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Discovery and characterization of novel genetic markers for use in the management of Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*)

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Abstract

The Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*) is threatened by habitat destruction, over-harvest and hybridization with nonnative trout. Currently, three Geographic Management Units (GMUs) are recognized within the taxon. Here, we describe a suite of 68 single-nucleotide polymorphism (SNP) genetic markers for use in the study and management of Lahontan cutthroat trout and a closely related subspecies, the Paiute cutthroat trout (*O. c. seleneris*). These include markers variable within the two subspecies (*n* = 35), diagnostic for the two subspecies (*n* = 23) and diagnostic for Yellowstone cutthroat trout (*O. c. bouvieri*) and other closely related subspecies (*n* = 10). Sixty-three markers were discovered by Sanger sequencing of 171 EST loci in an ascertainment panel including Lahontan cutthroat trout from four populations representing all GMUs. Five markers were identified in a secondary sequencing effort with a single population of Lahontan cutthroat trout. TaqMan assays were validated on six Lahontan cutthroat trout populations and a diverse panel of other trout. Over 90% of the markers variable in Lahontan cutthroat trout were polymorphic in at least two populations, and 66% were variable within all three GMUs. All Lahontan diagnostic markers were also fixed for the Lahontan allele in Paiute cutthroat trout. Most of the Yellowstone diagnostic markers can also be used for this purpose in other cutthroat trout subspecies. This is the first set of SNP markers to be developed for Lahontan cutthroat trout, and will be an important tool for conservation and management.

Keywords: hybridization, Lahontan cutthroat trout, *Oncorhynchus clarkii*, single nucleotide polymorphisms

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Introduction

The cutthroat trout (*Oncorhynchus clarkii*) is a polytypic species native to western North America. It is generally considered to consist of four major evolutionary lineages (Behnke 2002; but see Loxterman & Keeley 2012): coastal cutthroat trout (*O. c. clarkii*) – the sole taxon with an anadromous form, westslope cutthroat trout (*O. c. lewisi*), the 'Lahontan group' – Lahontan (*O. c. henshawi*) and Paiute cutthroat trout (*O. c. seleneris*), and the 'Yellowstone group' – Yellowstone (*O. c. bouvieri)*, Colorado River (*O. c. pleuriticus*), greenback (*O. c. stomias*), Bonneville (*O. c. utah*) and Rio Grande cutthroat trout (*O. c. virginalis*). All cutthroat trout subspecies have declined dramatically over the past 150 years as a result of habitat loss, overfishing, and the impacts of nonnative trout introduction. Cutthroat trout hybridize with rainbow trout/steelhead (*O. mykiss*) and widespread stocking of this species has led to the replacement of many native cutthroat trout populations with hybrid swarms. In addition, transplantation of cutthroat trout into the geographic range of other subspecies can also lead to hybridization or the establishment of naturalized nonnative populations. Genetic analysis is an important tool to guide cutthroat trout conservation and management, allowing investigation of population structure and evolutionary history, estimation of contemporary population sizes and migration rates, as well as the rate and magnitude of genetic introgression by nonnative taxa.

The Lahontan cutthroat trout is native to the Lahontan Basin of northern Nevada, southeastern Oregon and

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northeastern California and is believed to have evolved in the Pleistocene in large, pluvial Lake Lahontan and associated satellite basins (Behnke 2002). When these lakes desiccated after the Pleistocene, populations were left in fluvial systems in the eastern Lahontan basin and fluvial and lacustrine systems in the western basin. Currently, three Geographic Management Units (GMUs; Fig. 1) are recognized and are based on meristic, allozyme, mtDNA and microsatellite data (Coffin & Cowan 1995; Nielsen & Sage 2002; Peacock & Kirchoff 2007): Northwestern (Quinn River drainage, Coyote Basin, Summit Lake), Eastern (Humboldt and Reese river drainages) and Western (Carson, Walker and Truckee river drainages). A morphologically distinct taxon native to a single subbasin in the Carson River drainage is recognized as a separate subspecies, Paiute cutthroat trout (Behnke 2002). Peacock *et al.* (2010) suggested on the basis of microsatellite data that the Coyote Basin group be designated a fourth GMU, while Behnke (2002) proposed, on the basis of meristics and evolutionary history, that the Eastern GMU and Coyote Basin populations be raised to subspecific status. Historically, Lahontan cutthroat trout in Lake Tahoe, Pyramid Lake and Walker Lake (Western GMU) grew to unusually large size (up to 1 m in length) and supported important fisheries (Behnke 2002). However, these populations, along with many others, were lost during the 20th century as a result of the factors mentioned above and the subspecies is currently estimated to occupy less than 10% of its historic range. As a result of their decline, Lahontan cutthroat trout are currently listed as 'Threatened' under the U.S. Endangered Species Act. In addition, many remnant populations are believed to have hybridized with, or are threatened by, *O. mykiss*. Yellowstone cutthroat trout have also been stocked within the native range of Lahontan cutthroat trout, but the extent to which hybridization has occurred is not known (Coffin & Cowan 1995). Conversely, historic transplantation of Lahontan cutthroat trout has led to the establishment of populations outside their native range. For example, streams in the Pilot Peak mountain range in the state of Utah contain transplanted Lahontan cutthroat trout that appear to be descended from the original Pyramid Lake strain and these are now used as broodstock for reintroduction into their native habitat (Hickman & Behnke 1979; Peacock & Kirchoff 2007). In addition, Lahontan cutthroat trout are tolerant of high pH and are stocked into alkaline lakes, including Lake Lenore in Washington State, for sport fisheries.

Most previous genetic work on Lahontan cutthroat trout has used mtDNA or microsatellite markers (e.g. Nielsen & Sage 2002; Peacock & Kirchoff 2004). However, single-nucleotide polymorphisms (SNPs) have several advantages over other types of genetic markers, including high density across the genome, low mutation and genotyping error rates and portability of data among researchers and genotyping platforms (Morin *et al.* 2004). Because of these advantages, as well as rapid advances in cost-effective SNP discovery and high-throughput genotyping, SNPs are fast becoming the marker of choice for population genetic studies. Indeed, several sets of SNP markers with applications to the management of cutthroat trout have recently been described and include markers diagnostic between *O. mykiss* and all or most subspecies of *O. clarkii* (e.g. Harwood & Phillips 2011; Pritchard *et al.* 2012), and markers developed for studies of westslope cutthroat trout, including some that are diagnostic for introgression from Yellowstone cutthroat trout into a wider range of subspecies (e.g. Kalinowski *et al.* 2010; Campbell *et al.* 2012). Here, we present a suite of 68 novel markers, identified from sequencing of ESTs isolated in *O. mykiss*, designed to address management questions in Lahontan cutthroat trout. They include markers variable within Lahontan cutthroat trout, markers to discriminate Lahontan cutthroat trout from all other cutthroat trout subspecies (and from *O. mykiss*) and markers to identify introgression into Lahontan cutthroat trout from Yellowstone cutthroat trout.

Methods

Variable nucleotide sites were identified using a Sanger chain-termination sequencing approach, with initial PCR performed using 242 primer pairs designed from randomly chosen nuclear tentative consensus (TC) sequences generated from *O. mykiss* expressed sequence tags and catalogued by the Harvard DFCI Gene Index project (http://compbio.dfci.harvard.edu/tgi/tgipage.html; accessed on December 8, 2006). Of these, 225 had successfully been used to generate DNA sequence in *O. mykiss* (Abadía-Cardoso *et al.* 2011); the remainder had failed in *O. mykiss* but had successfully generated sequence in both *O. tshawytscha* and *O. kisutch*. PCR and subsequent visualization, purification and sequencing steps were as described in Pritchard *et al.* (2012).

The SNP ascertainment panel, described in detail in Pritchard *et al.* (2012), included fish from four populations of Lahontan cutthroat trout, encompassing all currently recognized GMUs: Eastern – North Fork, Humboldt River; Northwestern – Three Mile Creek, Quinn River; Western – Independence Lake and Pilot Peak, believed to be derived from Pyramid Lake. Sequence data were collected for five Lahontan cutthroat trout individuals, including one for each population except Pilot Peak, for which a second individual was added for 71 loci to improve the discovery of sites polymorphic in the broodstock at the Lahontan National Fish Hatchery, which is derived from this population. The ascertainment

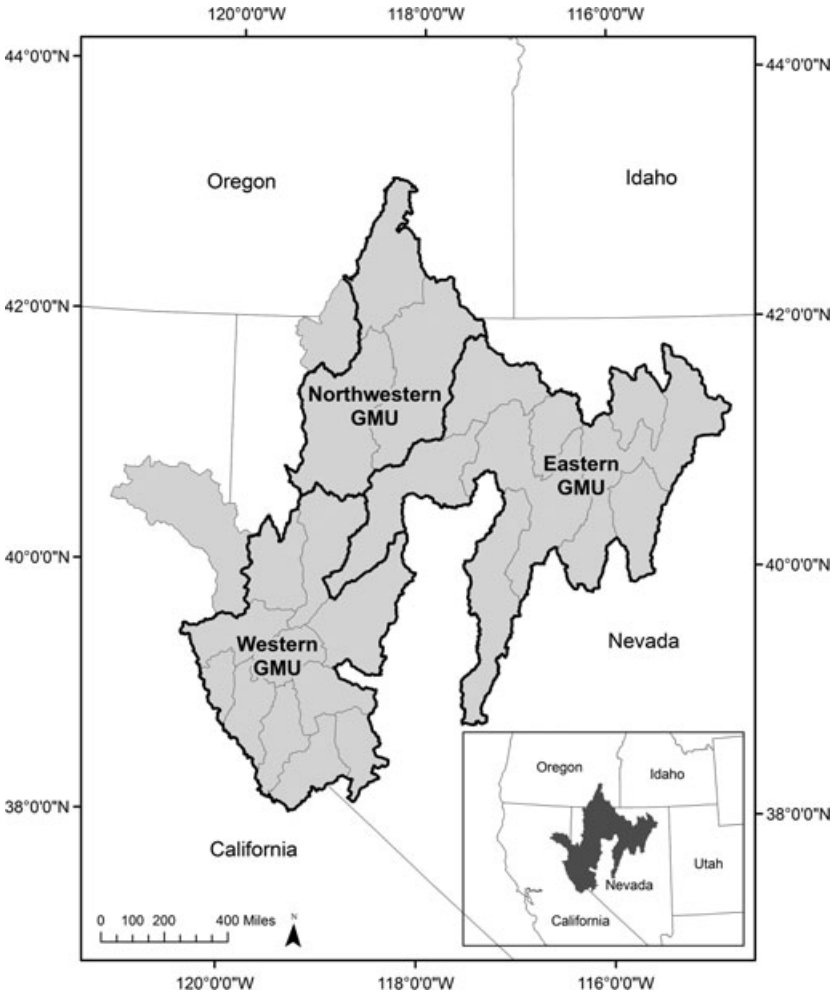

Fig. 1 The Lahontan hydrographic basin (grey shading) with the three Lahontan cutthroat trout GMUs designated. The shaded subbasin outside the Western GMU in California is part of the historic range of LCT but does not currently support populations of Lahontan cutthroat trout. The Alvord subbasin west of the Northwestern GMU spanning Nevada and Oregon is the historic distribution for the extinct Alvord cutthroat trout. Paiute cutthroat trout are found in Silver King Creek in the Carson River drainage of California, located in the extreme southwestern portion of the western GMU.

panel also included fish from populations of: coastal cutthroat trout (n = 3), westslope cutthroat trout (n = 4) and the closely related 'Yellowstone group' of subspecies: Yellowstone (n = 3), Colorado River (n = 1), greenback (n = 3), Bonneville (n = 2) and Rio Grande (n = 3) cutthroat trout.

Forward and reverse sequences for each locus were pruned, aligned and assembled into contigs using Sequencher 4.10.1 (Gene Codes Corporation). When available, homologous genomic sequences for *O. mykiss* (Abadía-Cardoso *et al.* 2011) were also included in the alignments. Chromatograms were visually examined to confirm potentially variable sites. Sites where variation was due to a single heterozygote identified in a single sequence read, without confirmation from a sequence read in the opposite direction, were excluded from the analysis. Sequence variation was summarized for each locus and examined for the presence of (i) polymorphic sites within Lahontan cutthroat trout, (ii) fixed substitutions (single base, multibase or indel) distinguishing Lahontan cutthroat trout from all other taxa, and (iii) fixed substitutions distinguishing Yellowstone cutthroat trout from all other taxa. This third category arose due to the discovery of introgression by Yellowstone cutthroat trout into one of the Lahontan cutthroat trout ascertainment populations (see below), which indicated that the detection of such introgression should be a management goal for Lahontan cutthroat trout.

Additional SNPs were identified from sequence data obtained in a second Sanger sequencing effort, described in detail in Campbell *et al.* (2012), that targeted a set of loci with known coding functions in *O. mykiss*. Lahontan cutthroat trout were represented in this secondary ascertainment panel by two individuals from the introduced population in Lake Lenore.

A total of 80 TaqMan (5′ nuclease) genotyping assays were designed and manufactured (Applied Biosystems Inc.). Of these, 42 targeted sites variable within Lahontan cutthroat trout, 26 targeted substitutions putatively distinguishing Lahontan cutthroat trout from all other taxa and 12 targeted substitutions putatively distinguishing Yellowstone cutthroat trout from all other taxa. No more than one assay for a given purpose was designed from each locus, to minimize linkage disequilibrium. None of

the assays targeted the same nucleotide sites as those described in Pritchard *et al.* (2012) or in Campbell *et al.* (2012), but 70% of the targeted sites were in the same locus as an assay described in one of those publications.

Assays were validated on a panel consisting of 141 Lahontan cutthroat trout from six populations across the subspecies' range, 22 Paiute cutthroat trout from two populations, 79 individuals representing all other cutthroat trout subspecies and 19 *O. mykiss* (Table 1). Genotyping was carried out in 96.96 Dynamic Genotyping Arrays on an EP1 Genotyping System (Fluidigm Corporation) with a preamplification step, following the manufacturer's protocols. Genotype calls were determined using the Fluidigm SNP Genotyping Analysis software (v 3.0.2), with confidence threshold set to 80%. Only samples successfully called for at least 90% of the loci in a plate were retained in subsequent analyses, as evidence suggested that DNA quality affected both genotype call rate and accuracy. Observed and expected heterozygosity were calculated using Genetix (Belkhir *et al.* 1996–2004) and, where applicable, conformation to Hardy–Weinberg and linkage equilibria within each population evaluated with exact tests implemented in GenePop (Raymond & Rousset 1995). Correction for multiple tests used the False Discovery Rate (FDR) approach of Benjamini & Hochberg (1995) with $\alpha = 0.05$.

Putative function for each sequenced locus was investigated by running a Blastx search on the National Center for Biotechnology Information (NCBI) nonredundant protein sequence database with the TC sequence from which primers were designed. Intron regions and gene features in the sequences generated for SNP discovery were identified by aligning them with the annotated TC sequences associated with that EST.

Results

Of the 242 primer pairs applied to the primary ascertainment panel, 210 generated a PCR product potentially amenable to sequencing. Of these, 171 produced high quality sequence data in Lahontan and at least six other cutthroat trout subspecies. In total, this represented 90 730 bp of consensus sequence with a mean of 4.2 Lahontan cutthroat trout and 18.0 other cutthroat trout individuals sequenced for each locus. Homologous *O. mykiss* genomic sequence was available for 164 of these loci.

Visual examination of the sequence data from individuals sampled in the Three Mile Creek population revealed multiple single nucleotide polymorphisms absent in other Lahontan cutthroat trout populations but matching those observed within the Yellowstone group of cutthroat trout subspecies (V. L. Pritchard & J. C. Garza, unpublished data). Such polymorphisms

Table 1

Taxon	Drainage/location	Population	*n*
Lahontan cutthroat *O. c. henshawi*	Humboldt R. (E)	West Mary R.	24
	Humboldt R. (E)	Frazer Ck	24
	Quinn R. (NW)	Line Ck	23
	Summit Lk (NW)	Summit Lk	18
	Independence Lk (W)	Independence Lk, CA	26
	Pilot Peak (W, trans.)	Pilot Peak, UT	26
Paiute cutthroat *O. c. seleneris*	Cottonwood Ck (trans.)	Cottonwood Ck	11
	San Joaquin R. (trans.)	Stairway Ck	11
Coastal cutthroat *O. c. clarki*	Columbia R.	Abernathy Ck	4
	Columbia R.	SF Mill Ck	4
	Humboldt Bay	Jolly Giant Ck	4
	Margaret Lk	Margaret Lk, AK	2
Westslope cutthroat *O. c. lewisi*	Kootenai R.	McGuire Ck	2
	Kootenai R.	Trout Ck	2
	Salmon R.	Cache Ck	2
	Salmon R.	Wilson Ck	2
Bonneville cutthroat *O. c. utah*	Glenwood Hatchery	Hatchery stock	4
	Bear R.	Deep Ck	1
Yellowstone cutthroat *O. c. bouvieri*	LeHardy Rapids	Hatchery stock	4
	Snake R.	Salt R.	1
	Yellowstone Lk	Hatchery Ck	2
	Yellowstone R.	McBride Lk	1
	Snake R.	Barnes Ck	5
	Portneuf R.	Gibson Jack Ck	2
Colorado River cutthroat *O. c. pleuriticus*	Colorado R.	SF Parachute Ck	2
	Colorado R.	SF Slater Ck	3
	Lk Nanita	Lk Nanita, CO	2
	San Juan R.	Navajo Ck	1
	South Platte R.	Hunter's Ck	3
	Williamson Lk	Williamson Lk	2
Greenback cutthroat *O. c. stomias*	Arkansas R.	Bear Ck	2
	Arkansas R.	Severy Ck	3
	Colorado R.	Bobtail Ck	1
	Colorado R.	Cunningham Ck	2
	Gunnison R.	W Antelope Ck	1
Rio Grande cutthroat *O. c. virginalis*	Canadian R.	McCrystal Ck	3
	Rio Grande	Columbine Ck	3
	Rio Grande	El Rito Ck	3
	Rio Grande	Osier Ck	2
	Rio Grande	U Comanche Ck	2
	Rio Mora	Rio Mora	2
Rainbow trout *O. mykiss*	American R. Hatchery	Eagle Lk Strain	2
	Fillmore Hatchery	Shasta Strain	2
	Fillmore Hatchery	Virginia Strain	2
	Fillmore Hatchery	Whitney Strain	2
	Hot Ck Hatchery	Kamloops Strain	2
Steelhead *O. mykiss*	Klamath R.	SF Trinity R.	2
	Mad R.	Mad R.	2
	Russian R.	EF Russian R.	2
	Santa Ynez R.	Hilton Ck	1
	Scott Ck	Big Ck	2

Ck, Creek; Lk, Lake; NF, North Fork; R., River; SF, South Fork; U, Upper; W, West.

occurred in approximately 10% of loci. This suggested that this population contained hybrids between Lahontan cutthroat trout and, most likely, Yellowstone cutthroat trout. Sequence data from the Three Mile Creek population were retained both for their utility in the identification of novel markers diagnostic between Lahontan cutthroat trout and members of the Yellowstone group, and because they were still informative for the discovery of sites variable in pure Lahontan cutthroat trout populations.

Markers putatively polymorphic in Lahontan cutthroat trout

There were 72 sites polymorphic within or between the Lahontan cutthroat trout samples in the primary ascertainment panel (69 SNPs and three indels, within 52 loci), excluding variants shared between Three Mile Creek and the Yellowstone group. Assays were successfully designed for 35 of these sites. There were also 10 sites (within seven loci) polymorphic in Lahontan cutthroat trout in the secondary ascertainment panel, and assays were designed for seven of these.

Following validation, five of these 42 assays were rejected due to failure of amplification ($n = 2$), invariant genotype calls ($n = 2$), or difficulty in discriminating genotypes ($n = 1$). Three assays designed from sites discovered in the secondary ascertainment panel were not polymorphic in Lahontan cutthroat trout; one was diagnostic between the Yellowstone group and all other taxa assayed (Ocl_parp3Y), and the other two were variable in taxa other than Lahontan cutthroat trout (data not shown). All 34 remaining assays were found to be variable in at least one of the Lahontan or Paiute cutthroat trout validation samples (Table 2).

Markers putatively diagnostic for Lahontan cutthroat trout

Excluding the Three Mile Creek population, 131 substitutions (123 SNPs and eight indels, within 86 loci) diagnostic between Lahontan cutthroat trout and all other individuals in the primary ascertainment panel were observed. Of these, 94 (within 67 loci) were also fixed in the Three Mile Creek sample and assays were designed for 26 of these polymorphisms. One of these assays actually targeted a dinucleotide site and one targeted a trinucleotide site, as there were two polymorphic sites directly adjacent to each other and in complete disequilibrium in the sequence data. These multiple nucleotide polymorphism markers performed identically to the SNP markers within our validation panel and are referred to here as SNPs for convenience. However, recombination within such multi-nucleotide regions might occasionally produce genotypes for which assays have not been designed, leading to spurious results.

Validation confirmed 20 of these 26 polymorphisms as completely diagnostic markers distinguishing Lahontan and Paiute cutthroat trout from all other taxa, although one assay (Ocl_123044H) failed to amplify in *O. mykiss* (Table 3). One of the remaining six assays (Ocl_106560H) was polymorphic in coastal cutthroat trout; a second (Ocl_97077H) exhibited a single 'Lahontan' allele copy in both the coastal cutthroat trout and *O. mykiss* validation samples; and a third (Ocl_96899H) appeared diagnostic except for a single allele copy observed in one Lahontan population and a polymorphism shared with greenback cutthroat trout. The other three were all polymorphic within Lahontan cutthroat trout. One of these (Ocl_114448h) was retained and the other two discarded due to problems discriminating genotypes.

Markers putatively diagnostic for the 'Yellowstone group'

A further 40 polymorphic sites (39 SNPs and one indel, within 17 loci) were observed with an allele shared between Three Mile Creek and one or more taxa in the Yellowstone group. Assays were successfully designed for 12 of these sites.

Validation confirmed that none of the 12 assays produced variable genotype calls in the pure Lahontan cutthroat trout populations tested (Table 3). Six assays were diagnostic between all subspecies of the 'Yellowstone group' and all other taxa (with the exception of a single allele observed at Ocl_118205Y). Two further assays were diagnostic between Yellowstone cutthroat trout and all other cutthroat subspecies except Rio Grande. An additional two assays were diagnostic between Yellowstone and Lahontan cutthroat trout but polymorphic or fixed for alternative alleles in other taxa. The remaining two assays (not included in Table 3) were fixed in Lahontan but variable within subspecies of the Yellowstone group.

Complete assay set

The set of 68 markers retained following validation included 35 that were variable within Lahontan cutthroat trout, 23 that were diagnostic between Lahontan cutthroat trout and all other taxa and 10 that were diagnostic between Lahontan and Yellowstone cutthroat trout (Table 4). Some markers that were designed for different purposes occurred within the same locus (which can be identified by the numeric portion of the marker name, Tables 2 and 3), and were therefore physically linked. The mean call rate over all loci was 96.6% (range = 96.4–100%), excluding the two cases where a locus failed

Table 2 Minor allele frequency (MAF), unbiased expected heterozygosity (H_e) and observed heterozygosity (H_o) of Lahontan cutthroat trout SNPs in Lahontan and Paiute cutthroat trout validation populations. Lahontan cutthroat trout Geographic Management Units are indicated by: (E) Eastern; (W) Western; (NW) Northwestern. Significant deviations from Hardy–Weinberg proportions are indicated by bold type. The minor allele is defined by its frequency over all Lahontan cutthroat trout samples. NC, no call

	West Mary (E), n = 24			Frazer (E), n = 24			Line (NW), n = 23			Summit (NW), n = 18			Independence (W), n = 26			Pilot Peak (W), n = 26			Paiute, n = 22		
Locus	MAF	H_e	H_o	MAF	H_e	H_o	MAF	H_e	H_o	MAF	H_e	H_o	MAF	H_e	H_o	MAF	H_e	H_o	MAF	H_e	H_o
Ocl_96376h	0.60	0.49	0.54	0.83	0.28	0.25	0.80	0.32	0.39	0.61	0.49	0.44	0.38	0.48	0.54	0.15	0.27	0.23	0.00	—	—
Ocl_96899h	0.00	—	—	0.22	0.35	0.26	0.00	—	—	0.00	—	—	0.00	—	—	0.00	—	—	0.00	—	—
Ocl_99550h	0.46	0.51	0.39	0.16	0.27	0.23	0.52	0.51	0.61	0.56	0.51	0.63	0.58	0.50	0.46	0.00	—	—	0.00	—	—
Ocl_105115h	0.50	0.51	0.58	0.36	0.47	0.45	0.26	0.39	0.35	0.00	—	—	0.74	0.39	0.36	0.00	—	—	0.00	—	—
Ocl_105407h	0.13	0.22	0.25	0.30	0.43	0.35	0.20	0.32	0.30	0.00	—	—	0.81	0.32	0.38	0.02	0.04	0.04	0.00	—	—
Ocl_105768h	0.00	—	—	0.00	—	—	0.00	—	—	0.00	—	—	0.00	—	—	0.13	0.24	0.27	0.00	—	—
Ocl_106313h	0.56	0.50	0.54	0.56	0.50	0.54	0.00	—	—	0.19	0.32	0.28	0.31	0.43	0.46	0.31	0.43	0.46	0.00	—	—
Ocl_106419h	0.30	0.43	0.59	0.25	0.38	0.42	0.00	—	—	0.00	—	—	0.00	—	—	0.42	0.50	0.46	0.00	—	—
Ocl_106747h	0.34	0.46	0.32	0.57	0.50	0.43	0.33	0.45	0.57	0.89	0.20	0.22	0.30	0.43	0.44	0.00	—	—	0.00	—	—
Ocl_107074h	0.00	—	—	0.00	—	—	0.00	—	—	0.11	0.20	0.22	0.42	0.50	0.38	0.71	0.42	0.35	0.00	—	—
Ocl_107336h	0.10	0.19	0.21	0.02	0.04	0.04	0.26	0.39	0.35	0.25	0.39	0.39	0.40	0.49	0.50	0.00	—	—	0.00	—	—
Ocl_107607h	0.33	0.45	0.42	0.56	0.50	0.71	0.00	—	—	0.03	0.06	0.06	0.58	0.50	0.46	0.00	—	—	0.00	—	—
Ocl_108210h	0.00	—	—	0.00	—	—	0.00	—	—	0.03	0.06	0.06	0.31	0.43	0.62	0.15	0.27	0.31	0.00	—	—
Ocl_110064h	0.00	—	—	0.00	—	—	0.61	0.49	0.43	0.11	0.20	0.22	0.44	0.50	0.58	0.44	0.50	0.50	0.90	0.18	0.19
Ocl_111312h	0.06	0.12	0.13	0.10	0.19	0.21	0.52	0.51	0.50	0.08	0.16	0.17	0.02	0.04	0.04	0.19	0.32	0.38	0.00	—	—
Ocl_114250h	0.29	0.42	0.50	0.52	0.51	0.67	0.02	0.04	0.04	0.86	0.25	0.28	0.22	0.35	0.20	0.00	—	—	0.00	—	—
Ocl_114315h	0.48	0.51	0.38	0.44	0.50	0.63	0.00	—	—	0.17	0.29	0.33	0.23	0.36	0.38	0.15	0.27	0.15	0.00	—	—
Ocl_114448h	0.29	0.42	0.19	0.13	0.22	0.25	0.00	—	—	0.81	0.31	0.25	0.21	0.34	0.33	0.18	0.30	0.36	1.00	—	—
Ocl_116865h	0.28	0.41	0.30	0.38	0.48	0.58	0.00	—	—	0.14	0.25	0.17	0.12	0.21	0.23	0.00	—	—	0.00	—	—
Ocl_117138h	0.00	—	—	0.00	—	—	0.61	0.49	0.43	0.11	0.20	0.22	0.44	0.50	0.58	0.44	0.50	0.50	0.91	0.17	0.18
Ocl_117815h	0.00	—	—	0.26	0.39	0.43	0.00	—	—	0.00	—	—	0.17	0.29	0.27	0.88	0.21	0.23	0.07	0.13	0.14
Ocl_120751h	0.27	0.40	0.38	0.10	0.19	0.21	0.00	—	—	0.67	0.46	0.44	0.17	0.29	0.35	0.13	0.24	0.27	1.00	—	—
Ocl_123470h	0.00	—	—	0.00	—	—	0.11	0.20	0.22	0.14	0.25	0.28	0.40	0.49	0.58	0.63	0.47	0.50	0.00	—	—
Ocl_126160h	0.06	0.12	0.13	0.00	—	—	0.02	0.04	0.04	0.06	0.11	0.11	0.25	0.38	0.35	0.60	0.49	0.50	0.00	—	—
Ocl_127645h	0.29	0.42	0.58	0.13	0.22	0.25	0.00	—	—	0.00	—	—	0.02	0.04	0.04	0.00	—	—	0.00	—	—
Ocl_128302h	0.31	0.44	0.54	0.35	0.47	0.46	0.41	0.50	0.48	0.67	0.46	0.33	0.69	0.43	0.46	0.38	0.48	0.54	0.00	—	—
Ocl_128757h	0.25	0.38	0.50	0.52	0.51	0.46	0.72	0.41	0.48	0.14	0.25	0.28	0.13	0.24	0.27	0.12	0.21	0.23	0.00	—	—
Ocl_129144h	0.00	—	—	0.00	—	—	0.00	—	—	0.75	0.39	0.50	0.83	0.29	0.35	0.31	0.43	0.46	1.00	—	—
Ocl_130295h	0.00	—	—	0.00	—	—	0.00	—	—	0.00	—	—	0.25	0.38	0.35	0.00	—	—	0.00	—	—
Ocl_131460h	0.65	0.47	0.46	0.57	0.50	0.52	0.11	0.20	0.22	0.11	0.20	0.22	0.40	0.49	0.42	0.21	0.34	0.27	1.00	—	—
Ocl_131785h	0.35	0.47	0.54	0.55	0.51	0.45	0.43	0.50	0.43	0.00	—	—	0.33	0.45	0.42	0.27	0.40	0.46	1.00	—	—
Ocl_gNa11h	0.13	0.22	0.25	0.15	0.25	0.29	0.00	—	—	0.19	0.32	0.39	0.60	0.49	0.35	0.46	0.51	0.62	0.00	—	—
Ocl_Nipsh	0.00	—	—	0.00	—	—	NC	—	—	0.25	0.39	0.28	0.50	**0.51**	**0.13**	0.17	0.29	0.19	1.00	—	—
Ocl_pNpOh	0.21	0.34	0.33	0.00	—	—	0.00	—	—	0.44	0.51	0.56	0.02	0.04	0.04	0.44	0.50	0.58	0.00	—	—
Ocl_rbm4bh	0.00	—	—	0.00	—	—	0.00	—	—	0.44	0.51	0.33	0.00	—	—	0.00	—	—	0.00	—	—

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Table 3 Observed frequencies of putative Lahontan diagnostic alleles and Yellowstone diagnostic alleles for each taxon in the validation panel. LCT, Lahontan cutthroat trout; PCT, Paiute cutthroat trout; CCT, coastal cutthroat trout; WCT, westslope cutthroat trout; BCT, Bonneville cutthroat trout; YCT, Yellowstone cutthroat trout; CRCT, Colorado River cutthroat trout; GCT, greenback cutthroat trout; RGCT, Rio Grande cutthroat trout; OM, *O. mykiss*; NC, no call; nr, assay not run or failed over entire Fluidigm plate

	LCT: WMary, $n = 22$	LCT: Frazer, $n = 24$	LCT: Line, $n = 21$	LCT: Summit, $n = 18$	LCT: Indep., $n = 24$	LCT: Pilot, $n = 22$	PCT, $n = 22$	CCT, $n = 14$	WCT, $n = 8$	BCT, $n = 5$	YCT, $n = 15$	CRCT, $n = 13$	GCT, $n = 9$	RGCT, $n = 15$	OM, $n = 19$
Ocl_96899H	1.00	1.00	1.00	0.94	1.00	1.00	1.00	0	0	0	0	0	1.00	0	0
Ocl_97077H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.04	0	0	0	0	0	0	0.02
Ocl_98409H	1.00	1.00	nr	nr	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_99550H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_101554H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_102420H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_104515H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_105385H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_105897H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_106747H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_106560H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.48	0	0	0	0	0	0	0
Ocl_110064H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_110495H	1.00	1.00	nr	nr	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_110571H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_112820H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_113600H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_115987H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_117242H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_123044H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	NC
Ocl_128693H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_129870H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_130524H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_131460H	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0	0	0	0	0	0	0	0
Ocl_119108Y	0	0	0	0	0	0	0	1.00	0	1.00	1.00	1.00	1.00	1.00	NC
Ocl_120102Y	0	0	0	0	0	0	0	0.36	0.93	0.50	1.00	0.42	0	0	0.53
Ocl_123921Y	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0.13	0
Ocl_102420Y	0	0	0	0	0	0	0	0	0	0	1.00	0	0	0.13	NC
Ocl_107974Y	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0
Ocl_111681Y	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0
Ocl_118205Y	0	0	0	0	0	0	0	0	0	1.00	0.96	1.00	1.00	1.00	0
Ocl_123048Y	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0
Ocl_128693Y	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0
Ocl_parp3Y	0	0	0	0	0	0	0	0	0	1.00	1.00	1.00	1.00	1.00	0

completely for a taxon or population. The marker Ocl_Nipsh exhibited a strong deviation from Hardy–Weinberg equilibrium in Independence Lake ($P = 0.0005$) and failed to amplify in Line Canyon Creek, suggesting the presence of null alleles in some populations. Two of the Lahontan variable markers (Ocl_110064h and Ocl_117038h) were found to be in strong linkage disequilibrium ($P < 0.0001$) in all populations. Following FDR correction, no significant deviations from Hardy–Weinberg or linkage equilibria were observed for any other loci. Putative functions were identified for 88% of the ESTs containing these markers (Table S1, Supporting information). Three Lahontan variable and two Lahontan diagnostic assays were identified as targeting nonsynonymous substitutions.

Discussion

We describe the discovery and validation of the first SNP markers for use in the study of the threatened Lahontan cutthroat trout. These 68 novel genetic markers and assays represent a major addition to the genetic tools previously available for study of this group of cutthroat trout (Peacock *et al.* 2004; Robinson *et al.* 2009). The suite of assays interrogate nucleotide sites polymorphic within Lahontan and the closely related Paiute cutthroat trout, as well as sites that are diagnostic for either Lahontan and Paiute cutthroat trout or members of the 'Yellowstone group' of cutthroat trout subspecies. Together with a large number of SNP markers recently developed to identify hybridization between rainbow trout and cutthroat trout (e.g. Pritchard *et al.* 2012), these new markers will be an important tool to inform genetic management of both Lahontan and Paiute cutthroat trout.

The primary SNP discovery ascertainment panel included five Lahontan cutthroat trout individuals, originating from the three currently recognized GMUs. There were a large number of nucleotide sites ($n = 131$) diagnostic between these Lahontan cutthroat trout and all other taxa in the panel, confirming the phylogenetic distinctiveness of this group. All of the assays diagnostic for Lahontan cutthroat trout were also fixed for the Lahontan allele in Paiute cutthroat trout, demonstrating the close affiliation of these two groups. In contrast, there were relatively few sites within the sequence data that were variable in Lahontan cutthroat trout samples ($n = 72$). The probability of identifying variable sites within a taxon depends both on its evolutionary history and on its representation in the ascertainment panel, which in this case included relatively few samples. Nevertheless, the frequency of Lahontan cutthroat trout variable sites observed per individual sequenced was comparable to that seen in Rio Grande cutthroat trout, considered a 'minor subspecies' of the Yellowstone group (Behnke 2002; 58 variable sites in three ascertainment individuals, unpublished data). Taken together, these results suggest that the amount of genetic divergence between Lahontan and Paiute cutthroat trout, as well as between the Eastern GMU (proposed as a separate subspecies, 'Humboldt cutthroat trout', Behnke 2002) and other Lahontan cutthroat trout GMUs, is relatively small.

The identification of variable sites within Lahontan cutthroat trout was complicated by the presence of genetic material from Yellowstone cutthroat trout, or a closely related subspecies, in the Three Mile Creek population. In addition, one variable site identified in the Lake Lenore samples was found during validation to be diagnostic for the Yellowstone group, suggesting that this population may also contain genetic material from other subspecies. Such results once again emphasize the need to account for introduction histories when performing population genetic analyses on cutthroat trout and other species that have been impacted by anthropogenic transplants and that can hybridize with closely related taxa (e.g. Metcalf *et al.* 2007). Yellowstone cutthroat trout were stocked extensively throughout the western United States from the late 19th century onward, and this is not the first study to identify the presence of their genetic material outside the native range (Pritchard *et al.* 2007). Nevertheless, most population genetic studies of cutthroat trout focus on identifying and excluding nonnative genetic material from *O. mykiss* alone and ignore potential introgression from conspecifics. Here, eight SNPs are described that identify ancestry from members of the Yellowstone group that have introgressed Lahontan, westslope, and coastal cutthroat trout populations. Two additional assays are also useful for identifying introgression from the Yellowstone group into Lahontan cutthroat trout, although interpretation of results from these loci may be complicated by shared alleles with *O. mykiss* or other cutthroat trout subspecies.

Assays were successfully developed for 35 sites variable within Lahontan cutthroat trout. These SNPs differed widely in their level of polymorphism. Four loci were variable in only one of the validation populations, although this population was different for each locus. The Western GMU exhibited the highest number of variable loci ($n = 33$), followed by the Northwestern GMU ($n = 29$) and the Eastern GMU ($n = 25$). This pattern probably at least partly reflects ascertainment bias, due to a larger number of individuals from the Western GMU in the ascertainment panel. However, it also suggests that the inclusion of an introgressed population (Three Mile Creek) as the representative of the Northwestern GMU did not substantially curtail SNP discovery for this GMU. The four SNPs that were identified in the single population of the secondary ascertainment panel (Ocl_gNa11h, Ocl_Nipsh, Ocl_pNpOh and Ocl_rbm4bh)

Table 4 Assay name, NCBI database accession number, polymorphism, forward and reverse amplification primers and labelled TaqMan probes for the markers described in this study. For Lahontan cutthroat trout diagnostic alleles, nucleotide targets are shown as Lahontan/other. For Yellowstone diagnostic alleles, nucleotide targets are shown as Yellowstone/other

Assay	NCBI ss #	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_96376h	491231994	A/G	F: GAGGAGGGTCTTGAGAACATGT	VIC: CTGCACGTAAACTC
			R: ACTAAATCGTCTGCATGTGATCTTTTC	FAM: CTGCACATAAACTC
Ocl_96899h	491231995	C/T	F: GGAGCAACCCCTTGACCTT	VIC: CAGGTCAGAACACCCAC
			R: TGTGGTCATCTGACTGTTTTCTCAA	FAM: CAGGTCAGAATACCCAC
Ocl_99550h	491231996	C/T	F: GAGAGTTACTGATACTGAAGTGTGAAAGAG	VIC: CGTACTGATACAGACCTCACAG
			R: ACTGTGTGTAACTCCTTCCACTAGA	FAM: CGTACTGATACAGACCCCACAG
Ocl_105115h	491231997	A/T	F: TCTCGAAGAGGAGCTGAGATTTCTT	VIC: AGGGAGCATATGATG
			R: CAGCTATTTTTGCCCCATGCA	FAM: AGGGAGCATTTGATG
Ocl_105407h	491231998	A/G	F: ACGCAAAGAGAACATGGATAATCTTCT	VIC: CAATATTTACGCGGATGTAC
			R: CTCACAACTCTTCATCAACCATTCC	FAM: AATATTTACGCAGATGTAC
Ocl_105768h	491231999	C/T	F: GTACAGGACTAGACCTCAGAGACA	VIC: CTACTGTATAGTTAACCTCTGCAAT
			R: ATGCACTTAATTAGTTAGCACCTTTGAGA	FAM: CTACTGTATAGTTAACCTCTGTAAT
Ocl_106313h	491232000	G/T	F: GGTACAGTAAATCAAGTTTCCCCATTACA	VIC: ACTTACCAACGGTTGTGTC
			R: CCAAACCCTGTGTGAAAATGTGAAA	FAM: ACTTACCAACTGTTGTGTC
Ocl_106419h	491232001	A/T	F: ACACCTGGAGGGTGAAGGATTAAT	VIC: TGCCAATGAGCCCTT
			R: CAGTCAATTCAGGAAGTACCTTGCT	FAM: TGCCAATGTGCCCTT
Ocl_106747h	491232002	A/G	F: GTGTGTCTGTTTATGTTCCAGGAAAT	VIC: AAGGTCTTGTTGTCAGAGAA
			R: CTGTCGCCCTCCCATGTT	FAM: AAGGTCTTGTTGTCAGGGAA
Ocl_107074h	491232003	A/G	F: CCGTAGCAGCACCTGGTA	VIC: AGCCTGAAGCCCC
			R: GTCAGCAGCCCACTTCCA	FAM: AGCCTAAAGCCCC
Ocl_107336h	491232004	G/T	F: CCTCAGGCCTTGTGTTTTAGCA	VIC: AGCTGGTAGAACCTC
			R: GCCTAAACCAGGGCTTTGACAA	FAM: CAGCTGGTATAACCTC
Ocl_107607h	491232005	A/G	F: TTTTAGACACCAATGTTCCGACCAT	VIC: CTGATAGTGTGCATTGCA
			R: CGATGTAGGCTACTCCAGATCAG	FAM: CTGATAGTGTGCGTTGCA
Ocl_108210h	491232006	C/G	F: AACCCAACCTCACACACTATTTCA	VIC: TTGTCATGAAGGAGACGTA
			R: GTCGATATTCTGCGTGTACATTCTG	FAM: TGTCATGAAGCAGACGTA
Ocl_110064h	491232007	A/T	F: ACTGCAGGTGGACTCTTGATAAATT	VIC: ACATGTGCTGGCCTGT
			R: TCTGGAACAATGGCCTGGTTA	FAM: CATGTGCAGGCCTGT
Ocl_111312h	491232008	C/T	F: AAAGGCAATCCACTGGTTCTCAT	VIC: CCAATCAGAGATTACCGC
			R: GCAGAACTATACCCATACCCAAAGG	FAM: CCAATCAGAGATTACTGC
Ocl_114250h	491232009	A/G	F: CCAACCAAAACTGTCACTGTGTATG	VIC: CCATAGCAACATAGCCACAT
			R: TGGTGTAGGTCTAGCTGTCAGTTAA	FAM: CCATAGCAACATAACCACAT
Ocl_114315h	491232010	C/T	F: AACGCACCCTTCTGAAGCT	VIC: CTTCCGCGCTTATC
			R: TCACAGGTCAAGGGTCACTCA	FAM: CCTTCTGCGCTTATC
Ocl_114448h	491232011	T/C	F: CATCCTCAAAGTCCAATGAACTCAGT	VIC: CTTCTCACGTAATGATTC
			R: GCATTACACACCAATAGAGGGTACA	FAM: TCACGCAATGATTC
Ocl_116865h	491232012	A/T	F: AGCTATTTTATACAGTTGAGTCATCAAACCA	VIC: CCACAGCAGATAGAT
			R: AAAGTAGGTCCATAGAAACCAAATAAAATCCA	FAM: CCACAGCTGATAGAT
Ocl_117138h	491232013	G/T	F: GGAGAGAGAGAACAGAAAAACAAGGT	VIC: CTCCTGTCATGACTGTT
			R: TGGTTGCAAGGAGGGAAATTAGTT	FAM: TCTCCTGTCATTACTGTT

Table 4 (Continued)

Assay	NCBI ss #	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_117815h	491232014	A/G	F: ATGATGCAATGGTAGGACTTCTTGT	VIC: ACAAAATCTGGAAAACT
			R: AGCAAATGGTTAAGCTACATCAGGAT	FAM: AACAAAATCTAGAAAACT
Ocl_120751h	491232015	A/G	F: GTGCTGCCCAGCATTCC	VIC: CTGTCAGTCAGTCTTTCA
			R: AGGGCTGAGCACTTCTCTCT	FAM: TGTCAGTCAATCTTTCA
Ocl_123470h	491232016	A/G	F: GGATTTGTGCCCATGTCTCATACTT	VIC: AATGGTTGTGACAGAGCA
			R: AATTTTGCATTAAGGAGTTGATGATATATTAGCA	FAM: TGGTTGTGGCAGAGCA
Ocl_126160h	491232017	A/C	F: GTGTTGGTGAGCAAGATAATTGTGT	VIC: TGTAAACAGTGAGCTAACA
			R: GCACAATTTTGTATAGAAAACACCTTTTGG	FAM: AAACAGTGCGCTAACA
Ocl_127645h	491232018	C/T	F: GGTGGACAGGAGAATCAACAAAGAG	VIC: AACTTTGATCGCTTTTATT
			R: GGTGCCTCTCGTGGCATAAAA	FAM: AACTTTGATCGTTTTTATT
Ocl_128302h	491232019	C/T	F: GCACCCTAACATGTCATCAAGATCT	VIC: CAGTCATCTACATGTAGCTGA
			R: TGAGATCCACAACTGATGAAAGACATG	FAM: TCATCTACATGCAGCTGA
Ocl_128757h	491232020	A/T	F: TCATCCTTGGTTCGATAAAAAAATATCTGACA	VIC: TGGGTATGTTAATCATATTACCA
			R: GAAAAATGCACAACCTTTTTTTGTTTTTAC	FAM: TGGGTATGTTAATCATTTTACCA
Ocl_129144h	491232021	C/T	F: CAGCCTGGCATCAACATCAC	VIC: CCTGGGCTTCCCTTC
			R: GGCTTCGTGGCCCTTGA	FAM: CTGGGCTCCCCTTC
Ocl_130295h	491232022	A/T	F: ACATTCTGTCCAAACCTCTGCAA	VIC: CCATTCTCTGAGATCAGTG
			R: ACTCTGAGCAACGCTTGTCAT	FAM: CCATTCTCTGAGTTCAGTG
Ocl_131460h	491232023	G/T	F: AGGAATGGAGGAGTACAGTAATACAGTAATAAA	VIC: CCACAAATGATCGTCCTGT
			R: GAAGTTCAGGTTGCATTACTCTGCTA	FAM: CCACAAATGATCTTCCTGT
Ocl_131785h	491232024	A/G	F: ACCCGCACTCACCTAAATATGTTTT	VIC: CCACCCAATGACCTTT
			R: CCAGGACTGGGAGATCTGTTATG	FAM: CCACCCAATAACCTTT
Ocl_gNa11h	491232025	A/G	F: GCTACAATAAACACTAGGTTGTTAAATTGCA	VIC: ATGACAAGATTCGATGATTT
			R: TTCTGATCTACTAATTTAAATGGGTGGTGATC	FAM: ATGACAAGATTCAATGATTT
Ocl_Nipsh	491232026	A/C	F: AGGAGTTTCAGCAATGACATCAACA	VIC: TGCTTTCACTTATATATTTTTG
			R: ATGCCGACACAGGAAAGACAA	FAM: TGCTTTCACTTATATCTTTTTG
Ocl_pNpoh	491232027	G/T	F: GCAGTAGGAGCCTCTCATGTT	VIC: TGAATAACTACGTGCTATATG
			R: CCTTGATTGTTATTAAACAAGAACGATTGGA	FAM: TTGAATAACTACGTTCTATATG
Ocl_rbm4bh	491232028	A/T	F: GGCTCCCAGAAGCCTTTTAGT	VIC: CAGTCGTTTTTTTAATTTT
			R: CAGCAAAAGTTTGTATCATGACACGTT	FAM: AGTCGTTTTTTAAATTTT
Ocl_96899H	537150606	A/G	F: TGTTTTGTGTGTGCGTGGGATATA	VIC: CATAGTACACACAGATGCAT
			R: CCTGCAGCGCACCTGATA	FAM: TAGTACACACGGATGCAT
Ocl_97077H	491232029	T/A	F: GTTCAGGTACCCATACATTTCCAAGA	VIC: AGTACACAAACATTTTTTT
			R: CAGGGCACAGGTAGGTTAAAAGAG	FAM: AGTACACAAACATATTTTT
Ocl_98409H	491232030	T/G	F: GTTGCAAAATTCCTGGGACTTTCAT	VIC: AAATCCTGGTTGTATGATT
			R: CTGGTTGGAGGGTTCCAGAT	FAM: CCTGGTTGGATGATT
Ocl_99550H	537150608	T/C	F: CTCTGTGCTTTGCAAAGTTTAAGC	VIC: CTTCTAGCTCGTCTAAGAGA
			R: TTTGGGCTTAGCTAGTTAAAGGTGAA	FAM: CTAGCTCGTCCAAGAGA
Ocl_101554H	491232031	T/C	F: CTAACCACTTTACACTCTTCTCATACTGT	VIC: CTGTTGACAACTTTATTCT
			R: TGCACTGACCTGGACTGAGA	FAM: CTGTTGACAACTCTATTCT
Ocl_102420H	491232032	T/C	F: CACATGCCAGTAGACTCCTAAAGC	VIC: CTCAGGTGTGTATTACGAC

Table 4 (Continued)

Assay	NCBI ss #	Target	Primers (5′–3′)	Probes (5′–3′)
			R: GTTGATGTCATCCTTGTTGCAGTTG	FAM: TCAGGTGTGTACTACGAC
Ocl_104515H	491232033	TT/CC	F: GGCAACACTGACAGAAAGACATG	VIC: TGCGTTAAGGTTCC
			R: ACAGTTAACGCCTTCGGTCAAG	FAM: CGCCAAGGTTCC
Ocl_105385H	491232034	G/T	F: TGAGAAAGTTATTGCCTGACAGAAGT	VIC: CTTTGTAAGTACTCTGGGTGTGT
			R: GGAGGCTACAGACTGCTCAGA	FAM: CTTTGTAAGTACTCTGTGTGTGT
Ocl_105897H	491232035	T/C	F: ATTGGCTTCAAATAACTACTGTTGTGA	VIC: TAGCCTCCACTGTGTTC
			R: CTAGGATTCTGTTCCTTTGCCTCAA	FAM: TAGCCTCCACCGTGTTC
Ocl_106560H	537150610	A/G	F: TGATGTAGGCCCCGATGGTA	VIC: CTGGACAAGGCTCCTC
			R: GAACTCTGGGAAGCTGATGTCA	FAM: CTGGACAAAGCTCCTC
Ocl_106747H	537150599	T/C	F: GGTGGTGGGTCTAACTACAATGTAA	VIC: CACCATGTAAACGTTTAGAG
			R: ACGCCATTCTACTCCAACCA	FAM: ACCATGTAAACGTTCAGAG
Ocl_110064H	537150602	A/C	F: AGAGTGCAAAGCAAACCAATAGTCT	VIC: CTCAAGTAGCATATTCC
			R: TCCAGAATGCATTCTTTTGGTTTCA	FAM: CTCAAGTAGCCTATTCC
Ocl_110495H	491232036	T/C	F: GGGTTTTCCTGTGAAGTTTCGTACA	VIC: CAACCCCATTCCATACGA
			R: GCTAATTCTGCTGCACTAATGTTATGG	FAM: ACCCCATCCCATACGA
Ocl_110571H	491232037	G/A	F: TGGGTCTGTAGACTGAGGTCATTT	VIC: TTTCGGGCGCATCTA
			R: TGTCTGCACAAATCAAATTGCCAAA	FAM: TTCGGGCACATCTA
Ocl_112820H	491232038	T/C	F: CATACCACTGCTAACTCTGCCTAAT	VIC: CCATGACGATGACCTTA
			R: AACAATTTGACCGCGCCG	FAM: CATGACGACGACCTTA
Ocl_113600H	491232039	A/G	F: GACCCCAGATCAGCCAGTAG	VIC: CATGCTGGCTATATCATAT
			R: GAGAGAAGCATCTCACCTTGATGAC	FAM: ATGCTGGCTATGTCATAT
Ocl_115987H	491232040	A/G	F: GAAAAGGTGAAGCACTGCAATTGTA	VIC: ATCTACCAGAAACTAAAC
			R: TTCCAGACTGCGCAATCCA	FAM: TCTACCAGAGACTAAAC
Ocl_117242H	491232041	T/C	F: GGACATGGGACAGGACAGC	VIC: CCCCCAGATTCCCACC
			R: CTATCAGCTCTGATGGATGCATCTG	FAM: CCCCAGACTCCCACC
Ocl_123044H	491232042	C/A	F: CATCTGGAAGCTGGTCTGCTAA	VIC: TGCCCCTTCGGCCTT
			R: AACCTTGTCGGCAGTATGTATCATT	FAM: TGCCCCTTAGGCCTT
Ocl_128693H	491232043	AAA/TAC	F: GGCGGATATGGGAATGTTAACCA	VIC: AATTCAGAATAAAATTTTTC
			R: TCCATGTCCCCACCAAACTG	FAM: TTCAGAATTACATTTTTC
Ocl_129870H	491232045	G/T	F: GAACGGCAAAGCCAGACAAAATTAT	VIC: TCGCGGGACATTT
			R: GTTTTGTGACTATCACTCAGCTACACA	FAM: CTCGCGGTACATTT
Ocl_130524H	491232046	T/C	F: TGCTGCCTTGTGCACTCT	VIC: CTGCTCACCACTATGTGT
			R: AAGCACACAGCAGAACAGACATA	FAM: CTGCTCACCACTACGTGT
Ocl_131460H	537150604	G/C	F: CGAAGGTCATTTGGCACATGAAA	VIC: CTGTTTCTTACCTGCCAGTTG
			R: GTGCTGTGAAATAGTCAATACTCGGA	FAM: CTGTTTCTTACCTCCCAGTTG
Ocl_102420Y	491232050	A/G	F: ATCAGGTAACACGTTTCACACTGT	VIC: AGAGAATGAATCGTGATTTA
			R: CATCATGATGTAGCCCTGTTTGC	FAM: AAGAGAATGAATCATGATTTA
Ocl_107974Y	491232051	C/T	F: CCATTTTGGCTTTGTTTGGTAGACA	VIC: CCAACTGTCAATCTC
			R: TGCCACAGCCAAGTGCTA	FAM: CAACTGCCAATCTC
Ocl_111681Y	491232052	A/G	F: ATTTTGTACCGGTGGTTCTGGAA	VIC: CCTGTCACCAACACAG
			R: CACCTAGCGAGCATGATCCA	FAM: CCTGTCACCGACACAG

Table 4 (Continued)

Assay	NCBI ss #	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_118205Y	491232053	C/T	F: CATAAGCAACACCGGAAGTTGTAG	VIC: CAAAAACCTTTGTAAGGGAA
			R: ACAGACAGTTGTCCAAGTTAATTACATG	FAM: AAACCTTTGCAAGGGAA
Ocl_119108Y	491232047	A/C	F: CACAAAAACAATGGGAACGTCTGA	VIC: CGCCTGTATCAGACTAT
			R: ACCAGCAGAAAGCAGTGTCATT	FAM: CGCCTGTATCCGACTAT
Ocl_120102Y	491232048	A/G	F: ACTCCTGTAGATGTCACTGACTGA	VIC: CTGGTTTATACCAAACATT
			R: ATTACATTATTTGGTCACTATTACATTATCAGTTATGT	FAM: TGGTTTATACCGAACATT
Ocl_123048Y	491232054	G/T	F: GGGCAAGTGCTTTTGTATTTTTTT	VIC: TGGCTGTATTGAATGACCTAG
			R: TCAGATCAATGGCACCTCACA	FAM: TGGCTGTATTGAATTACCTAG
Ocl_123921Y	491232049	A/G	F: CCCAACTTGCAACTTAAATCCAATGA	VIC: ATTAACTAACGTGAAATCA
			R: CAGACGTCAGTTCAACTTCTACTTTTG	FAM: ATTAACTAACATGAAATCA
Ocl_128693Y	491232055	A/C	F: GGACATGGAATTCTTAGAGTCAGGT	VIC: TAGGGACTCTGGTATTTGA
			R: GGCACTAGGGTGTGATAGAGAAG	FAM: TAGGGACTCTGGTCTTTGA
Ocl_parp3Y	491232056	A/G	F: GTTTAATCAGTTGTCATATTACTAACTTTTGCTGA	VIC: ACCAGACAGACATTGAA
			R: GAGAAAGGCAACAGTGCACAAG	FAM: CAGACAGGCATTGAA

were not markedly less variable than others. However, Ocl_Nipsh failed to amplify in two of the validation populations, probably due to a polymorphism in the region flanking the SNP, and results from this marker should be interpreted with caution. The majority of Lahontan cutthroat trout SNPs were invariant in the Paiute cutthroat trout sample, with only three of the loci exhibiting more than one allele. Six of the invariant loci were fixed for an allele that was also observed in Lahontan cutthroat trout but absent from all other taxa in the validation panel. This lack of variation within Paiute cutthroat trout could again reflect ascertainment bias, due to the lack of a Paiute cutthroat trout sample in the ascertainment panel. However, it is also not unexpected given the known history of the group, originally restricted to 16 km of a single stream (Behnke 2002), and the history of the populations represented in the validation sample, both of which are anthropogenic transplants.

Two Lahontan SNPs, Ocl_110064h and Ocl_117038h, were in strong linkage disequilibrium, displaying identical genotypes in all individuals tested, despite the fact that the SNPs were identified from sequences corresponding to different ESTs. This suggests that these two randomly chosen ESTs are either separated by a short physical distance, that there is strong selection acting on a nearby genomic site, or that they co-occur on a chromosomal region with suppressed recombination. Supporting the latter hypothesis is that such regions are known to exist in the *O. mykiss* genome (Phillips *et al*. 2009) and a locus containing one of these SNPs (Ocl_110064h) was also found to be in complete linkage disequilibrium with a third gene (SUMO-conjugating enzyme UBC9) in an *O. clarkii–O. mykiss* mapping cross (unpublished data). Although this tight linkage reduces the effective number of markers available for population genetic analyses within the subspecies to 34, it also suggests a future line of inquiry regarding organization of the Lahontan cutthroat trout genome, work which may be aided by the rapid advancement of next-generation sequencing technologies.

Management of species of conservation concern is increasingly dependent upon molecular genetic techniques. The availability of multiple SNP markers for the Lahontan cutthroat trout will facilitate improved management by increasing information on population genetic structure as well as inter- and intraspecific hybridization. Future efforts to propagate and reintroduce cutthroat trout to streams where they have been extirpated will require such data to maintain the evolutionary integrity of the species.

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Data accessibility

DNA Sequences: NCBI (dbSNP) accession numbers in Table 4.
Genotype data: Dryad entry doi:10.5061/dryad.6nc19.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Putative functions of loci containing SNPs and SNP location with respect to gene features. Superscripts indicate species in which the best Blast match was found: [1]*O. mykiss*; [2]*Salmo salar*; [3]*Danio rerio*; [4]other. Only matches with an E-value $\leq 1e^{-15}$ are shown. Location abbreviations refer to the following: UTR, untranslated region; CDS, protein coding sequence; ss, synonymous polymorphism; ns, nonsynonymous polymorphism; n/a, not applicable (sequencing primers designed from known gene). Amino acid codes show changes generated by nonsynonymous substitutions.