative fitness impacts from inbreeding for populations that are experiencing small sizes presently (Van Oosterhout 2020). It appears that these were not formerly enormous populations that went through an intense bottleneck prior to 10,000 years ago; therefore, the current risk of exposing formerly masked deleterious alleles is lower than for a scenario in which they experienced a significant bottleneck event, as prolonged small population sizes are associated with purging of deleterious alleles (Robinson et al. 2018; Van Oosterhout 2020). As such, the observed current low heterozygosity and population size estimates may not be immediately problematic in the context of negative inbreeding risk as even most unrelated individuals in a given population likely harbor similar alleles that can become fixed in small populations due to genetic drift (Van Oosterhout 2020). However, mean fitness is generally lower in small populations compared to large ones, even in populations that have been small for a long time (Van Oosterhout 2020), and while we did not observe intense declines prior to 10,000 ypb, it is well known that Cutthroat Trout have experienced marked declines in the last 150 years across their range (Fausch et al. 2009). As mentioned above, negative fitness consequences have already been documented in GBCT broodstock programs (Rogers et al. 2022), so it is likely that other wild populations in this study with lower heterozygosity estimates than observed in GBCT may also be experiencing reduced fitness. This is an area where further genomic investigation on the relationship between observed heterozygosity and measures of fitness in the wild will be increasingly important for guiding management actions.

Another possible management implication of this finding is the risk of outbreeding depression when stocking genetically distinct individuals into a population of concern due to the potential for introducing alleles that are neutral in the source population but possibly deleterious in the target population (Fitzpatrick and Funk 2019; Fitzpatrick et al. 2020). In this context, when stocking is needed for managers to protect very small and genetically pure conservation populations such as has been considered with GBCT (Rogers et al. 2022), using a nearest neighbor approach - moving individuals from a geographically close conservation population of the same lineage - or screening native populations for local adaptations when possible before genetic rescue is attempted may reduce outbreeding depression risk (Frankham et al. 2011; Fitzpatrick and Funk 2019; Fitzpatrick et al. 2020).

Conclusion

It is important to recognize that we are taking a snapshot of diversity in evolutionary time, and that anthropogenic events have affected the evolutionary trajectory of the Cutthroat Trout species group. To borrow a phrase from the computational community: all models are wrong, but some are useful. Our nuclear models, in particular the phylogenetic tree presented here, represent the current relationships between Cutthroat Trout populations included in this study. It is possible that we will never fully understand the evolutionary processes that led to the Cutthroat Trout lineages we see on the landscape today because their intermediaries were lost to evolution, and their closely related lineages were lost to the movement of trout across North America. However, this does not necessitate that we give up on conserving the diversity of Cutthroat Trout still present on the landscape today. To echo Dr. Robert Behnke, a scientist who devoted his career to this species complex, “My advice to fishery biologists, managers, and administrators is to avoid taxonomic anxiety and concentrate instead on recognizing that particular forms of trout are native to particular areas, and that these forms are differentiated from each other. The sum total of this differentiation represents the biodiversity of western trout – a genetic resource still to be integrated into fisheries management programs” (Behnke 1992).

Table 1. Sampled populations with 3 letter codes, *a priori* lineage, and location.

Population	Code	Lineage	Basin	Slope	LAT	LON	Year	N
Navajo River	NAV	Green River	San Juan	West	37.19	-106.67	2006	2
Williamson Lakes	WLM	Green River	California	West	36.66	-118.33	2006	2
Steelman Creek	STL	Uncompahgre	Colorado	West	39.76	-105.93	2006	2
Lake Nanita	NAN	Green River	South Platte	East	40.26	-105.72	2008	2
Como Creek	COM	Uncompahgre	South Platte	East	40.02	-105.52	2008	2
South Hayden Creek	HAY	Uncompahgre	Arkansas	East	38.30	-105.82	2010	2
Severy Creek	SEV	Uncompahgre	Arkansas	East	38.90	-104.99	2010	2
Abrams Creek	ABR	Uncompahgre	Colorado	West	39.59	-106.86	2010	2
E Fk Piedra River	PIE	Green River	San Juan	West	37.49	-107.09	2010	2
W Fk Boulder Creek	WFB	Green River	Colorado	West	38.05	-111.49	2010	2
Kelso Creek	KEL	Uncompahgre	Gunnison	West	38.55	-108.57	2011	2
Roan Creek	ROA	Uncompahgre	Colorado	West	39.56	-108.63	2014	2
Dry Gulch	DRY	Green River	South Platte	East	39.70	-105.89	2016	2
S Twin Creek	TWN	Uncompahgre	Gunnison	West	39.20	-107.43	2017	2
Hunter Creek	HUN	Uncompahgre	Colorado	West	39.21	-106.65	2019	2
West Antelope Creek	ANT	Uncompahgre	Gunnison	West	38.62	-107.04	2022	2
Yellowstone	YSN	Yellowstone	Yellowstone	East	44.61	-110.38	2005	2
San Juan	SJN	San Juan	San Juan	West	37.59	-107.97	2007	2
Bonneville	BON	Bonneville	Bonneville	West	38.49	-112.07	2004	2
Greenback	GBK	Greenback	Arkansas	East	38.81	-104.90	2010	2
Lahontan	LAH	Lahontan	Lahontan	West	40.04	-119.64	2023	2
Coastal	COA	Coastal	Columbia	West	44.41	-123.99	2014	2
Westslope	WES	Westslope	Clark Fork	West	47.32	-113.29	2015	2
Rio Grande	RIO	Rio Grande	Rio Grande	East	37.02	-106.33	2016	2

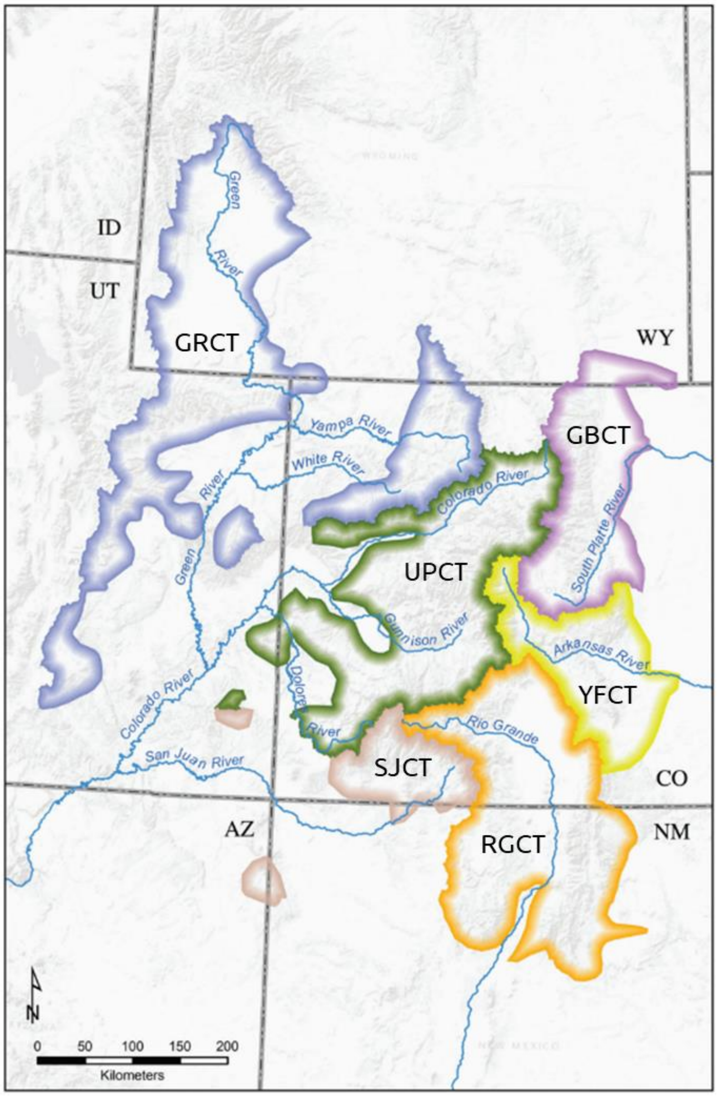

Figure 1. The putative native ranges of Cutthroat Trout clades in the Southern Rocky Mountains are generally aligned with major drainage basins and shown in blue (Green River Cutthroat Trout, GRCT), green (Uncompahgre Cutthroat Trout, UPCT), rust (San Juan Cutthroat Trout, SJCT), orange (including the headwaters of the Pecos and Canadian River basins; Rio Grande Cutthroat Trout, RGCT), gold (Yellowfin Cutthroat Trout, YFCT, extinct), and purple (Greenback Cutthroat Trout, GBCT). Adapted from Rogers et al. 2018.

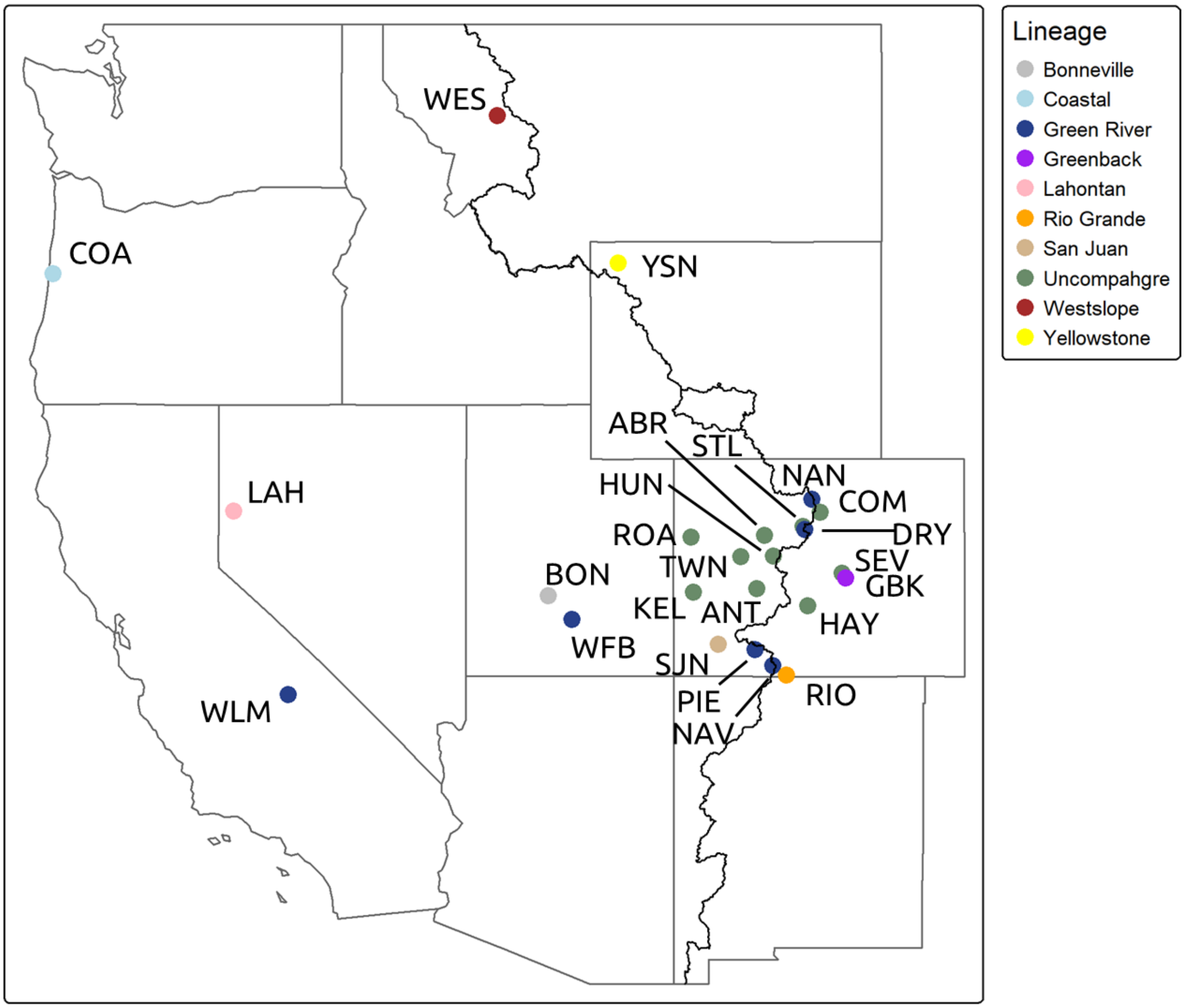

Figure 2. Map of population sampling locations with U.S. state borders in gray and the Continental Divide in black. The *a priori* lineage of each population is denoted by color. Populations are labeled with the associated 3 letter code from Table 1.

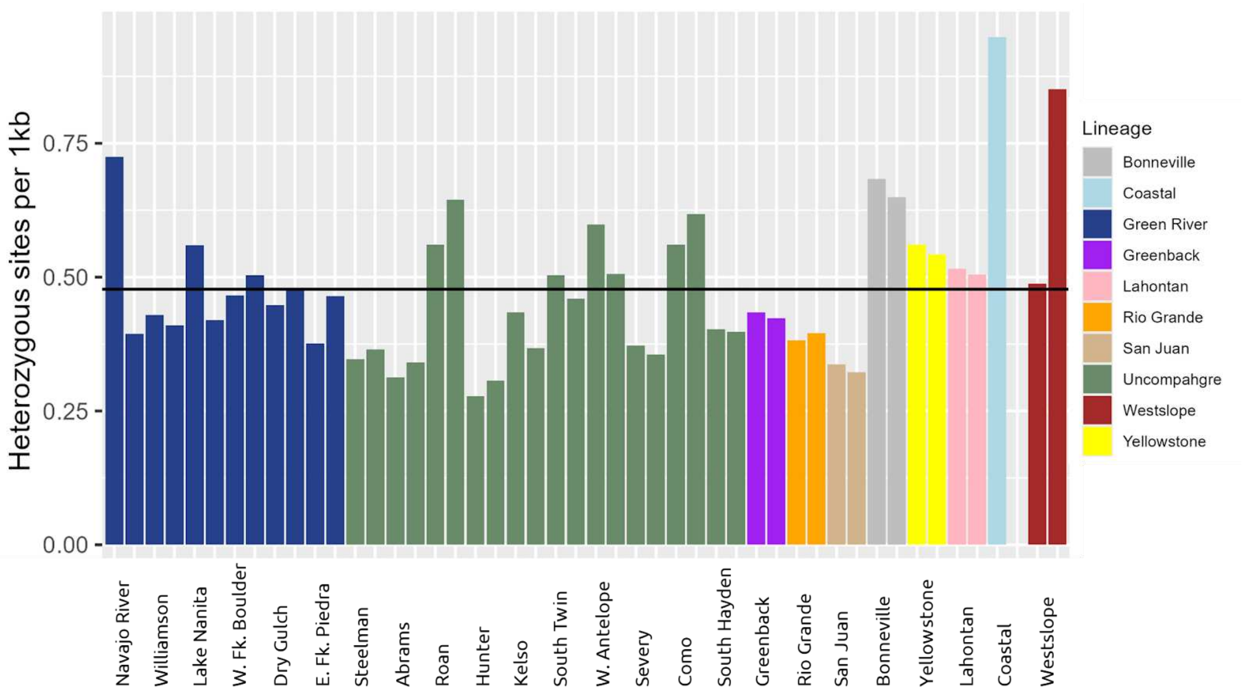

Figure 3. Individual autosomal heterozygosity proportions per 1 kilobase. The horizontal black line is the mean heterozygosity proportion (0.0179) across all samples after removing the Coastal outlier (C132065).

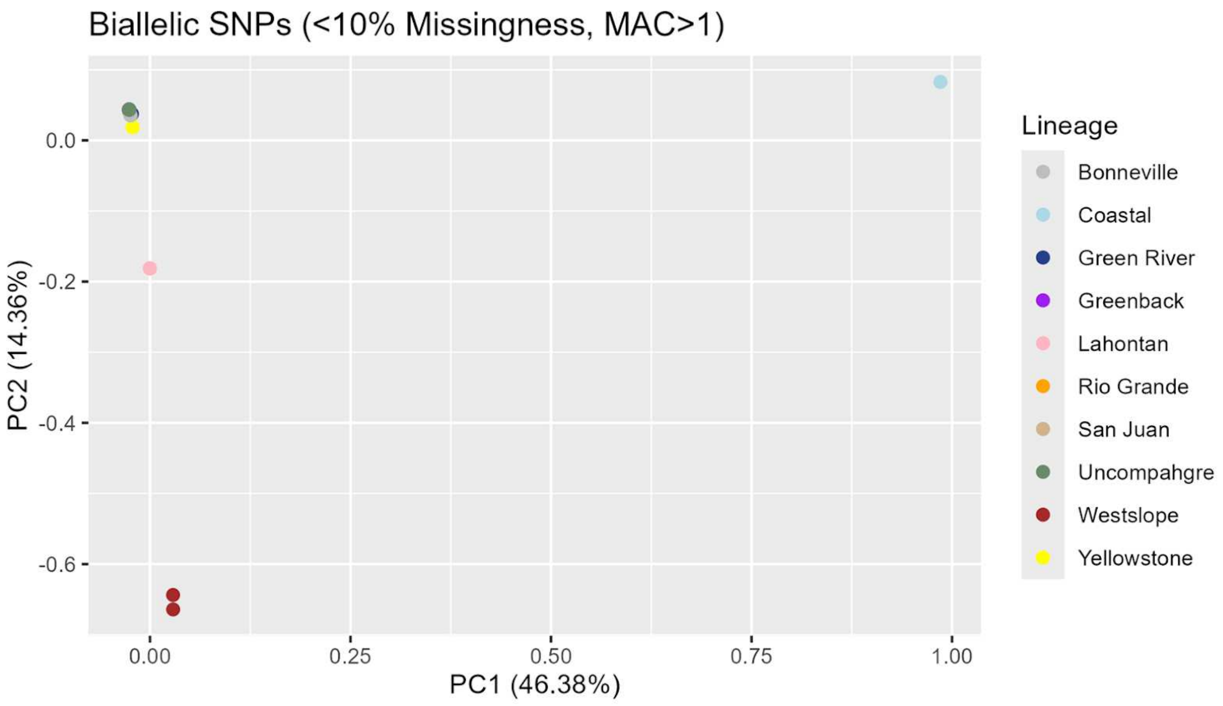

Figure 4. First and second principal component axes for the variant set with all samples. Colors correspond to *a priori* lineage.

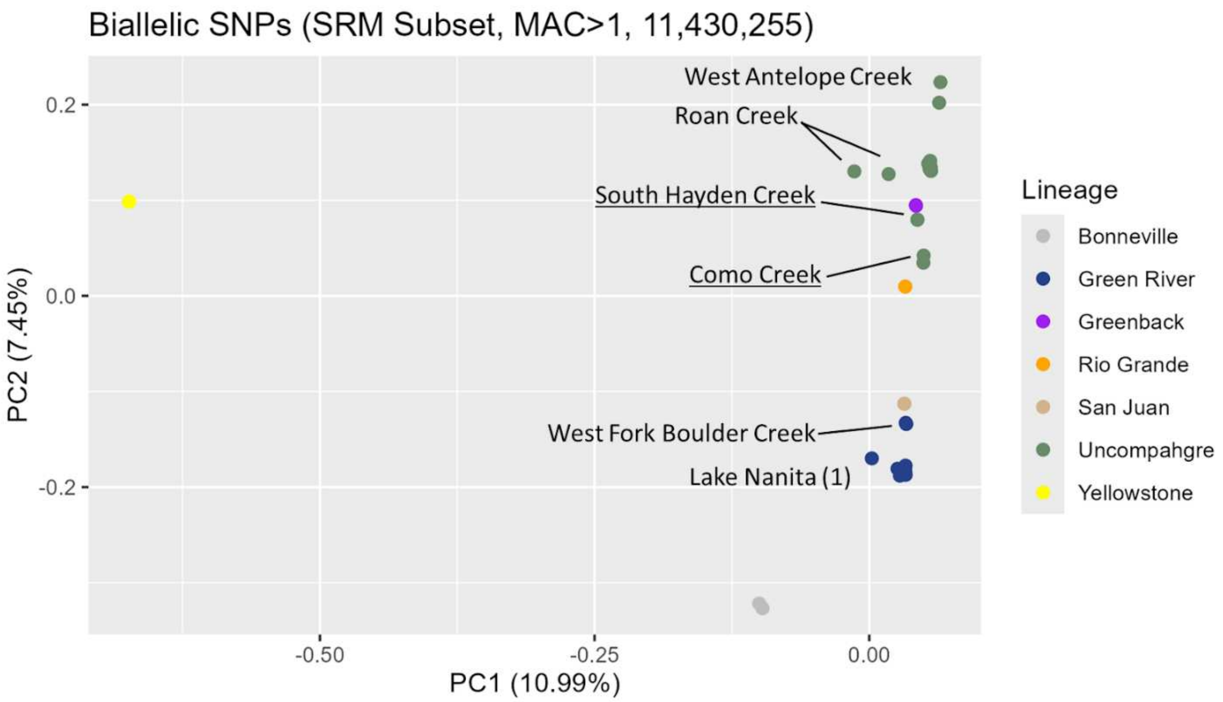

Figure 5. First and second principal component axes for variants from the Rocky Mountain populations. Colors correspond to *a priori* lineage, and populations or individuals of note are labeled. Como Creek and South Hayden Creek (underlined) are *a priori* UPCT populations located on the East slope. Only one individual (1) from Lake Nanita was distanced from the GRCT cluster.

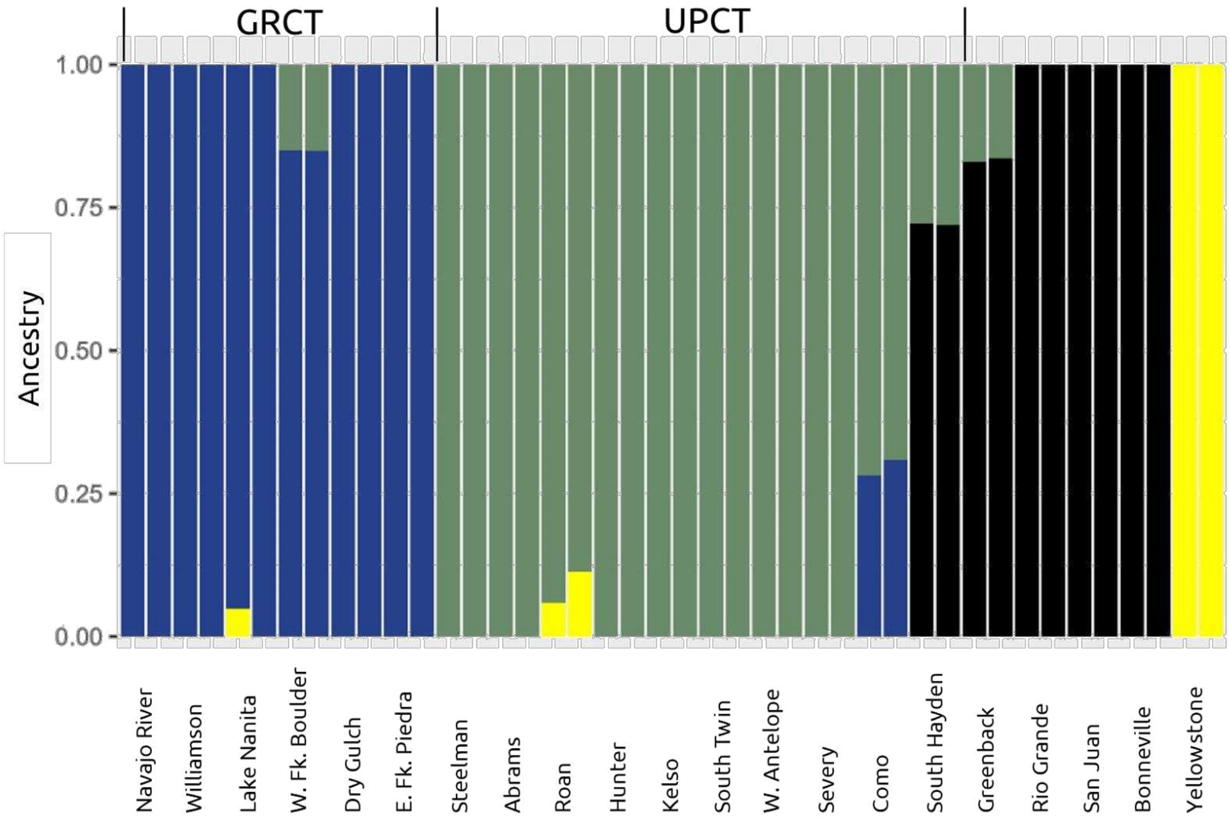

Figure 6. Ancestry proportions under K=4 generated from a subset of variants from the Rocky Mountain group. Each bar is an individual sample, and the colors represent the portion of an individual's ancestry that is assigned to that group. K=4 was identified as the best supported K based on the low coefficient of variation value (CV=0.453) for the minor allele count > 1 filter. GRCT and UPCT populations are denoted.

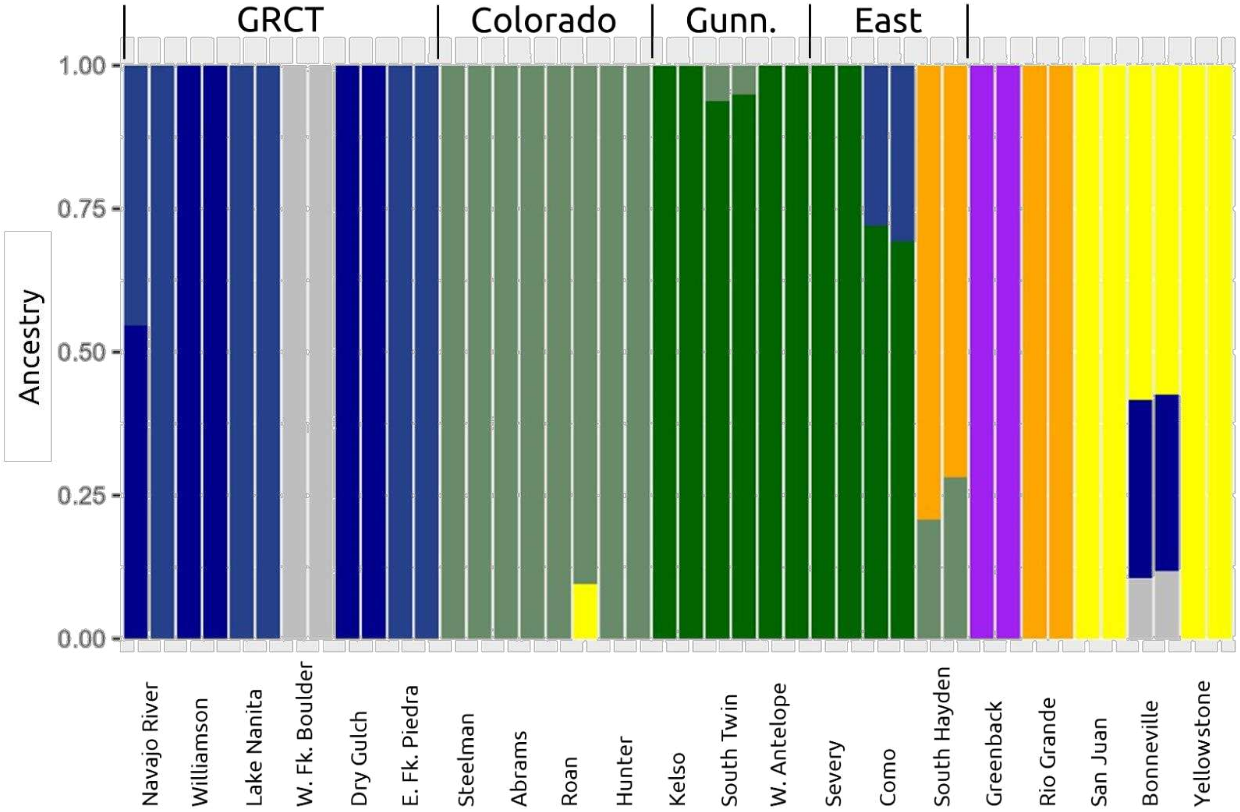

Figure 7. Ancestry proportions under K=9 generated from a subset of variants from the Rocky Mountain group. K=9 was the next best K after 4, with a coefficient of variation value slightly higher (CV=0.468) for the minor allele count > 1 filter. GRCT populations are denoted, with *a priori* UPCT populations from the Colorado, Gunnison (Gunn.), and basins on the East slope denoted as well.

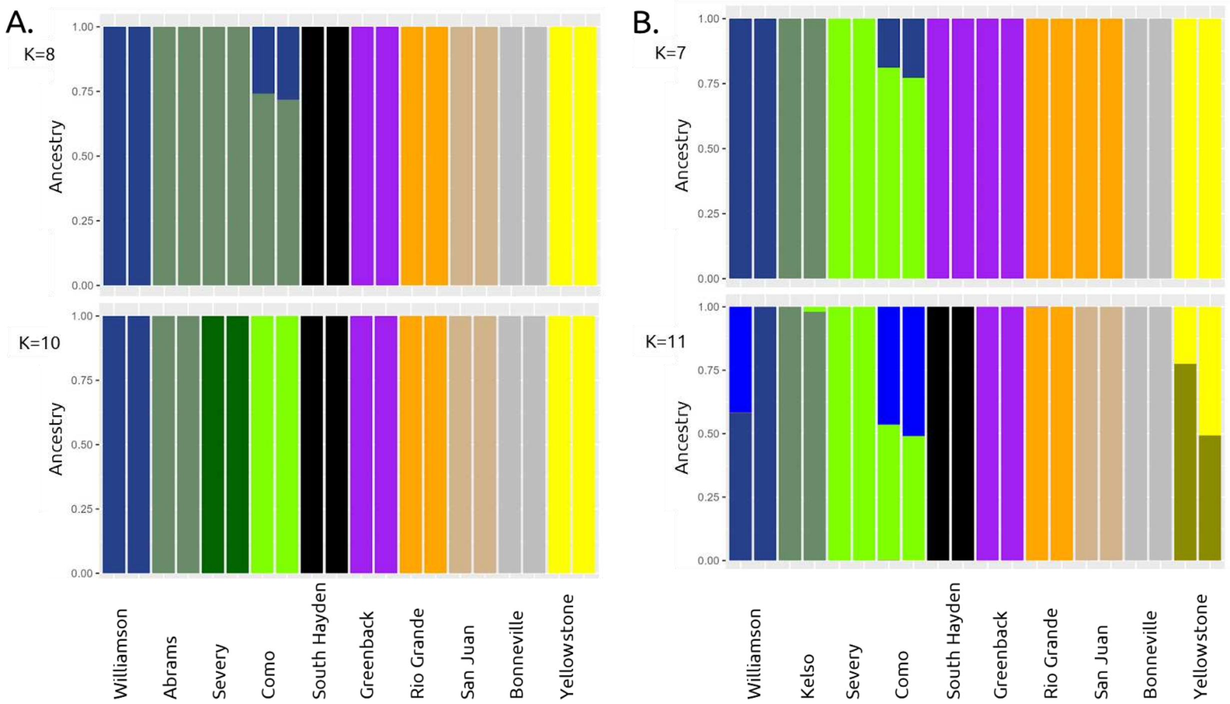

Figure 8. Ancestry proportions after filtering to only a single population per each lineage of the Rocky Mountain group: Williamson Lakes for GRCT in both, Abrams Creek for UPCT in panel A, and Kelso Creek for UPCT in panel B for UPCT. The top figure in each panel is the best K and the bottom figure in each panel is the second-best K value for the two subset scenarios. A. First (K=8, CV=0.478) and second-best K (K=10, CV=0.579) for Abrams Creek (Colorado Basin) as the representative population of the UPCT. B. First (K=7, CV=0.737) and second-best K (K=11, CV=0.763) for Kelso Creek (Gunnison Basin) as the representative population of the UPCT.

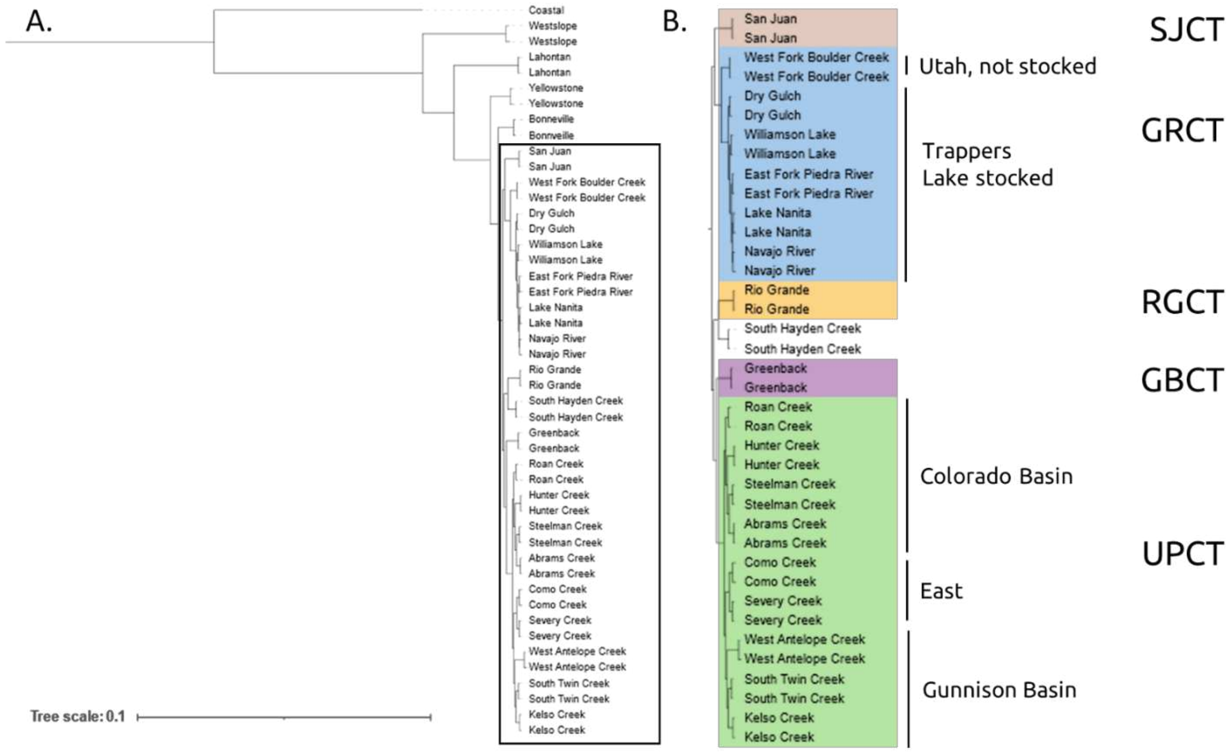

Figure 9. A. The maximum likelihood tree with all samples and a minor allele count > 1 filtering threshold. The consensus tree had the same topology; all branches had 100% bootstrap support in both the consensus and maximum likelihood trees. B. Branches contained within the black box of A. Populations within monophyletic lineages are highlighted in associated colors.

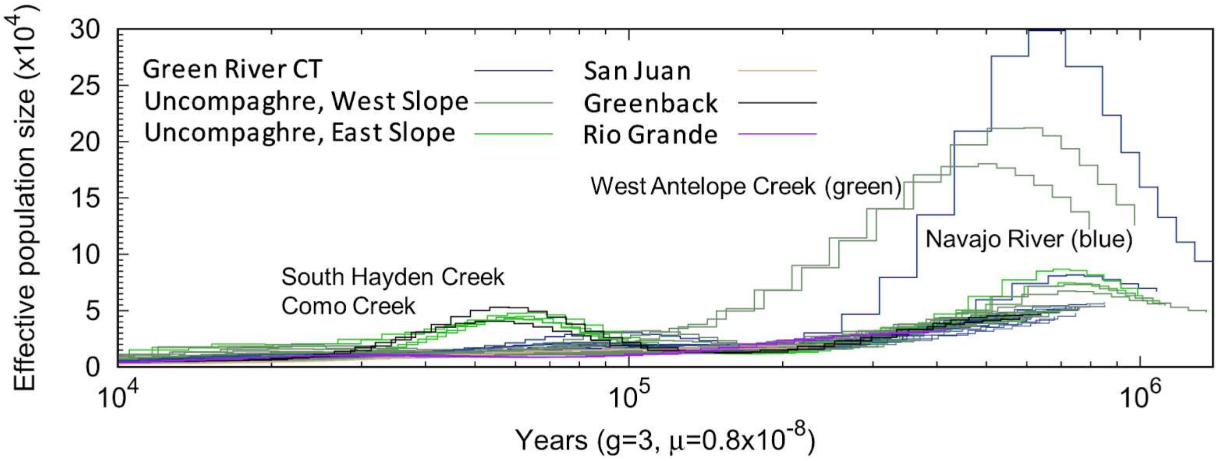

Figure 10. Pairwise sequentially Markovian coalescent (PSMC) curves of estimated effective population size over time. Each line represents the estimated ancestral effective population size from one sample genome. The x-axis is reverse time, with more recent (10,000 years ago) on the left and more ancient (1 MYA) on the right. g is the generation time, and μ is the mutation rate (bp / generation). Colors correspond to the *a priori* lineage to which an individual belongs. Populations with noteworthy PSMC curves are labeled.

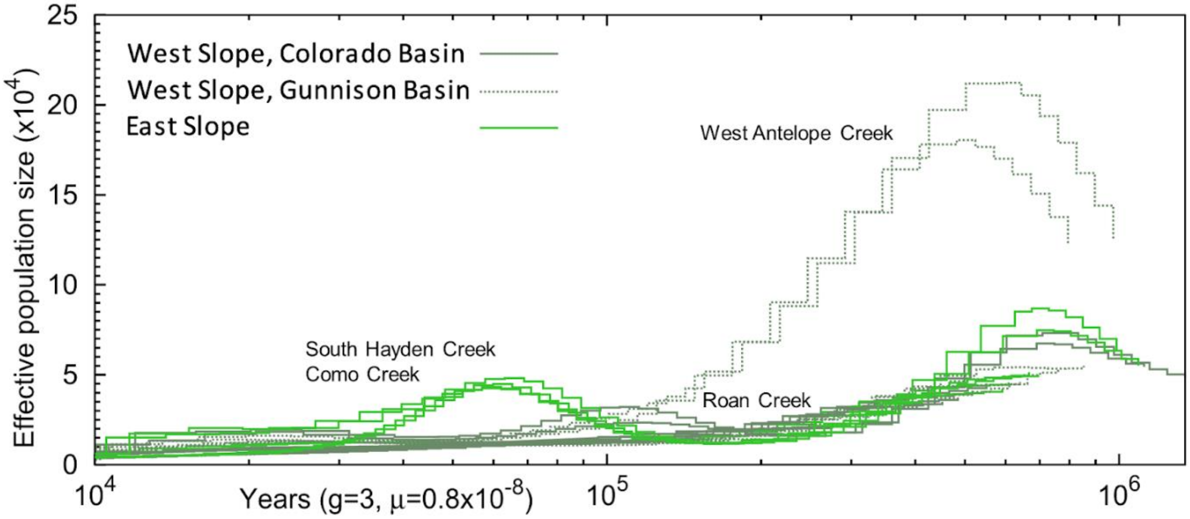

Figure 11. PSMC estimates subset to only putative UPCT populations. Light green corresponds to populations from the East slope, while solid dark green marks populations from the Colorado basin and dotted dark green mark populations from the Gunnison basin. Populations with noteworthy PSMC curves are labeled.

References

Al-Chokhachy, R., and A. J. Sepulveda. 2019. Impacts of Nonnative Brown Trout on Yellowstone Cutthroat Trout in a Tributary Stream. North American Journal of Fisheries Management 39(1):17–28.

Alexander, D. H., J. Novembre, and K. Lange. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome Research 19(9):1655–1664.

Allendorf, F. W., P. A. Hohenlohe, and G. Luikart. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics 11(10):697–709.

Auwera, G. V. der, and B. D. O'Connor. 2020. Genomics in the cloud: using Docker, GATK, and WDL in TerraFirst edition. O'Reilly, Beijing Boston Farnham Sebastopol Tokyo.

Behjati, S., and P. S. Tarpey. 2013. What is next generation sequencing? Archives of disease in childhood - Education & practice edition 98(6):236–238.

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

Beichman, A. C., T. N. Phung, and K. E. Lohmueller. 2017. Comparison of Single Genome and Allele Frequency Data Reveals Discordant Demographic Histories. Genes, Genomics, Genetics 7:3605–3620.

Benjamin, J. R., and C. V. Baxter. 2012. Is a trout a trout? A range-wide comparison shows nonnative brook trout exhibit greater density, biomass, and production than native inland cutthroat trout. Biological Invasions 14(9):1865–1879.

Bestgen, K. R., K. B. Rogers, and R. Granger. 2019. Distinct Phenotypes of Native Cutthroat Trout Emerge under a Molecular Model of Lineage Distributions. Transactions of the American Fisheries Society (148):442–463.

Bestgen, K., K. Rogers, and R. Granger. 2013. Phenotype predicts genotype for lineages of native cutthroat trout in the Southern Rocky Mountains.

Bi, C., N. Lu, Z. Huang, J. Chen, C. He, and Z. Lu. 2020. Whole-genome resequencing reveals the pleistocene temporal dynamics of Branchiostoma belcheri and Branchiostoma floridae populations. Ecology and Evolution 10(15):8210–8224.

Brüniche-Olsen, A., J. W. Bickham, C. A. Godard-Codding, V. A. Brykov, K. F. Kellner, J. Urban, and J. A. DeWoody. 2021. Influence of Holocene habitat availability on Pacific gray whale (Eschrichtius robustus) population dynamics as inferred from whole mitochondrial genome sequences and environmental niche modeling. Journal of Mammalogy 102(4):986–999.

Cahill, J. A., A. E. R. Soares, R. E. Green, and B. Shapiro. 2016. Inferring species divergence times using pairwise sequential Markovian coalescent modelling and low-coverage genomic data. Philosophical Transactions of the Royal Society B: Biological Sciences 371(1699):20150138.

Campbell, N. R., S. J. Amish, V. L. Pritchard, K. S. McKELVEY, M. K. Young, M. K. Schwartz, J. C. Garza, G. Luikart, and S. R. Narum. 2012. Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. Molecular Ecology Resources 12(5):942–949.

Campton, D. E., and L. R. Kaeding. 2005. Westslope Cutthroat Trout, Hybridization, and the U.S. Endangered Species Act. Conservation Biology 19(4):1323–1325.

Carpenter, M. L., J. D. Buenrostro, C. Valdiosera, H. Schroeder, M. E. Allentoft, M. Sikora, M. Rasmussen, S. Gravel, S. Guillén, G. Nekhrizov, K. Leshtakov, D. Dimitrova, N. Theodossiev, D. Pettener, D. Luiselli, K. Sandoval, A. Moreno-Estrada, Y. Li, J. Wang, M. T. P. Gilbert, E. Willerslev, W. J. Greenleaf, and C. D. Bustamante. 2013. Pulling out the 1%: Whole-Genome Capture for the Targeted Enrichment of Ancient DNA Sequencing Libraries. The American Journal of Human Genetics 93(5):852–864.

Chang, C. C., C. C. Chow, L. C. Tellier, S. Vattikuti, S. M. Purcell, and J. J. Lee. 2015. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience 4(1):s13742-015-0047–8.

Chen, S., Y. Zhou, Y. Chen, and J. Gu. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34(17):i884–i890.

Clemento, A. J., E. C. Anderson, D. Boughton, D. Girman, and J. C. Garza. 2009. Population genetic structure and ancestry of Oncorhynchus mykiss populations above and below dams in south-central California. Conservation Genetics 10(5):1321–1336.

Corsi, M. P., L. A. Eby, and C. A. Barfoot. 2013. Hybridization with rainbow trout alters life history traits of native westslope cutthroat trout. Canadian Journal of Fisheries and Aquatic Sciences 70(6):895–904.

Danecek, P., J. K. Bonfield, J. Liddle, J. Marshall, V. Ohan, M. O. Pollard, A. Whitwham, T. Keane, S. A. McCarthy, R. M. Davies, and H. Li. 2021. Twelve years of SAMtools and BCFtools. GigaScience 10(2):giab008.

DeRaad, D. A., J. M. McCullough, L. H. DeCicco, P. M. Hime, L. Joseph, M. J. Andersen, and R. G. Moyle. 2023. Mitonuclear discordance results from incomplete lineage sorting, with no detectable evidence for gene flow, in a rapid radiation of Todiramphus kingfishers. Molecular Ecology 32(17):4844–4862.

Després, L. 2019. One, two or more species? Mitonuclear discordance and species delimitation. Molecular Ecology 28(17):3845–3847.

Donnelly, P., and S. Tavaré. 1986. The ages of alleles and a coalescent. Advances in Applied Probability 18(1):1–19.

Downs, C. C., R. G. White, and B. B. Shepard. 1997. Age at Sexual Maturity, Sex Ratio, Fecundity, and Longevity of Isolated Headwater Populations of Westslope Cutthroat Trout. North American Journal of Fisheries Management 17(1):85–92.

Eaton, K. R., J. L. Loxterman, and E. R. Keeley. 2018. Connections and containers: Using genetic data to understand how watershed evolution and human activities influence cutthroat trout biogeography. PLOS ONE 13(8):e0202043.

Escalante, M. A., C. Perrier, F. J. García-De León, A. Ruiz-Luna, E. Ortega-Abboud, and S. Manel. 2020. Genotyping-by-sequencing reveals the effects of riverscape, climate and interspecific introgression on the genetic diversity and local adaptation of the endangered Mexican golden trout (Oncorhynchus chrysogaster). Conservation Genetics 21(5):907–926.

Fausch, K. D., B. E. Rieman, J. B. Dunham, M. K. Young, and D. P. Peterson. 2009. Invasion versus Isolation: Trade-Offs in Managing Native Salmonids with Barriers to Upstream Movement. Conservation Biology 23(4):859–870.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to Riverscapes: Bridging the Gap between Research and Conservation of Stream Fishes. BioScience 52(6):483.

Fitzpatrick, S. W., G. S. Bradburd, C. T. Kremer, P. E. Salerno, L. M. Angeloni, and W. C. Funk. 2020. Genomic and Fitness Consequences of Genetic Rescue in Wild Populations. Current Biology 30(3):517-522.e5.

Fitzpatrick, S. W., and W. C. Funk. 2019. Genomics for Genetic Rescue. Pages 437–471 in P. A. Hohenlohe and O. P. Rajora, editors. Population Genomics: Wildlife. Springer International Publishing, Cham.

Frankham, R., J. D. Ballou, M. D. B. Eldridge, R. C. Lacy, K. Ralls, M. R. Dudash, and C. B. Fenster. 2011. Predicting the Probability of Outbreeding Depression. Conservation Biology 25(3):465–475.

Freedman, A. H., I. Gronau, R. M. Schweizer, D. Ortega-Del Vecchyo, E. Han, P. M. Silva, M. Galaverni, Z. Fan, P. Marx, B. Lorente-Galdos, H. Beale, O. Ramirez, F. Hormozdiari, C. Alkan, C. Vilà, K. Squire, E. Geffen, J. Kusak, A. R. Boyko, H. G. Parker, C. Lee, V. Tadigotla, A. Siepel, C. D. Bustamante, T. T. Harkins, S. F. Nelson, E. A. Ostrander, T. Marques-Bonet, R. K. Wayne, and J. Novembre. 2014. Genome Sequencing Highlights the Dynamic Early History of Dogs. PLoS Genetics 10(1):e1004016.

Funk, W. C., J. K. McKay, P. A. Hohenlohe, and F. W. Allendorf. 2012. Harnessing genomics for delineating conservation units. Trends in Ecology & Evolution 27(9):489–496.

Galland, L. M., T. L. Parchman, and M. M. Peacock. 2024. Assessing the population genetic structure of introduced rainbow trout (Oncorhynchus mykiss) in the Lake Tahoe basin: implications for hybridization potential during the reintroduction of native Lahontan cutthroat trout (O. clarkii henshawi). Hydrobiologia.

Gao, G., S. Magadan, G. C. Waldbieser, R. C. Youngblood, P. A. Wheeler, B. E. Scheffler, G. H. Thorgaard, and Y. Palti. 2021. A long reads-based de-novo assembly of the genome of the Arlee homozygous line reveals chromosomal rearrangements in rainbow trout. G3 Genes|Genomes|Genetics 11(4):jkab052.

Gu, X., Y. X. Fu, and W. H. Li. 1995. Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. Molecular Biology and Evolution.

Hand, B. K., T. D. Hether, R. P. Kovach, C. C. Muhlfeld, S. J. Amish, M. C. Boyer, S. M. O'rourke, M. R. Miller, W. H. Lowe, P. A. Hohenlohe, and G. Luikart. 2015. Genomics and introgression: Discovery and mapping of thousands of species-diagnostic SNPs using RAD sequencing. Current Zoology 61(1):146–154.

Hernández-Alonso, G., J. Ramos-Madrigal, X. Sun, C. H. Scharff-Olsen, M. S. Sinding, N. F. Martins, M. M. Ciucani, S. S. T. Mak, L. T. Lanigan, C. G. Clausen, J. Bhak, S. Jeon, C. Kim, K. Y. Eo, S. Cho, B. Boldgiv, G. Gantulga, Z. Unudbayasgalan, P. A. Kosintsev, H. K. Stenøien, M. T. P. Gilbert, and S. Gopalakrishnan. 2023. Conservation implications of elucidating the Korean wolf taxonomic ambiguity through whole-genome sequencing. Ecology and Evolution 13(8):e10404.

Hoang, D. T., O. Chernomor, A. Von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35(2):518–522.

Horan, D. L., J. L. Kershner, C. P. Hawkins, and T. A. Crowl. 2000. Effects of Habitat Area and Complexity on Colorado River Cutthroat Trout Density in Uinta Mountain Streams. Transactions of the American Fisheries Society 129(6):1250–1263.

Jeon, H.-B., M. Yates, B. K. Gallagher, and D. J. Fraser. 2025. Life's a ditch: demographic history and environmental factors shape fine-scale local adaptation within small populations of brook trout. Canadian Journal of Fisheries and Aquatic Sciences 82:1–13.

Jiménez-Mena, B., H. Flávio, R. Henriques, A. Manuzzi, M. Ramos, D. Meldrup, J. Edson, S. Pálsson, G. Ásta Ólafsdóttir, J. R. Ovenden, and E. E. Nielsen. 2022. Fishing for DNA? Designing baits for population genetics in target enrichment experiments: Guidelines, considerations and the new tool supeRbaits. Molecular Ecology Resources 22(5):2105–2119.

Juday, C. 1906. A study of Twin Lakes, Colorado, with especial considertion of the food of the trouts. Pages 147–178. United States Bureau of Fisheries, Bulletin of the Bureau of Fisheries 616.

Jun, G., M. K. Wing, G. R. Abecasis, and H. M. Kang. 2015. An efficient and scalable analysis framework for variant extraction and refinement from population-scale DNA sequence data. Genome Research 25(6):918–925.

Kokkonen, A. L., P. C. Searle, D. K. Shiozawa, and R. P. Evans. 2024. Using de novo transcriptomes to decipher the relationships in cutthroat trout subspecies (Oncorhynchus clarkii). Evolutionary Applications 17(7):e13735.

Kovach, R. P., R. Al-Chokhachy, and T. Stephens. 2018. Proactive Rainbow Trout Suppression Reduces Threat of Hybridization in the Upper Snake River Basin. North American Journal of Fisheries Management 38(4):811–819.

Kovach, R. P., R. F. Leary, D. A. Bell, S. Painter, A. Lodmell, and A. R. Whiteley. 2022. Genetic variation in westslope cutthroat trout reveals that widespread genetic rescue is warranted. Canadian Journal of Fisheries and Aquatic Sciences 79(6):936–946.

Leliaert, F., H. Verbruggen, P. Vanormelingen, F. Steen, J. M. López-Bautista, G. C. Zuccarello, and O. De Clerck. 2014. DNA-based species delimitation in algae. European Journal of Phycology 49(2):179–196.

Letcher, B. H., K. H. Nislow, J. A. Coombs, M. J. O'Donnell, and T. L. Dubreuil. 2007. Population Response to Habitat Fragmentation in a Stream-Dwelling Brook Trout Population. PLoS ONE 2(11):e1139.

Letunic, I., and P. Bork. 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Research 52(W1):W78–W82.

Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics 27(21):2987–2993.

Li, H., and R. Durbin. 2011. Inference of human population history from individual whole-genome sequences. Nature 475(7357):493–496.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, and 1000 Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25(16):2078–2079.

Lucena-Perez, M., E. Marmesat, D. Kleinman-Ruiz, B. Martínez-Cruz, K. Węcek, A. P. Saveljev, I. V. Seryodkin, I. Okhlopkov, M. G. Dvornikov, J. Ozolins, N. Galsandorj, M. Paunovic, M. Ratkiewicz, K. Schmidt, and J. A. Godoy. 2020. Genomic patterns in the widespread Eurasian lynx shaped by Late Quaternary climatic fluctuations and anthropogenic impacts. Molecular Ecology 29(4):812–828.

Mace, G. M. 2004. The role of taxonomy in species conservation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 359(1444):711–719.

Mather, N., S. M. Traves, and S. Y. W. Ho. 2020. A practical introduction to sequentially Markovian coalescent methods for estimating demographic history from genomic data. Ecology and Evolution 10:579–589.

Metcalf, J. L., S. Love Stowell, C. M. Kennedy, K. B. Rogers, D. McDonald, J. Epp, K. Keepers, A. Cooper, J. J. Austin, and A. P. Martin. 2012. Historical stocking data and 19th century DNA reveal human-induced changes to native diversity and distribution of cutthroat trout. Molecular Ecology 21(21):5194–5207.

Metcalf, J. L., V. L. Pritchard, S. M. Silvestri, J. B. Jenkins, J. S. Wood, D. E. Cowley, R. P. Evans, D. K. Shiozawa, and A. P. Martin. 2007. Across the great divide: genetic forensics reveals misidentification of endangered cutthroat trout populations. Molecular Ecology.

Minh, B. Q., M. A. T. Nguyen, and A. Von Haeseler. 2013. Ultrafast Approximation for Phylogenetic Bootstrap. Molecular Biology and Evolution 30(5):1188–1195.

Minh, B. Q., H. A. Schmidt, O. Chernomor, D. Schrempf, M. D. Woodhams, A. Von Haeseler, and R. Lanfear. 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. Molecular Biology and Evolution 37(5):1530–1534.

Morin, P. A., G. Luikart, R. K. Wayne, and The Snp Workshop Group. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution 19(4):208–216.

Muhlfeld, C. C., V. S. D'Angelo, C. Downs, J. Powell, S. Amish, G. Luikart, R. Kovach, M. Boyer, and S. Kalinowski. 2016. Genetic Status and Conservation of Westslope Cutthroat Trout in Glacier National Park. Transactions of the American Fisheries Society 145(5):1093–1109.

Nadachowska-Brzyska, K., R. Burri, L. Smeds, and H. Ellegren. 2016. PSMC analysis of effective population sizes in molecular ecology and its application to black-and-white Ficedula flycatchers. Molecular Ecology 25(5):1058–1072.

Nordberg, B. J., E. G. Mandeville, A. W. Walters, J. C. Burckhardt, and C. E. Wagner. 2021. Historical Data Provide Important Context for Understanding Declines in Cutthroat Trout. North American Journal of Fisheries Management 41(3):809–819.

Olden, J. D., M. J. Kennard, F. Leprieur, P. A. Tedesco, K. O. Winemiller, and E. García-Berthou. 2010. Conservation biogeography of freshwater fishes: recent progress and future challenges: Conservation biogeography of freshwater fishes. Diversity and Distributions 16(3):496–513.

Ovchinnikov, I. V., and B. McCann. 2023. Mitogenomes revealed the history of bison colonization of Northern Plains after the Last Glacial Maximum. Scientific Reports 13(1):11417.

Patton, A. H., M. J. Marges, A. R. Stahlke, S. Hendricks, K. Lewallen, R. K. Hamede, M. Ruiz-Aravena, O. Ryder, H. I. McCallum, M. E. Jones, P. A. Hohenlohe, and A. Storfer. 2019. Contemporary Demographic Reconstruction Methods Are Robust to Genome Assembly Quality: A Case Study in Tasmanian Devils. Molecular Biology and Evolution 36(12):2906–2921.

Peacock, M. M., E. R. Hekkala, V. S. Kirchoff, and L. G. Heki. 2017. Return of a giant: DNA from archival museum samples helps to identify a unique cutthroat trout lineage formerly thought to be extinct. Royal Society Open Science 4(11):171253.

Peterson, D. P., K. D. Fausch, and G. C. White. 2004. Population ecology of an invasion: effects of Brook Trout on Native Cutthroat Trout. Ecological Applications 14(3):754–772.

Poplin, R., V. Ruano-Rubio, M. A. DePristo, T. J. Fennell, M. O. Carneiro, G. A. Van Der Auwera, D. E. Kling, L. D. Gauthier, A. Levy-Moonshine, D. Roazen, K. Shakir, J. Thibault, S. Chandran, C. Whelan, M. Lek, S. Gabriel, M. J. Daly, B. Neale, D. G. MacArthur, and E. Banks. 2017, November 14. Scaling accurate genetic variant discovery to tens of thousands of samples.

Pritchard, V. L., K. Jones, and D. E. Cowley. 2007. Genetic Diversity within Fragmented Cutthroat Trout Populations. Transactions of the American Fisheries Society 136(3):606–623.

Pritchard, V. L., J. L. Metcalf, K. Jones, A. P. Martin, and D. E. Cowley. 2009. Population structure and genetic management of Rio Grande cutthroat trout (Oncorhynchus clarkii virginalis). Conservation Genetics 10(5):1209–1221.

Quattrini, A. M., K. E. Snyder, R. Purow-Ruderman, I. G. L. Seiblitz, J. Hoang, N. Floerke, N. I. Ramos, H. H. Wirshing, E. Rodriguez, and C. S. McFadden. 2023. Mito-nuclear discordance within Anthozoa, with notes on unique properties of their mitochondrial genomes. Scientific Reports 13(1):7443.

Robinson, J. A., C. Brown, B. Y. Kim, K. E. Lohmueller, and R. K. Wayne. 2018. Purging of Strongly Deleterious Mutations Explains Long-Term Persistence and Absence of Inbreeding Depression in Island Foxes. Current Biology 28(21):3487-3494.e4.

Rogers, K. B., S. E. Albeke, D. Zafft, and R. Henderson. 2025. Range-wide status of Colorado River Cutthroat Trout (Oncorhynchus clarkii pleuriticus): 2020. Colorado River Cutthroat Trout Conservation Team Report, Colorado Parks and Wildlife, Fort Collins.

Rogers, K. B., J. R. Anderson, S. F. Brinkman, and A. P. Martin. 2022. Inbreeding Depression Reduces Fitness in Colorado’s Last Remaining Greenback Cutthroat Trout: Consequences for

Management. Pages 185–194 in J. S. Gregory, editor. Proceedings of Wild Trout XIII Symposium: reducing the gap between science and public opinion.

Rogers, K. B., K. R. Bestgen, S. M. Love Stowell, and A. P. Martin. 2018. Cutthroat Trout Diversity in the Southern Rocky Mountains. Pages 323–341 in P. Trotter, P. Bisson, L. Schultz, and R. Brett, editors. Cutthroat Trout: evolutionary biology and taxonomy.

Rondeau, E. B., K. A. Christensen, D. R. Minkley, J. S. Leong, M. T. T. Chan, C. A. Despins, A. Mueller, D. Sakhrani, C. A. Biagi, Q. Rougemont, E. Normandeau, S. J. M. Jones, R. H. Devlin, R. E. Withler, T. D. Beacham, K. A. Naish, J. M. Yáñez, R. Neira, L. Bernatchez, W. S. Davidson, and B. F. Koop. 2023. Population-size history inferences from the coho salmon (Oncorhynchus kisutch) genome. G3: Genes, Genomes, Genetics 13(4):jkad033.

Rosenthal, W. C., J. M. Fennell, E. G. Mandeville, J. C. Burckhardt, A. W. Walters, and C. E. Wagner. 2022. Hybridization decreases native cutthroat trout reproductive fitness. Molecular Ecology 31(16):4224–4241.

Rougemont, Q., T. Leroy, E. B. Rondeau, B. Koop, and L. Bernatchez. 2023. Allele surfing causes maladaptation in a Pacific salmon of conservation concern. PLOS Genetics 19(9):e1010918.

Rougemont, Q., J.-S. Moore, T. Leroy, E. Normandeau, E. B. Rondeau, R. E. Withler, D. M. Van Doornik, P. A. Crane, K. A. Naish, J. C. Garza, T. D. Beacham, B. F. Koop, and L. Bernatchez. 2021. Correction: Demographic history shaped geographical patterns of deleterious mutation load in a broadly distributed Pacific Salmon. PLOS Genetics 17(2):e1009397.

Saglam, I. K., D. J. Prince, M. Meek, O. A. Ali, M. R. Miller, M. Peacock, H. Neville, A. Goodbla, C. Mellison, W. Somer, B. May, and A. J. Finger. 2017. Genomic Analysis Reveals Genetic Distinctiveness of the Paiute Cutthroat Trout Oncorhynchus clarkii seleniris. Transactions of the American Fisheries Society 146(6):1291–1302.

Segelbacher, G., M. Bosse, P. Burger, P. Galbusera, J. A. Godoy, P. Helsen, C. Hvilsom, L. Iacolina, A. Kahric, C. Manfrin, M. Nonic, D. Thizy, I. Tsvetkov, N. Veličković, C. Vilà, S. M. Wisely, and E. Buzan. 2022. New developments in the field of genomic technologies and their relevance to conservation management. Conservation Genetics 23(2):217–242.

Shiozawa, D. K., R. P. Evans, D. D. Houston, and P. J. Unmack. 2018. Geographic Variation, Isolation, and Evolution of Cutthroat Trout with Comments on Future Directions for Management and Research. Page in P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: Evolutionary Biology and Taxonomy. American Fisheries Society.

Smith, G. R., and R. F. Stearley. 2018. The Fossil Record of Cutthroat Trout: Implications for Evolution and Conservation. Page in P. Trotter, P. Bisson, L. Schultz, and B. Roper, editors. Cutthroat Trout: Evolutionary Biology and Taxonomy. American Fisheries Society.

Stack, T., M. P. Fairchild, R. Geiger, S. J. Oyler-McCance, J. A. Fike, C. M. Kennedy, D. L. Winkelman, and Y. Kanno. 2024. A genetic assessment of natural barriers for isolating a proposed Greenback Cutthroat Trout reintroduction area. North American Journal of Fisheries Management 44(5):1062–1072.

Stearley, R. F., and G. R. Smith. 2016. Salmonid Fishes from Mio-Pliocene Lake Sediment in the Western Snake River Plain and the Great Basin. Miscellaneous Publications, Museum of Zoology, University of Michigan 204(Fishes of the Mio-Pliocene Western Snake River Plain and Vicinity).

Trotter, P., P. A. Bisson, L. D. Schultz, and B. B. Roper, editors. 2018. Cutthroat trout: evolutionary biology and taxonomy. American Fisheries Society, Bethesda, Maryland.

Tsuboi, J., K. Morita, Y. Koseki, S. Endo, G. Sahashi, D. Kishi, T. Kikko, D. Ishizaki, M. Nunokawa, and Y. Kanno. 2022. Small giants: Tributaries rescue spatially structured populations from extirpation in a highly fragmented stream. Journal of Applied Ecology 59(8):1997–2009.

United States 117th Congress. 2022. Endangered Species Act of 1973 as amended by Public Law 117-286.

USFWS, (U.S. Fish and Wildlife Service). 2003. Endangered and threatened wildlife and plants: reconsidered finding for an amended petition to list the westslope cutthroat trout as threatened throughout its range. Federal Register 68:46989–47009.

Van Oosterhout, C. 2020. Mutation load is the spectre of species conservation. Nature Ecology & Evolution 4(8):1004–1006.

Vasimuddin, Md., S. Misra, H. Li, and S. Aluru. 2019. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. Pages 314–324 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS). IEEE, Rio de Janeiro, Brazil.

Vences, M., A. Miralles, and C. Dufresnes. 2024. Next-generation species delimitation and taxonomy: Implications for biogeography. Journal of Biogeography 51(9):1709–1722.

Vilaça, S. T., M. E. Donaldson, A. Benazzo, T. J. Wheeldon, M. T. Vizzari, G. Bertorelle, B. R. Patterson, and C. J. Kyle. 2023. Tracing Eastern Wolf Origins From Whole-Genome Data in Context of Extensive Hybridization. Molecular Biology and Evolution 40(4).

Vilaça, S. T., A. T. Hahn, E. Naro-Maciel, F. A. Abreu-Grobois, B. W. Bowen, J. C. Castilhos, C. Ciofi, N. N. FitzSimmons, M. P. Jensen, A. Formia, C. J. Limpus, C. Natali, L. S. Soares, B. De Thoisy, S. D. Whiting, and S. L. Bonatto. 2022. Global phylogeography of ridley sea turtles (Lepidochelys spp.): evolution, demography, connectivity, and conservation. Conservation Genetics 23(6):995–1010.

Vogel Ely, C., S. A. D. L. Bordignon, R. Trevisan, and I. I. Boldrini. 2017. Implications of poor taxonomy in conservation. Journal for Nature Conservation 36:10–13.

Weckworth, B. V., N. G. Dawson, S. L. Talbot, and J. A. Cook. 2015. Genetic Distinctiveness of Alexander Archipelago Wolves (Canis lupus ligoni). Journal of Heredity 106(4):412–414.

Wenne, R. 2023. Single Nucleotide Polymorphism Markers with Applications in Conservation and Exploitation of Aquatic Natural Populations. Animals 13(6):1089.

Whiteley, A. R., K. Hastings, J. K. Wenburg, C. A. Frissell, J. C. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of coastal cutthroat trout. Conservation Genetics 11(5):1929–1943.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Wilson, E. O. 2017. Biodiversity research requires more boots on the ground. Nature Ecology & Evolution 1(11):1590–1591.

Young, M. K. 2008. Colorado River cutthroat trout: a technical conservation assessment. Page RMRS-GTR-207. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, RMRS-GTR-207, Ft. Collins, CO.

Young, M. K., R. N. Schmal, T. W. Kohley, and V. G. Leonard. 1996, August. Conservation Status of Colorado River Cutthroat Trout. USDA Forest Service.

APPENDIX 1

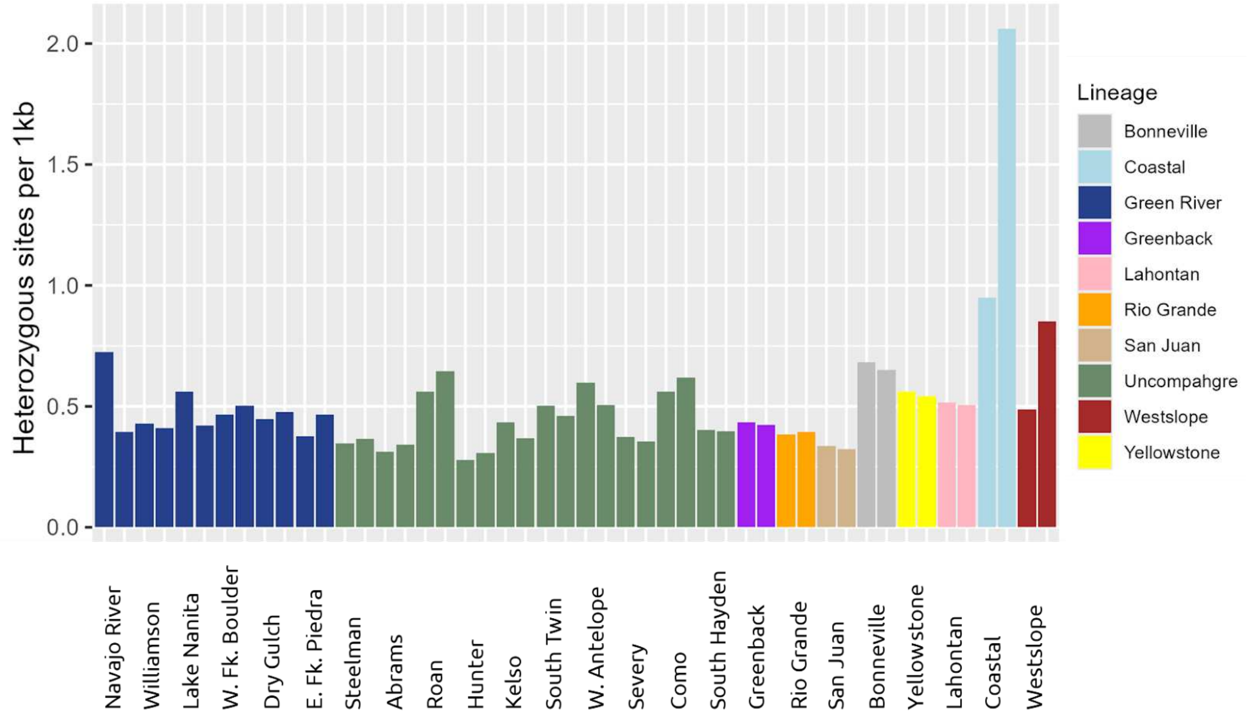

Figure A1. Individual autosomal heterozygosity proportions per 1 kilobase with the Coastal CT outlier (C132065) included.

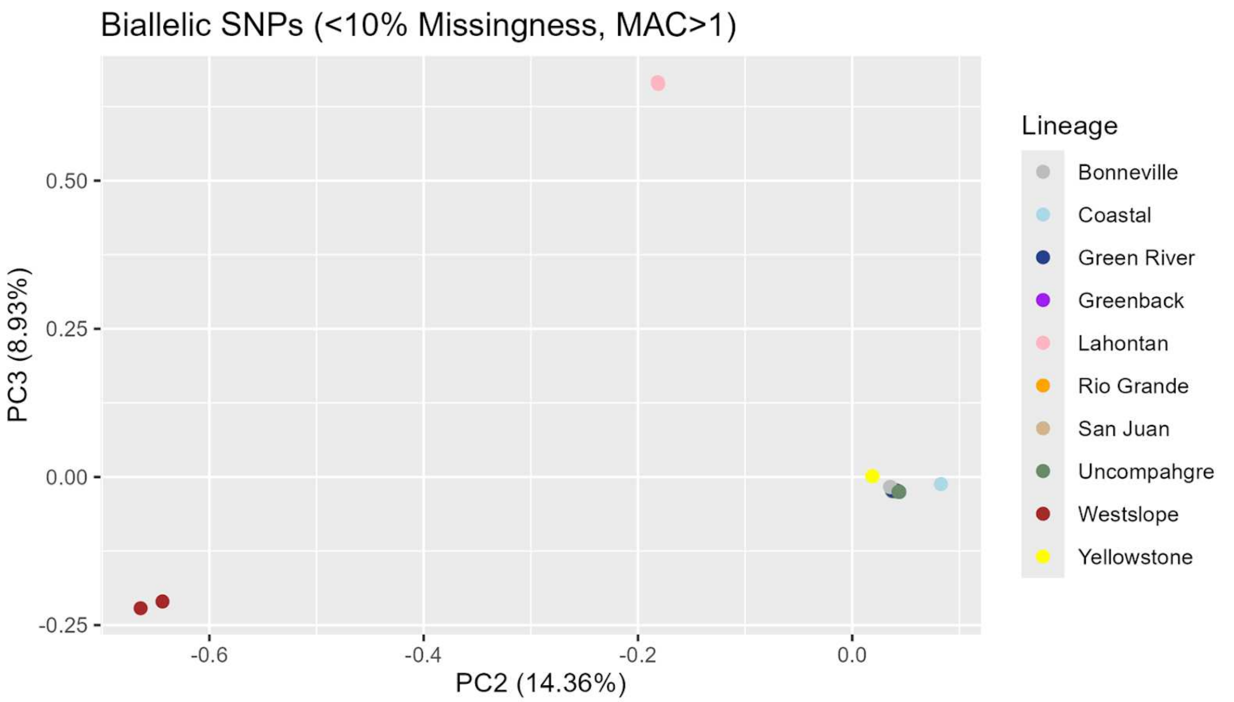

Figure A2. Second and third principal component axes for variant set with all samples at a minor allele count > 1 threshold. Colors correspond to *a priori* lineage.

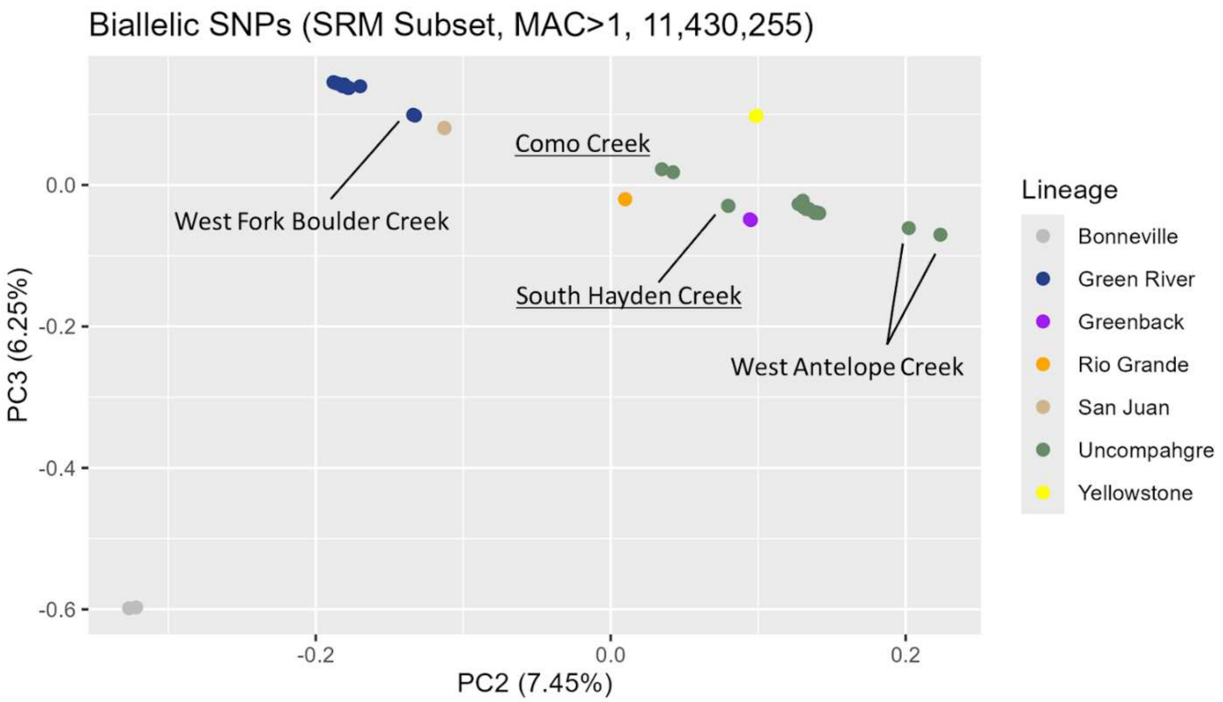

Figure A3. Second and third principal component axes for variants from the Rocky Mountain group populations filtered with a minor allele count > 1 threshold. Colors correspond to *a priori* lineage, and populations or individuals of note are labeled.

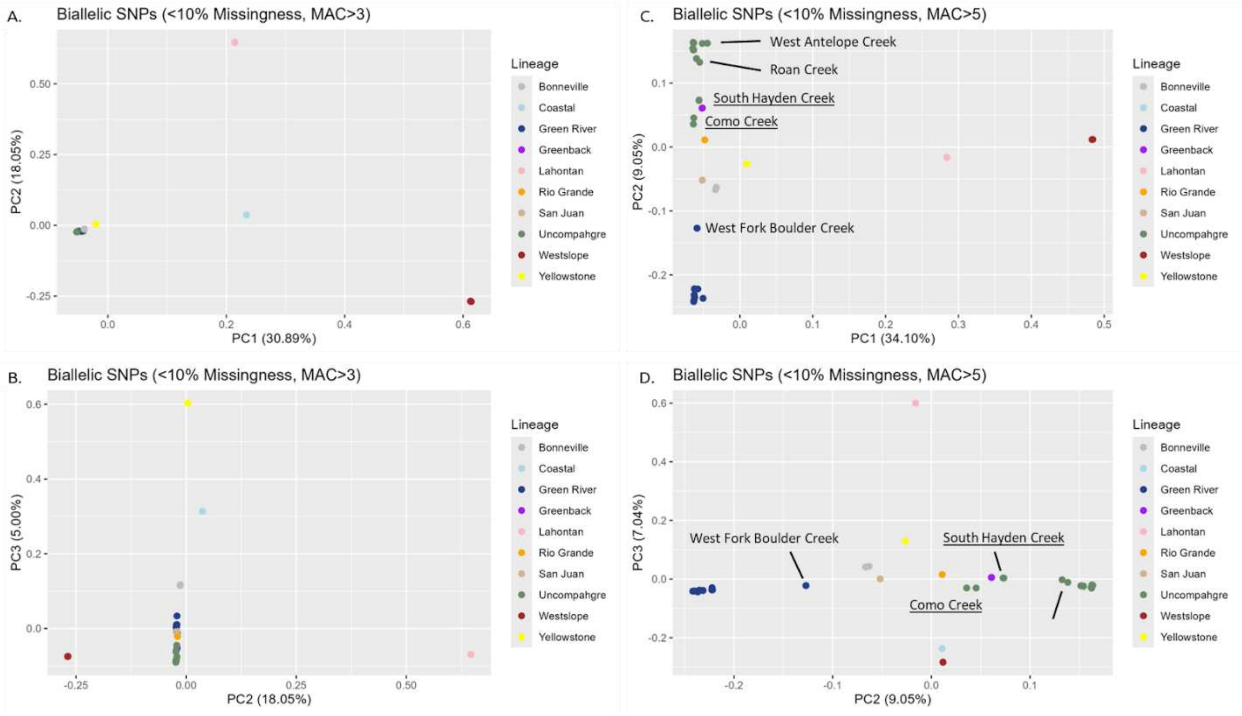

Figure A4. First, second, and third principal component axes for all variants at a minor allele count > 3 (A, B) and minor allele count > 5 (C, D) threshold. Colors correspond to a priori lineage, and populations or individuals of note are labeled on MAC>5 PCA (C, D).

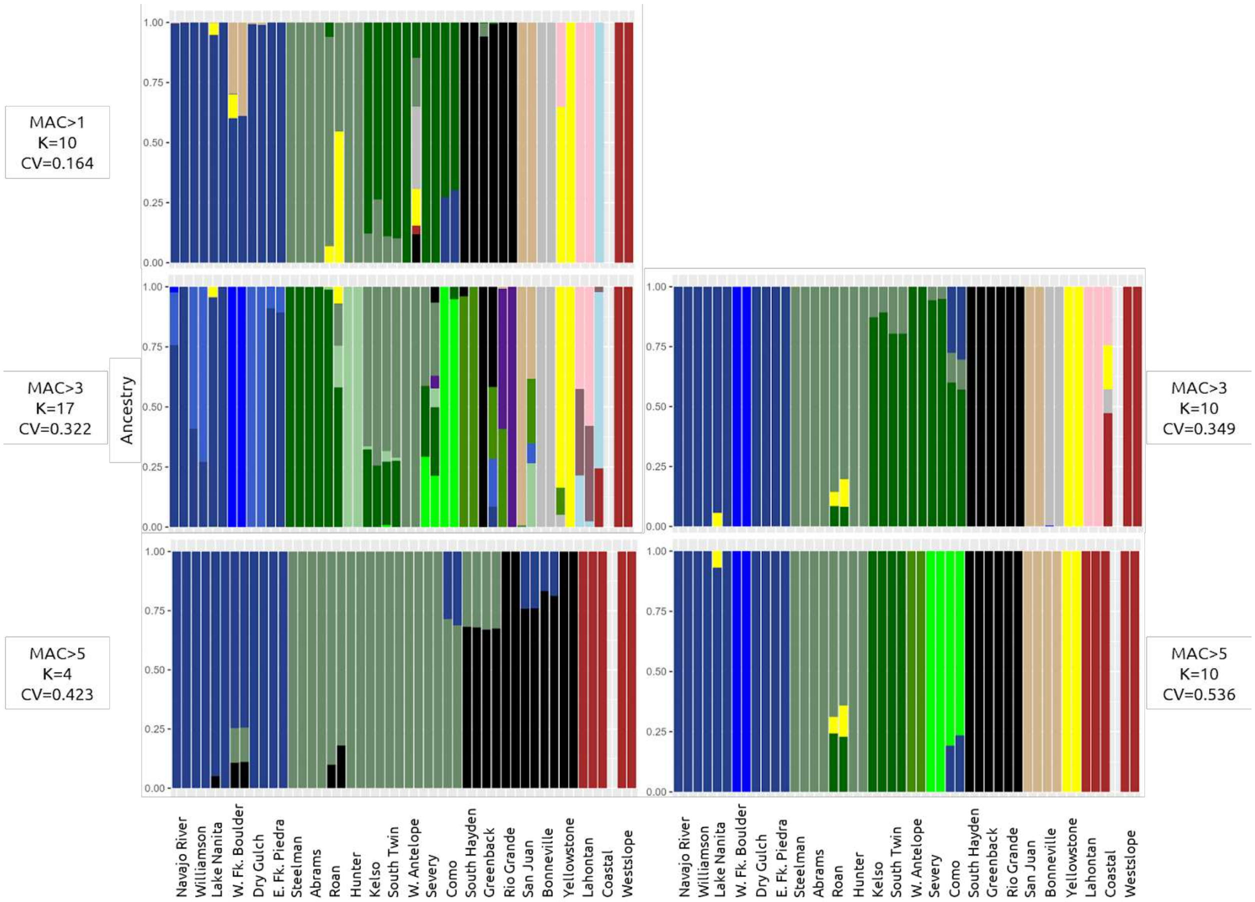

Figure A5. Ancestry proportion plots for three filtering thresholds at the best K (left column) and K=10 (right column), which is the *a priori* number of lineages.

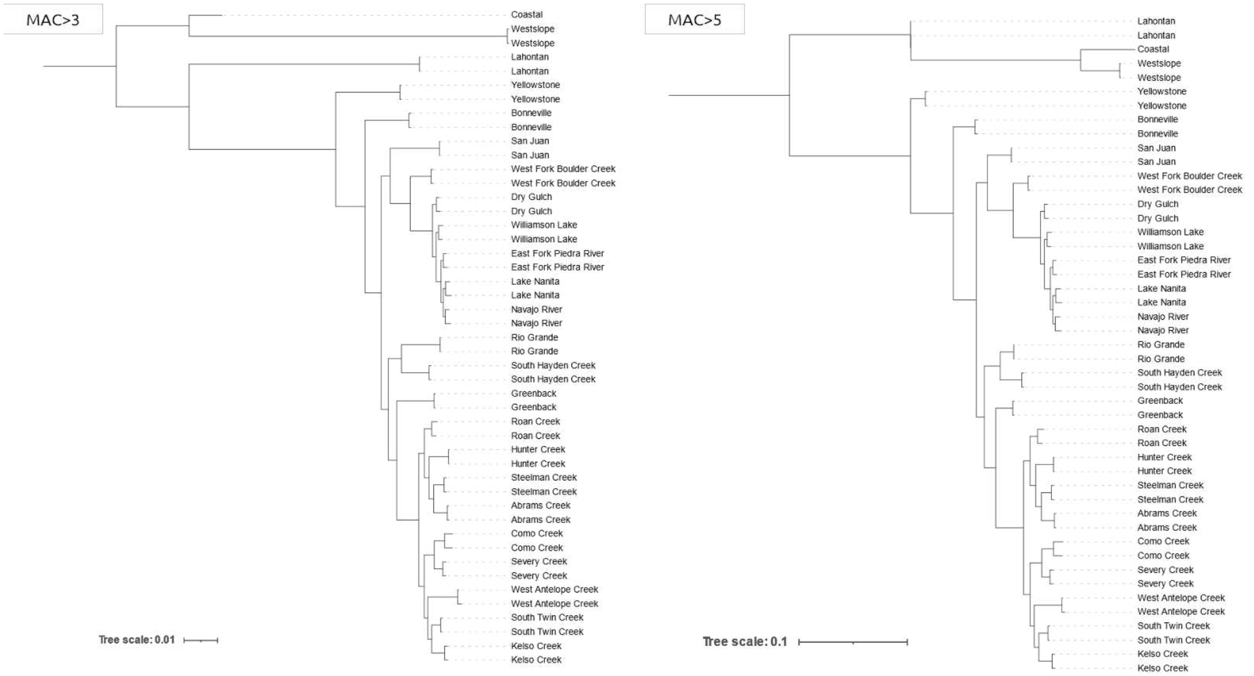

Figure A6. Maximum likelihood tree for all samples at the minor allele count > 3 (left) and minor allele count > 5 (right) thresholds. The tree topology is similar for the Rocky Mountain group populations across all three filtering thresholds, but the topology for the Coastal CT, Westslope CT, and Lahontan CT differs.

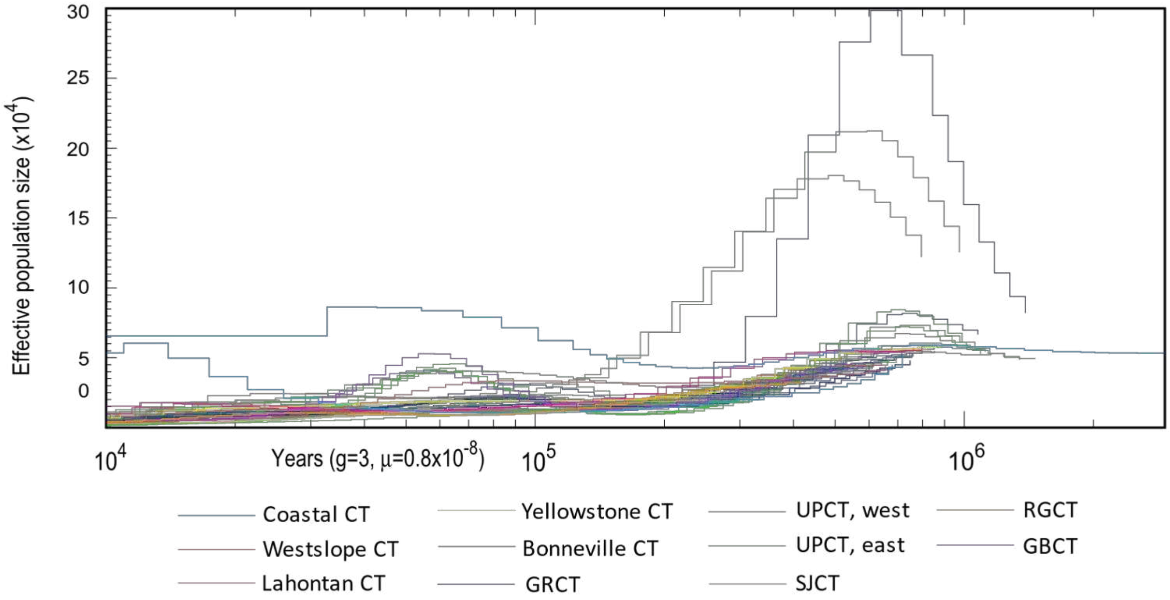

Figure A7. Pairwise sequentially Markovian coalescent (PSMC) curves of estimated effective population size for all 48 samples over time. Each line represents the estimated ancestral effective population size from one sample genome. The x-axis is reverse time, with more recent (10,000 years ago) on the left and more ancient (1 MYA) on the right. g is the generation time, and μ is the mutation rate (bp / generation). Colors correspond to the *a priori* lineage to which an individual belongs.