

PHYLOGEOGRAPHIC PATTERNS IN CALIFORNIA STEELHEAD AS DETERMINED BY MTDNA AND MICROSATELLITE ANALYSES

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EXTENDED ABSTRACT

Polymerase chain reaction (PCR) and direct sequencing of mtDNA and microsatellites were used to test genetic diversity and biogeographic distributions in putative wild ($N = 32$) and hatchery ($N = 6$) populations of steelhead trout (*Oncorhynchus mykiss*) in California. Total genomic mtDNA was extracted noninvasively from fin tissue (2 mm²) taken from 426 wild fish and 66 hatchery fish throughout California. Extraction, amplification, and visualization of mtDNA followed methods given in Nielsen et al. (1994). Mitochondrial types were derived from analysis of base pair differences found in a highly variable region of the 3' end of the salmonid

mtDNA control region (196 bp) and a 5 bp section of the adjacent phenylalanine tRNA. Nucleotide variation was found at nine sites (4.5%), and 13 different steelhead mtDNA types were identified in California (table 1). Distribution of steelhead mtDNA types in wild fish captured along the coast of California showed a distinct biogeographic frequency gradient, with different mtDNA types dominating broad geographic areas (figure 1). Hatchery populations in the same geographic areas lacked any similar biogeographic cline (Nielsen et al. 1994).

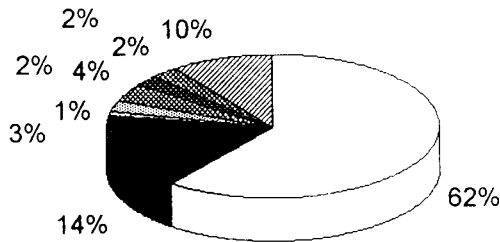
A subset of the same wild individuals used for mtDNA analysis ($N = 144$) was investigated for dispersed repetitive nuclear DNAs by means of PCR amplification and

TABLE 1
 Steelhead (*Oncorhynchus mykiss*) mtDNA Types Found in 32 Streams and 6 Hatcheries throughout California, 1990–1993

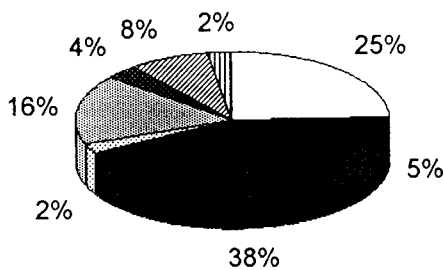
mtDNA type	N	Variable sites								
		63	86	94	96	130	148	151	154	194
ST1	161	T	G	T	T	T	A	A	G	G
ST2	33	C	G	T	T	T	A	A	G	G
ST3	88	T	G	T	T	T	A	A	A	G
ST4	7	T	G	T	T	C	G	A	G	G
ST5	59	T	G	T	T	C	G	*	G	A
ST6	10	T	G	C	T	C	G	*	G	A
ST7	9	T	G	T	T	C	A	A	G	A
ST8	69	T	G	T	T	C	A	*	G	A
ST9	9	T	G	T	T	T	A	A	G	A
ST10	1	T	G	T	C	T	A	A	A	G
ST11	0	T	A	T	T	T	A	A	G	G
ST12	30	T	G	T	T	C	A	*	G	G
ST13	15	T	G	T	T	C	G	*	G	G
ST14	1	C	G	T	T	T	A	A	A	G
TOTAL	492	California steelhead: hatchery and wild								
	426	California steelhead: wild stocks only								

The number of fish (N) found in this study is given for each type. An asterisk (*) represents a nucleotide deletion. Type #11 was found only in steelhead outside of California.

Northern California



Central California



Southern California

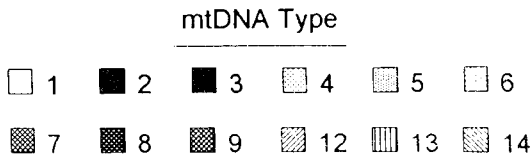
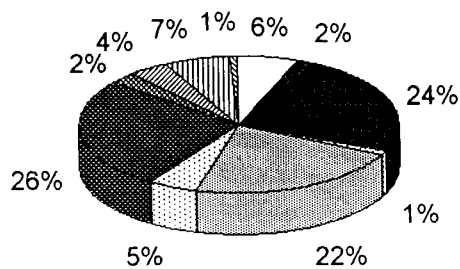


Figure 1. Frequency distributions of wild steelhead mtDNA types found throughout California. The northern range extends from the mouth of the Eel River (Humboldt County) to the Navarro River (Mendocino County); the central range is from the Russian River (Sonoma County) to the Carmel River (Monterey County); the southern range runs from Santa Rosa Creek (San Luis Obispo County) to Malibu Creek (Los Angeles County).

a microsatellite probe (OMY77) developed in the Marine Gene Probe Laboratory at Dalhousie University by J. M. Wright. This probe disclosed twenty microsatellite alleles, ranging in size from 80 to 141 bp, in California steelhead (J. L. Nielsen, unpublished data). The bimodal

Microsatellite Alleles - OMY77

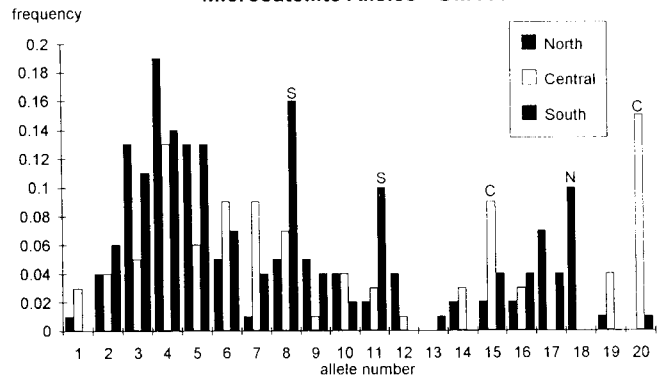


Figure 2. Frequency distribution of microsatellite alleles (given sequentially by size) obtained from analysis of 144 wild California steelhead, using the microsatellite probe OMY77, developed at Dalhousie University by J. M. Wright. Geographic ranges used to compare frequencies were the same as those given in figure 1. The letters indicate alleles where southern (S), central (C), or northern (N) frequencies were significantly different from those calculated with chi-square analysis ($p < 0.05$) for the other geographic areas.

frequency distribution of nuclear alleles, amplified by OMY77, was biogeographically distinct for alleles and geographic location (figure 2; likelihood $\chi^2 = 129.7$; d.f. = 38, $p < 0.001$). Chi-square analysis of frequency distributions within each microsatellite allele showed five alleles that occur at significantly different frequencies (contingency chi-square, $p < 0.05$) for different geographic areas. Further nuclear biogeographic resolution may be gained with additional microsatellite probes under investigation in our laboratory.

Parsimony analysis of the anadromous steelhead populations (PAUP V3.0) showed no significant geographic monophyletic relationships and supported significant gene flow between steelhead populations along the California coast (J. L. Nielsen, unpublished data). Some mtDNA types (#5, #6, and #8), however, remain relatively isolated in southern California. An area cladogram using character compatibility was run on 17 anadromous steelhead streams in California (CAFCA; M. Zandee, Dept. Theoretical Biology, P. O. Box 9518, 2300 RA Leiden, Netherlands.) This program combines a maximum parsimony tree with a presence-absence matrix based on geographic location. CAFCA added resolution to the hypothesis of recent reproductive isolation of southern steelhead populations by portraying all the southern steelhead streams as a single biogeographic clade (figure 3).

Ocean conditions contributing to the relative reproductive isolation of California steelhead genotypes leading to the mtDNA biogeographic cline remain speculative. An aquatic biogeographic species boundary running southwest from around Point Conception into the Pacific Ocean (Hedgecock et al. 1992) parallels the northern division of steelhead genotypes found primar-

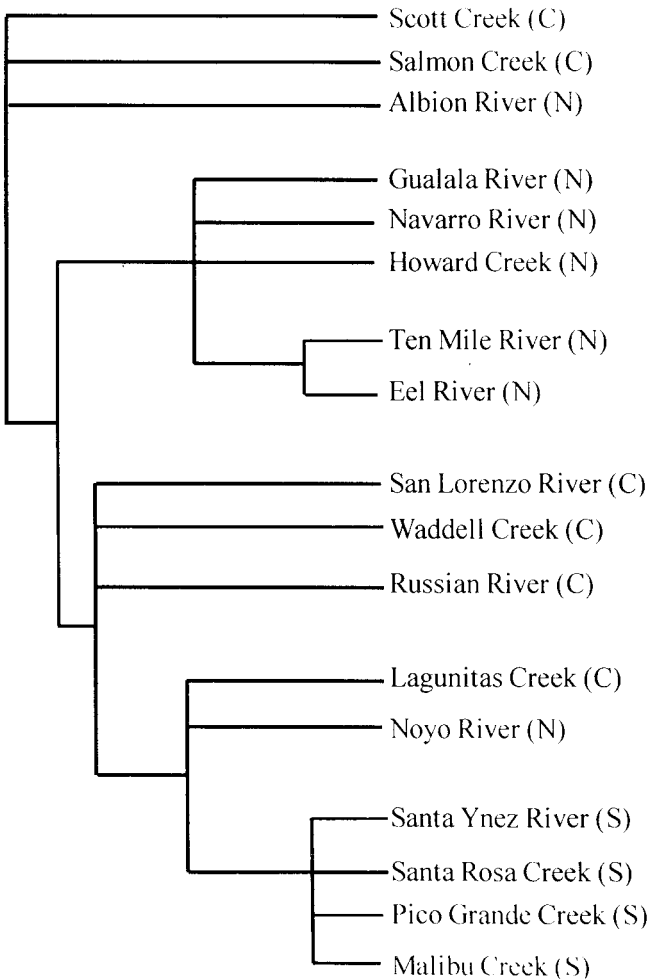


Figure 3. Area cladogram showing biogeographic character compatibility in 17 California steelhead streams from different geographic areas. Results were plotted after analysis of a maximum parsimony tree using PAUP V3.0 and the biogeographic function of CAFCA (available from M. Zandee, see text for address). Geographic stream locations are given as letter codes: N = northern; C = central; S = southern.

ily in southern California. This suggests that ocean currents along the southern California coast may have played a role in the distribution of southern steelhead lineages found in this study. At about 32° N the California Current turns eastward, flowing into the Southern California

Bight, creating an oceanic gyre that ends in a strong oligotrophic front (the Ensenada Front) at the center of the bight (Lynn and Simpson 1987; Thomas and Strub 1990). Unique southern steelhead mtDNA types are found in streams that enter the ocean from about 36°30' N (Santa Rosa Creek) to 34° N (Malibu Creek); however, their historic distribution extended farther south into Baja California (about 32° N). Their distribution remains within the richer nutrient areas of the gyre along the southern California coast (Strub et al. 1991). Spring and summer nearshore movements of steelhead smolts as they enter the ocean environment in southern California may remain localized in these areas. Subsequent adult oceanic migration behavior based on prevailing oceanic currents may also have contributed to the isolation of southern steelhead genotypes in California. The presence of northern genotypes, in low frequencies, in most southern anadromous steelhead populations suggests continued gene flow from north to south along the coast following the general direction of the California Current.

Putative relic steelhead populations, separated from the ocean by water impoundment dams built at the turn of the century, were found in our study to have genotypes endemic to their local geographic range as shown in contemporary anadromous stocks (Santa Ynez River, Sespe Creek, Matilija Creek). This suggests that southern steelhead stocks are not the result of recent anthropomorphic manipulation, and must be viewed in an evolutionary context.

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