

Population structure and ancestry of *O. mykiss* populations in South-Central California
based on genetic analysis of microsatellite data

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Introduction

Steelhead (*Oncorhynchus mykiss*) populations in California south of Monterey Bay are divided into two Distinct Population Segments (DPS), formerly Evolutionarily Significant Units (ESUs). 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, 2004). 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. A primary limiting factor for steelhead populations in southern California is access to freshwater habitat due to dams and water diversions, which are common in the region. Most of these barriers lack fish passage structures that prevent upstream migration. When fish from the species *O. mykiss* are currently found above such barriers they are considered to be resident rainbow trout, regardless of ancestry, and are not afforded protection under the ESA.

The recovery planning process for steelhead in these two southernmost DPSs is currently underway, yet several important questions regarding population structure of coastal trout in southern California remain. To provide insight into questions of population structure in this geographic area, we have performed genetic analysis of samples from 7 basins in the two DPSs using microsatellite DNA, highly variable genetic markers that can be used to trace ancestry and evaluate even small genetic distinction among populations. Microsatellites, also known as simple tandem repeat loci, have been

used in numerous studies of salmonids and have proven to be a valuable tool for elucidating genetic structure (Carlsson & Nilsson, 2001; Castric et al. 2001; Spidle et al. 2001; Wenberg & Bentzen, 2001; Docker and Heath, 2003; Olsen et al. 2003; Deiner, 2004; Garza et al. 2004; Poissant et al. 2005; Crispo et al. 2006).

Previous genetic work on genetic 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 gene 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, particularly on a relatively small geographic scale. However, recent work on *O. mykiss* in northern California using a large number of microsatellite loci has demonstrated that genetic structure can be easily identified with such data both at larger scales (Aguilar and Garza, 2006; Garza et al. in review) and at relatively fine ones (Deiner, 2004; Deiner et al. in press; Pearse et al. in press). For example, *O. mykiss* populations in the Russian River separated by waterfalls were highly genetically distinct, whereas those found above and below the two major dams (Warm Springs and Coyote) were found to show low levels of genetic distinction (Deiner et al. in press). In the Klamath River, genetic relationships of trout populations above barriers with those below barriers do not vary with geographic distance, whereas genetic relationships between populations below barriers do (Pearse et al. in press), a pattern referred to as isolation by distance.

In this study, we employ a collection of microsatellite loci to examine the genetic structure of *O. mykiss* in the two southernmost DPSs in California, with a focus on relationships between populations above and below dams. We analyze samples collected

in a systematic effort in 2003 from 5 watersheds: the Salinas, Arroyo Grande, Santa Ynez, Ventura and Santa Clara Rivers. We also analyze samples collected opportunistically and in small numbers from the southernmost extent of the range. These include samples from Malibu and Topanga Creeks in Los Angeles County, the Santa Ana and Arroyo Trabuco basins in Orange County, and San Mateo Creek and the Sweetwater River in San Diego County. We also analyze 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 part of the state. In some analyses, we use data from a previous study of *O. mykiss* in the northern part of the state (Garza et al. 2004) to provide a comparative phylogeographic framework.

We then use the results of the genetic analyses to address several aspects of the population structure of *O. mykiss* in this region that may be helpful in the management of this species. First, we evaluate recent ancestry of *O. mykiss* populations in streams above dams in multiple basins 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, we evaluate whether population genetic structure in the region 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, we evaluate patterns of genetic differentiation and genetic diversity between sites to provide insight into the levels of recent gene flow and demographic history.

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 sampled non-lethally by biologists from the National Marine Fisheries Service Santa Cruz Laboratory and the University of California Santa Cruz using a backpack electrofisher and the protocol described in Garza et al. (in review) to stratify sampling within the stream and minimize collection of tissue from siblings (Table 1, Figure 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. The reservoirs created by dams in this region have been stocked with trout from the Fillmore Hatchery, located on the Santa Clara River in Ventura County. Since population genetic structure may be influenced by hatchery *O. mykiss* plantings, we also collected and analyzed samples from all distinct Fillmore Hatchery strains.

South-Central California

Two river systems were sampled in the SCCC Steelhead DPS (Figure 1). On the Salinas River, “above-barrier” samples were acquired from Nacimiento Creek, above Nacimiento Dam (1957), and San Antonio Creek, above San Antonio Dam (1965). Tassajara Creek in the Arroyo Seco River drainage and Tassajara Creek in the extreme upper Salinas drainage, were sampled as the below-barrier populations for comparison.

Fish were also sampled from three sites in the Arroyo Grande River drainage, two sites below Lopez Dam, constructed in 1954 by the Army Corp of Engineers for flood control, and one site above. The Lopez Canyon site is located upstream of the county recreation area, above the dam, and the Los Berros Creek and lower mainstem Arroyo Grande sites are below any major barriers to anadromous migration. However, successful passage through the lower river is highly dependent on the timing and magnitude of water releases from the dam and downstream diversions, as the lower section of the river is often dewatered for part of the year.

Southern California

Three drainages were sampled in the SC steelhead DPS, with collections occurring sites in the Santa Ynez, Ventura, and Santa Clara River drainages (Table 1, Figure 1). On the Santa Ynez River, Santa Cruz Creek flows into Lake Cachuma upstream of Bradbury Dam (constructed in 1953), and the North Fork Juncal Creek site lies further upstream above both Gibraltar and Juncal Dams. Hilton Creek is the last tributary below Bradbury Dam, which is the first barrier to upstream migration on the Santa Ynez River, and Salsipuedes Creek is a large tributary in the lower Santa Ynez River. Additional sites were sampled, however, the sample size collected at each site was too small to provide accurate population genetic inference. These sites include: Devil's Canyon (N=3), Indian Creek (N=2) and the mainstem Santa Ynez (N=12) between the Gibraltar and Bradbury Dams. They are analyzed only using model-based assignment clustering techniques described below.

Four sites were sampled on the Ventura River, with two “above-barrier” sites upstream of Matilija Dam (constructed in 1947) and two sites below the dam. The first

site is on the mainstem of Matilija Creek just above the reservoir and the second site is on the Upper North Fork of Matilija Creek. The two below barrier sites are on the North Fork Matilija Creek, which is distinct from the Upper North Fork and has its confluence just downstream of the Matilija Dam, and Bear Creek which is a tributary to the North Fork of Matilija Creek.

The Santa Clara River is the largest drainage in the SC DPS that is consistently occupied by steelhead and also the furthest south of the systematically sampled basins. Three locations were sampled in the Piru Creek drainage upstream of Santa Felicia Dam (constructed in 1954): Lockwood Creek, Piru Creek at Gold Hill, and Piru Creek at Frenchman's Flat. The first two sites are also above Pyramid Dam and its associated reservoir. The two below-barrier sites are Santa Paula Creek and Lion Canyon in the Sespe Creek drainage. An additional site (Blue Point on Piru Creek, N=12) was sampled in this basin but contained too few samples for population analysis. It is analyzed only using model-based assignment clustering techniques described below.

There are numerous drainages further south than the Santa Clara River in the geographic range of the SC steelhead DPS, including some very large ones (e.g. Los Angeles, San Gabriel Rivers), and a number of smaller drainages in the Santa Monica and Santa Ana Mountains. However, most are very heavily impacted by anthropogenic activity and/or without ocean access for most of the year. Because of this, the Santa Clara River is the southernmost relatively large drainage with substantial anadromous fish habitat and population samples could not be collected from further south. However, tissues were obtained and analyzed from smaller numbers of individuals from several drainages in Los Angeles, Orange and San Diego counties collected over a number of

years, 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). However, one of the Topanga Creek fish was not used due to poor sample quality.

DNA Collection and Extraction

The non-lethally collected tissue samples consisted of small caudal fin clips (2-5mm²) that were placed on blotter paper, inserted into coin envelopes and dried thoroughly in a dessicator. DNA was extracted using the Qiagen Dneasy Tissue Kit, following the manufacturer's recommended protocol for animal tissues and using a BioRobot 3000 (Qiagen Inc.). Approximately 2mm² of tissue was digested in 180µL of Qiagen buffer ATL and 20µL proteinase K and kept overnight in a shaking incubator at 55°C. The DNA was then bound to the Dneasy silica-gel membrane with the addition of 200µL Qiagen buffer AL and 200µL of ethanol, washed with 500µL each of Qiagen buffer AW1 and AW2, and finally eluted in 200µL buffer AE (Qiagen, 2000). Extracted DNA was kept frozen at 20°C until it was diluted (20:1 with autoclaved, distilled water) and distributed to 96 well plates for microsatellite amplification via polymerase chain reaction (PCR).

Genotyping

Individuals were successfully genotyped at 18 microsatellite loci in all population samples collected and were therefore used in the population genetic analyses (Table 2). PCR reactions were carried out in 15µL aliquots containing 4µL purified and diluted

template DNA, 6.35 μ L H₂O, 1.5 μ L ABI 10X II PCR buffer, 0.9 μ L MgCl₂, 1.2 μ L dNTPs, 0.05 μ L ABI Amplitaq DNA polymerase, and 1 μ L fluorescent-labeled oligonucleotide primers (Integrated DNA Technologies, Inc.). Promega, Inc. reagents were used for Omy1011 reactions due to more consistent and reliable amplification. Multiple thermal cycler (MJ Research PTC 225) routines were employed to maximize PCR product. The typical profile consisted of a two minute pre-denaturation at 95 C, then two amplification stages: (a) 10 cycles of denaturation at 95 C for 15s, annealing at 53 C for 15s, and extension at 72C for 45s; (b) 25 cycles at 89 C for 15s, 55 C for 15s, and 72C for 45s. The routine concluded with a final extension phase of 72 C for 5 minutes and indefinite hold at 4C. PCR products were pooled to equalize peak heights and take advantage of multiple label colors and two non-overlapping ends of the measurable size range (50bp-500bp) within each lane. A mix of Formamide, loading dye and internal size standard was added to the pooled PCR product, denatured at 95 C for 3 minutes and immediately transferred to ice. The samples were electrophoresed with either an ABI Prism 377 DNA sequencer or an ABI 3100 genetic analyzer. Gel imaging, lane tracking and allele size for loci run with the ABI 377 were scored with GENESCAN version 3.1.2 and GENOTYPER version 2.1 software (Applied Biosystems). Loci analyzed with the ABI 3100 were analyzed with Gene Mapper version 4.0 software. 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 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 sample population. 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 2001). FSTAT was also used to calculate the population inbreeding coefficient (F_{IS}), with the probability of significance determined by 10,000 permutations. 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). The alternative hypotheses (H_1) of heterozygote deficiency and heterozygote excess were both tested with Markov chain parameters of 10000 (dememorization), 1000 (batches) and 1000 (iterations per batch). Linkage (gametic phase) disequilibrium was also evaluated to ensure segregation independence of the 18 microsatellite loci in each of the sample populations and using the same type of MCMC approximation of an exact tests as implemented in GENEPOP. Markov chain parameters were the same as those used for the heterozygosity exact tests.

The mean ratio M , the number of alleles/(range in allele size + 1), was also calculated 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 (see Garza and Williamson 2001). During a population decline, alleles are lost and gaps appear in the allele frequency distributions. As a result, the number of alleles decreases more rapidly than the range in allele size and M decreases (Garza and Williamson 2001). When 18 variable loci are assayed and conservative assumptions

about the mutation process are made, a value of $M < 0.71$ indicates that the population under study has experienced a recent reduction in effective population size.

Genetic differentiation between sample populations was examined with several methods. Using the test for genic differentiation in GENEPOP version 3.4, a Fisher's exact test was employed to calculate the probability of the null hypothesis (H_0) that allele frequencies were identical across populations (Raymond & Rousset 2001). Pairwise differentiation between all pairs of populations was also quantified using F_{ST} , as estimated by Weir and Cockerham's (1984) Θ estimator, and significance (> 0) assessed by the permutation algorithm in ARLEQUIN (Excoffier et al. 2005) with 10,000 replicates.

The distribution of molecular variation was assessed to identify informative groupings of sample populations using the Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) option in ARLEQUIN version 3.0. Molecular variance was partitioned into components of among groups (F_{CT}), among populations within groups (F_{ST}) and within sample populations (F_{SC}). Statistical significance of variance components was assessed with 10,000 permutations and p-values defined as the percent of random values greater than the observed value. All drainages with multiple above and below barrier populations were included in the analysis ($N=4$). Within-drainage comparisons were made between above and below barrier groups.

Individual-based assignment tests were used to further evaluate the degree of recent gene flow between sample populations of trout. Fish were assigned to their most likely population of origin, utilizing the Bayesian algorithm of Rannala and Mountain (1997) as implemented in GeneClass version 2.0.g (Piry 2004). Although application of

assignment tests can be used to detect first generation migrants (Rannala and Mountain 1997), misassignment, or assignment of an individual to a population other than that of its sampled location, should not be interpreted as migration with juvenile fish, but as a signal of recent ancestry. Since such long-distance migration events are infrequent, patterns of misassigned fish highlight similarities in genetic composition (allele frequencies) between sample populations/locations. Misassignments may also occur randomly if an individual expresses a genotype composed of alleles that are common to many groups. This type of analysis was the only one possible with the smaller samples of fish from the southernmost basins described above, as well as the samples from sites in systematically sampled basins that were not of sufficient size for population level analyses. In these analyses, only the larger population samples and the Fillmore Hatchery strains were used as reference populations for potential assignment. The other type of analysis applied to all of the samples is the Bayesian model-based individual clustering method implemented in the program structure (Pritchard et al. 2000). In this analysis, a prior hypothesis about the number of genetic “populations” is used to partition the dataset into clusters and then fractionally assign the ancestry of each individual fish to each of the clusters without regard to geographic location of origin.

Phylogeographic trees were constructed using matrices of Cavalli-Sforza & 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 consensus tree was assembled from 1,000 bootstraps of the distance matrix with the PHYLIP CONSENSE component. Internal branch lengths on the consensus tree are scaled by the number of times that relationship was found in the neighbor-joining trees

constructed with the bootstrap samples, which is a measure of confidence in that branch. These analyses were also carried out after combining the population samples described here with the 60 population samples analyzed by Garza et al. (in review), but only with the 15 loci where the data could be easily combined, due to differences in original data collection methods.

Results

The results described here generally only include the sites with adequate sample sizes for population genetic analyses (see above and Table 1). These results describe the population genetic structure found among watersheds as well as across barriers within basins. Combination of the new data with those from 60 additional population samples previously described (Garza et al. in review) provides broader geographic context for interpretation of genetic relationships. The phylogeographic results also bear upon the issue of introgression and/or hybridization of planted hatchery trout with native trout in the basins under study. In addition, the results provide a comparison of levels of genetic diversity among the sites sampled for this study.

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 (Figure 2a). The pattern of population clustering (topology) of the tree has several salient features. First, population samples from the Ventura, Santa Clara and Salinas Rivers, both those sampled above and below barriers, form monophyletic

lineages on the tree, whereas the population samples from the Santa Ynez and Arroyo Grande Rivers are interspersed with one another and in a central position in the tree. The Fillmore Hatchery strains all clustered together, and are separated by a long internal branch from all of the naturally spawned population.

The bootstrap consensus tree (Figure 2b) had very similar topology to the chord distance/neighbor joining tree, clustering the populations from the Salinas, Ventura and Santa Clara Rivers, as well as the hatchery populations, with moderate bootstrap support for monophyletic lineages, and interspersed and sparse bootstrap support for monophyletic lineages of the Santa Ynez and Arroyo Grande populations. 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 always clustered together, as did the two samples above both Pyramid and Santa Felicia Dams. It is important to point out that, although these groups were most closely related in our study, the next most similar population samples were those on the other side of the dam. In addition, samples from the late and early components of the Mt. Whitney trout strain from Fillmore Hatchery always clustered together, as did samples from two consecutive year-classes of the Hot Creek strain. The lack of interspersed 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 reproduction in trout population in streams above or below the dam reservoirs.

Evaluation of the 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 chord distance/neighbor joining tree and the bootstrap consensus tree are presented in Figure 3. Several features of the trees stand out. First, all of the southern steelhead population samples described here cluster 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 is no strong signal of geographically-based reductions in gene flow in the southern populations above the level of the basin. That is, there are not internal branches that separate populations into groups that correspond to the three currently recognized DPSs in this region. This is consistent with the results of Garza et al. (in review), 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 is concordant with the earlier work is 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.

Exact tests identified significant differences in allele frequencies between all pairs of sample sites, although only marginally so for two pairs. Similarly, pairwise F_{ST} , the proportion of genetic variation that separates the population samples, was significantly ($p < 0.001$) different from zero for all but two comparisons following correction for multiple comparisons (Appendix 1), the Lockwood Creek and Piru Creek at Gold Hill sampling sites ($F_{ST} = 0.004$, $p = 0.002$), above both dams on the Piru Creek tributary of the Santa Clara River, and the 2002 and 2003 year classes of the Hot Creek Virginia strain

($F_{ST}=0.009$, $p=0.008$) from the Fillmore Hatchery. Thus, sites from Lockwood Creek and Piru Creek at Gold Hill were combined for further analyses of genetic structure (e.g. AMOVA) among geographic sites. Overall, the mean value of F_{ST} was 0.124, indicating that approximately 12% of all of the genetic variation in the dataset was partitioned between population samples. Mean F_{ST} for within-basin comparisons was 0.086 while the mean value for between-basin comparisons was 0.108. 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.

AMOVA analysis indicated that within-population variation was the dominant component of molecular variance for all population groupings evaluated in this study (Table 3a, b). The molecular variance was generally greater among populations within groups than between groups, indicating substantial differentiation between sample sites and a generally lack of elevated differentiation between pairs of populations above and below dams. Differences between basins accounted for 3.42% of the overall variation, when only below-barrier populations were considered and 2.5% when all populations were considered (Table 3a). 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 that between-basin geographic and genetic population structure are not strictly concordant, 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 not significantly different from 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 were significantly different from 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 (Figure 2, Appendix 1).

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 93% and to the basin of origin with 98.9% accuracy (Table 4). Only 105 fish of 1505 were misassigned to a population location other than the one where they were sampled and, of those, only 16 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. One-third of fish at these sites were misassigned which is only marginally better than the one half expected with random assignment. This is consistent

with the non-significant differentiation (F_{ST}) value between them, indicating that they are not separate populations. When these samples are combined, the total assignment accuracy to population climbed to 96.5%. Only two fish from rivers assigned to the various hatchery strains, one each from the Salinas and Arroyo Grande (Table 4).

The individual assignment tests were also performed with the fish collected from locations where insufficient numbers were present for full population genetic analyses using the 5 basin population samples and the Fillmore Hatchery strains as potential populations of origin. The results of these assignment tests are presented in Table 5. A larger proportion of fish from these southern basins assigned to Fillmore Hatchery Strains than from basins further north (29.3% [12 of 41] in the south vs. 0.1% [2 of 1505] in the northern 5 basins). These values are not strictly comparable, because the population of origin was not available for assignment with the small samples. However, the second most likely population for assignment for the fish in the northern 5 basins included a Fillmore Hatchery stock as second choice less than 1% (11 of 1505) of the time. The one fish from San Juan Creek (Arroyo Trabuco) was assigned to hatchery stocks, as were almost half of the fish from Topanga Creek (8 of 17) and the Sweetwater River (3 of 7).

Genetic Diversity

Expected heterozygosity ranged from 0.582 to 0.707 with a mean value of 0.634 over the 20 sample populations evaluated (Table 1). After adjusting the mean number of alleles per population for the smallest sample size ($N=23$), mean allelic richness was 5.6 over all samples. Both the number of alleles observed (9.7) and allelic richness (6.7) 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 (4.9) and allelic richness (4.6). 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 60 populations surveyed in the two populations 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 by Garza et al. (in review) 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. Values for the M-Ratio were all significant, and ranged from 0.505 to 0.667 and averaged 0.582 over all sample populations, indicating widespread effective population size reductions.

Values of F_{IS} were found to be significantly greater than zero ($p < 0.01$) in five of the sample populations, suggesting potential inbreeding, sampling of two distinct populations or null alleles (Table 1). However, statistical evaluation of heterozygote excess and deficiency yielded only 26 of 453 significant tests ($p < 0.001$), which appeared randomly distributed across loci and populations. Similarly, tests for linkage disequilibrium revealed that over all populations, only 11 loci pairs (out of 153) showed significant disequilibrium ($p < 0.001$). Overall, deviations from equilibrium were similar to that expected by chance alone and were not expected to impact the other analyses.

Discussion

Genetic analyses of microsatellite data presented here successfully address questions regarding population genetic structure in the South Central California Coast and Southern California 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.

Specifically, no substantial genetic differentiation was found between trout populations above and below dams in the 5 river basins studied, which indicates that populations of trout breeding in streams tributary to the dam reservoirs are recently derived from a common ancestral population with trout populations breeding below the dams. This suggests that breeding populations in these upstream tributaries are likely dominated by trout descended from steelhead isolated above the dams following their construction.

Phylogeographic and AMOVA analyses also failed to find a signal of reduced gene flow at any point on the coast, including between the Santa Ynez and Arroyo Grande Rivers, the location of the current boundary between the two steelhead DPSs in this region. This indicates that there is no significant genetic differentiation separating populations to the north or south of any point in the geographic region of study here and indicates that there is no genetic basis for delineation of these two distinct population segments. Several different types of analysis indicated that the primary level of structure for steelhead in the southern part of California is the local population, followed by the river basin, then the region. Evaluation of the results of phylogeographic analyses of the

current dataset with that of Garza et al. (in review) indicates that there is no evidence for any geographically defined “breaks” in gene flow, that result in genetically distinct lineages including multiple river basins. To the extent that a DPS is intended to represent a unique genetic lineage within a species, the current delineations south of the Golden Gate are not consistent with population genetic structure.

Finally, several analyses indicate that trout populations breeding in streams above dam reservoirs and those breeding below dams have not been heavily introgressed by planted trout from Fillmore Hatchery or any other hatchery stock that is derived from out of basin broodstock. This does not mean that there has been no influence of hatchery plants on any of the populations surveyed here, or that their will not be in the future, but only that the vast majority of fish sampled for this study are not directly descended from hatchery raised fish or their recent progeny. However, use of individual-based assignment analyses to identify the recent ancestry of small numbers of fish from basins in Los Angeles, Orange and San Diego Counties found a substantial signal of hatchery ancestry, particularly in Topanga Creek.

Each of these areas of inference is described in more detail below.

Overall Genetic Structure

Analysis of population genetic structure found evidence for hierarchical structure similar to that found in steelhead populations further to the north (Garza et al. in review). The majority of genetic variation was at the level of individual local population with multiple analyses. Tests of genetic differentiation were significant for nearly every

location sampled and the differentiated populations were represented by relatively long terminal branches on the phylogeographic trees. In the AMOVA analyses approximately 90% of the molecular variance was partitioned among individual populations in almost all analytical frameworks evaluated. These results are also consistent with the very high assignment accuracy (>95%) to differentiated populations and the almost perfect 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 not separated by a dam, help delineate the lower geographic limit at which population structure might be observed.

Analysis of population structure at a higher spatial scale found variable results. 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, as do the Salinas River populations from above San Antonio Dam and below it, but relatively far downstream, in Tassajara Creek in the Arroyo Seco drainage. All population samples, both above and below dams, from the Salinas, Ventura, and Santa Clara Rivers formed basin-specific lineages in some of 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 (Figure 4). 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.

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. (in review) for 60 populations of steelhead from the Oregon border to Morro Bay and is indicative of relatively high levels of gene flow (straying and subsequent reproduction) between basins separated by small coastline distances. 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 18 loci and closely related populations, so particular emphases in 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. In the AMOVA, the proportion of molecular genetic variance partitioned between populations in the two DPSs was only 1.61% of the total variation and was only marginally significantly different from zero (Table 3, Grouping 5). The grouping that separated the Santa Clara drainages from all others had the highest proportion of genetic variation partitioned 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 of the total molecular variation. The phylogeographic trees 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 evolutionary histories 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 evolutionary history, similar to that assumed for an ESU, in steelhead in northern California with the approaches used (Garza et al. in review). 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 our 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 that a non-significant proportion 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 Santa Clara and Ventura drainages, the proportion of genetic variation explained by the presence of dams was significantly different than zero and average $F_{ST}=0.109$ for sites above and below the dams in the Santa Clara drainage (when the Lockwood and Gold Hill samples are combined) and 0.100 for the Ventura drainage (Appendix 1). Although differentiation within groups still explained a greater percentage of the overall variation, pairwise F_{ST} values were generally lower between above-barrier sites (average $F_{ST}=0.069$) and below-barrier sites (average $F_{ST}=0.059$) than for comparisons of an above-barrier and a below-barrier site (see above).

For comparison, Deiner et al. (in press), using the same microsatellites, found comparisons of trout populations above Coyote and Warm Springs dams in the Russian River with populations below to be very similar (average $F_{ST}=0.057$) to both that observed between populations below barriers in the 5 basins studied here and to differentiation observed between eight sites below barriers in the Russian River. In contrast, they also found differentiation between *O. mykiss* populations above and below

natural barriers (e.g. waterfalls) to be considerably higher (average $F_{ST} = 0.158$) than those found across the dams in the Santa Clara and Ventura drainages (average $F_{ST} = 0.105$). However, pairwise F_{ST} is highly correlated with genetic diversity and, therefore, population size in California steelhead (e.g. Garza et al. in review). Many of the population sizes in areas above waterfalls in the Russian River basin are highly constrained by available habitat area, whereas population sizes above dams in the Ventura and Santa Clara Rivers appear to be less constrained, based on genetic diversity, so it is hard to conclude anything from this comparison.

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 trout either above or below the dams. Individual-based assignment tests identified only two fish sampled from wild populations 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 and model-based clustering (Figure 2, 3 and 4) clearly identified the Fillmore hatchery strains as highly divergent from the wild *O. mykiss* populations sampled. It is worth noting that this does not mean that there has been no introgression of 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 hatchery ancestry. For example, it is known that steelhead from various other rivers tributary 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, personal communication). However, they do not maintain a hatchery strain and it seems unlikely that there has been substantial activity of this nature further south, as it is clear that there is little genetic influence of the hatchery strains that are currently commonly raised in California.

A previous study of trout in the Santa Ynez drainage suggested that there was significant introgression of native fish with hatchery fish in the upper basin (Greenwald and Campton 2005). However, this is likely an artifact of the weak power associated with using a single mitochondrial locus. Overall, fish sampled from these sites in southern California 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 a substantial signal 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 (Figure 4b). Likewise, the fish

from the Sweetwater River appear to be primarily of hatchery origin, although individual assignments (Table 5) do suggest that there is some native steelhead ancestry. Finally, the fish from San Juan Creek (Arroyo Trabuco) is of clear hatchery ancestry, whereas those from Malibu, San Gabriel and San Mateo Creeks are clearly not (Figure 4b, Table 5).

The contrasting results for the Sweetwater River fish with the two assignment methods are most likely a consequence of the way that the two analyses are conducted. In the probabilistic method (Table 5), each of the individual population samples is a potential source and the allele frequencies in each are estimated from only those individuals in the population, whereas in the other, semi-Bayesian method (Figure 4) the data are partitioned so as to assign ancestry of each individual fish to one of the three primary clusters, which are constructed without geographic information and characterized by the allele frequencies from all of the constituent fish. In this latter analysis, there are only three choices, the cluster comprising only the Fillmore strains, the one dominated by the Santa Clara River and the one that includes most individuals in all of the other rivers surveyed. When there are only these three choices, then the most similar cluster for all of the Sweetwater fish is the one dominated by Fillmore Hatchery strains. When all of the individual population samples are potential sources for these fish, then four of the Sweetwater fish appear more similar to a coastal steelhead population. However, both analyses force each fish to assign to one of the provided source populations, even when the actual source is not present. It is possible that reason for this discrepancy between the two methods in the primary affinity for four of the seven Sweetwater River fish is because the actual source, or a genetically similar proxy population, is not available for assignment. This unrepresented source population would almost definitely have to be

from an isolated rainbow trout lineage, as the coastal steelhead lineages used in the analyses encompass the full range of genetic variation likely to be present in any steelhead-derived fish in Southern California. This hypothesis is supported by the semi-Bayesian assignment analysis that uses as a potential source population all of the hatchery rainbow trout strains, thereby encompassing a broader sample of the genetic variation present in California rainbow trout, and finds all of the Sweetwater fish to be more similar to the hatchery rainbow trout than either of the two Southern California steelhead lineages. Analysis of more samples from California rainbow trout populations, as well as evaluation of additional genetic haplotype markers currently under development by the authors, should provide greater resolution of this issue in the future.

Genetic Diversity

Genetic diversity was variable between sample sites, with heterozygosity varying by as much as 20% between the Ventura-Bear and Arroyo Grande- Lopez Canyon sites, and allelic richness by almost 50% between the same 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. in review, Deiner et al. in press). 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 these 18 microsatellite loci in the 60 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, 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. (in review), so the prospect of inbreeding, and consequent inbreeding depression, in these hatchery strains and any populations established from them are of concern. Moreover, although the populations studied here appear to have experienced little introgression with 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 retain and recover viable populations.

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Figure 1: Map showing the approximate locations of the samples systematically collected for this study. The 5 basins sampled are highlighted in color.

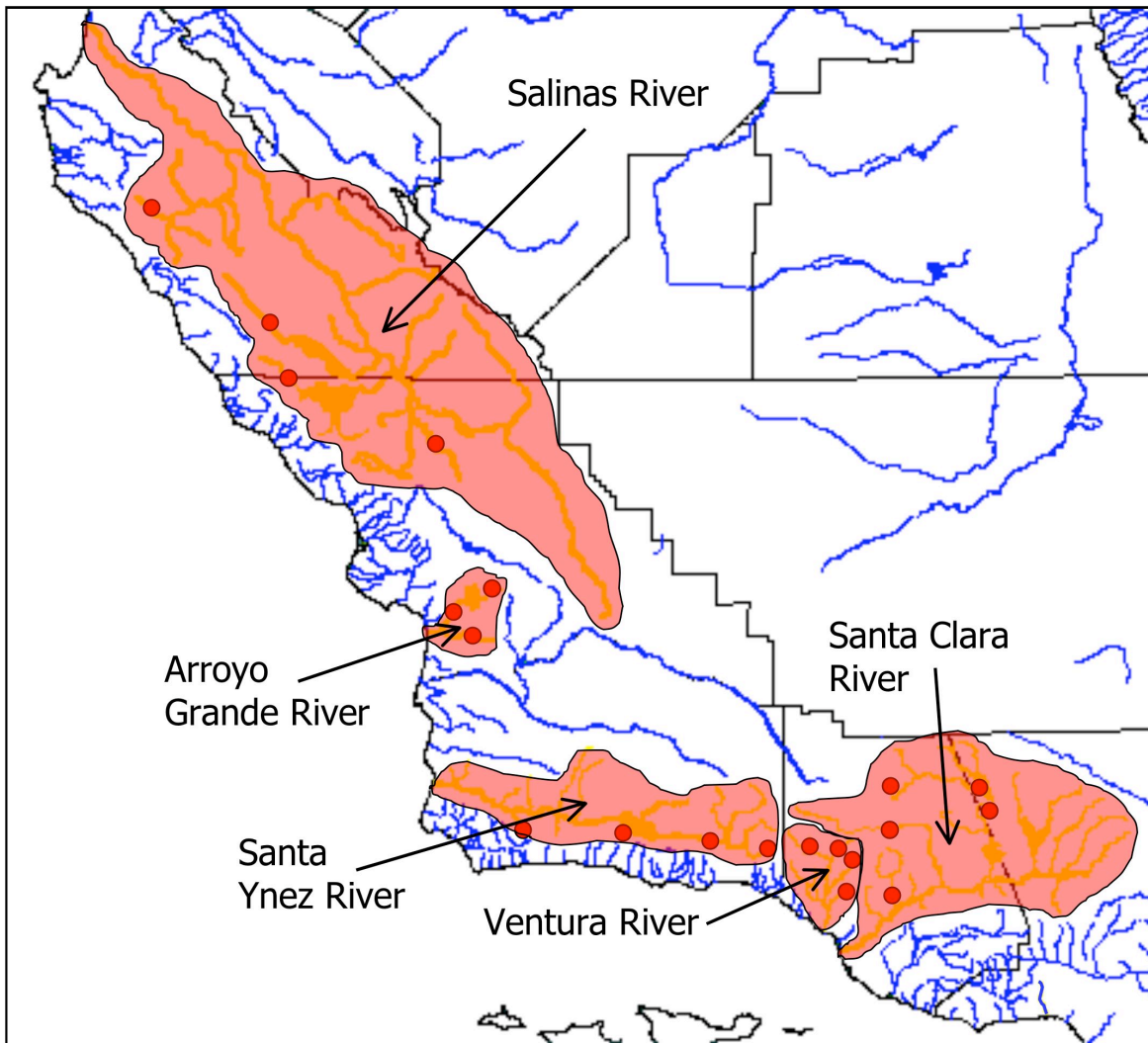


Figure 2. a) A neighbor-joining tree depicting relationships between populations samples constructed using chord genetic (Cavalli-Sforza and Edwards) distances. Branch lengths are proportional to genetic distance.

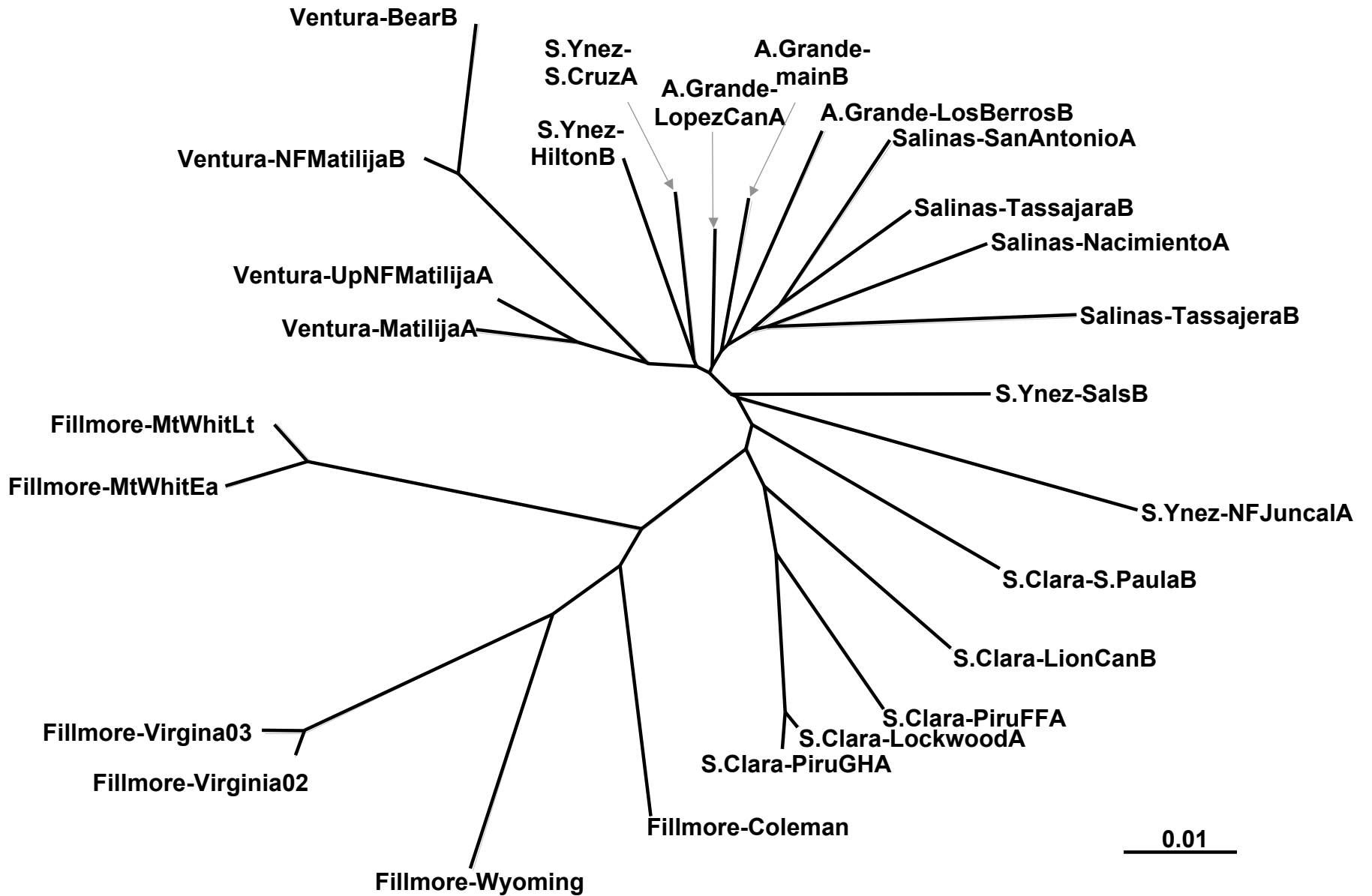


Figure 2. b) Majority rule consensus of neighbor-joining trees constructed with chord distances calculated from 1000 bootstrap datasets. Numbers on internal branches are the percentage of bootstrap replicates in which that grouping was found.

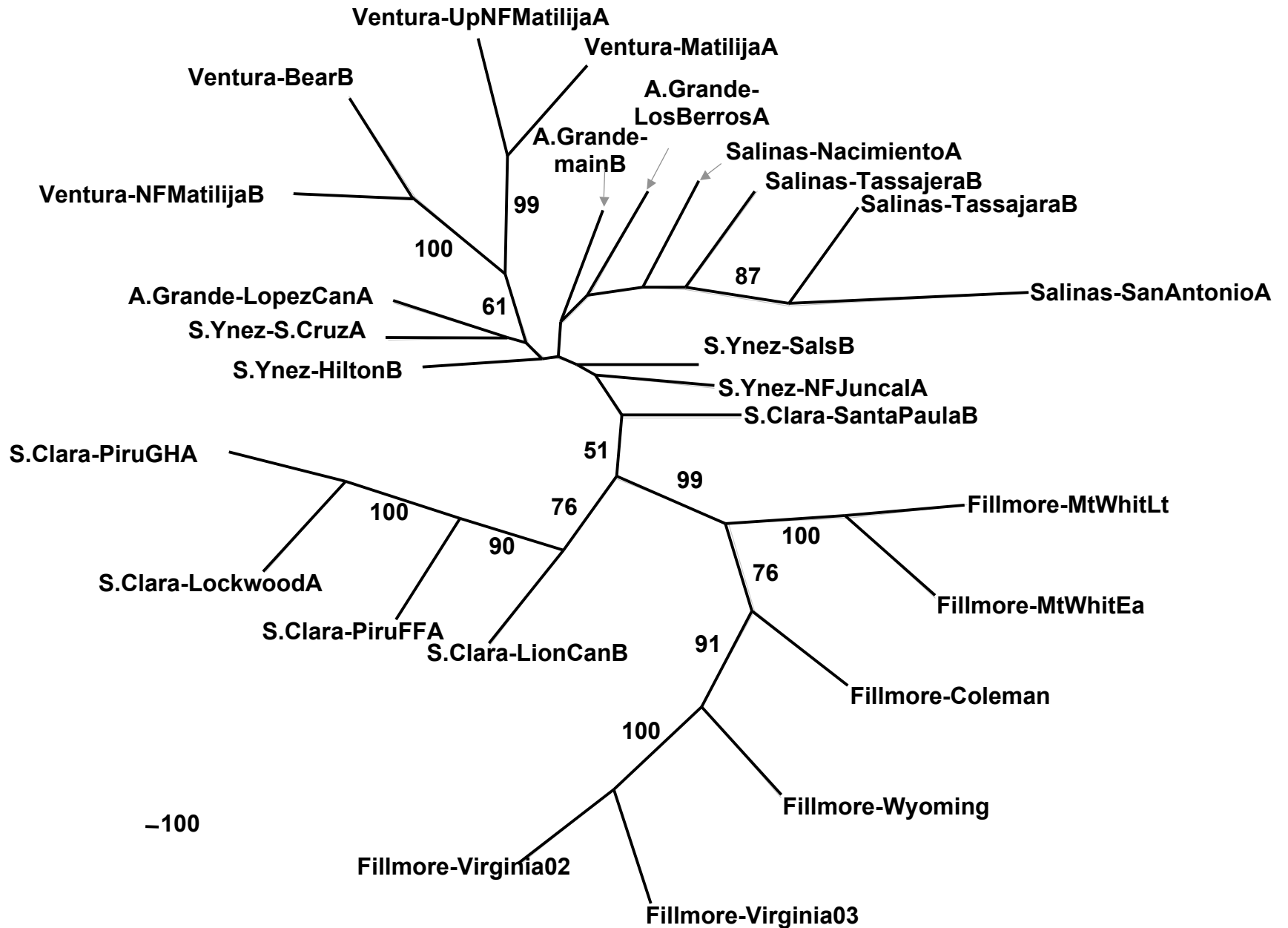


Figure 3. a) Neighbor-joining tree constructed with chord distances calculated from combined northern and southern California steelhead population data from 15 microsatellite loci. Branch lengths are proportional to genetic distance.

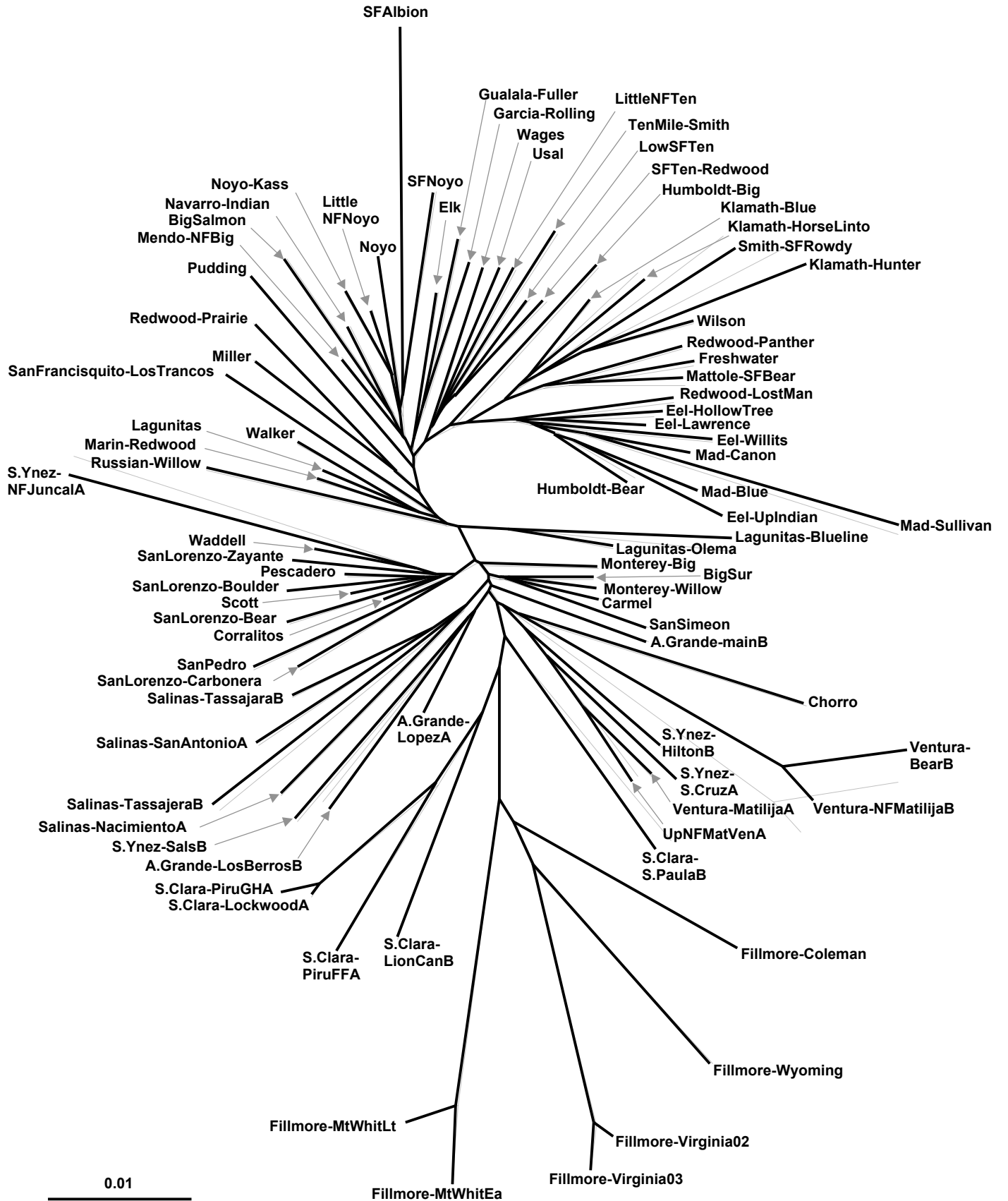


Figure 3 (cont.). b) Majority rule consensus of neighbor-joining trees constructed with chord distances from 100 bootstrap samples of the combined northern and southern California steelhead populations dataset of 15 microsatellite loci. Numbers on internal branches are the percentage of bootstrap replicates in which grouping was found.

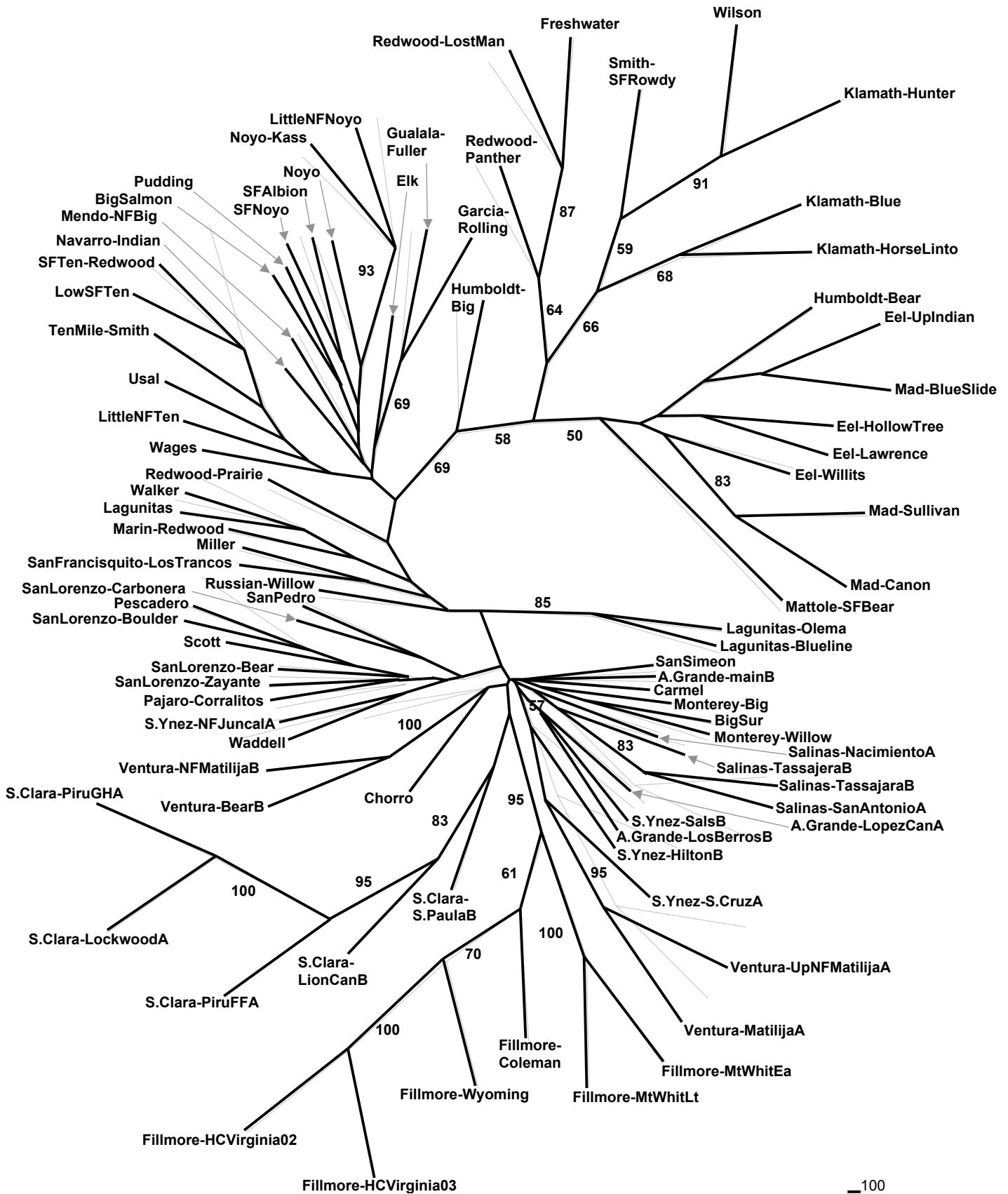


Figure 4. a) Summary of results for individual ancestry assignment using the model-based clustering method implemented in the software package *structure*. Green, blue and red represent the fractional ancestry of each fish as it is assigned to each of the 3 clusters. Groups 1-4, Salinas River; 5-8, Arroyo Grande; 9-15 Santa Ynez; 16-21, Ventura; 22-27, Santa Clara; 28-33, Fillmore Hatchery; 34-South of Santa Clara.

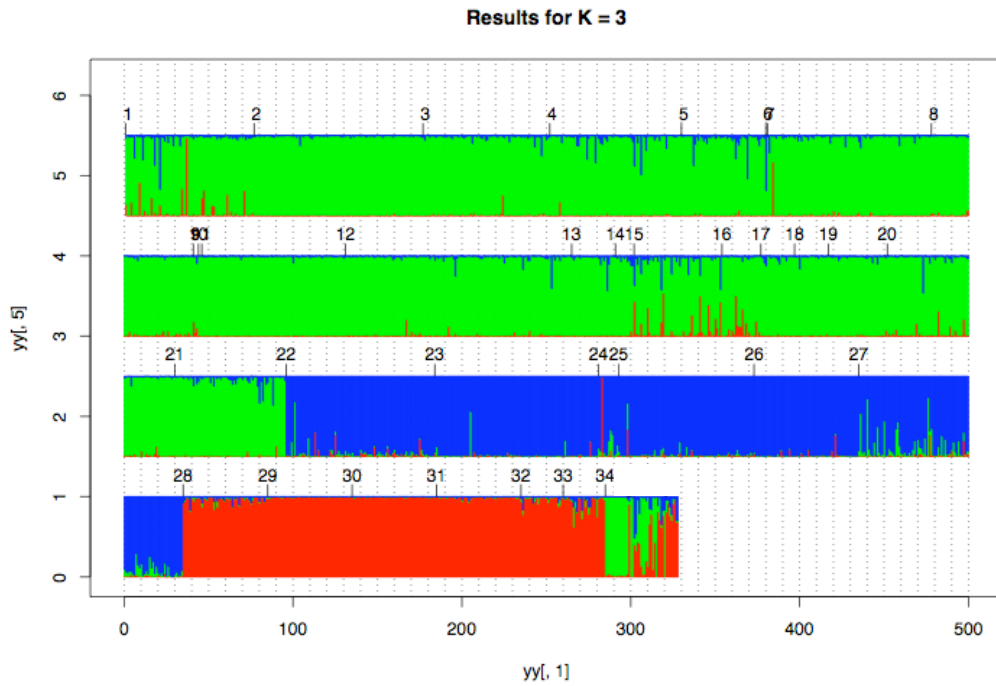


Figure 4. b) Expanded view of ancestry results for fish south of Santa Clara River.

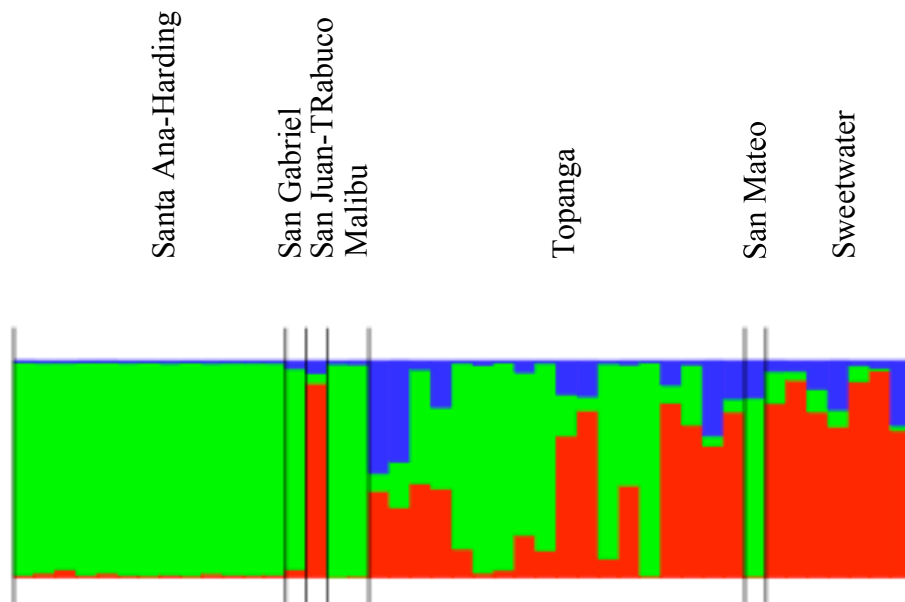


Table 1: Descriptive statistics of 20 populations of *O. mykiss* from 5 basins and 6 samples from Fillmore Hatchery strains. N=Sample size, He=Expected heterozygosity, Ho=Observed heterozygosity, A=Observed no. of alleles, R=Allelic richness

Drainage	Population	Barrier	N	He	Ho	A	R	Fis	M-Ratio
Salinas	Nacimiento	A	76	0.649	0.660	8.5	6.1	-0.018	0.558
	SanAntonio	A	100	0.637	0.596	7.9	5.8	0.065	0.548
	Tassajara	B	75	0.649	0.638	8.8	6.0	0.017	0.611
	Tassajera	B	78	0.632	0.610	7.3	5.6	0.035	0.565
Arroyo Grande	ArrGrande	B	51	0.673	0.662	8.5	6.6	0.017	0.599
	LopezCan	A	97	0.704	0.728	9.7	6.7	-0.034	0.653
	LosBerros	B	63	0.667	0.671	7.2	5.9	-0.006	0.585
Santa Ynez	Hilton	B	52	0.626	0.589	7.3	5.7	0.060	0.528
	NFJuncal	A	85	0.585	0.611	6.2	4.8	-0.045	0.548
	Salsipuedes	B	134	0.615	0.588	7.6	5.3	0.044	0.505
	SantaCruz	A	26	0.652	0.660	6.6	5.9	-0.012	0.533
Ventura	Bear	B	23	0.617	0.599	4.9	4.6	0.030	0.513
	Matilija	A	75	0.642	0.606	7.6	5.6	0.057	0.592
	NFMatilija	B	78	0.631	0.623	6.2	5.0	0.013	0.605
	UpNFMatilija	A	66	0.651	0.667	7.6	5.8	-0.024	0.591
Santa Clara	LionCan	B	88	0.600	0.606	8.3	6.0	-0.010	0.667
	Lockwood	A	97	0.586	0.592	7.1	5.3	-0.009	0.552
	PiruFF	A	80	0.606	0.616	6.8	5.1	-0.018	0.578
	PiruGH	A	62	0.582	0.579	6.9	5.3	0.006	0.612
	SantaPaula	B	100	0.707	0.691	8.2	6.4	0.023	0.544
Fillmore Hatchery	Coleman	n/a	50	0.637	0.643	7.3	5.8	-0.010	0.629
	HCVirginiaA	n/a	50	0.647	0.612	6.5	5.2	0.054	0.631
	HCVirginiaB	n/a	50	0.655	0.651	5.5	4.9	0.006	0.624
	HCWyoming	n/a	50	0.666	0.673	6.7	5.5	-0.011	0.570
	MtWhitEa	n/a	25	0.597	0.629	6.1	5.3	-0.055	0.614
	MtWhitLt	n/a	25	0.601	0.613	5.8	5.1	-0.020	0.617
		Mean	66	0.634	0.634	7.1	5.5	0.001	0.582
		SD	29	0.033	0.038	1.2	0.6	0.042	0.042
		Var	812.62	0.001	0.001	1.6	0.4	0.002	0.002

A= above barrier, B = below barrier

Table 2. The microsatellite loci studied with the species in which they were originally described and the original reference.

Locus	Species	Reference
Omy 27	<i>O. mykiss</i>	McConnell et al. 1995
Omy 77	<i>O. mykiss</i>	Morris et al. 1996
Omy 1011	<i>O. mykiss</i>	Morris et al. 1996
One 11b	<i>O. nerka</i>	Scribner et al. 1996
One 13b	<i>O. nerka</i>	Scribner et al. 1996
Ots 1b	<i>O. tshawytscha</i>	Banks et al. 1999
Ots G3	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots G43	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots G85	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots 103	<i>O. tshawytscha</i>	Small et al. 1998
Ots G243	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots 249b	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots 253b	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots 401	<i>O. tshawytscha</i>	Williamson et al. 2001
Ots 409	<i>O. tshawytscha</i>	Williamson et al. 2001
Oki 23	<i>O. kisutch</i>	Smith et al. 1998
Ssa 85	<i>Salmo salar</i>	O'Reilly et al. 1996
Ssa 289	<i>Salmo salar</i>	McConnell et al. 1995

Table 3. (a Analysis of molecular variance results with various hierarchical groupings of sites above and below dams. Details of groupings are shown in Table 3b. Groupings 8-12 consist of pooled above-barrier sites vs. pooled below-barrier sites for each drainage.

Grouping	Description	Nb	Among Groups			Among Populations within Groups			Within populations		
			Var	%	F _{CT}	Var	%	F _{ST}	Var	%	F _{SC}
1	Interdrainage - below only	5	0.217	3.42	0.034	0.474	7.46	0.077	5.663	89.11	0.109
2	Interdrainage - all pops	5	0.158	2.50	0.025	0.535	8.46	0.087	5.635	89.04	0.110
3	All above - all below	2	0.019	0.31	0.003*	0.660	10.45	0.105	5.630	89.24	0.108
4	Salinas River Break	2	0.098	1.53	0.015*	0.632	9.88	0.100	5.663	88.58	0.114
5	Arroyo Grande break	2	0.103	1.61	0.016	0.615	9.63	0.098	5.663	88.76	0.112
6	Santa Ynez break	2	0.106	1.66	0.017	0.611	9.58	0.097	5.663	88.76	0.112
7	Ventura River break	2	0.136	2.13	0.021	0.610	9.51	0.097	5.663	88.36	0.116
8	Salinas -above, below	2	-0.043	-0.70	-0.007*	0.498	8.06	0.080	5.725	92.64	0.074
9	Arroyo Grande- above, below	2	0.030	0.46	0.005*	0.309	4.80	0.048	6.090	94.74	0.053
10	Santa Ynez - above, below	2	0.032	0.52	0.005*	0.712	11.76	0.118	5.310	87.72	0.123
11	Ventura - above, below	2	0.389	6.16	0.062	0.206	3.26	0.035	5.719	90.57	0.094
12	Santa Clara - above, below	2	0.258	4.16	0.042	0.419	6.75	0.075	5.524	89.08	0.109

* non-significant genetic differences among groupings

Table 3. (b The groups of population samples included in each of the AMOVA hierarchical partitions in Table 3a. Number next to each site indicates which group in Table 3a. Number of groups for each test is also indicated.

1. Interdrainage - below only	2. Interdrainage - all pops	3. All above - all below	4. Salinas/Arroyo Grande break	5. Arroyo/Santa Ynez break	6. Santa Ynez/ Ventura break	7. Ventura/ S.Clara break
# Groups = 5	# Groups = 5	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2
1 SLTassajaraB	1 SLTassajaraB	1 SLNacimientoA	1 SLTassajaraB	1 SLTassajaraB	1 SLTassajaraB	1 SLTassajaraB
1 SLTassajeraB	1 SLTassajeraB	1 SLSanAntonioA	1 SLTassajeraB	1 SLTassajeraB	1 SLTassajeraB	1 SLTassajeraB
2 AGArrGrandeB	1 SLNacimientoA	1 AGLopezCanA	2 AGArrGrandeB	1 AGArrGrandeB	1 AGArrGrandeB	1 AGArrGrandeB
2 AGLosBerrosB	1 SLSanAntonioA	1 SYNfJuncalA	2 AGLosBerrosB	1 AGLosBerrosB	1 AGLosBerrosB	1 AGLosBerrosB
3 SYHiltonB	2 AGArrGrandeB	1 SYSantaCruzA	2 SYHiltonB	2 SYHiltonB	1 SYHiltonB	1 SYHiltonB
3 SYSalsB	2 AGLosBerrosB	1 VTMatilijaA	2 SYSalsB	2 SYSalsB	1 SYSalsB	1 SYSalsB
4 VTNFMatilijaB	2 AGLopezCanA	1 VTUNFMatilijaA	2 VTNFMatilijaB	2 VTNFMatilijaB	2 VTNFMatilijaB	1 VTNFMatilijaB
4 VTBearB	3 SYHiltonB	1 SCLockwoodA	2 VTBearB	2 VTBearB	2 VTBearB	1 VTBearB
5 SCLionCanB	3 SYSalsB	1 SCPiruFFA	2 SCLionCanB	2 SCLionCanB	2 SCLionCanB	2 SCLionCanB
5 SCSantaPaulaB	3 SYNfJuncalA	1 SCPiruGHA	2 SCSantaPaulaB	2 SCSantaPaulaB	2 SCSantaPaulaB	2 SCSantaPaulaB
	3 SYSantaCruzA	2 AGArrGrandeB				
	4 VTBearB	2 SLTassajaraB				
	4 VTNFMatilijaB	2 SLTassajeraB				
	4 VTMatilijaA	2 AGLosBerrosB				
	4 VTUpNFMatilijaA	2 SYHilton				
	5 SCLionCanB	2 SYSals				
	5 SCSantaPaulaB	2 VTNFMatilijaB				
	5 SCPiruLockA	2 VTBearB				
	5 SCPiruFFA	2 SCLionCanB				
	5 SCPiruGHA	2 SCSantaPaulaB				

8. Salinas - above, below	9. Arroyo Grande - above, below	10. Santa Ynez - above, below	11. Ventura - above, below	12. Santa Clara - above, below
# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2	# Groups = 2
1 SLTassajaraB	1 AGArrGrandeB	1 SYHiltonB	1 VTNFMatilijaB	1 SCLionCanB
1 SLTassajeraB	1 AGLosBerrosB	1 SYSalsB	1 VTBearB	1 SCSantaPaulaB
2 SLNacimientoA	2 AGLopezCanA	2 SYNfJuncalA	2 VTMatilijaA	2 SCPiruLockA
2 SLSanAntonioA		2 SYSantaCruzA	2 VTUpNFMatilijaA	2 SCPiruFFA

Table 4. The results of assignment tests are presented with misassignments off of the diagonal. Rows represent distribution of assignments for all fish collected at that site.

	SLNacimientoA	SLSanAntonioA	SLTassajaraB	SLTassajeraB	AGArrGrandeB	AGLopezCanA	AGLosBerrosB	SYHiltonB	SYNFJuncalA	SYSalsB	SYSantaCruza	VTBearB	VTMatilijaA	VTNFMatilijaB	VtUpNFMatilijaA	SCLionCanB	SCPiruLockA	SCPiruFFA	SCPiruGHA	SCSantaPaulaB	FHColeman	FHVirginia02	FHVirginia03	FHWyoming	FHMtWhitEa	FHMtWhitLt								
SLNacimientoA	75																														1			
SLSanAntonioA	99	1																																
SLTassajaraB	1	70	1		2	1																												
SLTassajeraB				78																														
AGArrGrandeB					48	1					1		1																					
AGLopezCanA						94					1		1																			1		
AGLosBerrosB						1	62																											
SYHiltonB						1	1	45		1	3					1																		
SYNFJuncalA									85																									
SYSalsB						2				132																								
SYSantaCruza					1						25																							
VTBearB												18		5																				
VTMatilijaA											1		63		11																			
VTNFMatilijaB					1							3	1	73																				
VTUpNFMatilijaA											1		7		58																			
SCLionCanB																88																		
SCPiruLockA																	69		28															
SCPiruFFA																		77	2															
SCPiruGHA																		25	37															
SCSantaPaulaB																																	100	
FHColeman																						48			2									
FHVirginia02																																	1	
FHVirginia03																																		
FHWyoming																																		50
FHMtWhitEa																																	16	9
FHMtWhitLt																																	9	16

Table 5: Assignments of individual fish from small populations in Los Angeles, Orange and San Diego Counties. The top three choices for assignment as population of origin are displayed, along with the probability of assignment to that population. Fish assigned to Fillmore Hatchery strains are in bold type.

Sample assigned	Assigned to:	Probability	Second choice	Probability	Third Choice	Probability
Malibu	SLTassajaraB	52.758	AGArrGrandeB	45.059	VTNFMatilijaB	2.18
Malibu	AGArrGrandeB	92.454	AGLopezCanA	6.393	SYHiltonB	0.439
Topanga	FHColeman	53.736	AGArrGrandeB	32.124	SCLionCanB	14.129
Topanga	AGLopezCanA	84.858	FHColeman	15.124	SLTassajaraB	0.018
Topanga	FHColeman	77.651	AGLopezCanA	21.9	SCLionCanB	0.232
Topanga	AGLopezCanA	99.187	AGArrGrandeB	0.79	SLSanAntonioA	0.015
Topanga	AGLopezCanA	99.156	AGArrGrandeB	0.732	SYHiltonB	0.098
Topanga	AGLopezCanA	46.963	SLTassajaraB	41.806	AGArrGrandeB	11.008
Topanga	AGLopezCanA	76.743	SYSantaCruzA	11.536	SYHiltonB	6.794
Topanga	AGLopezCanA	94.65	AGArrGrandeB	5.294	SLNacimientoA	0.034
Topanga	FHColeman	100	SCLionCanB	0	SCPiruFFA	0
Topanga	FHColeman	100	SCPiruFFA	0	SCLionCanB	0
Topanga	AGArrGrandeB	99.201	SYHiltonB	0.489	AGLopezCanA	0.302
Topanga	SLNacimientoA	97.002	SYHiltonB	1.229	AGLopezCanA	0.798
Topanga	AGArrGrandeB	90.548	SLNacimientoA	9.449	SLTassajaraB	0.003
Topanga	FHColeman	99.735	SCLionCanB	0.261	FHWyoming	0.004
Topanga	FHColeman	100	AGLopezCanA	0	SLSanAntonioA	0
Topanga	FHColeman	100	SCLionCanB	0	FHWyoming	0
Topanga	FHColeman	99.815	FHWyoming	0.176	AGLopezCanA	0.004
San Gabriel	AGArrGrandeB	99.443	SLTassajaraB	0.491	AGLopezCanA	0.066

Table 5 (cont.)

Sample assigned	Assigned to:	Probability	Second choice	Probability	Third Choice	Probability
Santa Ana-Harding	AGLopezCanA	99.917	SYHiltonB	0.033	SLSanAntonioA	0.027
Santa Ana-Harding	AGLopezCanA	95.85	SYHiltonB	3.106	VTUpNFMatilijaA	0.529
Santa Ana-Harding	AGLopezCanA	99.037	SLSanAntonioA	0.827	SLTassajaraB	0.136
Santa Ana-Harding	AGLopezCanA	90.149	SYHiltonB	9.779	SLSanAntonioA	0.032
Santa Ana-Harding	AGLopezCanA	99.837	SLSanAntonioA	0.114	SLTassajaraB	0.05
Santa Ana-Harding	SYHiltonB	98.447	AGLopezCanA	1.544	SLTassajaraB	0.004
Santa Ana-Harding	AGLopezCanA	98.311	SYHiltonB	1.092	SLTassajaraB	0.59
Santa Ana-Harding	AGLopezCanA	96.493	SYHiltonB	3.388	SLSanAntonioA	0.102
Santa Ana-Harding	AGLopezCanA	99.899	SLTassajaraB	0.075	SYHiltonB	0.021
Santa Ana-Harding	AGLopezCanA	92.828	SYHiltonB	6.732	SLTassajaraB	0.233
Santa Ana-Harding	AGLopezCanA	99.744	SLSanAntonioA	0.25	SYHiltonB	0.004
Santa Ana-Harding	AGLopezCanA	99.959	SLTassajaraB	0.023	SLSanAntonioA	0.01
Santa Ana-Harding	AGLopezCanA	99.394	SLSanAntonioA	0.35	SLTassajaraB	0.241
San Juan-Trabuco	FHVirginia02	72.683	FHVirginia03	22.052	SCSantaPaulaB	5.26
San Mateo	AGLopezCanA	58.792	SLTassajaraB	17.472	SLNacimientoA	12.502
Sweetwater	AGLosBerrosB	93.096	FHMtWhitLt	6.731	FHVirginia02	0.092
Sweetwater	FHMtWhitLt	96.525	FHMtWhitEa	3.432	FHWyoming	0.031
Sweetwater	SLTassajaraB	51.678	AGArrGrandeB	27.378	SLNacimientoA	17.361
Sweetwater	SYnezHilton	98.229	SCLionCanB	1.084	VTBearB	0.672
Sweetwater	FHMtWhitEa	52.908	FHMtWhitLt	47.092	FHColeman	0
Sweetwater	FHColeman	66.218	FHMtWhitEa	23.739	SCLionCanB	4.418
Sweetwater	SCLionCanB	100	FHMtWhitLt	0	FHMtWhitEa	0

