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RESEARCH ARTICLE

Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in south-central California

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Abstract Genetic analyses of coastal *Oncorhynchus mykiss*, commonly known as steelhead/rainbow trout, at the southern extreme of their geographic range in California are used to evaluate ancestry and genetic relationships of populations both above and below large dams. Juvenile fish from 20 locations and strains of rainbow trout commonly planted in reservoirs in the five study basins were evaluated at 24 microsatellite loci. Phylogeographic trees and analysis of molecular variance demonstrated that populations within a basin, both above and below dams, were generally each other's closest relatives. Absence of hatchery fish or their progeny in the tributaries above dams indicates that they are not commonly spawning and that above-barrier fish are descended from coastal steelhead trapped at dam construction. Finally, no genetic basis was found for the division of populations from this region into two distinct biological groups, contrary to current classification under the US and California Endangered Species Acts.

Keywords Steelhead · Rainbow trout · Introgression · Anadromy · Dams

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Introduction

The anthropogenic introduction of barriers to dispersal for migratory species can have many consequences. Such disruption of dispersal can cause extinction in species such as anadromous fishes that require migration as part of their life cycle. Barriers can also cause rapid evolution if there is sufficient life history plasticity and if evolutionary pressures change following the loss of dispersal opportunities. In species that were previously widespread, fragmentation will lead to an increase in genetic drift, decreasing genetic variation in each of the resulting populations. In many areas of the world, recently connected aquatic habitat has been highly fragmented due to the construction of dams and diversion of water for human use. The consequences for aquatic species are not always easily predictable, particularly for species with life history plasticity.

Steelhead (*Oncorhynchus mykiss*) historically occurred around the North Pacific Ocean from northwestern Mexico in North America to eastern Russia in Asia. Today, steelhead reach the southern limit of their natural distribution in southern California in the vicinity of Los Angeles, one of the world's largest urban areas. Steelhead are the anadromous form of rainbow trout and are assigned to the same species. In general, fish from the species *O. mykiss* found below barriers to anadromy, with presumptive access to the ocean, are referred to as steelhead and classified as such. Steelhead and rainbow trout are also the target of one of the world's largest recreational fisheries. This has led to the production and planting of billions of trout in lakes and reservoirs in California over the last century. Rainbow trout is also one of the world's most important aquaculture species, which has led to the development of extensive genomic resources and kindled interest in understanding the population structure of the species in its native range.

Populations of *O. mykiss* in California south of Monterey Bay and below barriers to anadromy are administratively divided into two Distinct Population Segments (DPSs), formerly Evolutionarily Significant Units (ESU). In the South Central California Coast (SCCC) DPS, which extends south from the Pajaro River in Monterey Bay to just north of the Santa Maria River in San Luis Obispo County, steelhead were listed as Threatened under the US Endangered Species Act (ESA) in 1997 (National Oceanic and Atmospheric Administration 1997). At the same time, steelhead in the Southern California (SC) DPS were ESA-listed as Endangered. At the time of ESA listing, this group included fish in coastal drainages from the Santa Maria River to Malibu Creek, but in 2002 it was extended to the Mexican border in San Diego County (National Oceanic and Atmospheric Administration 2002).

A primary limiting factor for steelhead populations in southern California is blocked access to freshwater habitat due to dams and water diversions, which are common in this semi-arid region. Most of these barriers lack fish passage structures to allow upstream migration. When fish from the species *O. mykiss* are currently found above such barriers they are considered to be resident rainbow trout and are not afforded protection under the ESA. To provide insight into questions of *O. mykiss* population structure in central and southern coastal California, genetic analysis of population samples from 5 basins in the two southernmost DPSs were performed using microsatellite DNA marker data. These highly variable genetic markers can be used to trace ancestry and evaluate genetic distinctions among populations of salmonids at multiple geographic scales (Carlsson and Nilsson 2001; Castric et al. 2001; Spidle et al. 2001; Wenburg and Bentzen 2001; Olsen et al. 2003; Poissant et al. 2005; Crispo et al. 2006). Previous genetic work on population structure of steelhead in this region has relied primarily on mitochondrial DNA (e.g., Berg and Gall 1988; Nielsen et al. 1997), which is a single locus that is often not reflective of population history or true relationships (Chan and Levin 2005), or small numbers of microsatellite loci and inadequate population sampling, which can also lead to inaccurate inference regarding population structure.

In this study, a large number of microsatellite loci and large population samples were employed to examine the genetic structure of *O. mykiss* in southern California, with a focus on relationships between populations above and below dams. Samples were collected with a standardized stream sampling protocol from five of the largest basins in the region: the Salinas, Arroyo Grande, Santa Ynez, Ventura and Santa Clara Rivers. Fish sampled opportunistically and in small numbers from the southernmost extent of the range were also analyzed. These include fish from Malibu and Topanga Creeks and the San Gabriel River in Los Angeles County, the Santa Ana and San Juan Creek basins in Orange County, and San Mateo Creek and the Sweetwater River in San Diego County. Samples of the *O. mykiss* strains raised at Fillmore Hatchery on the Santa Clara River and used in stocking of trout in reservoirs throughout the southern and central part of the state are also analyzed. In some analyses, data from a previous study of *O. mykiss* in the northern part of the state (Garza et al. 2004) are used to provide a comparative phylogeographic framework.

The results of the genetic analyses are then used to address several aspects of the population structure of *O. mykiss* that will help to inform the conservation and management of this species in southern California. First, recent ancestry of *O. mykiss* populations and individuals in streams above dams is evaluated to determine if they appear to have been derived from a coastal steelhead lineage or from planted hatchery trout derived from out-of-basin broodstock. This analysis also evaluates whether there is evidence of strong Fillmore Hatchery influence in the current genetic composition of naturally spawned populations in these streams. Second, population genetic structure in the region is examined to evaluate if it is consistent with the delineation of the two DPSs south of Monterey Bay. That is, do the sampled populations form distinct genetic lineages that reflect different demographic and evolutionary trajectories. Finally, patterns of genetic differentiation and genetic diversity between sites are summarized to provide insight into the levels of recent gene flow and demographic history of the species.

Methods

Sampling sites

Juvenile *O. mykiss* samples from 20 sites in southern California, representing five major drainages from Monterey Bay south to Ventura County, were collected nonlethally using a backpack electrofisher and a protocol to stratify sampling within the stream and minimize collection of tissue from siblings (Fig. 1; Table 1). Drainages were selected to provide spatial coverage across the current range of steelhead in southern California. Sampling specifically targeted watersheds with large impassible dams, which effectively stop upstream migration into the reservoir from populations downstream of the dam. From north to south, these basins are the Salinas River (Nacimiento Dam 1957; San Antonio Dam 1965) and Arroyo Grande (Lopez Dam 1954), which are assigned to the SCCC Steelhead DPS, and the Santa Ynez (Bradbury Dam 1953), Ventura (Matilija Dam 1947), and Santa Clara (Santa Felicia Dam 1954) Rivers, which are assigned to the SC Steelhead DPS. On the Santa Ynez River, the Juncal Creek

sample population is above two additional dams (Gibraltar 1920; Juncal 1930) and on the Santa Clara River, the Piru Creek-Gold Hill and Piru Creek-Lockwood population samples are above one additional dam (Pyramid Dam 1970). Since population genetic structure may be influenced by hatchery plantings, samples from the four distinct strains of trout raised at Fillmore Hatchery, located on the Santa Clara River, were also analyzed. Since it opened in 1940, Fillmore Hatchery has been the source of most rainbow trout that have been planted in southern and central California rivers and reservoirs.

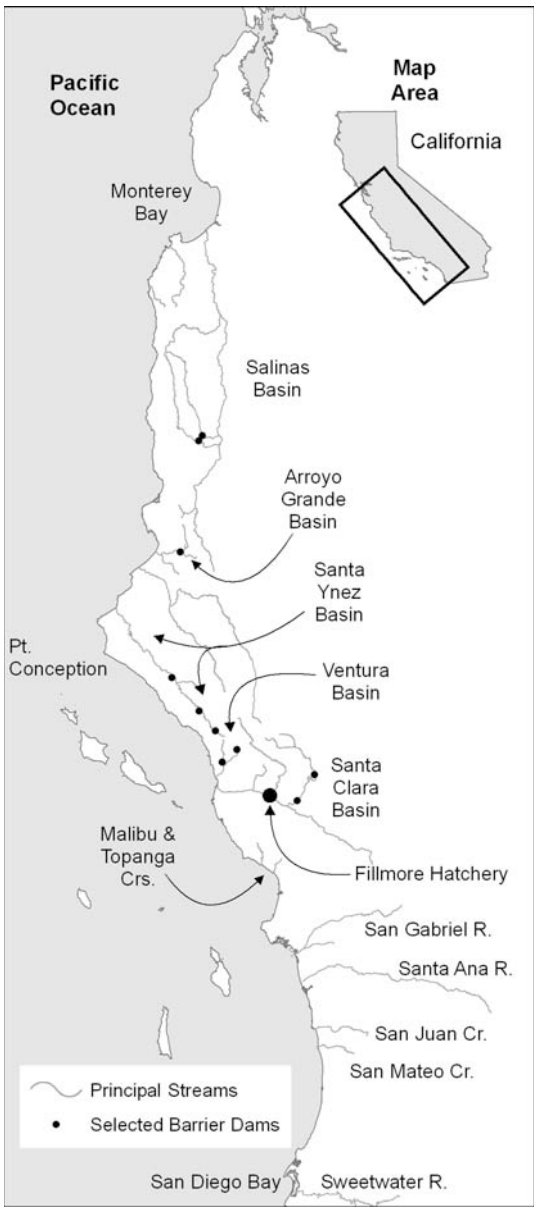

Fig. 1 Map of 20 sites from 5 major drainages in southern California where *O. mykiss* populations were sampled. Samples were also collected from four rainbow trout strains at the Fillmore Hatchery, located on the Santa Clara River

There are numerous drainages to the south of the Santa Clara River in the geographic range of the SC steelhead DPS, including large basins like the Los Angeles and San Gabriel Rivers, and many smaller basins draining the Santa Monica and Santa Ana Mountains. Most of these basins are heavily impacted by anthropogenic activity and/or are without ocean access most years or for most of the basin. However, tissues were collected opportunistically over a number of years from these southern basins, including Malibu Creek ($N = 2$), Topanga Creek ($N = 18$), the San Gabriel River ($N = 1$), the Santa Ana River ($N = 13$), San Juan Creek ($N = 1$), San Mateo Creek ($N = 1$) and the Sweetwater River ($N = 7$), near the border with Mexico. None of these samples were of sufficient size to accurately estimate population genetic parameters, but were analyzed with individual-based assignment tests.

DNA collection and extraction

Tissue samples consisted of small caudal fin clips (up to 10 mm^2) that were collected non-lethally and dried on blotter paper. DNA was extracted from approximately 2 mm^2 using the Qiagen DNeasy Tissue Kit, following the manufacturer's recommended protocol for animal tissues and using a BioRobot 3000 (Qiagen Inc.). Extracted DNA was kept frozen at −20°C until microsatellite amplification via polymerase chain reaction (PCR).

All individuals were genotyped at 24 microsatellite loci (Table 2). These include 18 loci that have been used extensively to study population structure of steelhead (Garza et al. 2004; Aguilar and Garza 2006) and six loci that have been shown to be linked to quantitative trait loci (QTL) for ecologically important traits (Perry et al. 2001; O'Malley et al. 2002; Jackson et al. 1998; Aguilar and Garza 2006). Multiple analyses showed no consistent evidence of recent selection acting on these loci in the populations under study here (data not shown), so all loci were used in the population genetic analyses. Moreover, all of the analyses described below yielded qualitatively equivalent results with these loci either included or excluded. For technical reasons, the small collections from the southernmost basins were not genotyped with the six loci described above. PCR was carried out in 15 µl volumes containing 4 µl template DNA and standard reagent concentrations. Multiple thermal cycling routines were employed to maximize PCR product yield for individual loci. Specific thermal cycling conditions are available from the authors upon request. PCR products were mixed with formamide, loading dye and internal size standard, denatured at 95°C for 3 min and electrophoresed on either an ABI 377 or an ABI 3100 genetic analyzer. Allele sizes were determined with Genotyper software (version 2.1, Applied Biosystems) or GeneMapper software (version

Table 1 Descriptive summary statistics for 20 population samples of *O. mykiss* from 5 river basins, as well as samples from four rainbow trout strains raised at Fillmore Hatchery

Basin	Tributary	Barrier[a]	*N*	H_e	H_o	*K*	A_r	HWE	LD (%)	*M*	FS	MFS
Salinas	Tassajara	B	75	0.685	0.688	9.7	7.6	2	30.4	0.592	23	2.92
	Tassajera	B	78	0.660	0.632	7.9	6.6	2	14.1	0.553	9	2.29
	SanAntonio	A	100	0.668	0.619	9.1	7.2	4	2.2	0.561	4	1.27
	Nacimiento	A	76	0.671	0.638	9.5	7.5	4	9.4	0.574	9	1.81
Arroyo Grande	ArroyoGrande-Main	B	51	0.682	0.677	9.0	7.7	1	14.1	0.590	9	2.55
	LosBerros	B	63	0.693	0.669	8.4	7.1	2	25.4	0.573	18	2.93
	LopezCanyon	A	97	0.722	0.712	10.8	8.1	4	23.2	0.660	16	1.61
Santa Ynez	Salsipuedes	B	133	0.627	0.573	8.1	6.1	7	4.0	0.530	2	1.05
	Hilton	B	51	0.649	0.580	8.0	6.7	5	7.2	0.547	5	1.15
	SantaCruz	A	26	0.663	0.649	7.0	6.9	0	0.0	0.534	1	1.00
	Juncal	A	82	0.614	0.632	6.8	5.6	0	2.5	0.525	8	1.71
Ventura	Bear	B	23	0.656	0.645	5.2	5.2	0	1.4	0.503	7	2.30
	Matilija-NorthFork	B	78	0.657	0.652	6.8	5.8	1	1.4	0.559	6	1.77
	Matilija	A	75	0.620	0.584	7.9	6.3	1	8.7	0.547	15	1.69
	Matilija-UpperNorthFork	A	66	0.664	0.665	8.4	6.9	1	8.3	0.565	19	1.78
Santa Clara	SantaPaula	B	100	0.723	0.689	9.0	7.6	3	1.4	0.551	4	1.29
	Sespe-LionCanyon	B	88	0.633	0.624	9.4	7.3	3	5.1	0.652	6	1.57
	Piru-FrenchmansFlat	A	79	0.641	0.645	7.7	6.2	0	0.4	0.562	3	1.14
	Piru-GoldHill	A	62	0.597	0.591	7.3	6.1	1	0.7	0.587	8	1.29
	Piru-Lockwood	A	96	0.604	0.598	7.9	6.2	1	1.1	0.565	3	1.14
Fillmore Hatchery	Coleman	n/a	50	0.664	0.654	7.7	6.6	0	1.1	0.615	3	1.30
	HotCkVirginia	n/a	100	0.641	0.615	7.1	5.6	5	1.1	0.627	4	1.25
	MtWhitney	n/a	50	0.624	0.623	7.5	6.2	0	0.4	0.611	3	1.14
	HotCkWyoming	n/a	50	0.661	0.661	6.8	6.1	0	0.4	0.550	2	1.22
			Mean	0.655	0.638	8.0	6.6	2.0	6.8	0.572	7.8	1.6
			Var	0.001	0.001	1.45	0.58	3.95	0.8	0.001	36.9	0.3

N = Sample size, H_e = Expected heterozygosity, H_o = Observed heterozygosity, K = Observed number of alleles, A_r = Allelic richness, HWE = Hardy–Weinberg equilibrium (number of loci with $P < 0.001$ for heterozygote deficit), LD = Linkage disequilibrium (proportion of pairwise comparisons of loci with $P < 0.0001$), M = M-ratio statistic of Garza and Williamson (2001), FS = size of the largest full-sib family, MFS = mean size of full-sib families in the sample

[a] Barrier indicates the location of the sampling site as either A = above or B = below one or multiple impassible dams

4.0, Applied Biosystems). At least two people performed all size scoring independently, discrepancies were identified, and if a resolution was not reached, the sample was rerun. If a discrepancy persisted through the second analysis, the fish was not scored at that locus. A representative fraction (5%) of samples were re-genotyped as a control for data quality.

Data analysis

Expected heterozygosity (Nei 1987), observed heterozygosity and number of alleles were calculated for each population sample. In order to compensate for variation in sample sizes, genetic diversity was also assessed using allelic richness as estimated with the rarefaction method in FSTAT version 2.9.3.2 (Goudet 1995). Three measures of genetic diversity, expected and observed heterozygosity and allelic richness, were evaluated for significant differences in the mean values for populations above and below dams using FSTAT. Deviations from Hardy–Weinberg equilibrium were examined utilizing the Markov Chain Monte Carlo (MCMC) approximation of an exact test (U test) implemented in the GENEPOP program (version 3.4; Raymond and Rousset 1995). To ensure segregation independence of the 24 microsatellite loci in each of the population samples, linkage (gametic phase) disequilibrium was also evaluated with GENEPOP. This method uses MCMC to generate an unbiased estimate of the P-value, as well as the standard error, for each locus pair in each population.

Each of the population samples from the 5 focal basins was examined for the presence of siblings using the program Colony (Wang 2004). This method uses a maximum likelihood algorithm to identify full-sib families nested

Table 2 Information for the 24 microsatellite loci used in this project, including the species in which the locus was described, original citation, forward (F) and reverse (R) primer sequences, number of observed alleles (A), and the observed size range (SR) of alleles

Locus	Species	Reference	Primer sequences	A	SR
Omy27	*O. mykiss*	McConnell et al. (1995)	F-TTTATGTCATGTCAGCCAGTG	6	97–109
			R-TTTATGGCTGGCAACTAATGT		
Omy77	*O. mykiss*	Morris et al. (1996)	F-CGTTCTCTACTGAGTCAT	23	80–144
			R-CCAAGAATTTTCTGATCCGGG		
Omy1011	*O. mykiss*	Morris et al. (1996)	F-AACTTGCTATGTGAATGTGC	24	132–256
			R-GACAAAAGTGACTGGTTGGT		
One11b	*O. nerka*	Scribner et al. (1996)	F-GTTTGGATGACTCAGATGGGACT	6	114–124
			R-CCTGCTGCCAACACTGTCAA		
One13b	*O. nerka*	Scribner et al. (1996)	F-TCATACCCCATGCCTCTTCTGTT	20	206–248
			R-GGGTGGAGAGACAGGTATCTTGTC		
Ots1b	*O. tshawytscha*	Banks et al. (1999)	F-GGAAAGAGCAGATGTTGTTAA	24	201–295
			R-CATGCTATTTCCAGACGGCA		
OtsG3	*O. tshawytscha*	Williamson et al. (2002)	F-GGACAGGACCGTCTGCTAAATGACTG	19	139–239
			R-GGATGGATTGATGAATGGGTGGG		
OtsG43	*O. tshawytscha*	Williamson et al. (2002)	F-AACTCCCGTTGACAATTTACTGTTG	24	133–205
			R-TTTTGGCAAAGTTGGCTACTCTG		
OtsG85	*O. tshawytscha*	Williamson et al. (2002)	F-CCATGTCAGCACTGACTTAAT	49	116–341
			R-GGATGTTGTTCCTAATGTTTT		
Ots103	*O. tshawytscha*	Small et al. (1998)	F-AGGCTCTGGGTCCGTG	6	58–116
			R-TGATATGGTGTGATAGCTGG		
OtsG243	*O. tshawytscha*	Williamson et al. (2002)	F-TTATTAAACTGCACTGTCTAACTACA	8	95–121
			R-GTATGCAGCAAGCCAGGTG		
Ots249b	*O. tshawytscha*	Williamson et al. (2002)	F-ATGGCAGTTAAGAGAACAAAAGTT	25	143–276
			R-GTACAACCCCTCTCACCTACCC		
OtsG253b	*O. tshawytscha*	Williamson et al. (2002)	F-CGCTGCAGAAACATTTTCGA	28	163–273
			R-AATTGGGTCATTAAGGCTCTGTGG		
OtsG401	*O. tshawytscha*	Williamson et al. (2002)	F-CTGCCCTGAGAAGCTGGAGTGCTC	22	165–233
			R-TTGCCCCACCCTTGCATCTATCCA		
OtsG409	*O. tshawytscha*	Williamson et al. (2002)	F-GTAGCCATTTGTGTCACCATCATT	3	86–90
			R-CATTCTCCTGCCTCACAGAGTTTA		
Oki23	*O. kisutch*	Smith et al. (1998)	F-TGTGCTATAGGGTGAATGTGC	24	118–218
			R-AACACAGGCATCCCCACTAA		
Ssa85	*Salmo salar*	O'Reilly et al. (1996)	F-AGGTGGGTCCTCCAAGCTAC	33	98–161
			R-ACCCGCTCCTCACTTAATC		
Ssa289	*Salmo salar*	McConnell et al. (1995)	F-CTTTACAAATAGACAGACT	10	107–125
			R-TCATACAGTCACTATCATC		
Ssa20.19.NUIG*	*Salmo salar*	Perry et al. (2001)	F-TCAACCTGGTCTGCTTCGAC	9	74–90
			R-CTAGTTTCCCCAGCACAGCC		
One112ADFG*	*O. nerka*	O'Malley et al. (2002)	F-GTGACCCAGACTCAGAGGAC	27	118–206
			R-CACAACCCATCACATGAAC		
OmyFGT12TUF*	*O. mykiss*	O'Malley et al. (2002)	F-CAGTGTTGGAACACGTCCTG	36	127–201
			R-TTGATTCTTGTGATGAAATCGC		
Omy325*	*O. mykiss*	Jackson et al. (1998)	F-TGTGAGACTGTCAGATTTTGC	28	93–151
			R-CGGGAGTCCGTATCCTTCCC		
OmyRGT31TUF*	*O. mykiss*	O'Malley et al. (2002)	F-TCTATGGAAGGTTCTGTTTGCA	10	204–250
			R-TTCCCCAACCCTCTCCTC		
OkeIGF-IIa*	*O. keta*	Aguilar and Garza (2006)	F-GCACATCTTTGTGTCTGTCA	28	140–196
			R-CGTCCACTCAGTAGTATCGC		

Loci with an asterisk have been described as linked to QTL in some rainbow trout crosses

within half-sib families. Genotyping error of 2% was assumed in sibship reconstruction, and allele frequencies were updated every 1,000 successful proposal configurations. Each population was run three times to ensure model convergence.

The mean ratio M, the number of alleles/(range in allele size + 1), was also estimated to test for recent reductions in effective population size and significance was evaluated with 10,000 simulated datasets from populations at equilibrium using the program M_P_Val (Garza and Williamson 2001). The mutation model parameter values used were 3.5 for the mean size of non-stepwise mutations and 0.1 for the proportion of non-stepwise mutations. Values of theta ($\theta = 4N_e\mu$) from 0.5 to 4 were examined, which correspond to pre-bottleneck effective population sizes of 250–2,000 individuals (when $\mu = 5 \times 10^{-4}$). For these parameter values, $M < 0.76$ indicates that the population under study has experienced a recent reduction in effective population size. Twenty-three loci were used in this analysis; Ssa85 was omitted due to the presence of 1 bp alleles, which violate the assumptions of the mutation model.

Genetic differentiation between population samples was examined with several methods. An MCMC approximation of a Fisher's exact test was employed to calculate the probability of the null hypothesis (H_O) that allele frequencies were identical across populations using the method for genic differentiation in GENEPOP (version 3.4a; Raymond and Rousset 1995). Pairwise differentiation between all pairs of populations was also evaluated using F_{ST}, as estimated by Weir and Cockerham's θ (1984), and significance ($P < 0.001$) assessed by the permutation algorithm in GENETIX (Belkhir et al. 1996–2004) with 10,000 replicates.

The distribution of molecular variation was assessed to identify informative groupings of population samples using the Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) option in ARLEQUIN (version 3.0, Excoffier et al. 2005). Molecular variance was partitioned into components of among groups (F_{CT}), among populations within groups (F_{ST}) and within populations (F_{SC}). Multiple definitions of groups of population samples were evaluated, including geographic partitions between each of the basins and partitions separating populations above and below barriers (see "Results").

To examine the influence of spatial distance on population structure in California steelhead, a matrix of geographic distance between population samples was calculated using the coastal contour distance between the river mouth of any two sites, plus the rivermile distance from the ocean. F_{ST} and $F_{ST}/(1 - F_{ST})$ were regressed on these geographic distances, as well as just on the coastal contour distances and on the natural logarithm of these distances, using the ISOLDE component of GENEPOP (Raymond and Rousset 1995).

Individual-based assignment tests were used to further evaluate the degree of recent gene flow between population samples. Fish were assigned to their most likely population of origin using the program GeneClass2 (Piry et al. 2004) with the Bayesian method for estimating population allele frequencies (Rannala and Mountain 1997). The method applies a "leave-one-out" procedure, in which the individual being assigned is excluded from the sample population before allele frequencies are calculated. In these analyses, only the 20 focal populations and the Fillmore Hatchery strains were used as reference populations for potential assignment. A Bayesian, model-based clustering method implemented in the program *structure* (version 2.2; Pritchard et al. 2000) was also used to assign individual fish to population of origin and to identify population structure. This analysis uses a prior hypothesis about the number of genetic "clusters" to fractionally assign the ancestry of individual fish to each of the clusters without regard to geographic location of origin. These two individual-based analyses were the only ones pursued with the smaller samples of fish from the southernmost basins described above.

Regional phylogeographic trees were constructed using matrices of Cavalli-Sforza and Edwards' (1967) chord distance (D_{CE}), using the software package PHYLIP (version 3.5c, Felsenstein 1993). The neighbor-joining algorithm was used to determine tree topology, and a majority-rule consensus tree was assembled from 10,000 bootstrap samples of the data with the PHYLIP CONSENSE component. Trees were visualized using TREEVIEW (Page 1996). A D_{CE}/neighbor-joining tree was also constructed with a larger dataset that included samples from 60 populations of coastal steelhead analyzed by Garza et al. (2004). These populations overlap with the geographic distribution of the populations sampled in the current study but are concentrated further north (to the border with the state of Oregon) and are mostly assigned to three more northern DPSs. The coastwide analyses used only the 15 loci for which the data could be easily combined, due to differences in original data collection methods.

Results

Genetic structure

The genetic structure of *O. mykiss* populations in the SCCC and SC DPSs is represented in an unrooted, neighbor-joining dendrogram with branch lengths scaled by chord distances (Fig. 2). The pattern of population clustering (topology) of the regional tree had several salient features. First, population samples from the Santa Clara and Salinas Rivers, both those sampled above and below barriers,

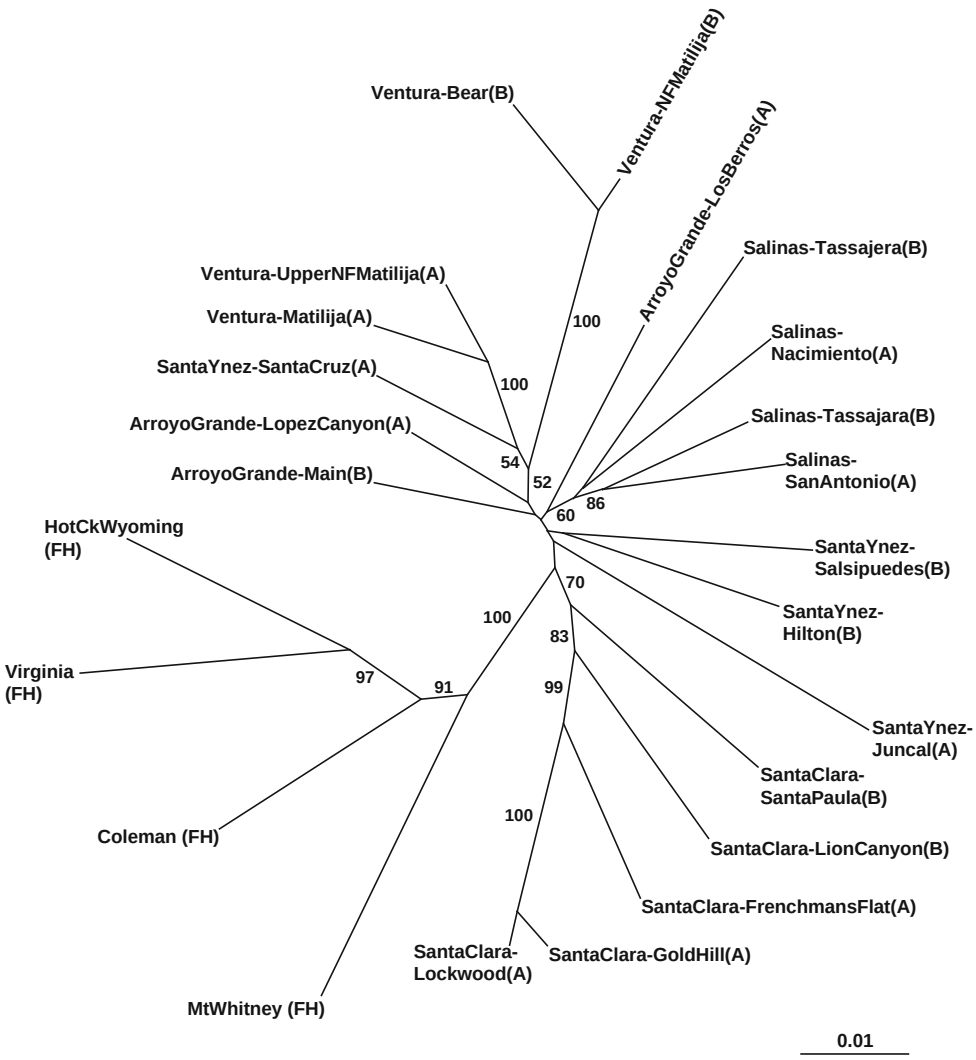

Fig. 2 Unrooted neighbor-joining tree (dendrogram) constructed with chord distances from the 24 sample collections representing naturally spawning populations in 5 major southern California drainages (A and B indicate above or below barrier, respectively) including 4 rainbow trout strains raised at Fillmore Hatchery (FH) and used in stocking throughout the region. Internal branches appearing in >50% of 10,000 bootstrap replicate trees are labeled with the percent bootstrap support

formed monophyletic lineages on the tree, whereas the population samples from the Santa Ynez and Arroyo Grande Rivers were interspersed with one another and in a central position in the tree. The Ventura River populations were nearly monophyletic, but one population from the Santa Ynez clustered with them. The Fillmore Hatchery strains all clustered together, and were separated by a long internal branch from all of the naturally spawned populations.

Regional structure

The regional bootstrap consensus tree had topology very similar to that of the D_{CE}/neighbor-joining tree, clustering the populations from the Salinas and Santa Clara Rivers, with moderate bootstrap support for monophyletic lineages (Fig. 2), and interspersion and sparse bootstrap support for monophyletic lineages of the Santa Ynez, Ventura and Arroyo Grande populations. Monophyly of the naturally spawned steelhead populations was strongly supported by the bootstrap analyses. In addition, very high bootstrap support (>80%) was observed for clusters of populations within some tributaries of the Salinas, Ventura and Santa Clara drainages. For example, the two sample locations below Matilija Dam on the Ventura River (Bear and North Fork Matilija) always clustered together, as did the two population samples above both Pyramid and Santa Felicia Dams on the Santa Clara River (Piru-Lockwood and Piru-GoldHill). It is important to point out that, although these groups were most closely related in the study, the next most similar population samples were those on the other side of the dam. The lack of interspersion of the hatchery strains with the wild populations in the trees and their separation by long internal branches with high bootstrap support indicates a general lack of contribution of fish planted from Fillmore Hatchery to the ancestry of and reproduction in trout populations in streams above or below the dam reservoirs.

Coastwide structure

Evaluation of the coastwide phylogeographic trees constructed with the combined dataset, which has dense coverage of coastal steelhead populations all the way to the Oregon border, provided geographic context for the analysis of population samples in the SCCC and SC steelhead DPSs. The D_{CE}/neighbor-joining tree is presented in Fig. 3. Several features of the trees stand out. First, all of the southern steelhead population samples described here clustered with all of the other populations from south of San Francisco Bay. These populations are separated from all of those north of San Francisco Bay (inclusive) by a relatively long internal branch. Second, there was no strong signal of geographically based reductions in gene flow in the southern populations above the level of the basin. That is, there were not internal branches that separated populations into groups that correspond to the three currently recognized DPSs in this region. This is consistent with the results of Garza et al. (2004), who found a similar lack of concordance with genetic structure and steelhead ESU/DPS boundaries in other parts of California. Another pattern evident in the combined phylogeographic trees that supports the earlier work was the general lack of strict concordance between geographic and genetic population structure at small spatial scales, and the overlapping genetic distances of population samples from the same basin with those from geographically proximate basins (Garza et al. 2004; Pearse et al. 2007).

Genetic differentiation

Exact tests identified significant differences in allele frequencies between all pairs of population samples. Similarly, pairwise F_{ST}, the proportion of genetic variation partitioned between population samples, was significantly different from zero ($P < 0.001$) for all comparisons, even following correction for multiple comparisons (Appendix).

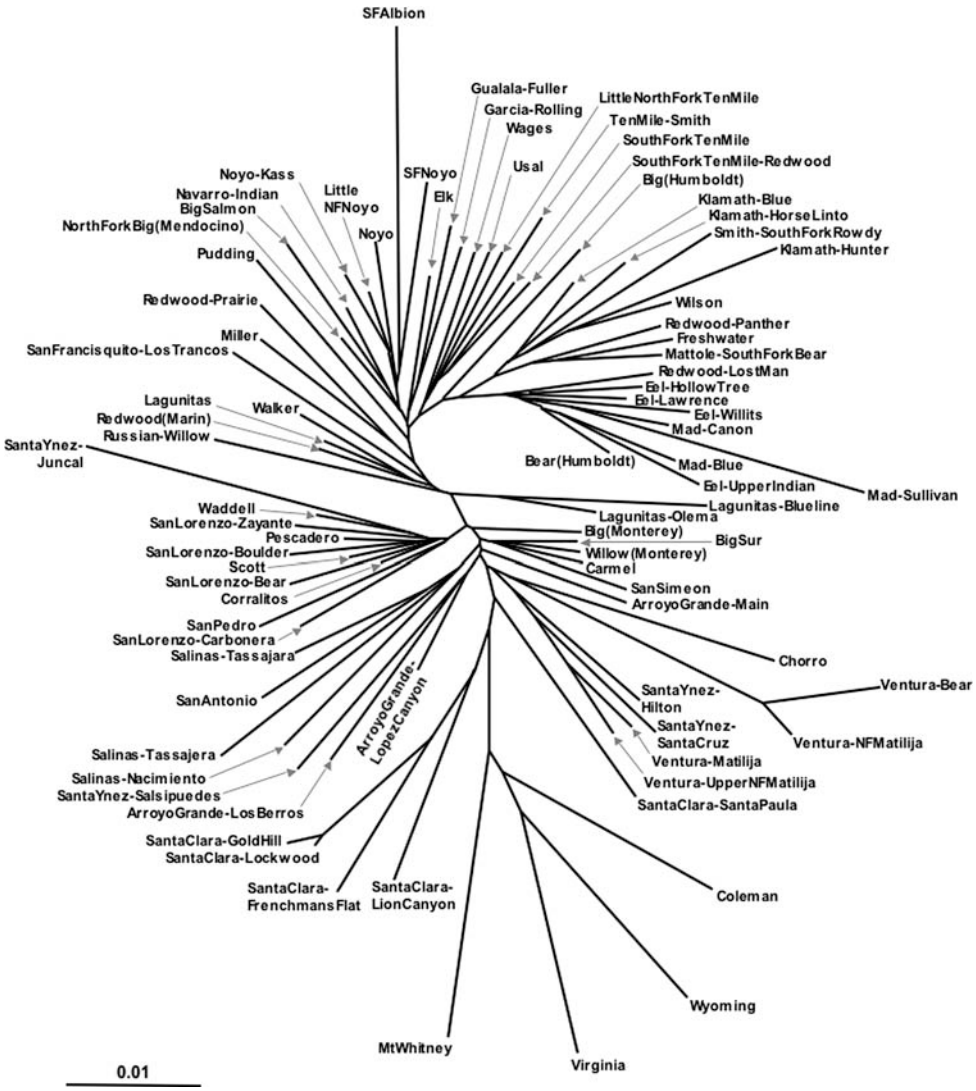

Fig. 3 Unrooted neighbor-joining chord distance tree of 84 *O. mykiss* populations from coastal California. Additional population samples are described in Garza et al. (2004)

Over all wild populations (excluding hatchery samples), the multilocus value of F_{ST} was 0.107, indicating that approximately 11% of all genetic variation in the dataset was partitioned between population samples. Mean F_{ST} for within-basin comparisons was 0.088 while the mean value for between-basin comparisons was 0.110. A two-tailed *t*-test found the distribution of between-basin comparisons to be significantly higher ($P < 0.001$) than the distribution of within-basin comparisons, although the observations are clearly not independent.

Tests for isolation by distance were not significant using either F_{ST} or $F_{ST}/(1 - F_{ST})$, or using the untransformed cumulative river and coastline distances, coastline distances only or their natural logs. Values of r^2 were less than 0.16 for all comparisons. Slightly higher r^2 values were observed when only below-barrier population samples were used, but the regressions remained non-significant.

Analysis of molecular variance

AMOVA indicated that within-population variation was the dominant component of molecular variance for all population groupings evaluated (Table 3). The molecular variance was greater among populations within groups than between groups for all basins except the Ventura River, indicating substantial differentiation between sample sites and a general lack of elevated differentiation between pairs of populations above and below dams. Differences between basins accounted for 3.22% of the overall variation when only below-barrier populations were considered, and 2.53% when all populations were considered (Table 3, Groupings 1 & 2). Grouping of all above-barrier populations and all below-barrier populations explained very little (0.30%) of the overall genetic variation (Table 3, Grouping 3). When groupings that separated populations to the north and south of a particular geographic point were considered (Table 3, Groupings 4–7), the Ventura River break (separating the Ventura and rivers to the north from the Santa Clara River) yielded the largest genetic differentiation between groups of basins. Grouping the sites according to the current DPS designation (the Arroyo Grande break) yielded a level of genetic variation between groups that was lower than that for divisions between most other groups of basins in the study area. However, none of the geographic groupings of population samples from different basins yielded results that explained more than about 2% of the total genetic variation in the study, indicating a lack of deep phylogeographic separation between the study basins, a result consistent with that found in the analysis of phylogeographic trees.

Evaluating the structure of molecular variation within each drainage separately (Table 3, Groupings 8–12), differences between above and below barrier groups were essentially zero for the Salinas, Arroyo Grande, and Santa Ynez Rivers. In contrast, differentiation between above-barrier and below-barrier sites in the Ventura and Santa Clara River basins was non-zero. However, only the Ventura River showed a greater proportion of variance between groups than within groups, suggesting a larger difference between above and below barrier populations. Even so, the proportion of molecular variation partitioned above and below Matilija Dam is still only ~6% and this is partially due to the great similarity between the two above-barrier populations with each other and the two below-barrier populations with each other (Fig. 2; Appendix).

Table 3 Results of several AMOVA evaluating different hypotheses regarding partitioning of genetic variation between different groupings of populations samples

Grouping	Description	Nb	Among groups			Among pops within groups			Within populations		
			Var	%	F_{CT}	Var	%	F_{SC}	Var	%	F_{ST}
1	Interdrainage below barriers	5	0.281	3.22	0.032	0.637	7.30	0.075	7.800	89.48	0.105
2	Interdrainage all	5	0.221	2.53	0.025	0.748	8.56	0.088	7.763	88.91	0.111
3	Above/below	2	0.026	0.30	0.003	0.918	10.55	0.106	7.763	89.16	0.108
4	Salinas River break	2	0.080	0.91	0.009	0.858	9.81	0.099	7.805	89.28	0.107
5	Arroyo Grande break	2	0.100	1.15	0.011	0.835	9.56	0.097	7.805	89.30	0.107
6	Santa Ynez break	2	0.166	1.89	0.019	0.798	9.10	0.093	7.805	89.01	0.110
7	Ventura River break	2	0.173	1.97	0.020	0.813	9.25	0.094	7.805	88.78	0.112
8	Salinas abv/blw	2	−0.051	−0.60	−0.006	0.655	7.61	0.076	8.000	92.99	0.070
9	Arroyo Grande abv/blw	2	−0.008	−0.09	−0.001	0.465	5.29	0.053	8.331	94.80	0.052
10	Santa Ynez above/below	2	0.006	0.07	0.001	0.967	11.79	0.118	7.228	88.13	0.119
11	Ventura above/below	2	0.550	6.36	0.064	0.362	4.18	0.045	7.739	89.46	0.105
12	Santa Clara above/below	2	0.335	3.88	0.039	0.624	7.22	0.075	7.679	88.90	0.111

Nb = Number of groups, Var = Covariance component, % = Percent of the overall genetic variance and *F*-statistics (F_{CT}, F_{SC}, F_{ST}) appropriate for each level of comparison. The different groupings are described in greater detail in the text

Individual assignments

Assignment tests readily distinguished individuals sampled from various river locations throughout southern California. Overall, fish were assigned to the location from which they were sampled with an accuracy of ~95% and to the basin of origin with 99.1% accuracy (Table 4). Only 80 fish of 1,499 (excluding hatchery samples) were misassigned to a population location other than the one where they were sampled and, of those, only 13 were assigned to a location outside of their sample drainage. The largest number of reciprocal misassignments was between the two sites on upper Piru Creek above both dams, Gold Hill and Lockwood Creek, where 23% of fish at these sites were misassigned. This is consistent with the small differentiation ($F_{ST} = 0.01$) value between them, indicating that they are likely not separate biological populations (see Hedrick 1999). Only 2 fish from rivers were assigned to the various hatchery strains, one each from the Salinas and Arroyo Grande basins (Table 4).

Individual assignment tests were also performed with the fish collected from the southernmost locations with insufficient numbers for full population genetic analyses. These analyses used the population samples from the 5 focal basins and the Fillmore Hatchery strains as potential populations of origin. The frequentist assignment method (GeneClass2) and the model-based clustering method (*structure*) yielded largely concordant results. With both methods, the two fish from Malibu Creek, the one fish from the San Gabriel River, the 14 fish from the Santa Ana River, and the one fish from San Mateo Creek were assigned to steelhead populations, while the one fish from San Juan Creek was assigned to hatchery stocks, all with high confidence. However, whereas the frequency-based method assigned 8 of 18 fish from Topanga Creek to hatchery stocks (data not shown), the model-based clustering method indicated that most of these fish are of hybrid ancestry (Fig. 4). Moreover, the two methods yielded somewhat discordant results for the 7 fish from the Sweetwater River. Frequency-based assignment identified

Table 4 Results of individual assignment tests

	Tassajara	Tassajera	SanAntonio	Nacimiento	ArroyoGrande-Main	LosBerros	LopezCanyon	Salsipuedes	Hilton	SantaCruz	Juncal	Bear	Matilija-NorthFork	Matilija	Matilija-UpperNorthFork	SantaPaula	Sespe-LionCanyon	Piru-FrenchmansFlat	Piru-GoldHill	Piru-Lockwood	Coleman	HotCkVirginia	MtWhitney	HotCkWyoming
Tassajara	74					1																		
Tassajera		78																						
SanAntonio			100																					
Nacimiento				75																		1		
ArroyoGrande-Main					48	1				1					1									
LosBerros					1	59	3																	
LopezCanyon							95			1												1		
Salsipuedes					1		1	130	1															
Hilton						1	1	1	45	3														
SantaCruz					1					25														
Juncal											82													
Bear												18	5											
Matilija-NorthFork												4	74											
Matilija														70	5									
Matilija-UpperNorthFork					1					1				6	58									
SantaPaula																100								
Sespe-LionCanyon																	88							
Piru-FrenchmansFlat																		79						
Piru-GoldHill																			43	19				
Piru-Lockwood																			18	78				
Coleman																					48			2
HotCkVirginia																						99	1	
MtWhitney																							50	
HotCkWyoming																								50

The semi-Bayesian method of Rannala and Mountain (1997), as implemented in GeneClass2 (Piry et al. 2004), was used to identify the most likely source population (top) for each individual fish sampled from the locations at left

Shaded values indicate within-basin assignments

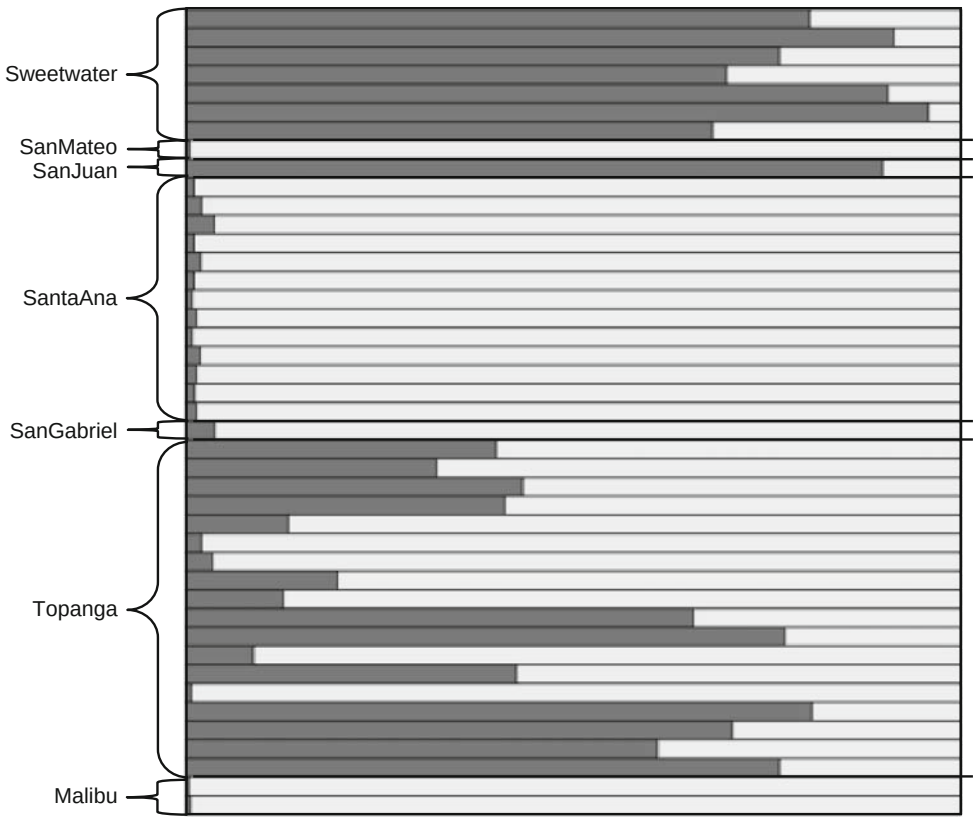

Fig. 4 Fractional ancestry estimates for 43 individuals opportunistically collected from 7 drainages at the extreme southern range of *O. mykiss* in California. *Horizontal bars* indicate the estimated fraction of ancestry from two inferred clusters ($K = 2$ using *structure*, Pritchard et al. 2000). *Black* corresponds to ancestry of Fillmore Hatchery rainbow trout strains, while *white* indicates coastal steelhead ancestry

4 of these fish as being of steelhead origin, whereas the model-based clustering method assigned most of their ancestry to hatchery stocks.

Genetic diversity

Expected heterozygosity ranged from 0.597 to 0.723 with a mean value of 0.655 over the 20 population samples evaluated (Table 1). After adjusting the mean number of alleles per population for the smallest sample size ($N = 23$), mean allelic richness was 6.6 over all samples. Both the number of alleles observed (10.8) and allelic richness (8.1) was highest in the Lopez Canyon population from Arroyo Grande, whereas the Bear Creek population from the Ventura River had both the lowest observed number of alleles (5.2) and allelic richness (5.2). An analysis of the number of alleles present in the combined data set of northern and southern California population samples found a significant pattern of reduction in diversity in the southern populations (data not shown). The 80 populations sampled in the two studies included 35 from south of the Golden Gate and 45 from further north in California. However, only 10 of the 35 lowest diversity values in the combined dataset were observed in populations from north of the Golden Gate. This is consistent with the pattern observed in the data of Garza et al. (2004) of a strong correlation between latitude and allelic diversity. It is worth noting that the Fillmore Hatchery strains, when included in this analysis, all had allelic diversity values that were among the very lowest observed. There were no significant differences in mean values of expected heterozygosity, observed heterozygosity or allelic richness for populations above and below dams.

Values for the *M*-ratio were all significant; they ranged from 0.503 to 0.660 and averaged 0.567 over all population samples. No significant differences in *M* were found between above- and below- dam sites. All values, including those for the hatchery populations, were below the critical value ($M = 0.76$), indicating widespread recent reductions in effective population size.

Statistical evaluation of heterozygote deficiency yielded 47 of 576 (8.2%) significant tests ($P < 0.001$), which appeared randomly distributed across loci and populations (Table 1). Only four significant tests ($P < 0.001$) for heterozygote excess were found, all at the locus *One13b*. Overall, deviations from equilibrium were similar to those expected by chance alone and were not expected to impact other analyses. Tests for linkage disequilibrium (LD) revealed that over all population samples, 681 pairs of loci out of 6,624 (10.3%) showed significant disequilibrium ($P < 0.001$). Even with this stringent significance level, some populations, particularly in the Salinas and Arroyo Grande drainages, still had a large number of significant tests (Table 1).

Since the presence of family groups can cause LD, the estimated distribution of full and half siblings in each population sample was assessed using the program Colony (Wang 2004) and the size of the largest full-sib family (FS) and the mean size of full-sib families (MFS) in each population sample are reported (Table 1). The Tassajara Creek sample contained the largest full-sib family in the study with 23 individuals, while the largest mean full-sib family size was 2.93 in the Los Berros Creek population sample from the Arroyo Grande basin. No full-sib families were detected in the Santa Cruz Creek population and, therefore, mean full-sib family size was one. As the samples represent an unknown fraction of the total population, little can be inferred directly from these sibship estimates. However, both the size of the largest full-sib family and the mean size of full-sib families explained a large proportion of the observed linkage disequilibrium ($r^2 = 0.72$ and 0.58, respectively).

To evaluate the extent to which the presence of full siblings in the population samples are responsible for the observed population genetic patterns, all but one individual from all full sibships identified in the analyses above were removed and the analyses redone. The deviations from LD and HWE were reduced to near zero in all cases. However, none of the other results, including topology and bootstrap values of the trees, individual assignment accuracy and F_{ST} estimates changed in any substantive way in analyses with this modified dataset (data not shown).

Discussion

Genetic analyses of microsatellite data presented here successfully address questions regarding population genetic structure in California coast steelhead DPSs. The data and analyses allow the evaluation of specific hypotheses regarding the impact of dams on the genetic structure of steelhead, the effects of large-scale stocking of rainbow trout in the reservoirs above these dams, and the concordance of genetic population structure with existing DPS boundaries.

Overall genetic structure

Analysis of population genetic structure found evidence for hierarchical structure similar to that found in steelhead populations farther to the north (Garza et al. 2004). Multiple analyses indicated that the majority of genetic variation was at the level of individual local population. Tests of genetic differentiation were significant for every location sampled and the differentiated populations were generally represented by relatively long terminal branches on the phylogeographic trees (Fig. 2). In the AMOVA analyses, approximately 90% of the molecular variance was partitioned among individual populations in all analytical frameworks evaluated. These results are also consistent with the very high assignment accuracy (>94%) to individual populations and the near perfect (>99%) assignment accuracy to basin of origin. This last result indicates that these data are useful as a reference baseline for genetic stock identification techniques to determine basin and tributary of origin for individual trout in management or forensic applications. In contrast, the high genetic similarity of the Gold Hill and Lockwood populations from upper Piru Creek (Santa Clara River), which were also the most spatially proximate samples in the study, may delineate the lower geographic limit at which population structure can be detected in a semi-continuously distributed species.

Analysis of population structure at a larger spatial scale found that certain populations from within a basin always clustered together with high bootstrap support, reflecting high levels of recent gene flow. For example, the three locations from above dams on Piru Creek always formed a well-supported cluster, as do the two populations above Matilija Dam on the Ventura River. In addition, the two Ventura River populations from below the dam form a well-supported cluster. All population samples, both above and below dams, from the Salinas and Santa Clara Rivers formed basin-specific lineages in the phylogeographic trees, although they were generally not supported by high bootstrap values. In contrast, the Santa Ynez and Arroyo Grande River population samples were interspersed and found basally in the trees, with populations separated by short internal branches. An alternative, Bayesian model-based clustering method that uses no prior information about geographic origin of the samples found that, with an hypothesis of three genetic groups present in the 20 population samples and the hatchery strains, the hatchery strains formed one group, the Santa Clara River populations formed another and all of the other population samples formed another (data not shown). This result is consistent with the AMOVA, which found the highest proportion of variance partitioned between regions when the framework separated the Santa Clara River from all others. These results together suggest that the Santa Clara River trout populations are the most distinct of the 5 basins studied here. This may be a consequence of greater influence of hatchery introgression on these populations, as they consistently cluster with Fillmore Hatchery strains on the trees and the hatchery is located on the Santa Clara River. However, the Santa Clara River *O. mykiss* populations are still much more closely related to other coastal steelhead populations than to the Fillmore Hatchery trout strains and still cluster with populations in the two southernmost steelhead DPSs when the present data are combined with

those from other coastal California steelhead populations (Fig. 3). Moreover, additional analyses that include unpublished data from California Central Valley *O. mykiss* population samples and hatchery strains clarify that the Fillmore Hatchery trout strains are not monophyletic or most closely related to the Santa Clara River populations, when analyzed within a broader geographic and phylogenetic context (data not shown).

The more general finding of lack of strict concordance of geographic and genetic clustering for populations from geographically proximate basins is consistent with the pattern found by Garza et al. (2004) for 60 populations of steelhead from the Oregon border to the Point Conception area and is indicative of relatively high levels of gene flow (straying and subsequent reproduction) between basins separated by small coastline distances. It is also likely that fragmentation (i.e., reductions in effective population size) and past hatchery stocking practices have somewhat disrupted this relationship, through use of out-of-basin broodstock and interbasin transfers of fish. It is also important to note that construction of such trees requires simultaneous estimation of many population relationships and it is expected that some of them will not be properly resolved with only 24 loci and closely related populations (Felsenstein 2004), so interpretation of the results should not focus on any particular population relationship, as estimated from either the trees or F_{ST} values.

Evaluation of distinction of SCCC and SC DPSs

The current analyses do not provide evidence for a significant genetic distinction between steelhead in the two southern California DPSs. The original delineation of the boundary between these two administrative units was based upon biogeographic considerations (Busby et al. 1996). The major rivers in the SC DPS drain the Transverse Coast Range, the only major mountain range in California on an east/west axis, whereas the rivers in the SCCC DPS drain the South Coast Range, further to the north. In the AMOVA, the proportion of molecular genetic variance partitioned between populations in the two DPSs was only 1.15% of the total variation (Table 3, Grouping 5). The grouping that separated the Santa Clara River drainages from all others had the highest proportion of partitioned genetic variation of any of the possible groupings of drainages to the north and south of any geographic point (Table 3), but it still explained a very small proportion ($\sim$2%) of the total molecular variation. The phylogeographic trees (Figs. 2, 3) also failed to yield branches that separated populations from the two DPSs into distinct genetic lineages. These analyses demonstrate that there are not substantial differences in the recent ancestry of populations in the SCCC and the SC DPSs. Such methods are, indeed, sufficiently powerful to detect structure above the level of a river basin that is reflective of distinct evolutionarily history, similar to that assumed for a DPS or ESU, in steelhead in coastal California with the approaches used (Garza et al. 2004). Nevertheless, further analyses with population samples from additional year-classes might be helpful in confirming this result.

Evaluation of distinction between above- and below- barrier sites

Examination of the phylogeographic trees indicates that trout above and below dams in the same basin are generally closely related and in many cases the most genetically similar populations in the study. However, the magnitude of differentiation between above- and below- barrier populations was variable in the five basins examined (Table 3, Groups 8–12). While all pairs of population samples were significantly differentiated, the AMOVA results found none of the genetic variation was due to differences between above- and below-dam populations in the Salinas, Arroyo Grande, and Santa Ynez basins. This indicates recent common ancestry for these populations and/or contemporary gene flow (through downstream migration or transplantation in either direction) across the dams. The genetic similarity of these populations indicates that there has not been substantial divergence of trout populations breeding in streams above dam reservoirs since they were isolated by construction of the dams decades ago.

In the Ventura and Santa Clara drainages, the proportion of genetic variation explained by the presence of dams was substantially higher than in the other basins, although still relatively small. However, differentiation between populations within a basin but not separated by a dam still explained a greater percentage of the overall variation in all but the Santa Clara drainage. In addition, average F_{ST} was 0.097 for sites separated by Matilija Dam in the Ventura drainage, while average F_{ST} was 0.110 for sites separated by dams in the Santa Clara drainage (Appendix).

Pairwise F_{ST} values were generally lower between sites above the same dam (mean $F_{ST} = 0.079$) and between sites below dams within the same drainage (mean $F_{ST} = 0.069$) than for comparisons within a drainage but separated by a dam (mean $F_{ST} = 0.096$), although these values overlapped extensively. However, analyses of isolation by distance did not yield any significant relationships either through regression or Mantel tests.

Impact of stocking of study basins with trout from Fillmore Hatchery

The results of this study indicate that trout raised at Fillmore Hatchery and planted extensively in dam reservoirs in

the study basins have not made a substantial contribution to reproduction in the populations of *O. mykiss* studied here. There is no evidence of widespread admixture or introgression of hatchery trout into breeding populations of naturally spawning fish either above or below the dams. Individual-based assignment tests identified only two fish sampled from the five study basins as belonging to hatchery lineages, and tests for population or genic differentiation were highly significant in all comparisons of hatchery and wild population/strain samples. In addition, phylogeographic tree analysis (Figs. 2, 3) and model-based clustering (results not shown) clearly identified the Fillmore Hatchery strains as highly divergent from the naturally spawning *O. mykiss* populations sampled. It is worth noting that this does not mean that there has been no introgression of genes from hatchery fish into populations of native trout in these basins. Small numbers of hatchery fish may achieve reproductive success in some local populations and/or in some years, including those studied here. Moreover, if hatchery strains much more genetically similar to the native populations in this area were raised in a hatchery and released in the study area at some point in the past, then it is possible that some of these populations have past contributions from fish born in a hatchery. For example, it is known that steelhead from various other tributaries to Monterey Bay have been raised at the Kingfisher Flat (Big Creek) Hatchery on Scott Creek in Santa Cruz County and released in the Arroyo Seco (including Tassajara Creek) drainage of the Salinas River (Dave Strieg, Monterey Bay Salmon and Trout Project-MBSTP, personal communication). However, MBSTP breeds only naturally spawned fish and there is no documentation of substantial hatchery rearing of steelhead farther south.

A previous study of trout in the Santa Ynez drainage suggested that there was significant introgression of hatchery fish into native *O. mykiss* populations within the upper basin (Greenwald and Campton 2005). However, this is likely at least partially an artifact of the weak power associated with using a single mitochondrial locus. Overall, the fish sampled from sites in southern California for this study appear to share little ancestry with the hatchery strains included in this study. This may be a consequence of simple differences in timing of reproductive maturity or behavior of the two types of fish, which may in turn be a result of either domestication selection or ancestral differences in these traits.

Analyses of the small numbers of fish collected south of the Santa Clara River did, however, reveal some signals of hatchery ancestry. The Topanga Creek fish sampled were a mixture of fish with either predominately hatchery or native steelhead genotypes as well as some fish that appear intermediate (Fig. 4). Likewise, the fish from the Sweetwater River appear to be partly of hatchery origin, although individual assignments suggest that there may be some native steelhead ancestry (Fig. 4). Finally, the fish from San Juan Creek (Arroyo Trabuco) is of clear hatchery ancestry, whereas those from Malibu Creek, the San Gabriel River, the Santa Ana River and San Mateo Creek are clearly not (Fig. 4).

Genetic diversity

Genetic diversity was variable between sample sites, with observed heterozygosity varying by nearly 20% between the Santa Ynez-Salsipuedes and Arroyo Grande-Lopez Canyon sites, and allelic richness by more than 35% between the Ventura-Bear and the Arroyo Grande-Lopez Canyon sites. The more variable sites had levels of genetic diversity similar to those found in steelhead populations in the northern part of coastal California (Garza et al. 2004; Deiner et al. 2007). However, the majority of the population samples examined here have levels of diversity that are among the lowest observed in California steelhead populations, falling in the lower part of the distribution of allelic diversity for 18 of these microsatellite loci in the 80 population samples from the two studies. Similarly, estimates of the *M*-ratio, which uses a comparison of two measures of genetic diversity that decline at different rates following a reduction in population size (Garza and Williamson 2001), suggest widespread, recent decreases in effective population size and consequent loss of genetic diversity in the populations examined here, although it is not clear of what magnitude. It is also worth noting that the hatchery stocks have among the lowest levels of genetic variation observed in this study or that of Garza et al. (2004), so the prospect of inbreeding, and consequent inbreeding depression, in these hatchery strains and any populations established from them is of concern. Moreover, although the populations studied here appear to have experienced little introgression from these hatchery strains, changes in environmental conditions or stocking practices in the future could result in such admixture, and the consequent reduction in effective population size that would occur (Ryman and Laikre 1991) would be of concern and possibly complicate efforts to establish and recover viable populations.

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Appendix

Appendix 1 Pairwise genetic differentiation (F_{ST}) between 20 population samples and four hatchery strains from Southern California

	Tassajera	SanAntonio	Nacimiento	ArroyoGrande-Main	LosBerros	LopezCanyon	Salsipuedes	Hilton	SantaCruz	Juncal	Bear	Matilija-NorthFork	Matilija	Matilija-UpperNorthFork	SantaPaula	Sespe-LionCanyon	Piru-FrenchmansFlat	Piru-GoldHill	Piru-Lockwood	Coleman	HotCkVirginia	MtWhitney	HotCkWyoming
Tassajara	0.077	0.053	0.065	0.054	0.066	0.050	0.093	0.068	0.075	0.102	0.116	0.107	0.122	0.079	0.074	0.115	0.091	0.132	0.131	0.111	0.162	0.141	0.152
Tassajera		0.080	0.082	0.082	0.079	0.081	0.140	0.092	0.097	0.134	0.147	0.137	0.135	0.103	0.106	0.140	0.114	0.140	0.144	0.134	0.195	0.167	0.171
SanAntonio			0.074	0.061	0.071	0.052	0.100	0.090	0.077	0.118	0.129	0.113	0.120	0.080	0.079	0.115	0.113	0.119	0.125	0.118	0.177	0.147	0.165
Nacimiento				0.071	0.074	0.064	0.114	0.093	0.073	0.125	0.143	0.133	0.120	0.091	0.088	0.115	0.091	0.129	0.132	0.114	0.166	0.145	0.158
ArroyoGrande-Main					0.054	0.046	0.086	0.067	0.060	0.097	0.100	0.085	0.093	0.050	0.073	0.112	0.082	0.117	0.119	0.108	0.164	0.139	0.150
LosBerros						0.056	0.094	0.071	0.073	0.115	0.111	0.104	0.114	0.087	0.086	0.125	0.111	0.129	0.133	0.094	0.154	0.138	0.134
LopezCanyon							0.073	0.062	0.050	0.094	0.098	0.084	0.084	0.053	0.056	0.104	0.092	0.116	0.117	0.103	0.145	0.127	0.135
Salsipuedes								0.103	0.099	0.128	0.150	0.125	0.124	0.104	0.097	0.133	0.140	0.167	0.166	0.138	0.186	0.142	0.181
Hilton									0.076	0.134	0.126	0.113	0.125	0.082	0.097	0.139	0.115	0.158	0.159	0.121	0.176	0.158	0.155
SantaCruz										0.141	0.111	0.104	0.072	0.061	0.067	0.120	0.094	0.130	0.133	0.125	0.174	0.160	0.158
Juncal											0.168	0.156	0.172	0.130	0.108	0.160	0.155	0.186	0.180	0.155	0.199	0.172	0.202
Bear												0.031	0.129	0.108	0.114	0.148	0.145	0.169	0.164	0.145	0.175	0.174	0.179
Matilija-NorthFork													0.110	0.090	0.102	0.126	0.133	0.160	0.158	0.141	0.170	0.156	0.171
Matilija														0.051	0.102	0.157	0.135	0.146	0.148	0.155	0.199	0.176	0.184
Matilija-UpperNorthFork															0.082	0.134	0.107	0.130	0.132	0.119	0.184	0.148	0.167
SantaPaula																0.080	0.091	0.118	0.117	0.115	0.147	0.132	0.146
Sespe-LionCanyon																	0.098	0.120	0.118	0.147	0.181	0.170	0.180
Piru-FrenchmansFlat																		0.094	0.102	0.125	0.174	0.160	0.163
Piru-GoldHill																			0.010	0.142	0.208	0.190	0.195
Piru-Lockwood																				0.143	0.207	0.183	0.202
Coleman																					0.127	0.114	0.117
HotCkVirginia																						0.151	0.104
MtWhitney																							0.149

Values are calculated using the θ estimator of Weir and Cockerham (1984). All values are significant ($P < 0.001$)

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