Title

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Journal

Conservation Genetics, 17(3)

ISSN

1566-0621

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Publication Date

2016-06-01

DOI

10.1007/s10592-016-0814-9

Peer reviewed

Conserv Genet
DOI 10.1007/s10592-016-0814-9

RESEARCH ARTICLE

Population genetic structure and ancestry of steelhead/rainbow trout (*Oncorhynchus mykiss*) at the extreme southern edge of their range in North America

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Received: 31 July 2015 / Accepted: 21 January 2016

Abstract Steelhead (*Oncorhynchus mykiss*) populations have declined dramatically in many parts of their range in North America, most critically in Southern California, where these anadromous trout are now classified as 'Endangered' under the United States Endangered Species Act. The widespread introduction of hatchery rainbow trout, the domesticated freshwater resident form of the species *O. mykiss*, is one factor threatening the long-term persistence of native steelhead and other trout populations. To identify where native fish of coastal steelhead lineage remained, we performed a population genetic analysis of microsatellite and SNP genotypes from *O. mykiss* populations at the extreme southern end of their range in Southern California, USA and Baja California, Mexico. In the northern part of this region, nearly all populations appeared to be primarily descendants of native coastal steelhead. However, in the southern, more urbanized part of this region, the majority of the sampled populations were derived primarily from hatchery trout, indicating either complete replacement of native fish or a strong signal of introgression overlaying native ancestry. Nevertheless, these genetically introgressed populations represent potentially critical genetic resources for the continued persistence of viable networks of *O. mykiss* populations, given the limited native ancestry uncovered in this region and the importance of genetic variation in adaptation. This study elucidates the geographic distribution of native trout populations in this region, and serves as a baseline for evaluating the impacts of hatchery trout on native *O. mykiss* populations and the success of steelhead conservation and recovery efforts.

Keywords *Oncorhynchus mykiss* · Rainbow trout · Steelhead · SNPs · Microsatellites · Introgression

This paper carries forward the memory of the late Skip Price who participated in this sampling effort and was an active member and leader in GSF and TU-Chapter 920. He is honored for his many contributions to conservation efforts throughout the region in fieldwork and educational programs.

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Introduction

The native distribution of the species *Oncorhynchus mykiss*, commonly known as rainbow trout, redband trout, or steelhead, extends from the Kamchatka Peninsula in northeastern Eurasia, across to North America, and south into northern Baja California, Mexico. While both trout and steelhead life-history forms reproduce in fresh water, steelhead are anadromous, while resident trout live entirely in freshwater (Busby et al. 1996). Steelhead numbers have declined dramatically in the past hundred years, especially at the southern end of the species' distribution and in heavily urbanized areas such as Southern California (Swift et al.

Published online: 01 February 2016

1993; Behnke 2002). A major contributor to this decline has been the creation of barriers to migration by dam construction and large-scale water development. This has isolated many *O. mykiss* populations in the remote headwaters of their native basins without access to the ocean, creating fragmented populations of native coastal steelhead ancestry that are forced to adopt a completely resident life history (Clemento et al. 2009). However, despite their native ancestry, all populations above complete natural or artificial barriers to anadromy are excluded from the steelhead Distinct Population Segments (DPSs) that are protected under the U.S. Endangered Species Act (ESA; NOAA 2006). Yet below-barrier spawning and rearing habitat accessible to anadromous steelhead has been extremely degraded, particularly in Southern California, leading to intensive efforts by federal and state agencies to restore habitat and protect them from extinction (NMFS 2012). Accordingly, the Southern California Coast Steelhead DPS, which extends from just north of Point Conception southward to the U.S.-Mexico border, is the only steelhead DPS listed as 'endangered', at the highest risk of extinction, under the U.S. Endangered Species Act (ESA; Good et al. 2005; NOAA 2006). Within this DPS, populations are further managed as five Biogeographic Population Groups (BPGs; NMFS 2012). Similarly, *O. mykiss* inhabiting the Río Santo Domingo basin in the Sierra de San Pedro Mártir in Baja California are considered of Special Concern and protected by Mexican laws (SEMARNAT 2010; Ruiz-Campos et al. 2014).

Although the species *O. mykiss* is native to the North Pacific and its tributaries, it has been introduced to every continent of the world, except Antarctica, due to intense demand for trout angling opportunities. This stocking of hatchery-produced rainbow trout has also occurred within the species native range, primarily in reservoirs above dams and in other locations where it was believed that anthropogenic disturbance would otherwise preclude trout angling (Clemento et al. 2009). This introduction of hatchery rainbow trout of diverse, but uniformly alien, ancestry into most Southern California streams and reservoirs presents an additional threat to the survival of native *O. mykiss* populations. Most hatchery rainbow trout strains used in California were domesticated from populations in the inland Sacramento River (Central Valley) basin (Busack and Gall 1980), a region in which the phylogenetically distinct steelhead are recognized as a separate DPS (Nielsen 1996, Nielsen et al. 1997b; NOAA 2006; Pearse and Garza 2015). From a conservation perspective, stocking hatchery trout can negatively impact native *O. mykiss* populations, because most hatchery rainbow trout strains are genetically depauperate and have experienced strong domestication selection (Busack and Gall 1980; Clemento et al. 2009; Pearse and Garza 2015). Thus, interbreeding with natural-origin populations can cause reduced fitness and maladaptation (Araki et al. 2007). Stocked trout may also compete with or predate upon native fish, potentially leading to complete replacement of the native population. Hence, genetic characterization of *O. mykiss* populations where stocking has occurred is needed to determine the extent of introgression by hatchery rainbow trout and identify extant natural-origin populations. Clemento et al. (2009) undertook such an evaluation in the northern part of the Southern California DPS, but ancestry of trout populations in the remainder of Southern California and Baja California has not been comprehensively examined.

Berg and Gall (1988) surveyed *O. mykiss* populations throughout California, including populations in the Southern California DPS, and found evidence of high genetic variability at 24 allozyme loci. A subsequent status review (Busby et al. 1996) also presented evidence for high genetic variability with allozymes in Southern California *O. mykiss* populations. Similar analyses with mitochondrial DNA control region sequences and nuclear microsatellite genotypes (Nielsen et al. 1994) again found higher variability than expected in these small populations. Further molecular genetic studies to define population structure of *O. mykiss* in the region have identified significant differentiation throughout Southern California and Mexico (Nielsen 1996; Nielsen et al. 1997b; Nielsen et al. 1998). However, none of this previous work attempted to distinguish native and hatchery-derived populations, and therefore could not determine whether the higher genetic diversity in the region was due to analysis of a combination of native and hatchery trout coming from diverse evolutionary lineages.

In recent years, a finer resolution view of *O. mykiss* population genetic structure in California has been developed through extensive analysis of both microsatellite and single nucleotide polymorphism (SNP) data (Abadía-Cardoso 2014; Aguilar and Garza 2006; Clemento et al. 2009; Garza et al. 2014; Pearse et al. 2007; Pearse and Garza 2015). Notably, in the Southern California and South Central California Coast steelhead DPSs, populations of *O. mykiss* have been found to have greater genetic similarity within a watershed than between proximate watersheds, even when separated by physical barriers (Clemento et al. 2009). In addition, the signal of high diversity in southern populations found by previous work has not been found when only native ancestry populations are analyzed. Also of relevance is a study of museum specimens from steelhead populations sampled in 1897 and 1909 (Pearse et al. 2011) that found a much stronger association between genetic distance and geographic distance separating populations historically than in the contemporary populations, presumably reduced due to anthropogenic influences such as construction of migration barriers, degradation of habitat and rainbow trout stocking.

Here, we conduct a large-scale population genetic analysis of *O. mykiss* at the extreme southern end of their

range, extending southward from the geographic focus area of Clemento et al. (2009). We used statistical analysis of data from more than 100 microsatellite and SNP loci to provide insight into the origins and ancestry of *O. mykiss* populations in the remaining aquatic habitat in this region. In addition, we evaluated data from two SNP loci located in a genomic region of *O. mykiss* chromosome Omy5 that has been shown to be associated with the anadromous and resident life-history strategies in the species (Pearse et al. 2014). These loci are in strong linkage disequilibrium in many coastal California *O. mykiss* populations, and show parallel changes in allele frequency in coastal *O. mykiss* populations isolated above natural and artificial barriers (Pearse et al. 2014). Our analysis provides greater insight into the adaptive landscape inhabited by Southern California *O. mykiss* and will be critical for informing recovery planning efforts for Southern California and Baja California *O. mykiss* populations.

Materials and Methods

Tissue sample collection

From 2009 to 2013, fish were captured by fly-fishing and netting in basins for which the waters with anadromous access are considered habitat of the Southern California Steelhead DPS (for details see Jacobson et al. 2014). A similar capture effort using hook and line was undertaken in 1994 in the Río Santo Domingo in the Sierra de San Pedro Mártir mountain range in Baja California, Mexico, the southernmost known population of *O. mykiss* in its native range. U.S. capture efforts occurred in 30 creeks and rivers spanning 10 watersheds extending south from the Santa Maria River to the Sweetwater River watershed immediately north of the U.S./Mexico international boundary (Fig. 1; Table 1). Approximately 600 fish total were captured and small caudal fin clips excised for genetic analysis before the fish were released in the location of capture. Tissue samples were also obtained from collections more than a decade before the current effort in three basins: Sespe Creek ($N = 40$), Pauma Creek ($N = 47$), and Sweetwater River ($N = 26$). These basins were resampled as part of the current study, providing temporal comparisons of genetic variation in these locations (Tables 1, 2).

DNA extraction and genotyping

Genomic DNA was extracted from dried fin clips using DNeasy 96 Tissue Kits (Qiagen Inc.) with a protocol modified for use on a BioRobot 3000 workstation (Qiagen). DNA was eluted in 200 µL Tris buffer for subsequent analyses. A total of 19 microsatellite loci used to study *O. mykiss* throughout California (Clemento et al. 2009; Garza

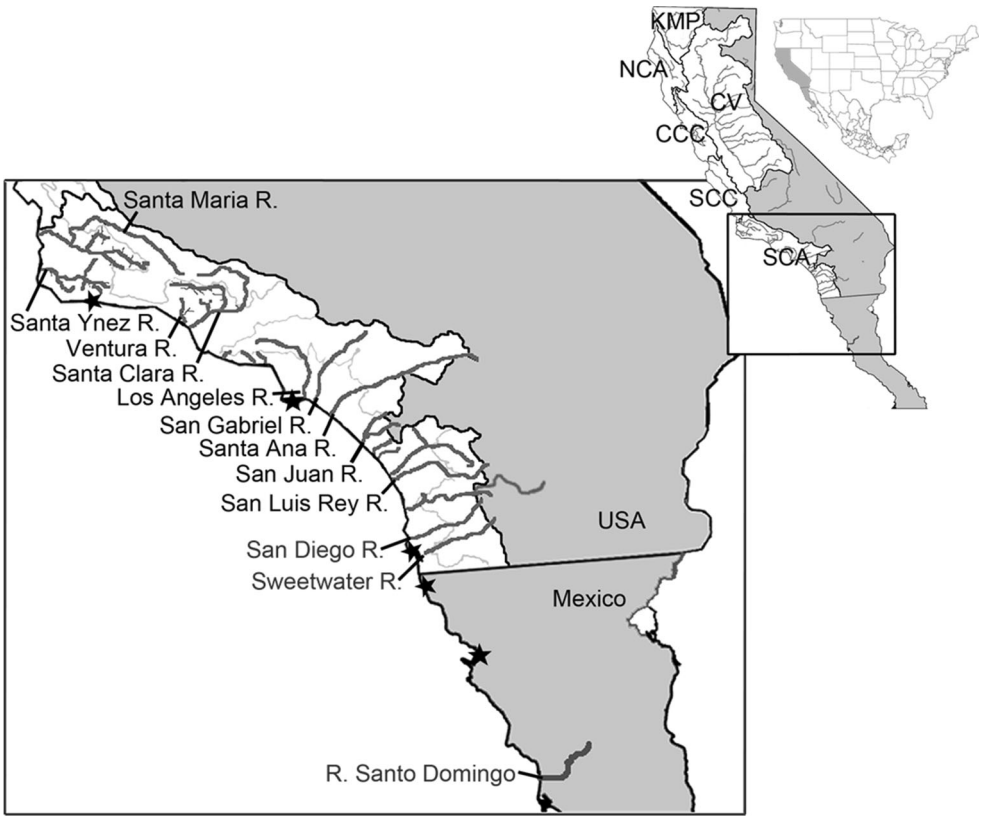

Fig. 1 Geographic location of the steelhead Distinct Population Segments in California. The *inset* shows the Southern California Steelhead DPS and the major watersheds analyzed in this study. *Stars* represent major cities: from north to south—Santa Barbara, Los Angeles, San Diego, Tijuana, and Ensenada. *KMP* Klamath Mountain Province, *NCA* Northern California Coast, *CV* California Central Valley, *CCC* Central California Coast, *SCC* South-Central California Coast, *SCA* Southern California

Table 1 Sample locations

DPS	County	Watershed	Tributary	Site	GPS coordinates	Population ID
Klamath Mountains Province						
	Del Norte	Klamath River		Blue Creek	41.442, −123.906	Klamath-Blue[a]
Northern California						
	Humboldt	Mattole River	Bear Creek	SF Bear Creek	40.033, −124.024	Mattole-Bear[a]
	Sonoma	Gualala River	SF Gualala River	Fuller Creek	38.698, −123.326	Gualala-Fuller[a]
Central Valley						
	San Francisco	Sacramento River	McCloud River	Claiborne Creek	41.057, −122.121	McCloud-Claiborne[a]
	San Francisco	Sacramento River		Battle Creek	40.384, −122.143	Sacramento-Battle[a]
	San Francisco	Sacramento River		Deer Creek	40.000, −121.966	Sacramento-Deer[a]
	San Francisco	Sacramento River	Feather River	Yuba River	39.570, −120.719	Sacramento-Feather-Yuba[a]
	San Francisco	Sacramento River	American River	NF American River	39.203, −120.613	American NF[a]
Central California Coast						
	San Mateo	San Francisquito Creek		Los Trancos Creek	37.407, −122.193	Los Trancos[a]
	Santa Cruz	Waddell Creek		Waddell Creek	37.116, −122.267	Waddell[a]
South-Central California Coast						
	Monterey	Carmel River		Carmel River	36.406, −121.679	Carmel[a]
	Monterey	Salinas River	Arroyo Seco Creek	Tassajara Creek	35.386, −120.681	Salinas-Tassajara[b]
	Monterey	Big Sur River		Big Sur River	36.245, −121.789	Big Sur[c]
	Monterey	Willow Creek		Willow Creek	35.894, −121.455	Willow[c]
	San Luis Obispo	Chorro Creek		Pennington Creek	35.342, −120.730	Chorro-Pennington[a]
	San Luis Obispo	San Simeon Creek		San Simeon Creek	35.618, −121.065	Sn Simeon[c]
Southern California						
	San Luis Obispo	Santa Maria River	Cuyama River	Reyes Creek	34.668, −119.306	Sta Maria-Cuyama-Reyes
	San Luis Obispo	Santa Maria River	Sisquoc River	Manzana Creek	34.735, −119.873	Sta Maria-Sisquoc-Manzana
	San Luis Obispo	Santa Maria River		Sisquoc River	34.877, −120.289	Sta Maria-Sisquoc[d]
	Santa Barbara	Montecito Creek		Montecito Creek	34.427, −119.640	Montecito
	Santa Barbara	Santa Ynez River	Quiota Creek	Quiota Creek	34.567, −120.094	Sta Ynez-Quiota
	Santa Barbara	Santa Ynez River		Santa Cruz Creek	34.633, −119.766	Sta Ynez-Sta Cruz[b]
	Santa Barbara	Santa Ynez River		Hilton Creek	34.580, −119.982	Sta Ynez-Hilton[b]
	Santa Barbara	Santa Ynez River		NF Juncal Creek	34.517, −119.501	Sta Ynez-Juncal[b]
	Santa Barbara	Santa Ynez River		Salsipuedes Creek	34.622, −120.392	Sta Ynez-Salsipuedes[b]
	Ventura	Ventura River		San Antonio Creek	34.381, −119.307	Ventura-Sn Antonio
	Ventura	Ventura River	Matilija Creek	NF Matilija Creek	34.506, −119.384	Ventura-Matilija[e]
	Ventura	Ventura River	NF Matilija Creek	Bear Creek	34.515, −119.267	Ventura-Matilija-Bear[b]
	Ventura	Santa Clara River		Santa Paula Creek	34.447, −119.067	Sta Clara-Sta Paula[b]

Table 1 continued

DPS	County	Watershed	Tributary	Site	GPS coordinates	Population ID
	Ventura	Santa Clara River		Piru Creek	34.706, −118.946	Sta Clara-Piru
	Ventura	Santa Clara River	Piru Creek	Buck Creek	34.662, −118.826	Sta Clara-Piru-Buck
	Ventura	Santa Clara River		Sespe Creek 1997	34.495, −118.945	Sta Clara-Sespe[d]
	Ventura	Santa Clara River	Sespe Creek	Piedra Blanca Creek	34.579, −119.167	Sta Clara-Sespe-Piedra Blanca
	Ventura	Santa Clara River	Sespe Creek	Lion Canyon Creek	34.380, −118.955	Sta Clara-Sespe-Lion Canyon[e]
	Los Angeles	Los Angeles River	Rio Hondo	Santa Anita Creek	34.201, −118.018	Los Angeles-Hondo-Sta Anita
	Los Angeles	San Gabriel River		NF San Gabriel River	34.291, −117.840	NF Sn Gabriel
	Los Angeles	San Gabriel River	WF San Gabriel River	Bear Creek	34.252, −117.884	WF Sn Gabriel-Bear
	Los Angeles	San Gabriel River	WF San Gabriel River	Devil's Canyon Creek	34.263, −117.973	WF Sn Gabriel-Devil's Canyon
	Los Angeles	San Gabriel River		WF San Gabriel River	34.246, −118.049	WF Sn Gabriel
	Los Angeles	San Gabriel River	EF San Gabriel River	Cattle Creek	34.230, −117.751	EF Sn Gabriel-Cattle
	Los Angeles	San Gabriel River	EF San Gabriel River	Fish Fork	34.308, −117.727	EF Sn Gabriel-Fish Fork
	Los Angeles	San Gabriel River	EF San Gabriel River	Iron Fork	34.311, −117.765	EF Sn Gabriel- SFIron Fork
	Los Angeles	San Gabriel River	EF San Gabriel River	EF Iron Fork	34.298, −117.752	EF Sn Gabriel- Iron Fork
	Los Angeles	San Gabriel River		EF San Gabriel River	34.284, −117.745	EF Sn Gabriel
	Los Angeles	San Gabriel River		Fish Canyon Creek	34.177, −117.929	Sn Gabriel-Fish Canyon
	Orange	Santa Ana River	Chino Creek	San Antonio Creek	34.238, −117.655	Sta Ana-Chino-Sn Antonio
	Orange	Santa Ana River		Bear Creek	34.154, −117.019	Sta Ana-Bear
	Orange	Santa Ana River	Temescal Wash	Coldwater Canyon Creek	33.754, −117.495	Sta Ana-Temescal-Coldwater Canyon
	Orange	Santa Ana River	Temescal Wash	Fuller Mill Creek	33.793, −116.749	Sta Ana-Temescal-Fuller Mill
	Orange	Santa Ana River	Temescal Wash	NF San Jacinto Creek 2009	33.793, −116.747	Sta Ana-Temescal-Sn Jacinto09
	Orange	Santa Ana River	Temescal Wash	NF San Jacinto Creek 2012	33.793, −116.749	Sta Ana-Temescal-Sn Jacinto12
	Orange	San Juan Creek		Arroyo Trabuco Creek	33.674, −117.526	Sn Juan-Arroyo Trabuco
	San Diego	San Luis Rey River		Doane Creek	33.349, −116.913	Sn Luis Rey-Doane
	San Luis Rey River		Pauma Creek 1997	33.331, −116.974	PaumaO (old 1997)[d]	
	San Diego	San Luis Rey River		Pauma Creek 2009 − 11	33.347, −116.925	PaumaR (recent 2009–2011)
	San Diego	San Luis Rey River		WF San Luis Rey	33.328, −116.801	WF Sn Luis Rey
	San Diego	San Diego River		Boulder Creek	32.972, −116.637	Sn Diego-Boulder
	San Diego	Sweetwater River		Sweetwater River 1997	32.913, −116.570	Sweetwater (old 1997)[e]
	San Diego	Sweetwater River		Sweetwater River 2010/2013	32.9080, −116.579	Sweetwater (recent 2010/2013)
O. mykiss nelsoni						
	Baja California	Río Santo Domingo	Río San Antonio	San Antonio/La Grulla	30.820, −115.625	*O. m. nelsoni*[d]
Hatchery strains						
				Fillmore Hatchery	N/A	FH-Coleman[b]
				Fillmore Hatchery	N/A	FH-Virginia[b]
				Fillmore Hatchery	N/A	FH-Whitney[f]

Table 1 continued

DPS	County	Watershed	Tributary	Site	GPS coordinates	Population ID
				Fillmore Hatchery	N/A	FH-Wyoming[b]
				American River Hatchery	N/A	ARH-Eagle[f]
				American River Hatchery	N/A	ARH-Shasta[d]
				Hot Creek Hatchery	N/A	HC-Kamloops[g]

County indicates location where a given watershed enters the ocean, not the specific collecting location within the watershed. Similarly, Distinct Population Segment (DPS) designation is the one applied where the watershed enters the ocean, regardless of whether fish from the specific sampling location are considered members NF=North Fork, SF=South Fork, EF=East Fork, WF=West Fork

N/A not applicable

[a] SNP genotypes from Abadía-Cardoso et al. (2015) and microsatellites from Garza et al. (2014)

[b] Microsatellite genotypes from Clemento et al. (2009)

[c] Microsatellite genotypes from Garza et al. (2014)

[d] SNP and microsatellite genotypes from Abadía-Cardoso et al. (2015)

[e] SNP genotypes from Abadía-Cardoso et al. (2015) and microsatellites from Clemento et al. (2009)

[f] SNP genotypes from Abadía-Cardoso et al. (2011) and microsatellites from Clemento et al. (2009)

[g] SNP genotypes from Abadía-Cardoso et al. (2011) and microsatellites from Abadía-Cardoso et al. (2015)

et al. 2014) were genotyped on all samples. Polymerase chain reaction (PCR) amplification was performed with conditions as reported by Clemento et al. (2009). PCR products were electrophoresed and detected on an ABI 3730XL DNA Analyzer (Applied Biosystems, Inc.) with the use of fluorescently labeled primers. Genotypes were called using GeneMapper (Applied Biosystems) and all allele calls checked for consistency with previously genotyped reference samples. To reduce error rate and control for bias in calling alleles, all genotypes were derived twice independently and two people resolved any discrepancies between first and second calls.

A total of 93 SNP loci were also genotyped on all samples evaluated in the current study. These SNP markers include loci from Aguilar and Garza (2008), Campbell et al. (2009), and Abadía-Cardoso et al. (2011) and were genotyped with 5′-nuclease chemistry (TaqMan™, Life Technologies, Inc.) on an EP1 system (Fluidigm Corporation). See Abadía-Cardoso et al. (2013) for details of the pre-amplification and genotyping conditions.

To provide a broader phylogeographic context, genotypes for the same microsatellite and SNP markers from other *O. mykiss* populations, including representatives of all six California steelhead DPSs ($N = 1152$) and seven hatchery rainbow trout strains ($N = 331$), were included in some analyses (Tables 1 and 2).

Population genetic diversity and structure

Observed (H_O) and expected (H_E) heterozygosity and deviations from Hardy–Weinberg (HW) equilibrium were estimated using GENEPOP (Rousset 2008). The percentage of polymorphic SNPs (P) at 0.95 and 0.99 allele frequency levels was estimated with the software GENETIX (Belkhir et al. 1996–2004), and microsatellite allelic richness (A_R) calculated using the rarefaction method implemented in HP-RARE (Kalinowski 2005).

The combined genetic data were analyzed using several methods for evaluating population structure and historical ancestry. First, the model-based clustering method employed in the software STRUCTURE (Pritchard et al. 2000) was used to investigate patterns of ancestry in all *O. mykiss* populations and in individual fish. This Bayesian analysis is based on individual multi-locus genotypes and uses hypotheses about the number of clusters (e.g., populations or lineages), K, represented by the dataset to infer ancestry and the level of interbreeding within and between groups without reference to any prior information about the geographic location or the population affiliation of any of the constituent samples. Values of $K = 2$–7 were investigated and five iterations were performed for each K value, with a burn-in period of 50,000 steps and 150,000 Markov chain Monte Carlo repetitions. The results were reordered and visualized

Table 2 Summary statistics of all populations analyzed

Population ID	*N*	H_O	H_E	*Ar*	*P*(0.95)	*P*(0.99)	r^2
Klamath-Blue	32	0.38	0.40	5.32	0.90	0.97	0.930
Mattole-Bear	31	0.41	0.40	4.57	0.88	0.97	0.999
Gualala-Fuller	61	0.45	0.44	4.78	0.98	0.99	0.999
McCloud-Claiborne	33	0.38	0.38	4.29	0.79	0.92	–
Sacramento-Battle	92	0.41	0.42	5.04	0.97	1.00	0.240
Sacramento-Deer	43	0.44	0.43	5.26	0.98	1.00	0.369
Sacramento-Feather-Yuba	27	0.44	0.44	4.78	0.94	1.00	0.996
American NF	48	0.39	0.39	4.36	0.91	0.98	0.672
Los Trancos	24	0.41	0.40	3.98	0.89	0.93	0.999
Waddell	31	0.41	0.41	3.96	0.95	0.96	0.997
Carmel	32	0.43	0.43	4.59	0.95	0.98	0.675
Salinas-Tassajara	46	0.42	0.42	4.16	0.95	0.99	0.877
Big Sur	31	0.43	0.43	4.77	0.98	0.99	0.999
Willow	27	0.39	0.41	4.80	0.94	1.00	1.000
Chorro-Pennington	30	0.40	0.37	3.67	0.86	0.90	0.999
Sn Simeon	31	0.44	0.42	4.58	0.95	0.98	0.942
Sta Maria-Cuyama-Reyes	47	0.41	0.41	4.08	0.94	0.99	1.000
Sta Maria-Sisquoc-Manzana	47	0.36	0.34	3.30	0.79	0.88	0.585
Sta Maria-Sisquoc	47	0.37	0.37	3.76	0.91	0.96	0.790
Montecito	5	0.37	0.32	–	–	–	–
Sta Ynez-Quiota	35	0.40	0.39	3.77	0.91	0.98	0.887
Sta Ynez-Sta Cruz	35	0.38	0.38	4.08	0.88	0.93	0.999
Sta Ynez-Hilton	42	0.40	0.40	3.93	0.90	0.98	0.379
Sta Ynez-Juncal	81	0.38	0.37	3.53	0.81	0.95	0.990
Sta Ynez-Salsipuedes	47	0.36	0.38	3.79	0.90	0.95	0.999
Ventura-Sn Antonio	5	0.37	0.37	–	–	–	–
Ventura-Matilija	46	0.38	0.37	3.70	0.86	0.96	0.732
Ventura-Matilija-Bear	14	0.37	0.35	3.47	0.79	0.86	0.882
Sta Clara-Sta Paula	45	0.41	0.42	4.34	0.93	0.97	1.000
Sta Clara-Piru	26	0.34	0.35	3.82	0.83	0.94	0.999
Sta Clara-Piru-Buck	16	0.36	0.37	3.61	0.88	0.93	0.997
Sta Clara-Sespe	39	0.36	0.37	4.13	0.90	0.98	0.999
Sta Clara-Sespe-Piedra Blanca	10	0.32	0.33	3.35	0.89	0.89	0.999
Sta Clara-Sespe-Lion Canyon	47	0.36	0.36	3.95	0.88	0.98	0.909
Los Angeles-Hondo-Sta Anita	23	0.25	0.33	2.83	0.79	0.90	0.890
NF Sn Gabriel	16	0.37	0.39	4.18	0.91	0.97	0.802
WF Sn Gabriel-Bear	22	0.38	0.38	4.06	0.89	0.98	0.832
WF Sn Gabriel-Devil's Canyon	13	0.33	0.31	2.65	0.69	0.71	–
WF Sn Gabriel	22	0.32	0.34	3.32	0.80	0.90	1.000
EF Sn Gabriel-Cattle	16	0.38	0.40	4.19	0.93	0.96	0.999
EF Sn Gabriel-Fish Fork	11	0.40	0.40	3.89	0.82	0.88	0.998
EF Sn Gabriel-SFIron Fork	15	0.37	0.35	2.91	0.80	0.82	0.000
EF Sn Gabriel-Iron Fork	28	0.37	0.37	3.52	0.91	0.97	0.350
EF Sn Gabriel	18	0.38	0.39	4.13	0.90	0.98	0.724
Sn Gabriel-Fish Canyon	5	0.36	0.35	–	–	–	0.999
Sta Ana-Chino-Sn Antonio	36	0.39	0.40	3.84	0.89	0.93	0.052
Sta Ana-Bear	22	0.38	0.40	4.57	0.83	0.94	0.645
Sta Ana-Temescal-Coldwater Canyon	19	0.13	0.12	1.76	0.30	0.32	0.485

Table 2 continued

Population ID	*N*	H_O	H_E	*Ar*	*P*(0.95)	*P*(0.99)	r^2
Sta Ana-Temescal-Fuller Mill	16	0.34	0.27	2.50	0.57	0.71	–
Sta Ana-Temescal-Sn Jacinto09	12	0.36	0.35	3.01	0.78	0.84	0.996
Sta Ana-Temescal-Sn Jacinto12	24	0.31	0.34	3.04	0.85	0.90	0.428
Sn Juan-Arroyo Trabuco	14	0.39	0.41	4.12	0.94	0.98	0.532
Sn Luis Rey-Doane	3	0.28	0.27	–	–	–	0.999
PaumaO (old 1997)	39	0.38	0.38	3.92	0.87	0.97	0.813
PaumaR (recent 2009–2011)	26	0.31	0.32	2.93	0.78	0.82	0.828
WF Sn Luis Rey	12	0.26	0.26	2.53	0.59	0.66	0.999
Sn Diego-Boulder	11	0.37	0.34	3.09	0.80	0.83	0.353
Sweetwater (old 1997)	26	0.36	0.37	3.58	0.86	0.94	0.229
Sweetwater (recent 2010/2013)	37	0.38	0.37	3.69	0.83	0.92	0.409
O. m. nelsoni	39	0.16	0.19	2.27	0.43	0.55	0.999
FH-Coleman	47	0.38	0.38	4.04	0.91	0.98	0.220
FH-Virginia	48	0.31	0.33	3.82	0.79	0.89	0.070
FH-Whitney	48	0.37	0.37	3.64	0.83	0.94	0.563
FH-Wyoming	47	0.39	0.39	3.96	0.89	0.94	0.100
ARH-Eagle	47	0.30	0.30	3.38	0.76	0.94	0.997
ARH-Shasta	47	0.35	0.36	3.32	0.81	0.92	0.121
HC-Kamloops	47	0.29	0.29	3.84	0.72	0.79	–

N number of samples genotyped for the genetic analysis, H_O Observed heterozygosity, H_E Expected heterozygosity, *Ar* Microsatellite allelic richness, *P* Percentage of polymorphic SNPs, r^2 linkage disequilibrium between the two Omy5 loci. – indicates that r^2 could not be calculated

using the software CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

Phylogeographic dendrograms were constructed using Cavalli-Sforza and Edwards (1967) chord distances and the neighbor-joining method (Takezaki and Nei 1996), with the software PHYLIP (Felsenstein 2005). Markers for which genotypes were lacking for any entire population were excluded, leaving 13 microsatellite and 90 SNP loci. For this analysis, population samples with mixed ancestry evident in the STRUCTURE results were first sub-divided to reflect differences detected among sampling sub-locations and times. Allele frequencies were bootstrapped 10,000 times and the resulting datasets used to construct dendrograms to evaluate statistical support for branching patterns.

Finally, a principal component analysis (PCA) was used to graphically explore differentiation and relationships among all populations and individuals using the R-based package *adegenet* 1.3-4 (Jombart 2008). Populations were plotted to indicate four groups (northern coastal steelhead, hatchery/Central Valley, *O. m. nelsoni*, and Southern California DPS basin populations).

Life history adaptation

Two of the SNP loci genotyped in all individuals are located on chromosome Omy5 in a genomic region strongly associated with the prevalence of resident or anadromous life history traits in coastal California *O. mykiss* populations (Pearse et al. 2014). Analysis of these two SNPs in Central Valley *O. mykiss* populations, from which the majority of hatchery rainbow trout strains used in California were derived, has shown similar patterns of allele frequency variation but with significantly reduced linkage disequilibrium relative to coastal populations (Pearse and Garza 2015). We examined patterns of variation in these two markers to (1) explore the association between adaptive variation at Omy5 and migratory life history patterns in Southern and Baja California *O. mykiss* and (2) further evaluate the extent of genetic introgression by hatchery trout in these populations, as inferred from linkage disequilibrium between the two markers using the allelic correlation coefficient (r^2) calculated in the R package *genetics* (Warnes 2003).

Results

Genotypes of 577 individual fish were combined with data from previously genotyped *O. mykiss* from throughout California, including seven hatchery rainbow trout strains, for a total of genotypes from 2109 fish (Table 2). These genotypes consisted of 106–110 loci per population, and genotypes of 25 fish were removed from all analyses due to missing data (≥10 microsatellite or SNP loci missing). This combined dataset provided increased resolution

relative to single marker-type datasets or those based on fewer loci (Narum et al. 2008).

Genetic diversity statistics

None of the microsatellite or SNP loci deviated significantly from HW equilibrium after correction for multiple comparisons. Southern California DPS populations generally had lower heterozygosity than northern populations, and had variation similar to the hatchery rainbow trout strains. The northernmost of the BPGs, the Monte Arido Highlands BPG, containing the Santa Maria, Santa Ynez, Ventura, and Santa Clara rivers, had H_O values that ranged from 0.32 to 0.41. Further south, the Mojave Rim BPG, containing the Los Angeles, San Gabriel, and Santa Ana rivers, had H_O values with a wider range (0.13–0.40), but the majority of samples came from tributaries of the San Gabriel River and values for those populations were generally high (0.33–0.40). Of note is that the southernmost sampled population in this BPG is in a small currently land-locked tributary of the Santa Ana River (Sta Ana-Temescal-Coldwater Canyon), and had strikingly low heterozygosity ($H_O = 0.13$ and $H_E = 0.12$; Table 2). The southernmost Santa Catalina Gulf Coast BPG, comprised of populations from Orange and San Diego counties, had H_O that ranged from 0.26 to 0.39. In contrast, H_O in coastal populations from the northern DPSs ranged from 0.38 to 0.45.

Consistent with the above observations, Northern California populations had higher microsatellite allele richness, and a higher proportion of polymorphic SNPs (Table 2). Microsatellite *Ar* was highest in the Klamath-Blue (5.32) and lowest in the Sta Ana-Temescal-Coldwater Canyon population (1.76). Gualala-Fuller, Sacramento-Deer and Big Sur had the highest proportion of polymorphic SNPs at 0.95 (0.98), while Sacramento-Feather-Yuba, Sacramento-Battle, Sacramento-Deer, and Willow were highest at 0.99 (1.00). Sta Ana-Temescal-Coldwater Canyon again had the lowest variation, with SNP polymorphism of $P(0.95) = 0.30$ and $P(0.99) = 0.32$.

Population genetics

Model-based clustering at low K values (Fig. 2a) gave an initial indication of whether populations and individuals were primarily of native coastal steelhead or hatchery rainbow trout ancestry. Populations of northern and central

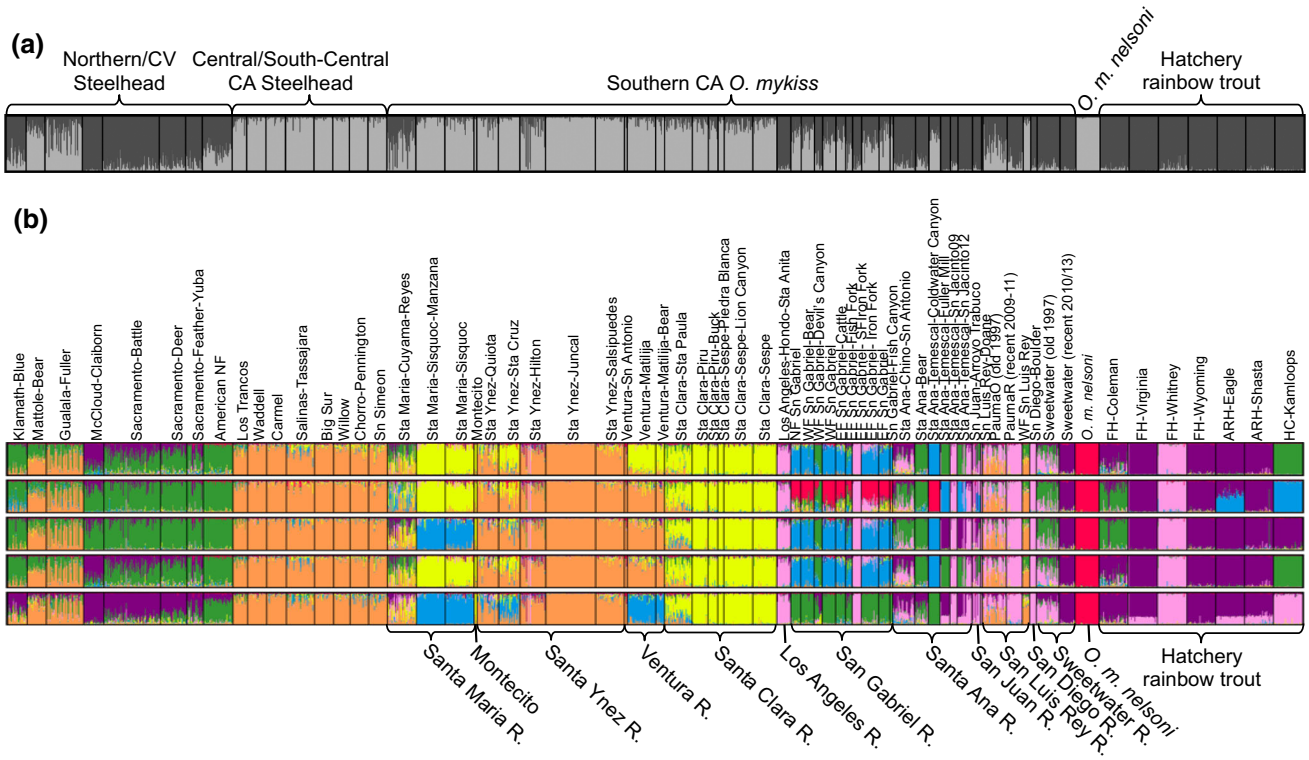

Fig. 2 Results from STRUCTURE showing individual fractional ancestry and genetic relationships. Each *vertical line* represents an individual's fractional assignment to each of K genetic lineages. Sampling locations are separated with *black lines*, ordered from north to south. **a** $K = 2$ genetic clusters in the data (five out of five replicates had the same pattern). Individuals represented in *dark grey* from Southern CA are derived primarily from hatchery rainbow trout lineages. Individuals with more *light grey* represent ancestry of coastal steelhead lineage, while intermediate values indicate introgression and shared ancestry from both lineages. **b** $K = 7$ genetic clusters, showing concordance and variability among five runs

California coastal steelhead were clearly differentiated from hatchery rainbow trout and Central Valley *O. mykiss*. The predominance of native coastal *O. mykiss* ancestry (light grey in Fig. 2a) in Southern California decreased in the more southern regions, with many populations of mixed or completely hatchery ancestry (dark grey in Fig. 2a).

At higher values of K, population structure within and between basins became evident, as did the distinctiveness of many of the populations. Examination of individual fractional ancestry at $K = 7$ clearly identified both native and hatchery origin/introgressed fish among the Southern California DPS (Fig. 2b). Individuals from the Santa Clara River tributaries (Piru, Santa Paula and Sespe creeks) were of native coastal steelhead descent, consistent with the results of Clemento et al. (2009). Further south, only four groups of contemporary fish contained significant native coastal steelhead ancestry: 1) populations from the San Gabriel River system, 2) Coldwater Canyon Creek in the Santa Ana River, 3) the West Fork San Luis Rey River, and 4) *O. mykiss nelsoni* from the Santo Domingo River in Baja California. Differences among some hatchery trout strains were also discernible, with apparent widespread stocking of the Mount Whitney strain (Fig. 2b). Moreover, *O. mykiss nelsoni* was consistently identified as distinct from all other population samples (Fig. 2b).

Population-level phylogeographic relationships, reconstructed with a neighbor-joining tree (Fig. 3), were consistent with the clustering results. The division between native coastal steelhead lineage populations and hatchery trout/Central Valley populations was clear and is summarized on

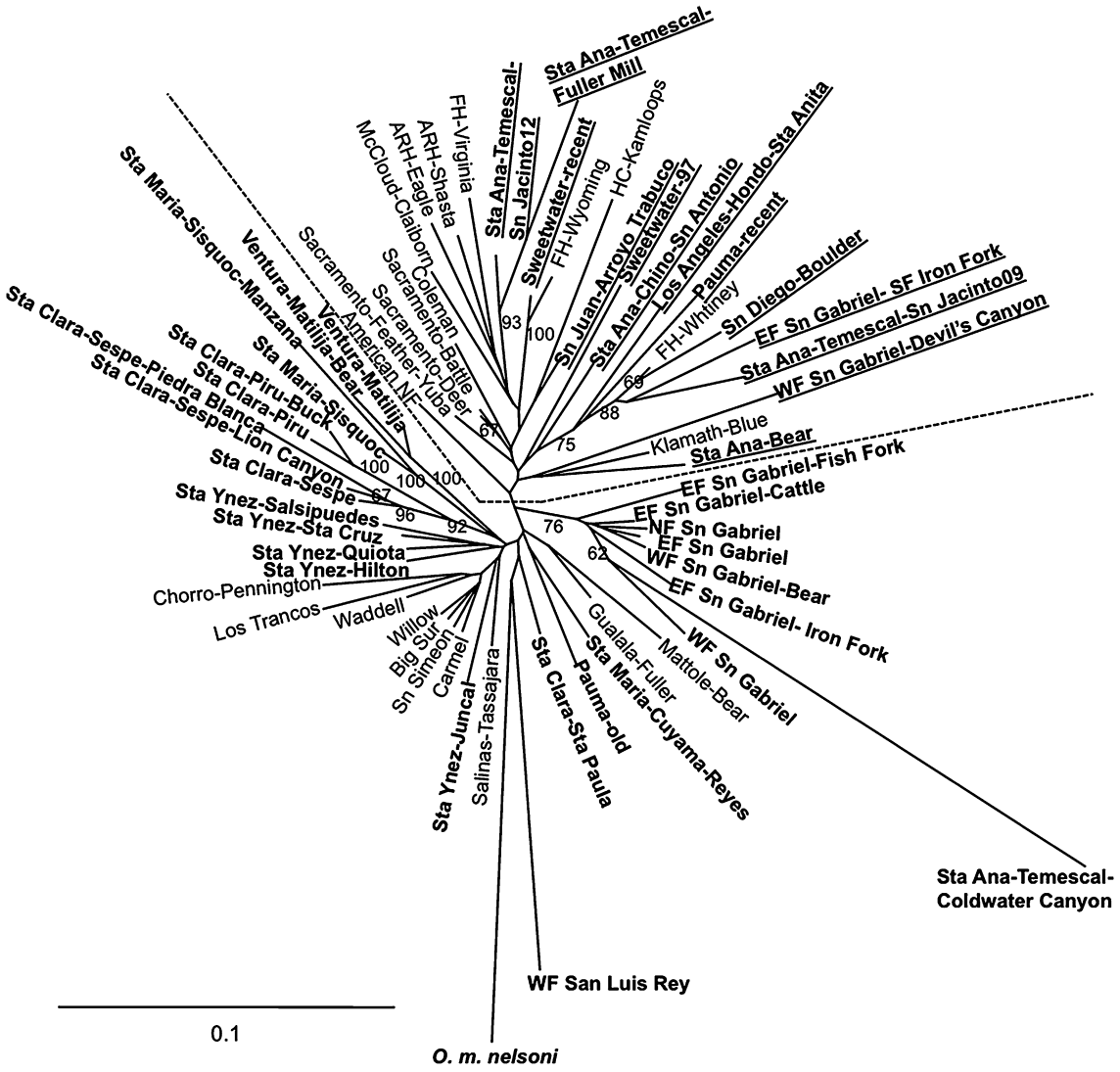

Fig. 3 Neighbor-joining tree of all populations included in the analysis, with bootstrap support for internal branches of > 60 % shown. Note that the *dashed line* almost perfectly divides coastal steelhead lineage populations (*lower section*) from those with significant hatchery rainbow trout/Central Valley ancestry (*upper section*). The Southern California DPS populations are indicated in *bold*, and those that cluster with the hatchery rainbow trout strains are *underlined*

the tree with a dashed line on the central branch that divides them (Fig. 3). Southern California populations of native coastal steelhead ancestry were also delineated as were the relationships between trout of hatchery/Central Valley lineage and populations in Southern California that were primarily the result of hatchery stocking (underlined). Populations from Los Angeles-Hondo-Sta Anita, Pauma-recent and Sn Diego-Boulder were most closely related to Whitney hatchery strain, while the Sn Juan-Arroyo Trabuco population was most closely related to the Kamloops strain. Moreover, population replacement over time was evident in the comparison of samples taken > 10 years apart in Pauma Creek and the Sweetwater River. The hatchery-origin population found in the upper reaches of Pauma Creek in the recent sampling apparently replaced the mostly native trout population sampled in 1997 (Pauma-old). These results are also consistent with the results from the STRUCTURE analysis (Fig. 2b).

Finally, the results from the PCA were consistent with those observed in the STRUCTURE analysis and the neighbor-joining tree. The PCA confirmed the division between the native coastal steelhead populations and the hatchery trout/Central Valley populations, as well as the distinctiveness of *O. mykiss nelsoni* (Fig. 4). The close relationship between some Southern California populations and the hatchery trout/Central Valley populations was also evident by the co-clustering of these lineages in the right half the plot (Fig. 4).

Life history patterns

Both the allele frequencies of the two Omy5 SNP loci and patterns of linkage disequilibrium between them provided

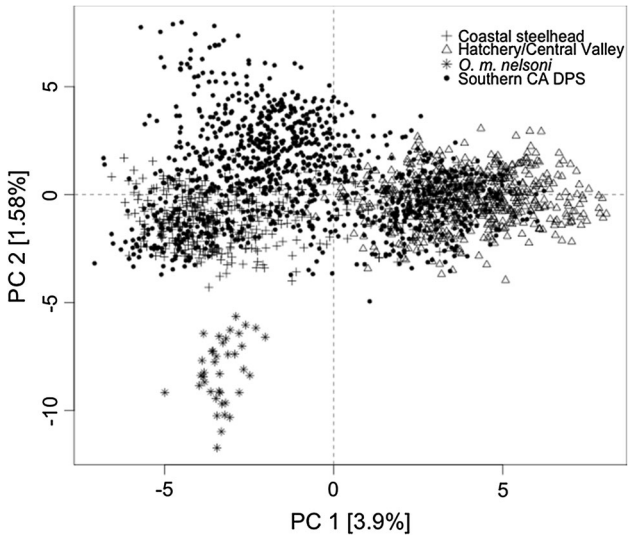

Fig. 4 Principal component analysis (PCA) based on allele frequencies from all 114 genetic markers. Populations were pooled into four major groups, representing coastal steelhead, hatchery/Central Valley *O. mykiss*, *O. m. nelsoni*, and Southern California DPS populations

inference about the ancestry and presumptive life history of Southern California *O. mykiss* populations. First, unlike northern coastal steelhead populations (Pearse et al. 2014), most populations in Southern California had very low frequencies of alleles associated with anadromy at both Omy5 loci (Fig. 5), consistent with their limited opportunities to express this life history. In addition, strong LD and concordant allele frequencies of both markers were present in most coastal California steelhead populations (Table 2), while inland rainbow trout populations, particularly those from the Central Valley, where most of the hatchery rainbow trout strains originated, showed strong discordance and lower LD (Pearse and Garza 2015). Southern California populations that were identified as having hatchery trout ancestry with the presumably neutral genetic loci also displayed low LD between the two Omy5 loci, providing additional evidence that these populations were composed of hatchery rainbow trout, their descendants or introgressed hybrids rather than native coastal steelhead origin fish (Fig. 5).

Discussion

The population genetic analysis of Southern and Baja California *O. mykiss* populations described here identified two major lineages of trout in this region: native coastal steelhead and introduced hatchery rainbow trout. Many populations fell into the second category, representing almost complete introgression or replacement of native fish by introduced hatchery trout. Among the populations in tributaries of rivers that run through the highly urbanized areas of Southern California, only three groups of populations contained significant evidence of native coastal steelhead ancestry: (1) populations from the San Gabriel River system, (2) Coldwater Canyon in the Santa Ana River, and (3) the San Luis Rey River. The analyses also further established the native origin of *O. mykiss nelsoni* from the Sierra San Pedro Martir in Baja California as the southernmost representative of the coastal steelhead lineage in North America (Abadía-Cardoso et al. 2015). The geographic distribution of the US populations in the context of their respective BPGs is shown in Jacobson et al. (2014). The three groups in Southern California with substantial native ancestry, inferred from concordant results of multiple analyses, should be prioritized for conservation planning so as to ensure their persistence. However, some other populations, most notably Bear Creek in the Santa Ana River and Devil's Canyon Creek in the West Fork San Gabriel River, contained remnants of native ancestry overlaid with substantial introgressive hybridization with hatchery rainbow trout. While these populations are not pure native Southern California trout, they may be self-sustaining and adapting to the current local environment.

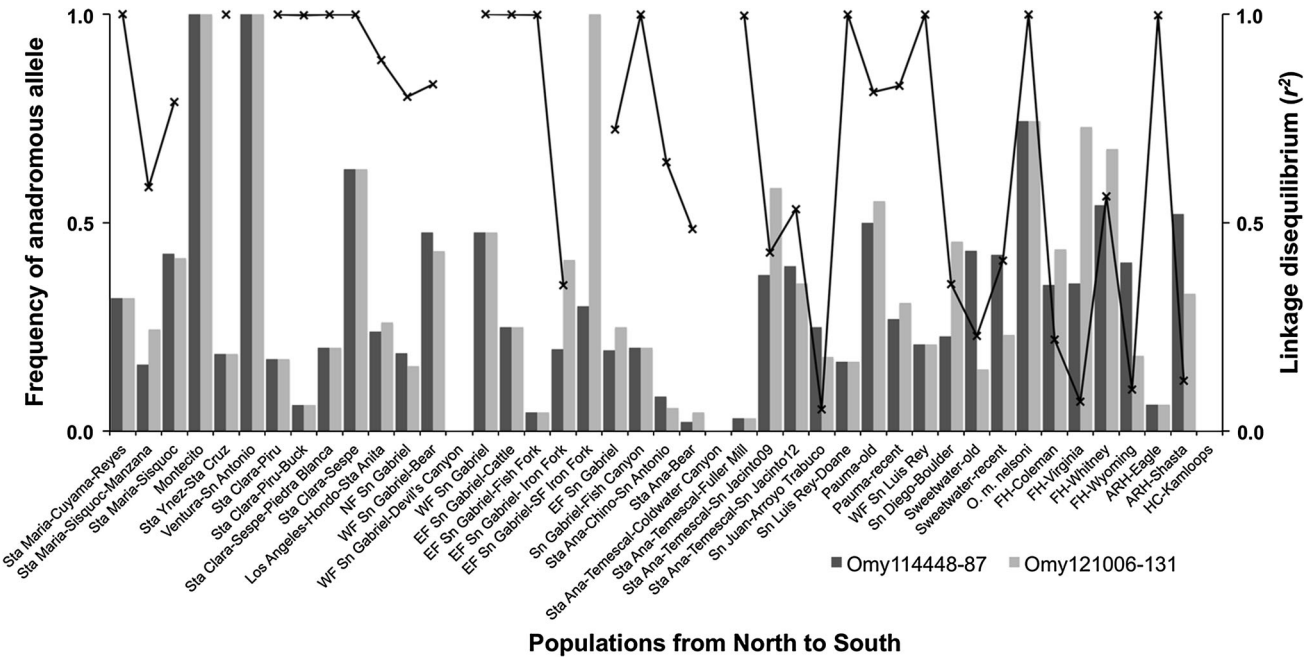

Fig. 5 Patterns of genetic variation by population for the two SNPs located on chromosome Omy5. *Left axis* frequency of the anadromous-associated allele (*bars*). *Right axis* linkage disequilibrium estimated using r^2

Therefore, they represent potentially important reservoirs of the very limited native ancestry remaining in the region, and should be considered as significant for the continuing persistence of viable networks of *O. mykiss* populations in Southern California. Moreover, as noted above, hatchery rainbow trout are members of the same species, so some introgression does not necessarily render such small populations less viable than purely native populations (Harbicht et al. 2014). In fact, the introduction of some novel genetic diversity from hatchery trout into these small, isolated, populations will likely increase heterozygosity, providing more variation to adapt to changing environmental conditions and reduce inbreeding. This may be particularly true for the populations in Coldwater Canyon Creek and West Fork San Luis Rey River that have particularly low heterozygosity and allelic richness (Table 2). Unlike native populations in the San Gabriel River basin, for which there is some prospect of natural gene flow between tributary populations, these populations are completely isolated from other potential native migrants, and managed gene flow between them and other native Southern California DPS populations may be a necessary step in ensuring their persistence. Further sampling throughout the watersheds inhabited by these populations will help to establish the extent of remaining diversity they harbor and determine whether the extremely low variability was due to sampling biases, effects of isolation in remote headwater canyons above barriers, or both. Regardless, the perilously low genetic variation found in the remaining native lineage populations in Southern California may not be enough to sustain them through environmental change. Indeed, genetic variation in bull trout populations has been positively correlated with habitat size and with gene flow, and negatively correlated with maximum summer temperature and frequency of winter flooding (Kovach et al. 2015), both of which are predicted to increase with global climate change.

The U.S. federal Recovery Plan for the Southern California Steelhead DPS (NMFS 2012) describes specific goals and strategies for augmenting steelhead populations within the Southern California area. The Recovery Plan addresses factors limiting the species' ability to survive and reproduce in the wild. A central tenet of the Recovery Plan is that a viable DPS will consist of a sufficient number of discrete viable populations, that may be spatially dispersed but nevertheless adequately connected through migration, to achieve the long-term persistence and evolutionary potential of the species. Thus, future efforts should identify additional populations with native ancestry, particularly in watersheds where mixed and purely native stocks have been identified, and establish connectivity between them. Furthermore, studies that quantitatively assess habitat characteristics and environmental cues that isolated populations experience will be important to more clearly understand relationships between genetic diversity and population persistence.

Previous studies of genetic structure of *O. mykiss* collected from freshwater locations above and below barriers in California indicated that contemporary populations were

dominated by native coastal steelhead lineage fish (Clemento et al. 2009; Deiner et al. 2007; Nielsen et al. 1997b, 2003; Pearse et al. 2009). Populations downstream of recent barriers in these regions were genetically similar to above-barrier populations in the same basins (Deiner et al. 2007; Clemento et al. 2009), suggesting that the latter have value in restoring steelhead populations below barriers (Boughton et al. 2006). Since anadromous fish are relatively rare in the highly disturbed watersheds of Southern California, the current resident trout populations with native ancestry may therefore be critical resources for steelhead recovery. Anadromous offspring of resident parents have been documented to occur with sufficient frequency that recently landlocked fish that are derived from a steelhead lineage should be considered integral components of steelhead recovery, particularly in Southern California (Courter et al. 2013; Kendall et al. 2015).

The population in the Sweetwater River, the southernmost in the U.S. with documented contemporary trout populations, was mainly of hatchery rainbow trout descent, both the fish sampled in 1997 and those sampled in 2010–2013. The population of native coastal steelhead lineage fish identified further north in the remote West Fork of the San Luis Rey River thus represents the southernmost documented population of native *O. mykiss* in the United States. The population of *O. mykiss nelsoni*, a subspecies found only in Baja California, Mexico (Ruiz-Campos and Pister 1995), was also found to be derived from the coastal steelhead lineage. Consistent with this native ancestry, these two southernmost documented populations of native *O. mykiss* are each other's closest relatives on the neighbor-joining tree.

Pauma Creek, another tributary of the San Luis Rey River, illustrates the challenges of maintaining native coastal steelhead populations. Comparison of samples from 1997 with those collected in 2009–2011 indicated that the Pauma Creek population went from a 'mixed' status to a completely hatchery trout-derived one over that time period, possibly through either competition or continued hatchery trout stocking, highlighting the risks to native *O. mykiss* of hatchery rainbow trout stocking. However, it is possible that the recent collections represented a different subpopulation than that from 1997, since the sampling was conducted by different people in slightly different habitat and using different methods, and native fish may still exist in the stream.

Most of the populations in the San Gabriel River showed clear evidence of native ancestry, and it was identified as a stronghold of native trout in the region. However, ancestry can change over very small spatial scales in this basin, with neighboring canyons in the West Fork San Gabriel River having fish of coastal steelhead lineage interspersed with those of hatchery descent (Fig. 2). For example, populations from the East, North and West forks of the San Gabriel River had native genetic ancestry, but nearby populations in the West Fork San Gabriel-Devil's Canyon and East Fork San Gabriel-Iron Fork showed hatchery introgression. The presence of hatchery ancestry in fish from Devil's Canyon was unexpected given its remote location.

Populations in the northernmost watersheds in the Southern California DPS, including the Santa Maria, Santa Ynez, Ventura and Santa Clara rivers, were clearly of native coastal steelhead ancestry, concordant with the results of Clemento et al. (2009) and Nielsen et al. (1997a). The recent collections from Piru and Sespe creeks in the Santa Clara River basin served as a positive control for this study, since they overlapped with the southernmost population samples analyzed by Clemento et al. (2009) and were analyzed in the same lab.

Recent studies using quantitative trait locus and association mapping have identified discrete genetic regions associated with the complex migratory behavior in *O. mykiss* (Hecht et al. 2012; Martínez et al. 2011; Miller et al. 2012; Nichols et al. 2008; Pearse et al. 2014). However, there is extensive variation among populations in the patterns of allele frequencies and the associations between them (Pearse et al. 2014; Pearse and Garza 2015), and these differences can be used to identify the ancestry of trout populations. In addition, the combined analysis of ancestry with the presumably neutral SNP markers and allele frequencies at life history-associated ones allows inference about recent selection on anadromy. Such analysis revealed, for example, that a diversion dam on the lower Santa Clara River probably acts as a functional barrier to anadromy, in spite of a fish passage facility (Pearse et al. 2014). In Southern California, the high variability in the frequency of alleles associated with anadromy suggests that many populations retain the ability to express this phenotype, but further analysis will be necessary to understand the implications of this variation for restoration of steelhead in the Southern California DPS.

This study provides the first detailed evaluation of population structure and ancestry of *O. mykiss* populations at the extreme southern end of the species distribution in North America, in an arid area where its continuing existence is threatened by urbanization, habitat change and water development. Population genetic analyses were used to document the remaining native steelhead lineage populations and the effects of widespread hatchery rainbow trout stocking in the region. The results establish a baseline for maintaining and augmenting the current distribution of native coastal steelhead lineage fish in Southern and Baja California and will be a valuable resource for setting priorities for conservation measures, as well as evaluating the success of ongoing and future steelhead recovery projects.

Acknowledgments The following people contributed effort, information and energy, without which this project would have been

impossible. California Department of Fish and Wildlife: S. Bankston, R. Barabe, H. Block, A. M. Eubanks, M. Larson, C. Lima, D. McCanne, C. McKibbin, J. O'Brien, and P. Riparetti; United States Forest Service: T. Bishop, E. Bracamonte, D. Brook, F. Duncan, C. Fong, R. Howell, A. Krist, M. McIntyre, T. Reeder, N. Sill, J. Sirski, J. Taylor, M. Thomas, L. Welch and C. Whelan; Marine Corps Base Camp Pendleton: M. Hamilton, M. Rouse, and B. Shemai; Los Angeles Department of Public Works: C. Ly; Riverside Corona Resource Conservation District: B. Mills, S. Pynn and K. Russell; Mountain Cove: M. Leach, P. Mallon, and L. Vasquez; NOAA Southwest Fisheries Science Center and University of California Santa Cruz: E. Anderson, V. Apkenas, M. Beakes, D. Boughton, A. Clemento, C. Columbus, H. Fish, C. Michel and J. Notch; Volunteers of Trout Unlimited (TU) Chapter 920 and the affiliated Golden State Flycasters (GSF, San Diego) and colleagues from related organizations: G. Applebee, B. Bechard, S. Burley, V. Carrasquillo, G. Gates, M. Hamilton, D. Irby, W. Johnson, M. McVay, J. Narkevitz, H. Pippen, J. Regan, S. Reynes, D. Shulze, N. Spitzer, G. Strawn, G. Sutherland, D. Volgarino, M. Wagner, B. Watkins and B. Weiler. The Pauma Creek 1997 samples were collected on tribal lands of the Pauma Band of Luiseño Indians and we thank them for allowing access. Many of the collections in Southern California were funded by the Fisheries Restoration Grant Program of the California Department of Fish and Wildlife/Game (Project no. 0950015). Trout collections in Baja California were funded by Consejo Nacional de Ciencia y Tecnología, México (Grant: 4311005-1993PN).

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