

Population Structure and History of Steelhead Trout in California

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Steelhead trout (*Oncorhynchus mykiss*) are the most widespread of the anadromous salmonids. In the western continental United States, 14 Evolutionarily Significant Units (ESUs) have been delineated on the basis of genetic, geographic and ecological variation. Of these 14 western ESUs, 11 have been listed as protected under the US Endangered Species Act (ESA). In California, there are six ESUs, 5 on the coast and 1 in the Central Valley. All but the Klamath Mountain Province ESU is listed, with the Southern California ESU classified as “Endangered” and the others “Threatened”.

Here, we investigate genetic population structure and demographic history of steelhead trout in coastal California using multilocus genetic data. We use size variation at highly variable microsatellite loci from populations at 62 sites from 41 basins, covering almost the entire range of the species in coastal California. The list of sites is found in Table 1. Results of phylogeographic analyses and assignment tests are described and the partitioning of variation at the tributary, river basin and ESU level is examined.

Table 1. Populations sampled in this study. Populations are listed south to north. Ho is observed heterozygosity. No of alleles is the mean across 18 loci. M is the mean M ratio = no. of alleles/(range in allele size+1). *=Significant values of the M-ratio.

Watershed	Ho	No. of alleles	M	Watershed	Ho	No. of alleles	M
Santa Ynez-Hilt	0.640	10.5	0.683*	Big River	0.648	12.3	0.702*
Santa Ynez-Sal	0.512	14.1	0.623*	Noyo	0.687	11.7	0.721*
Chorro Creek	0.687	5.8	0.629*	Noyo-Kass	0.756	12.7	0.733
San Simeon Ck	0.722	9.0	0.710*	Noyo-LNFk	0.722	12.8	0.696*
Willow Creek	0.666	12.1	0.674*	Noyo-SFk	0.709	11.5	0.653*
Big Creek	0.697	10.7	0.760	Pudding Creek	0.647	8.2	0.663*
Big Sur River	0.705	11.6	0.709*	TenMile-LtNFk	0.704	10.8	0.667*
Carmel River	0.696	8.1	0.715*	TenMile-LSFk	0.654	10.1	0.697*
Pajaro River	0.686	12.3	0.652*	TenMile-Redwd	0.682	11.5	0.708*
SLor-Bear	0.608	11.6	0.625*	TenMile-Smith	0.711	10.9	0.709*
SLor-Boulder	0.717	9.5	0.679*	Wages Creek	0.726	12.7	0.719*
SLor-CarbCk	0.673	8.2	0.636*	Usal Creek	0.685	12.9	0.725*
SLor-Zayante	0.724	10.2	0.679*	Big-Lost Coast	0.703	10.4	0.698*
Scott Creek	0.662	11.8	0.625*	Mattole River	0.689	10.5	0.705*
Waddell Creek	0.667	7.8	0.685*	Bear River	0.646	12.3	0.749
Pescadero	0.685	9.1	0.643*	Eel-Hollowtree	0.697	11.7	0.730*
San Pedro	0.692	8.1	0.668*	Eel-Indian	0.557	7.0	0.722
LTrancos-SF Bay	0.631	9.8	0.637*	Eel-Lawrence	0.612	13.3	0.795
Miller Ck-SF Bay	0.636	8.2	0.645*	Eel-Willits	0.687	10.5	0.655*
Redwood Creek	0.699	13.0	0.741	Freshwater Ck	0.675	11.0	0.753
Lagunitas	0.691	13.8	0.737	Mad-BlueSlide	0.657	10.8	0.695*
Lagunitas-Olema	0.697	12.0	0.698*	Mad-Canon	0.717	10.1	0.725*
Lagunitas-Bline	0.665	9.2	0.623*	Mad-Sullivan	0.707	7.1	0.591*
Walker Ck	0.679	9.4	0.674*	Redwd-LostMan	0.691	11.6	0.711*
Russian River	0.705	9.2	0.694*	Redwd-Panther	0.727	12.8	0.705*
Gualala River	0.664	8.4	0.720*	Redwd-Prairie	0.648	9.5	0.720*
Garcia River	0.645	9.9	0.726*	Klamath-Blue	0.727	12.7	0.794
Elk Creek	0.698	10.2	0.704*	Klamath-Hunter	0.614	10.3	0.694*
Navarro River	0.724	13.5	0.809	Klamath-HLinto	0.749	11.9	0.792
Big Salmon	0.690	12.5	0.708*	Wilson Creek	0.664	12.0	0.819
Albion River	0.692	8.5	0.648*	Smith River	0.751	11.7	0.801

Samples were collected from June to October 2001 by electrofishing. Small tissue clips were taken non-lethally from caudal fins of juvenile trout and dried on blotter paper. Samples were collected from five habitat units per site and only fish believed to represent young of the year were included in the analyses, in attempts to minimize the number of related individuals in the sample and include only one cohort. Steelhead are iteroparous and can have a highly variable life history strategy. When they are not anadromous, they are called rainbow trout. At present, all fish found in water bodies with ocean access are classified as steelhead, and those above barriers to anadromy as rainbow trout. We thus treat all sampled fish as steelhead, though we can not be certain that they are anadromous.

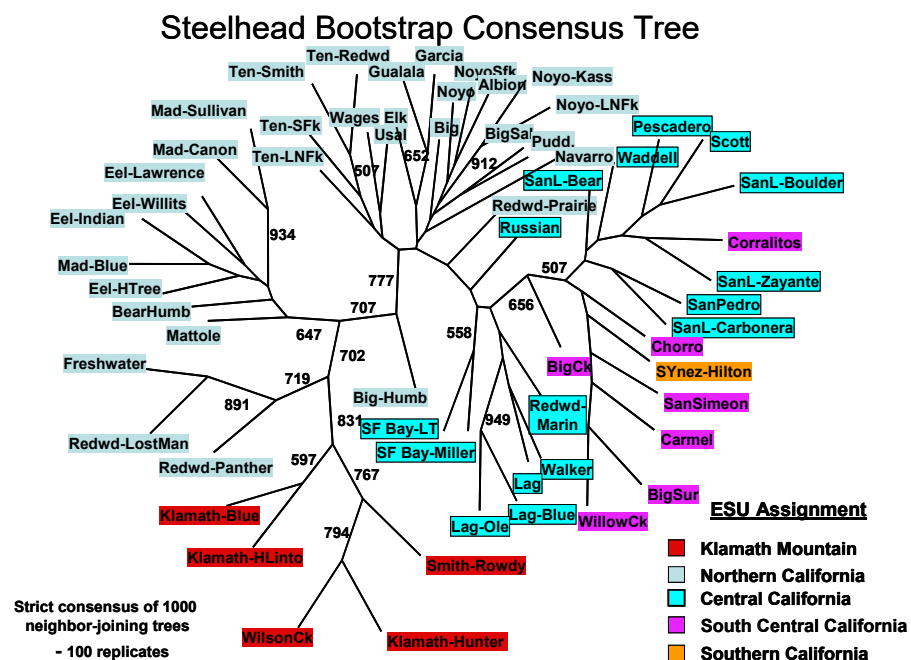
An average of 66 fish per site was analyzed with 18 microsatellite loci. They include genes with a wide range of variability (from 4 to 64 alleles) that were originally isolated in several species, both to minimize ascertainment biases. Genomic DNA was extracted from the dried fin clips using a semiautomated protocol. Genotypic data was generated via the PCR with fluorescent primers and electrophoresis was done on automated sequencers. Two people performed all allele calls independently and any discrepancies were resolved by mutual agreement.

We found substantial genetic variation in coastal California steelhead with a total of 540 alleles, as defined by number of repeats, at the 18 microsatellite loci assayed. The mean number of alleles per locus varied from 5.8 in Chorro Ck. to 13.8 in Lagunitas Ck (Table 1). Heterozygosity varied from 0.557 in Indian Ck (Eel River) to 0.756 in Kass Ck. (Noyo River). We also examined the population genetic data for evidence of recent reductions in population size, or bottlenecks, using the M-ratio method of Garza and Williamson (2001). In general, we found a trend of increasing evidence for bottlenecks from north to south, with no significant tests among the 5 non-ESA protected populations from the Klamath ESU (Table 1). We also examined independence of populations through use of tests of genic population differentiation (Raymond and Rousset 1995). Every pairwise comparison of samples from our study yielded a highly significant test.

To evaluate the relationships between sites, we calculated several population-based measures of genetic distance and population subdivision. These include F_{st} , the standardized variance in allele frequencies among sites, and Cavalli-Sforza and Edward's (1967; CSE) chord distance.

F_{st} was transformed as $F_{st}/(1-F_{st})$ and regressed on geographic distance as measured by rivermouth distance plus stream miles. The relationship was highly significant, with $R^2 = 0.204$. Thus, there is a strong signal that migration is dependent on distance, leading to a pattern that has classically been called isolation by distance, with geographic distance alone explaining about 20% of the genetic variation in the samples. This dependence of population structure on geographic distance was also evident in the matrices of genetic distance and the trees constructed with them. The consensus tree for 1,000 bootstrap replicates with CSE distances and the neighbor joining (NJ) tree-building algorithm is found in Fig. 1. The concordance with geography is very high, with the topology almost entirely consistent with geographic proximity. The CSE/NJ tree is also characterized by concordance with geography, and also by long terminal branch lengths.

Fig. 1. Bootstrap consensus tree for 62 steelhead populations. Genetic data were bootstrapped 1,000 times, CSE chord distances calculated and neighbor-joining trees constructed. The majority-rule tree consensus tree is shown with internal branches representing the number of times that grouping was found.



These long terminal branches are reflected in the power of assignment tests to assign individuals to their population of origin. We found that 76% of individuals were correctly assigned to their population of origin with our dataset. When assignment to another site within the same basin was not considered an error, this accuracy rose to over 80%. The frequency with which individuals were misassigned across ESU boundaries was well over 90%. It should be noted that this assignment accuracy is probably affected by the presence of related individuals in our sample and would thus decrease slightly with individuals from other year classes. However, the high assignment accuracies indicate that genetic data can be used to accurately assign individuals to river of origin, even on small spatial scales, and that a combination of limited migration and local adaptation is shaping steelhead population genetic structure in coastal California.

In summary, we have examined multilocus genetic data from 62 populations of steelhead trout in all five of the coastal California ESUs. The results of our work indicate that population structure of steelhead trout in coastal California has been largely unaffected by hatcheries and remains influenced primarily by migration, which is dependent on distance. The significant relationship between geographic and genetic distance, as well as the high concordance of geography with genealogy, are indicative of this. The long terminal branch lengths and high assignment accuracies indicate that, while migration is important, drift and local adaptation likely contribute to the differentiation between all populations in our study.

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