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SHORT COMMUNICATION

SNPs reveal previously undocumented non-native introgression within threatened trout populations

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Abstract Two questions relevant to the management of species threatened by introgressive hybridization are whether results from different genetic marker are comparable, and whether all sources of introgression have been identified. We used recently-developed SNP markers to quantify introgression from two non-native taxa: rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout of the Yellowstone evolutionary lineage (*O. clarkii* subspp.), into populations of threatened Lahontan cutthroat trout (*O. c. henshawi*). Results for *O. mykiss* introgression largely agreed with those of previous studies using different genetic markers. However, three populations contained much genetic material from the Yellowstone lineage, a source of introgression not previously examined. This included one population proposed to be a remnant of an extinct cutthroat trout lineage.

Keywords Alvord · Lahontan · Yellowstone · Cutthroat trout · Hybridization · *Oncorhynchus clarkii*

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Introduction

Introgressive hybridization as a result of anthropogenic movement of taxa is a threat to many species of conservation concern. Ongoing interbreeding between native and non-native individuals can replace a pure population with a hybrid swarm (Allendorf et al. 2012). Even small amounts of non-native genetic material might increase the extirpation risk of a population via outbreeding depression (Edmands 2007). Effective conservation of such threatened species requires genetic markers to discriminate the hybridizing genomes. As genetic technology has advanced, new marker types have become available. While early studies of introgressive hybridization utilized allozymes and maternally-inherited mtDNA, later studies used AFLPs, microsatellites, and related markers. Currently, single nucleotide polymorphisms (SNPs), co-dominant markers which can allow examination of a large part of the genome, are a marker of choice (Morin et al. 2004). One challenge that managers face is whether to examine previously tested populations using newer markers; it is therefore interesting to know whether different types of genetic marker give congruent results. Another challenge is identifying all sources of non-native introgression in a population. There may be more than one evolutionarily distinct lineage posing a hybridization threat (Kalinowski 2010); markers developed to identify one of these lineages are unlikely to discriminate genetic material from the other lineages, so threats may be missed.

One species greatly threatened by introgressive hybridization from introduced taxa is the cutthroat trout (*Oncorhynchus clarkii*) of western North America. *O. clarkii* is considered to comprise nine extant allopatric subspecies within four major evolutionary lineages (Behnke 2002): coastal cutthroat trout (*O. c. clarkii*);

westslope cutthroat trout (*O. c. lewisi*), the Lahontan lineage (*O. c. henshawi*, *O. c. seleneris*), and the Yellowstone lineage (*O. c. bouvieri*, *O. c. utah*, *O. c. pleuriticus*, *O. c. stomias*, *O. c. virginalis*). Cutthroat trout are fully interfertile with other *Oncorhynchus* trout. Rainbow trout (*O. mykiss*) have been planted in vast numbers throughout the historical range of cutthroat trout and hybridization is well-documented (e.g. Weigel et al. 2003; Rubidge and Taylor 2004; Gunnell et al. 2008). However, introduced non-native cutthroat subspecies also threaten the genetic integrity of native taxa. Millions of Yellowstone cutthroat trout (*O. c. bouvieri*) were transplanted across the American West in the early twentieth century (Gresswell and Varley 1988), and naturalized populations occur in many states (e.g. Pritchard et al. 2007; Neville and Dunham 2011). Other subspecies have been transplanted less widely, but have also become established outside their native range (Neville and Dunham 2011; Metcalf et al. 2007). Much progress has been made in the identification of genetic material from rainbow trout within indigenous cutthroat trout populations (e.g. Ostberg and Rodriguez 2002; Pritchard et al. 2012). However, less attention has been paid to the influx of genes from non-native conspecifics (but see Kanda et al. 2002; Kalinowski 2010).

The Lahontan cutthroat trout (*O. c. henshawi*) is native to the Lahontan hydrographic basin of Nevada, Oregon and California (Behnke 2002). The subspecies is currently divided into three Geographic Management Units (GMUs), based on drainage history, morphology, ecology and genetics (Coffin and Cowan 1995; Nielsen and Sage 2002; Peacock and Kirchoff 2007). A phenotypically distinct population in a single subbasin is currently considered a separate subspecies (*O. c. seleneris*). Other phenotypically distinct populations in the Alvord Basin of southern Oregon, now lost through hybridization with rainbow trout, were suggested to comprise a third subspecies ('Alvord cutthroat trout', Bartley and Gall 1991; Behnke 2002). Historically, cutthroat trout from the Alvord Basin may have been transplanted to Guano Creek, Oregon (Behnke 2007). Hence, this stream may hold a remnant of this 'extinct' lineage; however it has also received Lahontan cutthroat trout from at least two other locations (Oregon Department of Fish and Wildlife 2005). Conversely, Lahontan cutthroat trout from the neighbouring Coyote Lake sub-basin were transplanted into 'fishless' streams in the Alvord Basin in the late twentieth century (Peacock et al. 2011).

Currently, Lahontan cutthroat trout occupy <10 % of their historical range, and are listed as 'Threatened' under the U.S. Endangered Species Act. Hybridization with rainbow trout is an ongoing threat and most extant populations have been screened using genetic markers diagnostic between *O. clarkii* and *O. mykiss* (allozymes, mtDNA, microsatellites and SSR loci; Elliot and Layton 2004; Peacock and Kirchoff 2004). The potential for introgressive hybridization by Yellowstone cutthroat trout has largely been ignored. Here, we used recently-developed SNP markers to investigate Lahontan cutthroat trout populations for the presence of genetic material from rainbow trout and the Yellowstone evolutionary lineage. Our study had three aims: (i) to examine whether results from SNPs were congruent with those from other genetic markers; (ii) to examine whether historical introductions of Yellowstone cutthroat trout or related subspecies threaten the genetic integrity of Lahontan cutthroat trout populations; and (iii) to further investigate the putative 'Alvord cutthroat trout' remnant population in Guano Creek.

Methods

Fin clips were collected between 1996 and 2008 from 33 trout populations (Peacock and Kirchoff 2007; Peacock et al. 2011, Table 1). All were considered, from previous genetic testing, to be pure Lahontan cutthroat trout, with the exception of Cascade Lake and Crowley, Guano, Sage and Three-Mile Creeks (Sevon et al. 1999; Elliot and Layton 2004; Peacock and Kirchoff 2004; Peacock unpublished data). Lahontan National Fish Hatchery broodstock ($n = 22$), considered to be pure and used for restoration purposes, were included as a Lahontan cutthroat trout reference. Reference samples were also obtained for Yellowstone cutthroat trout (15 individuals from six populations across the subspecies' range) and *O. mykiss* (19 individuals, from five steelhead populations and five hatchery rainbow trout strains) (for details see Pritchard et al. 2013).

Individuals were genotyped for: (i) 22 SNPs considered diagnostic between the Lahontan cutthroat trout lineage and all other *Oncorhynchus* trout; (ii) 14 SNPs considered diagnostic between the Lahontan lineage and rainbow trout; (iii) eight SNPs considered diagnostic between the Lahontan lineage and the subspecies of the Yellowstone evolutionary lineage, and (iv) two SNPs considered diagnostic between the Lahontan lineage and Yellowstone cutthroat trout (Pritchard et al. 2012, 2013, Supplementary data). Genotyping was carried out using TaqMan assays (Applied Biosystems Inc.) in 96.96 Dynamic Arrays on an EP1 Genotyping System (Fluidigm Corporation), with a pre-amplification step, following the manufacturer's protocol. Calls were determined using Fluidigm SNP Genotyping Analysis software (v3.0.2), with confidence threshold at 80 %. For logistical reasons unrelated to sample quality or identity, five of the 33 test populations were genotyped for only 20 or 21 of the 22 Lahontan diagnostic SNPs, and seven were genotyped for only 10 of

Table 1 The proportion of genetic material in each test population inferred by STRUCTURE to derive from each reference source

GMU	Drainage	UTM-N	UTM-E	Population	n	LCT	RT	YCT
Eastern	Humboldt River	4587792	442744	Abel Creek	21	**1.00**	0.00	0.00
Eastern	Humboldt River	4619288	637744	Marys River Basin Creek	21	**1.00**	0.00	0.00
Eastern	Humboldt River	4545873	571334	Beaver Creek	24	0.98	**0.02**	0.00
Eastern	Humboldt River	4537408	565840	Coyote Creek	23	**1.00**	0.00	0.00
Eastern	Humboldt River	4622288	673336	East Mary River	24	**1.00**	0.00	0.00
Eastern	Humboldt River	4591744	584612	Foreman Creek	21	**1.00**	0.00	0.00
Eastern	Humboldt River	4568988	524432	Frazer Creek	23	**1.00**	0.00	0.00
Eastern	Humboldt River	4615947	448061	South Fork Little Humboldt	24	**1.00**	0.00	0.00
Eastern	Humboldt River	4531722	565668	Little Jack Creek	24	**1.00**	0.00	0.00
Eastern	Humboldt River	4316288	468144	Mohawk Creek	17	**1.00**	0.00	0.00
Eastern	Humboldt River	4608636	643072	T Creek	20	**1.00**	0.00	0.00
Northwestern	Quinn River	4631672	413000	Crowley Creek	24	0.94	**0.06**	0.00
Northwestern	Quinn River	4669303	448449	Sage Creek	21	0.99	**0.01**	0.00
Northwestern	Quinn River	4630048	447528	Three Mile Creek	24	0.87	0.00	**0.13**
Northwestern	Quinn River	463848	418144	Washburn Creek	22	**1.00**	0.00	0.00
Coyote	Coyote Basin	4700493	406348	Cottonwood (Coyote)	8	**1.00**	0.00	0.00
Coyote	Coyote Basin	4701169	389635	L Whitehorse Creek	8	**1.00**	0.00	0.00
Coyote	Coyote Basin	4678142	397147	Willow Creek (Coyote)	21	**1.00**	0.00	0.00
Coyote (t)	Alvord Basin	4723609	375496	Cottonwood (Alvord)	8	**1.00**	0.00	0.00
Coyote (t)	Alvord Basin	4717630	373099	Little Alvord Creek	8	**1.00**	0.00	0.00
Coyote (t)	Alvord Basin	4805361	336581	Little McCoy Creek	16	0.69	0.00	**0.31**
Coyote (t)	Alvord Basin	4728591	374409	Mosquito Creek	16	**1.00**	0.00	0.00
Coyote (t)	Alvord Basin	4683928	395072	Willow Creek (Alvord)	15	**1.00**	0.00	0.00
Western	Carson River	4291863	261353	East Fork Carson River	22	**1.00**	0.00	0.00
Western	Carson River	4263639	263417	Murray Canyon Creek	14	**1.00**	0.00	0.00
Western	Cascade Lake	4384136	740101	Cascade Lake	6	0.43	**0.57**	0.00
Western	Pyramid Lake	4435897	281176	Pyramid Lake	22	**1.00**	0.00	0.00
Western	Walker River	4238560	296625	By-Day Creek	22	**1.00**	0.00	0.00
Western	Walker River	4280098	278470	Slinkard Creek	23	**1.00**	0.00	0.00
Western (t)	Bonneville Basin	4549281	750079	Bettridge Creek	22	**1.00**	0.00	0.00
Western (t)	Mokelumne River	4264902	248976	Marshall Canyon Creek	8	**1.00**	0.00	0.00
Western (t)	Yuba River	4376377	705155	Macklin Creek	22	**1.00**	0.00	0.00
Mixed (t)	Catlow Basin	4711819	261405	Guano Creek	21	0.45	0.03	**0.52**

For the LCT column, bold indicates populations for which the estimated 95 % confidence limits of LCT ancestry overlap 1.00 for every individual

For the RT and YCT columns, bold indicates populations containing at least one individual for whom the 95 % confidence limits of RT or YCT ancestry do not overlap 0.00

LCT Lahontan lineage, *RT* rainbow trout, *YCT* Yellowstone lineage, *GMU* Geographic Management Unit, *UTM* Universal Transverse Mercator coordinate, *n* number of genotyped individuals, *t* transplanted population

the 14 Lahontan-rainbow diagnostic SNPs (Supplementary data). This slightly reduces our power to detect non-native introgression in these populations. Only samples with at least 90 % of loci successfully genotyped were retained for analysis (671 out of 689 total individuals).

Genetic ancestry of individuals within each test sample was investigated using a Bayesian clustering approach implemented in STRUCTURE (v2.3.4, Pritchard et al. 2000). We applied a model of three genetic clusters, corresponding to the Lahontan lineage, the Yellowstone lineage, and rainbow trout. Individuals within the reference samples were defined as having 100 % ancestry from their respective cluster, with test individuals allowed to have mixed ancestry. Allele frequencies were assumed to be uncorrelated between the three evolutionarily independent clusters. We used a burn-in period of 10,000 followed by 50,000 MCMC replicates. The analysis was performed four times to ensure consistency of results. STRUCTURE was also

used to estimate 95 % confidence intervals of ancestry from each cluster.

Results and discussion

STRUCTURE inferred 26 populations to be pure Lahontan cutthroat trout (Table 1; Fig. 1). Four of the remaining populations—Cascade Lake and Beaver, Crowley and Sage Creeks—were inferred to contain genetic material from rainbow trout. These results were generally in agreement with those from previous studies, with three of these populations known or suspected to be introgressed by this taxon (Sevon et al. 1999; Elliot and Layton 2004; Peacock and Kirchoff 2004; Peacock, unpublished data). However, the small amount of non-native genetic material in Beaver Creek was previously un-documented, suggesting a potential introgression threat to this population that may have arisen recently and warrants further investigation.

Three populations were inferred to contain genetic material from the Yellowstone lineage: Three Mile, Little McCoy, and Guano Creeks (Table 1; Fig. 1). Yellowstone

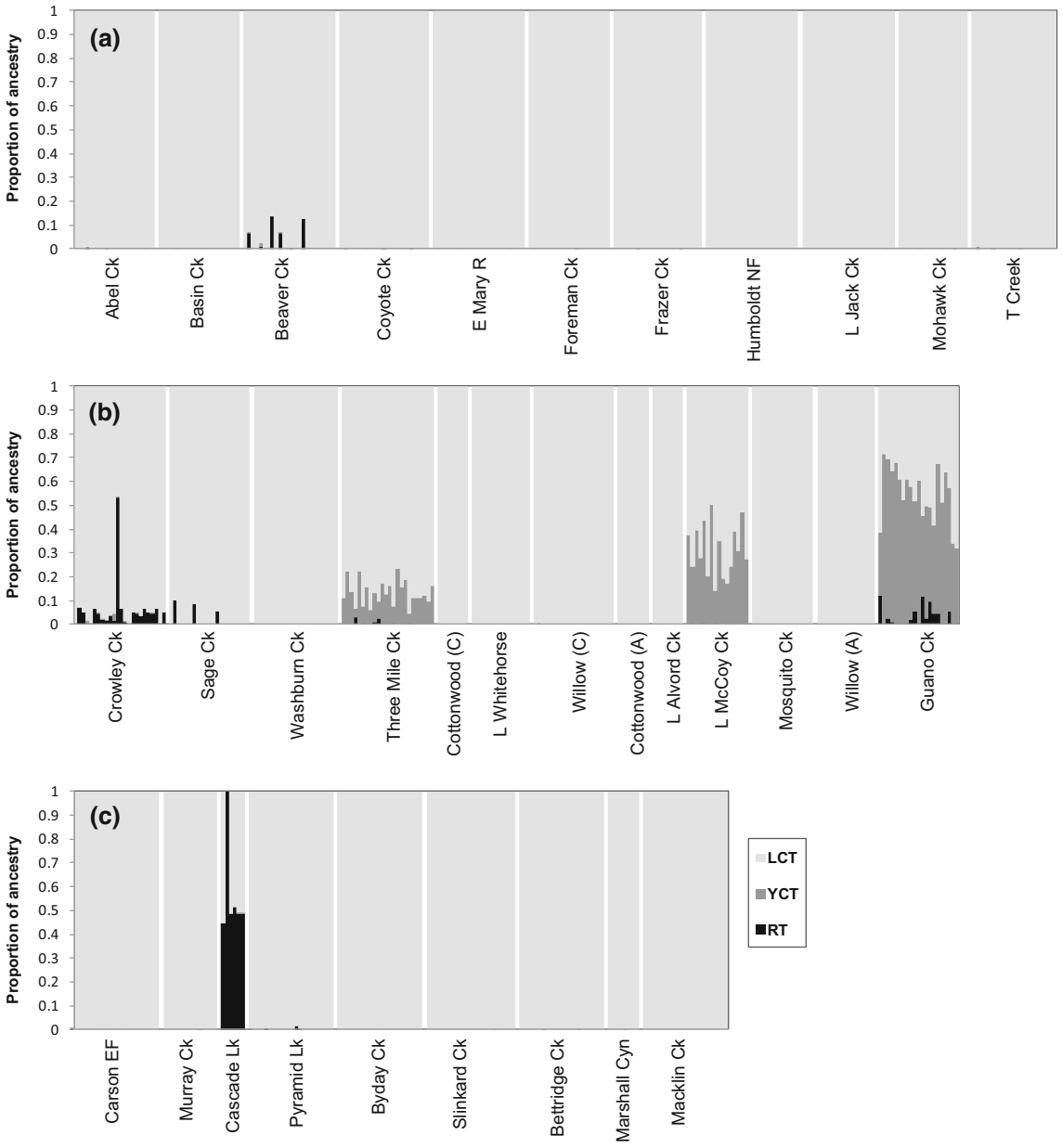

Fig. 1 Proportion of ancestry from the three reference populations, as estimated for each test individual using STRUCTURE. Test populations are ordered by Geographical Management Unit (GMU): **a** Eastern GMU; **b** Northwestern GMU including Coyote Lake Basin, and Guano Creek; **c** Western GMU. *LCT* Lahontan cutthroat trout, *YCT* Yellowstone lineage, *RT* rainbow trout

cutthroat trout mtDNA was documented in Three Mile Creek in 1985 (Sevon et al. 1999). Subsequent genetic analyses, investigating introgression from rainbow trout only, identified the population as 'pure' Lahontan, however we recently questioned this classification (Sevon et al. 1999; Pritchard et al. 2013). The Little McCoy Creek population in the Alvord Basin was founded in 1980 by a transplant from Willow Creek in the Coyote Lake Subbasin (Peacock et al. 2011). We found no evidence of Yellowstone lineage alleles within Willow Creek, nor within adjacent Alvord Basin populations, most of which were established in the same year from the same source population. This suggests either the existence of a trout population in Little McCoy Creek prior to transplantation, or a subsequent introduction. The presence of non-Lahontan genetic material in Little McCoy Creek may explain the elevated microsatellite diversity previously observed in pooled Alvord Basin transplant populations when compared with pooled Coyote Basin source populations (Peacock et al. 2011).

Guano Creek, suggested to harbour a remnant population of 'Alvord cutthroat trout', contained a large amount of genetic material from the Yellowstone lineage. This is most likely the result of undocumented Yellowstone cutthroat trout stocking. Nevertheless, we note that only two of our markers discriminate Yellowstone cutthroat trout from other members of the Yellowstone lineage. It follows that an evolutionary origin of 'Alvord cutthroat trout' within the Yellowstone rather than the Lahontan lineage would cause remnant Alvord genotypes, such as those proposed to be present in Guano Creek, and possibly also present in Little McCoy Creek, to assign to the Yellowstone cluster in the STRUCTURE analysis. Such an evolutionary affiliation appears unlikely, as trout in the neighbouring Coyote sub-basin are of Lahontan lineage and the two basins were intermittently connected during the Pleistocene (Carter et al. 2006). However, it has been suggested that the Coyote sub-basin populations are themselves transplants (Peacock and Kirchoff 2007). Genetic analysis of Alvord Basin museum samples and remnant hybridized cutthroat trout populations present there would further illuminate this issue.

In conclusion, estimates of rainbow trout introgression into Lahontan cutthroat trout populations, derived using newly developed SNP markers, were similar to earlier estimates using different markers. All but one of the 28 populations previously classified as pure Lahontan cutthroat trout were inferred to be free of rainbow trout ancestry in our new analysis. However, our finding of genetic material from the Yellowstone cutthroat trout lineage in several populations, including one previously believed to be pure Lahontan cutthroat trout, emphasizes the importance of considering all sources of non-native genetic material when managing taxa threatened by introgressive hybridization.

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