

UNIVERSITY OF CALIFORNIA
SANTA CRUZ
**PARALLEL EVOLUTION OF THE SUMMER STEELHEAD ECOTYPE
IN MULTIPLE POPULATIONS FROM OREGON AND
NORTHERN CALIFORNIA**

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Parallel evolution of the summer steelhead ecotype from multiple populations in Oregon and Northern California

Martha A. Arciniega-Hernández

Abstract

Parallel adaptive divergence of migratory and reproductive behavior can occur in multiple populations when similar selection is acting on these traits. Timing of migration, sexual maturity, and reproduction can have major impacts on the dynamics and viability of a population. Life-history variation in steelhead, *Oncorhynchus mykiss*, including variation in anadromous run timing, reproductive maturity and spawn timing, represents an important aspect of their biology and adaptation to local habitats. Here we present a genetic analysis of naturally spawning steelhead to evaluate the genetic relationships and ancestry of summer- and winter-run reproductive ecotypes from multiple river basins in Oregon and Northern California. We infer the phylogeographic relationships among populations of both summer- and winter-run steelhead ecotypes using 12 microsatellite loci and 90 single nucleotide polymorphisms. Phylogenetic trees and analysis of molecular genetic variance revealed that pairs of phenotypically and genetically distinct reproductive ecotypes within rivers were each other's closest relatives. Isolation by distance was also observed, confirming that genetic relatedness was strongly associated with geographic distance, and indicating limited migration or gene flow among river basins. These patterns support the hypothesis that the summer-run steelhead ecotype has repeatedly evolved through parallel evolution in multiple river basins. These results, together with further investigation of the underlying molecular basis for the divergence of

winter- and summer-run steelhead life-history traits, will inform management and conservation efforts for these ecotypes and improve our understanding of the role of adaptive variation in conservation genetics.

To my amazing parents

Laura H. Arciniega and Miguel Arciniega C.

To my awesome sisters

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To my dear grandparents

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Introduction

Variability in life-history traits can allow species to exploit diverse natural resources and may confer resilience to the potentially negative impacts of environmental change (eg. Price et al. 1984; Winemiller 1989; Dionne et al. 2008). Understanding the evolutionary basis of such variability is important for management and conservation purposes. However, changes in the expression of morphological, phenotypic, and behavioral traits can result from both plastic and evolutionary responses, making it difficult to identify the underlying genetic basis of phenotypic expression in an uncontrolled environment. Parallel evolution of phenotypic traits often occurs in association with similar selective pressures in distant habitats and can have a similar heritable component in closely related species (Haldane 1932). Similarities in the selective pressures experienced by populations or species can lead to convergent phenotypes (Pearse and Pogson 2000; Steiner et al. 2009; Rosenblum et al. 2010), although the underlying genetic change associated with the phenotypic trait may be the same (Cresko et al. 2004; Mundy et al. 2004; Colosimo et al. 2005; Gross et al. 2009; Miller et al. 2012; Hohenlohe et al. 2012; Protejo-Garcia et al. 2013; Pearse et al. 2014), or different (Jessen et al. 1991; Chen et al. 1997; Hoekstra and Nachman 2003). Thus, a first step in understanding the evolution of phenotypic variation in life-history traits is to compare the genetic relationships among populations with the same, and different, phenotypes. These relationships can then

provide a framework with which to test hypotheses about the evolutionary history of a specific phenotype (Harvey and Pagel 1991).

Species in the family Salmonidae are widely studied due to their importance in fisheries, aquaculture, and conservation. The complex spectrum of life-history traits in salmonid fishes (Quinn and Myers 2004) can be highly heritable (Bentzen et al. 2001; Nichols et al. 2008; Haidle et al. 2008; Miller et al. 2012; Abadía-Cardoso et al. 2013) and can change rapidly under strong selection (Hendry et al. 2000; Martínez et al. 2011; Pearse et al. 2014). Among the most variable and widely distributed of these species is *Oncorhynchus mykiss*, which is termed steelhead if it migrates to sea before returning to freshwater to spawn, and rainbow or redband trout when it does not. In California and Oregon, steelhead populations are categorized into distinct population segments (DPSs) for management and conservation purposes. The DPS boundaries are based on many factors, including geography, migratory behavior, spawn timing, and genetic structure (Busby et al. 1996; Garza et al. 2014). Because of spatial differences in temperature, water flow, sediment type, sedimentation rate, and other characteristics among rivers, divergent selection may drive the adaptation of steelhead to their local environment (Taylor 1991; Narum et al. 2008b; Narum et al. 2013; Matala et al. 2014). Instances of local adaptation in salmonid fishes have been found to occur at spatial scales of a few kilometers (Fraser et al. 2011; Bond et al. 2014) and have been shown to affect migratory timing (Major and Mighell 1966; Quinn and Adams 1996; Quinn et al. 1997; Robards and Quinn 2002). Thus, in order to manage *O. mykiss* effectively, it is important to understand both the geographic and

genetic structure of its life-history variation and the adaptive evolutionary processes that give rise to it.

Anadromous migratory behavior can also differ by the season in which adult fish return to freshwater to spawn, or run timing. Run timing is commonly used to describe populations of anadromous fish, and such temporal differences in migration and reproduction have been shown to reduce gene flow and increase divergence among populations within a basin (Quinn et al. 2000; Hendry et al. 2002; Waples et al. 2004; Clemente 2006). This temporal divergence in patterns of life-history traits may reflect either the effects of local adaptation or phenotypic plasticity, or some combination of the two. Winter-run steelhead typically return to their natal river already sexually mature during the months of November to May and spawn shortly after entry into freshwater (Shapovalov and Taft 1954; Withler 1966; Busby et al. 1996). In contrast, summer-run steelhead return sexually immature from April to June, hold in cool pools during summer and fall, then mature and spawn in the winter or the following spring, often moving upstream into headwater habitat as they do so (Everest 1973; Moyle 2002). Timing of migration is therefore decoupled from timing of sexual maturity for the summer-run ecotype, but not for the winter-run ecotype. Although these summer- and winter-run phenotypes usually occur within the same river, they may be effectively allopatric, due to spatial and temporal separation in migration timing and spawning habitat. Such spatial and temporal differences have been found to exist between winter- and summer-run steelhead populations in British Columbia (Smith 1968) and the Rogue River (Everest 1973), with earlier returning

fish spawning farther upstream than later returning fish (Briggs 1953). In the summer, stream conditions tend to restrict steelhead to the lower part of the river and, when water flow increases in the fall or winter, summer-run fish move further upstream (Behnke 1992). Although there is overlap in spawn time, winter-run fish are just entering the river when the summer-run fish are moving further upstream, creating spatial separation. In Capilano River, British Columbia, the two races of summer- and winter-run steelhead are mutually viable, however, their behavior would indicate that they do not interbreed and there are large differences in the fat and gonad weights between them (Smith 1969). The morphological appearance of summer steelhead, such as pronounced hooking of the snout and lower jaw, also suggests something about their behavior. It is possible that summer steelhead may get territorial and defend their spawning sites against winter steelhead (Smith 1969). Thus, pairs of populations of these two ecotypes from the same river offer the opportunity to investigate the genetic basis of migratory/reproductive strategies.

In California, summer-run steelhead are currently found primarily in the tributaries of the Eel and Klamath river basins, although historically they were more widespread (Bjorkstedt et al. 2005). Further to the north, populations of summer-run steelhead are more common, particularly in the Columbia River basin, where the inland populations are derived from a distinct lineage of the species (Allendorf 1975). If these ecotypes evolved independently multiple times, then the summer- and winter-run steelhead from the same river would be expected to be each other's closest relative. In the Middle Fork Eel (MFE) River, winter-run steelhead populations are

more closely related to the sympatric summer-run populations than to winter-run populations in neighboring tributaries (Clemento 2006), and the time of divergence between ecotypes has been estimated as 16,000-28,000 years (Nielsen and Fountain 1999). Additionally, Clemento (2006) suggested that the summer phenotype has a heritable component because multiple generations of summer steelhead were each other's closest relatives when compared to neighboring *O. mykiss* populations.

Although this evidence suggests that the summer ecotype has independently evolved within the Eel River, the population genetic relationships among sympatric summer- and winter-run steelhead populations in other basins remain unknown.

Microsatellite markers are highly polymorphic and informative, have a long history of use in population genetics (Sunnucks 2000), and have been used extensively to study *O. mykiss* populations (e.g. Heath et al. 2002; Hauser et al. 2006; Pearse et al. 2007, 2009; Clemento et al. 2009; Martínez et al. 2011; Garza et al. 2014). However, because of the ease of genotyping and portability of the resulting data, single nucleotide polymorphism (SNP) markers are increasingly used in population genetic investigations (Morin et al. 2004), including to assess population demography, estimate phylogenetic relationships and for parentage analysis (e.g. Anderson and Garza 2006; Morin et al. 2009; Abadía-Cardoso et al. 2011, 2013; Hansen et al. 2011; Helyar et al. 2011; Anderson 2012). However, Narum et al. (2008a) concluded that although microsatellites had a higher accuracy than SNPs alone, using both types of markers in combination can yield higher assignment accuracy and provide the most power to determine population genetic relationships.

Here we use 102 polymorphic markers, 12 microsatellites and 90 SNPs, to investigate the relationships between and evolution of summer- and winter-run ecotypes of naturally spawning steelhead. We examine the genetic structure of steelhead from summer- and winter-run populations in multiple rivers in Oregon and Northern California and place their relationships in a geographic context to test the hypothesis that the summer-run steelhead ecotype has evolved in parallel in multiple river basins from distinct local winter-run steelhead populations.

Methods

Sampling

Samples were obtained from four pairs of summer- and winter-run steelhead populations in the Columbia, Umpqua, Klamath, and Eel rivers (Table 1; Figure 1) in Oregon and Northern California through trapping and net capture of adults in holding pools or at weirs. Sampling at weirs was done during the peak time of river entry during each run, while summer-run adults were sampled in summer holding pools. For comparison of genetic distance and diversity, samples from winter-run steelhead from 17 additional locations in California were used (Table 1), as a way of visualizing how sympatric populations clustered relative to other coastal winter-run populations that are not in sympatry with summer steelhead.

Genetic Data Collection

DNA was extracted from dried fin clips using the DNeasy 96 filter-based nucleic acid extraction system on a BioRobot 3000 (Qiagen, Inc.), following the manufacturer's protocols. Extracted DNA was diluted 10:1 (microsatellites) or 2:1 (SNPs) with distilled water and used for polymerase chain reaction (PCR) amplification of 12 microsatellites and 90 SNPs (Supplemental Table 1). For microsatellites, PCR products of samples from the Columbia, Umpqua, Rogue and Trinity rivers were electrophoresed on ABI 377 sequencers (Applied Biosystems, Inc.). The same protocol and equipment was used to collect microsatellite genotypes for the other populations that were previously published based on data collected in the same laboratory (Clemento 2006; Garza et al. 2014). Genotypes were determined using Genescan 3.0 and Genotyper 2.1 software (Applied Biosystems). Two people scored all microsatellite genotypes independently, and discrepancies in the scores were resolved either by consensus or by re-genotyping. Otherwise, that genotype was deleted from the data set. SNPs were assayed with high throughput 96.96 dynamic genotyping arrays on an EP1 instrument (Fluidigm, Inc.), and genotypes called using Fluidigm SNP Genotyping Analysis software.

Data analysis

The microsatellite and SNP data were combined for all analyses. Basic population genetic statistics, and test for departures from Hardy-Weinberg and linkage equilibria were estimated using the GENEPOP program (v4.2; Rousset 2008). Allelic richness for microsatellites was calculated using FSTAT 2.9.3 (Goudet 2001).

Individual fish were assigned to their most likely population of origin and individual migration between populations assessed using the Bayesian method for estimating population allele frequencies (Rannala and Mountain 1997) implemented in the program GeneClass2 (Piry et al. 2004). Pairwise differentiation between all pairs of populations was evaluated using F_{ST} , as estimated by Weir and Cockerham (1984) in ARLEQUIN (v3.11, Excoffier et al. 2007). Cavalli-Sforza and Edwards (1967) chord distances were calculated and used to generate a neighbor joining phylogram of populations, with the statistical support for population relationships evaluated by taking 1000 bootstrap samples from the dataset, all with the software package PHYLIP (Felsenstein 2005). The resulting phylogram was visualized using Dendroscope 3 (Huson and Scornavacca 2012). Isolation by distance (IBD) analysis was performed using GENEPOP with a Mantel test (1000 iterations) used to evaluate significance of relationships between F_{ST} and distance (km). To evaluate fractional ancestry of individuals, clustering analyses were conducted using the program STRUCTURE (v2.0; Pritchard et al. 2000), which allows migrants and individuals of mixed ancestry to be identified without a priori designation of defined populations (Pritchard 2000; Pearse and Crandall 2004). Clustering analyses were done with a range of values for the number of genetic clusters ($K = 2 - 10$), with 10 runs of each K value to evaluate consistency of patterns of genetic association. Results from the STRUCTURE runs were compiled and visualized using CLUMPP (Rosenberg 2004) and DISTRUCT (Jakobsson and Rosenberg 2007). ARLEQUIN was used to perform an analysis of molecular variance (AMOVA; Excoffier et al. 1992) for different a

priori groupings of population samples to evaluate the distribution of genetic variation among and between basins and ecotypes. Finally, to evaluate the neutrality of our loci, F_{ST} outlier tests were done using FDIST2 (Beaumont & Nichols 1996) as implemented in LOSITAN (Antão et al. 2008).

Results

Genotyping and Population Statistics

A total of 730 individual fish were successfully genotyped, with samples sizes from each population ranged from 18 to 47, and averaged 29 individuals (Table 1). The missing data rate over all loci was <1.3%, and all individuals were genotyped at a minimum of 90 loci. No consistent, significant deviations from Hardy-Weinberg or linkage equilibria were found. Observed heterozygosity ranged from 0.263 to 0.448 across all loci, and allelic richness for the microsatellites ranged from 5.9 to 8.1 (Table 1).

Genetic structure

A combined dataset with 12 microsatellite loci and 88 SNPs was used to construct an unrooted neighbor-joining phylogram (Figure 2). Two SNP markers were excluded from this analysis because they failed entirely in more than one population. Phylograms were also constructed using the microsatellite and SNP loci separately, and revealed no significant differences in topology (results not shown). The population clustering pattern in all analyses was consistent with the geographic

locations of river basins (Figure 2). Summer- and winter-run steelhead from the same river basin were each other's closest relatives in all basins, and all steelhead populations generally clustered according to geographic proximity. For example, within the Eel River, winter-run steelhead from the upper mainstem (Van Arsdale) grouped more closely with summer-run steelhead from the Middle Fork Eel River than they did with winter-run steelhead from the Lawrence Creek and Hollow Tree Creek tributaries, which have their confluences with the mainstem Eel River much closer to its mouth. Similarly, winter-run steelhead from the South Fork Trinity River grouped more closely with summer-run steelhead from the New River tributary of the Trinity River than with winter-run steelhead in other basins. This pattern was also consistent for the Umpqua and Columbia River pairs of steelhead ecotypes.

Individual assignments

Assignment tests reliably distinguished individuals sampled from various river locations in Oregon and California. Using a 90% probability confidence criteria, the accuracy with which fish were assigned to their population and basin of origin was 90.7% and 95.8%, respectively (data not shown). Misassignments between ecotypes were reflective of the population divergence, with 11, 6, and 1 summer/winter cross-assignments in the Umpqua, Klamath, and Eel population pairs, respectively. When no probability criterion was used, mean self-assignment percentage to population was 87% and ranged from 42-100% (Table 1). In addition, 66 of 730 fish(9.0%) were

misassigned to a population location other than the one where they were sampled and of those, 38 (5.2%) were assigned to a location outside their basin of origin.

Genetic differentiation

The proportion of genetic variation partitioned between population samples, pairwise F_{ST} , was significantly different from zero ($p = 0.000$) for all comparisons except that between the Umpqua summer- and winter-run fish. The samples from the Umpqua River were collected at the same weir, so were separated only temporally. Pairwise F_{ST} values between all population pairs ranged from 0.037 - 0.312, while F_{ST} values between ecotypes within basins were generally lower (0 – 0.069; Table 2). A pattern of isolation by distance was indicated by a strong relationship between genetic and geographic distance (F_{ST} vs. distance, $r^2 = 0.479$, Mantel test: $P < 0.000$; Figure 3).

STRUCTURE analyses

Model-based clustering analyses with STRUCTURE of the four regional ecotype pairs revealed distinct genetic groupings (Figure 4), concordant with the groupings in the phylogenetic tree; in every case, clusters were defined geographically by basin and not by ecotype. Multiple iterations for each value of $K = 2 - 10$, showed consistent patterns of population genetic division. At low hypothesized K (i.e., < 5), geographic proximity was the primary determinant of genetic similarity

(Figure 4). As K increased to $K > 5$, subdivision and fractional ancestry that no longer made biological sense was apparent.

Analysis of molecular variance

AMOVA was used to partition and assess the significance of the variance at different levels of genetic structure with two hierarchal groupings. When populations were grouped by ecotype, the variance partitioned among groups was not significantly different from zero ($p = 0.943$), whereas when they were grouped by basin, it was 12.82% of the total genetic variance among groups, and highly significant ($p=0.009$; Table 3). Similarly, the variance among populations within groups categorized by ecotype and by basin were both significantly different from zero, representing 15.74 and 2.25% of the total variance, respectively (Table 3). Finally, the balance of the variance was partitioned within populations and accounted for 88.16 and 84.93% of the total variance for the ecotype and basin groups, respectively.

F_{ST} -outlier test

In order to identify loci that may not conform to neutral expectations, we conducted F_{ST} outlier tests on all genetic markers in this study. No consistent outlier loci were detected across multiple pairs of ecotype populations, confirming the

apparent selective neutrality of these loci and validating their use for population genetic inference (Supplemental table 2).

Discussion

In this study, the genetic structure of naturally spawning summer- and winter-run steelhead in multiple rivers of Oregon and Northern California was investigated using genotype data from 102 SNP and microsatellite loci. All analyses supported the hypothesis that populations of the two ecotypes within the same river basin are each other's closest relatives and that the summer-run life-history strategy has arisen multiple times independently in these basins. The close relationships of geographically proximate populations of both ecotypes indicate that local adaptation and genetic drift are more important for the evolution of the summer-run strategy than migration among populations of the same ecotype from different river basins. For example, the summer- and winter-run steelhead populations in the Klamath River basin are more closely related to each other than either one is to summer- and winter-run steelhead populations in the Eel River. These results suggest that isolation by distance (IBD) is a more important factor than ecotype in the genetic relationships of steelhead. IBD analysis found that almost 50% of the variation in genetic differentiation among populations from all sampling sites was explained by geographic distance (Figure 3). This pattern is consistent with previous studies of

coastal steelhead, which found similar signals of IBD (Pearse et al. 2011; Garza et al. 2014). A similar pattern of structuring by geography and not phenotype has also been observed in other anadromous and resident *O.mykiss* populations (Docker et al. 2003; Olsen et al. 2006; Pearse et al. 2007; Clemento et al. 2009).

The STRUCTURE analyses were concordant with the phylogram, with populations partitioned geographically by basin and not by ecotype. STRUCTURE can be used to identify recent migrants through individual assignment, but there was no evidence of recent migration of summer-run steelhead between rivers. The consistent grouping of the winter- and summer-run steelhead ecotypes within rivers with multiple iterations at different values of K is further evidence for a lack of gene flow among populations of the same ecotype from different rivers. The AMOVA analysis found that none of the total variation was distributed among groups when they are defined by ecotype, whereas when groups were defined by basins, a significant proportion was, concordant with the IBD and STRUCTURE results. In addition, approximately 85% of the molecular variance was contained within individual populations (Table 3). Finally, individual assignment tests yielded a high accuracy of assignment of individual samples to their corresponding populations of origin, with ~91% of fish accurately assigned to population and ~96% to basin of origin when a probability criterion was used. Additionally, the neutrality of the genetic markers used in this study was confirmed with the F_{ST} outlier test. Taken together, these results strongly support the hypothesis that the summer-run steelhead populations studied were more genetically similar to winter-run populations within

the same basin than to more geographically distant summer-run populations.

Similarly, winter-run populations grouped with other populations of either ecotype primarily on the basis of geographic distance.

The mechanisms by which parallel evolution of the summer steelhead ecotype has occurred are not known. However, as these summer, or “early”, returning steelhead populations colonized new habitat in the headwaters of these rivers, selection on migration timing, sexual maturity, and breeding date could potentially have been quite strong, with offspring viability increasing the earlier they returned from sea. Instances of adaptation of migration timing due to environmental change(s) are not uncommon in other salmonids. For example, pink salmon from Auke Creek, AK, have been observed to adapt to warmer water conditions by migrating earlier in the season in combination with a decrease in the number of very late returning fish (Kovach et al. 2012). Because of the tight coupling of run and spawn timing in Pacific salmonids (Flagg et al. 1995, Quinn et al. 2002), such divergent selection on run timing in may help facilitate differentiation between ecotypes, and the extent of reproductive isolation may be increased if individuals who disperse or reproduce at an intermediate time, especially hybrids, are maladapted and therefore have lower fitness (Crispo et al. 2006). Further work will help elucidate the underlying genetic basis of the observed differences in timing of return to freshwater and reproductive maturity.

Although summer- and winter-run steelhead appear to be discrete populations because of differences in timing of migration and spawning (Shapolov & Taft 1954; Royal 1972; Burgner et al. 1992; Hendry et al. 2005), as well as spawning location

(Everest 1973), the extent of gene flow between ecotypes in the same basin is unknown and the ecotypes have been found to be genetically very similar in some cases (Allendorf 1975; Nielsen & Fountain 1999; Clemente 2006). Chilcote et al. (1980) found that genetic heterogeneity did not differ significantly between summer- and winter-run steelhead in the Kalama River. In contrast, Leider et al. (1984) found genetic exchange was restricted between these populations because of spatial and temporal factors. In the Middle Fork Eel River, a landslide that formed a dam and paleolake during the Pleistocene era has been suggested to have facilitated gene flow between the two ecotypes by limiting upstream migration of summer-run steelhead and forcing both ecotypes to spawn in the same habitat (Mackey et al. 2011). However, the genetic divergence between summer- and winter-run steelhead populations in the other basins is similarly shallow, even though no such geologic explanation has been proposed, so it is not necessary to invoke a physical barrier to explain the contemporary genetic structure of steelhead ecotypes in the Eel River, even though gene flow may have been influenced by geologic events in the past.

Conservation Implications

Thus, the relationships between summer- and winter-run steelhead populations in the same basin have important management implications. In the United States, steelhead populations are administratively designated and managed as distinct population segments (DPSs), which are delineated on the basis of geographical, ecological, and genetic variation (Busby et al. 1996; Garza et al. 2014). Habitat loss,

poor water quality, and other factors have led to the decline of many steelhead populations and some populations are now listed as threatened species under the US Endangered Species Act (NOAA 2006). However, ecotypic run-timing differentiation is not specifically accounted for in considering DPS designations, despite the fact that access to summer-run habitat has been dramatically reduced by dam construction. Such habitat modifications may increase the potential for gene flow among ecotypes, leading to changes in their relationships. Finally, hatchery supplementation programs that support wild populations of summer or winter steelhead need to consider if individuals from different run timings represent distinct sub populations in order to effectively maintain this phenotypic variation in captive broodstock.

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Table 1. Summary statistics for all populations included in the present study. N = Sample size, Obs Hz = Observed Heterozygosity, A_r Mic = allelic richness for microsatellite loci only. Summer-run steelhead populations designated with (S).

DPS	Basin	Population	N	Loci typed	Obs Hz	A _r Mic	% self assignment
1 Lower Columbia	Columbia	Still Ck. (S)	24	101	0.298	7.7	100
2 Upper Willamette	Columbia	Wiley Ck.	23	101	0.263	6.0	95
3 Oregon Coast	Umpqua	Umpqua R.	23	102	0.328	8.1	75
4		Umpqua R. (S)	22	102	0.313	7.4	56
5 Klamath Mtn. Province	Rogue	Lobster Ck.	18	101	0.360	7.9	42
6		Lawson Ck.	23	101	0.361	8.0	86
7	Smith	Smith R.	32	102	0.361	7.9	97
8	Klamath	Blue Ck.	32	102	0.364	8.4	100
9		Hunter Ck.	28	102	0.384	6.5	92
10	Redwood	Lost Man Ck.	31	102	0.387	8.1	89
11	Trinity	New R. (S)	47	102	0.349	7.9	90
12		South Fork	47	102	0.340	8.1	83
13	Mad	Mad R.	28	102	0.410	6.9	88
14	Mattole	Mattole R.	31	102	0.401	6.8	96
15	Eel	Lawrence Ck.	30	102	0.402	7.8	88
16		Hollow Tree	28	102	0.390	7.4	79
18		Middle (S)	24	101	0.397	6.9	95
16		Van Aisdale	23	101	0.377	8.0	95
19	Noyo	Noyo R.	31	102	0.415	7.6	97
20	Gualala	Gualala R.	29	102	0.448	7.0	92
21 Central CA coast	Miller	Miller Ck.	31	102	0.407	5.9	100
22	Redwood	Redwood Ck.	30	102	0.438	7.6	83
23	San Lorenzo	San Lorenzo R.	32	102	0.431	6.2	88
24 South-Central CA coast	Carmel	Carmel R.	32	102	0.418	7.5	93
25	Big Sur	Big Sur R.	31	102	0.427	7.3	80

Table 2. Pairwise F_{ST} values for the eight focal winter- and summer-run steelhead population pairs. * = significant ($P < 0.05$). S = summer ecotype

Basin	Population	Wiley Ck.	Still Ck. (S)	Umpqua	Umpqua (S)	South Fork Trinity	New R. (S)	Middle Fork
Columbia	Wiley Ck.	-						
	Still Ck. (S)	*0.068	-					
Umpqua	Umpqua	*0.104	*0.037	-				
	Umpqua (S)	*0.121	*0.048	0.000	-			
Klamath	South Fork Trinity	*0.156	*0.085	*0.069	*0.076	-		
	New River (S)	*0.156	*0.092	*0.073	*0.081	*0.016	-	
Eel	Middle Fork (S)	*0.291	*0.236	*0.198	*0.198	*0.166	*0.168	-
	Van Arsdale	*0.312	*0.246	*0.210	*0.212	*0.185	*0.185	*0.034

Table 3. AMOVA results evaluating different hypotheses for groupings of the focal populations. Nb = number of groups, Var = covariance component, % = percent overall genetic variance, and F-statistics (F_{CT} , F_{SC} , F_{ST}) appropriate for each level of comparison. Bold values are significantly different from zero ($P < 0.05$).

Description	Nb	Among groups			Among populations groups			Within populations within groups		
		Var	%	FCT	Var	%	FSC	Var	%	FST
By ecotype	2	0.655	-3.90	-0.039	2.643	15.74	0.152	14.801	88.16	0.118
By basin	4	2.234	12.82	0.128	0.392	2.25	0.026	14.801	84.93	0.151

Figure 1

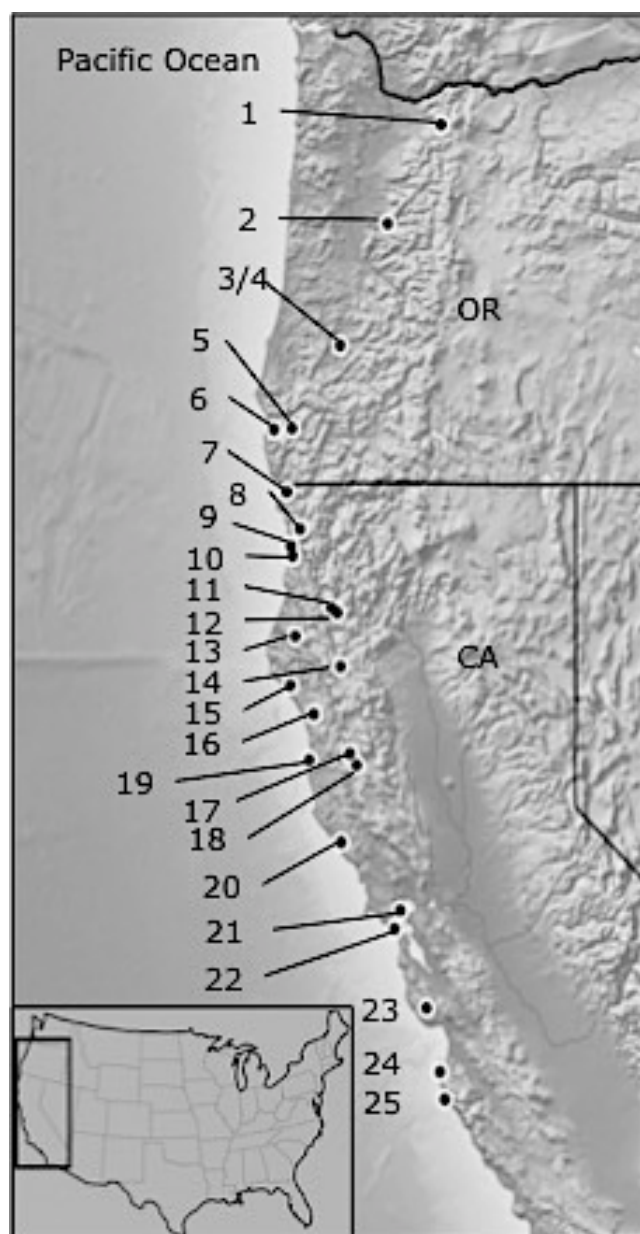


Figure 2

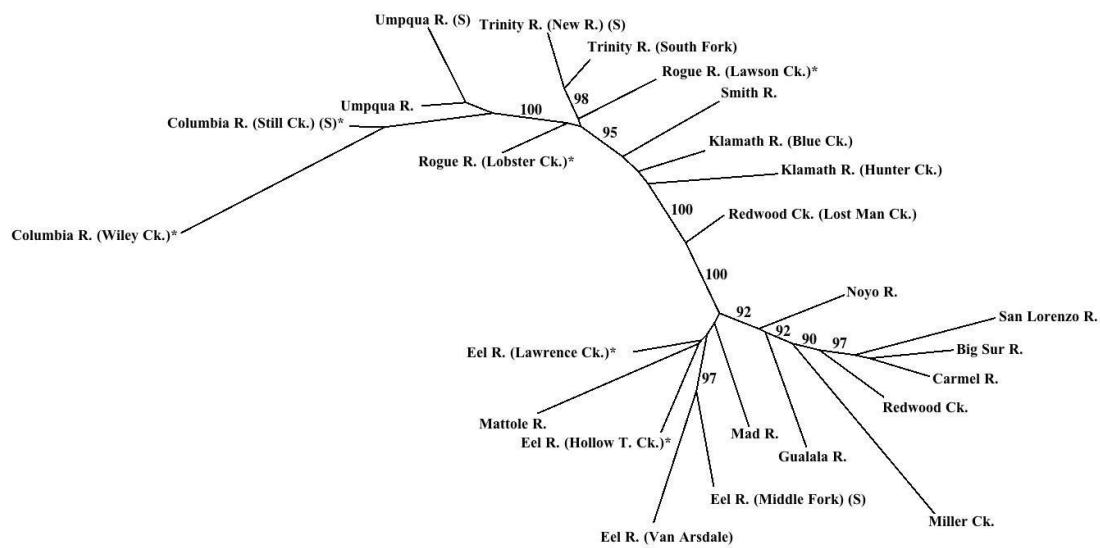
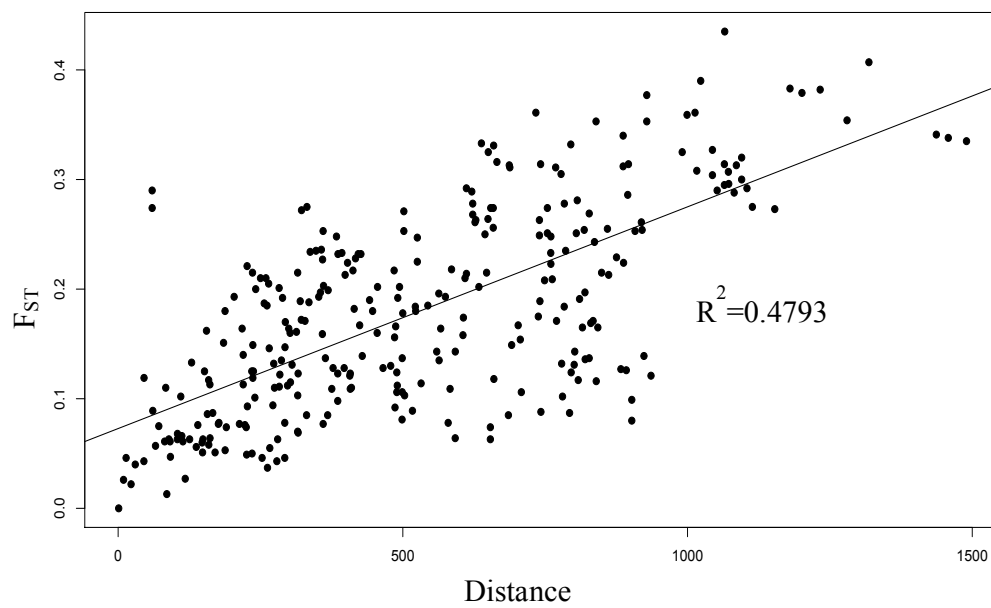


Figure 3



Supplemental Material Table 1.

Assay ID	Assay Target	Reference
<i>Omy1011</i>	(CAGA) ₁₁	Spies et al. 2005
<i>OtsG243</i>	(TAGA) ₆₃ (CAGA) ₁₂ (GACA) ₇ (GA) ₂₂	Williamson et al. 2002
<i>OtsG253B</i>	(GACA)10(GATA) ₁₄	Williamson et al. 2002
<i>One11</i>	(CA) ₁₃	Scribner et al. 1996
<i>OtsG249B</i>	(TAGA) ₁₉	Williamson et al. 2002
<i>OtsG43</i>	(GACA) ₁₃ (GATAGACA) ₂ (GATA) ₂₅	Williamson et al. 2002
<i>OtsG85</i>	(GATA) ₁₉	Williamson et al. 2002
<i>Oki23</i>		Spidle et al. unpublished, GenBank AF272822
<i>One13</i>	(GA) ₂₀	Scribner et al. 1996
<i>OtsG3</i>	(GATA) ₃₀ (TAGA) ₁	Williamson et al. 2002
<i>OtsG409</i>	(GA) ₉ (TAGA) ₆ -GGTA-(GATA) ₁₆	Williamson et al. 2002
<i>Ssa289</i>	(GT) ₁₂	McConnell et al. 1995
OMGHIPROM1-SNP1	A/T	Abadía-Cardoso <i>et al.</i> 2011
Omy_AldA	T/C	Aguilar & Garza 2008
Omy_arp-630	T/C	Campbell <i>et al.</i> 2009
Omy_aspAT-123	T/C	Campbell <i>et al.</i> 2009
Omy_COX1-221	T/A	Campbell <i>et al.</i> 2009
Omy_g12-82	G/A	WSU - J. DeKoning unpubl.
Omy_gh-475	G/A	Campbell <i>et al.</i> 2009
Omy_gsdf-291	T/C	WSU - J. DeKoning unpubl.
Omy_mapK3-103	T/A	CRITFC - N. Campbell unpubl.
Omy_mcsf-371	G/A	WSU - J. DeKoning unpubl.
Omy_nramp-146	T/C	Campbell <i>et al.</i> 2009
Omy_Ogo4-304	T/C	Campbell <i>et al.</i> 2009
OMY_PEPA-INT6	T/C	Aguilar & Garza 2008
ONMYCRBF_1-SNP1	T/C	Aguilar & Garza 2008
SH100771-63	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH100974-386	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH101554-306	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH101770-410	T/C	Abadía-Cardoso <i>et al.</i> 2011

Supplemental Material Table 1 continued from last page.

Assay ID	Assay Target	Reference
SH101832-195	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH101993-189	A/T	Abadía-Cardoso <i>et al.</i> 2011
SH102420-634	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH102505-102	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH102510-682	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH102867-443	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH103350-395	A/C	Abadía-Cardoso <i>et al.</i> 2011
SH103577-379	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH103705-558	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH104519-624	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH105075-162	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH105105-448	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH105115-367	C/G	Abadía-Cardoso <i>et al.</i> 2011
SH105385-406	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH105386-347	A/C	Abadía-Cardoso <i>et al.</i> 2011
SH105714-265	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH106172-332	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH106313-445	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH107074-217	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH107285-69	C/G	Abadía-Cardoso <i>et al.</i> 2011
SH108735-311	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH109243-222	A/C	Abadía-Cardoso <i>et al.</i> 2011
SH109525-403	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH109651-445	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH109693-461	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH109874-148	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH110064-419	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH110078-294	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH110201-359	T/G	Abadía-Cardoso <i>et al.</i> 2011

Supplemental Material Table 1. Table 1 continued from last page

Assay ID	Assay Target	Reference
SH110362-585	G/A	Abadía-Cardoso <i>et al.</i> 2011
SH110689-148	A/C	Abadía-Cardoso <i>et al.</i> 2011
SH111666-301	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH112208-328	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH112301-202	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH112820-82	G/A	Abadía-Cardoso <i>et al.</i> 2011
SH113109-205	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH113128-73	C/G	Abadía-Cardoso <i>et al.</i> 2011
SH114315-438	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH114587-480	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH114976-223	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH115987-812	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH116733-349	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH117259-96	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH117286-374	A/T	Abadía-Cardoso <i>et al.</i> 2011
SH117370-400	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH117540-259	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH117815-81	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH118175-396	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH118654-91	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH118938-341	A/T	Abadía-Cardoso <i>et al.</i> 2011
SH119108-357	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH119892-365	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH120255-332	A/T	Abadía-Cardoso <i>et al.</i> 2011
SH120950-569	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH123044-128	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH125998-61	T/G	Abadía-Cardoso <i>et al.</i> 2011
SH127510-920	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH128851-273	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH129870-756	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH130524-160	C/G	Abadía-Cardoso <i>et al.</i> 2011
SH130720-100	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH131460-646	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH131965-120	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH95318-147	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH95489-423	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH96222-125	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH97077-73	T/A	Abadía-Cardoso <i>et al.</i> 2011
SH97954-618	C/T	Abadía-Cardoso <i>et al.</i> 2011
SH98188-405	T/C	Abadía-Cardoso <i>et al.</i> 2011
SH98409-549	A/G	Abadía-Cardoso <i>et al.</i> 2011
SH98683-165	A/C	Abadía-Cardoso <i>et al.</i> 2011
SH99300-202	T/A	Abadía-Cardoso <i>et al.</i> 2011

Supplemental Material Table 2.

Drainage	Markers	Sim. Fst	Outliers (Y/N)	Loci [He/Fst/P]
Columbia	Msats	0.0676	N	N/A
Umpqua	Msats	0.0041	N	N/A
Trinity	Msats	0.0172	N	N/A
Eel	Msats	0.0266	N	N/A
Columbia	SNPs	0.0703	Y	SH106313-445 [0.08/0.064/1], SH110064-419 [0.10/0.085/1]
Umpqua	SNPs	0.0023	Y	Omy_nramp-146 [0.04/0.021/1], SH106172-332 [0.04/0.021/1]
Trinity	SNPs	0.0137	N	N/A
Eel	SNPs	0.0259	Y	SH112301-202 [0.25/0.234/0.999]
Columbia	Both	0.0758	Y	SH106313-445 [0.08/0.064/1]
Umpqua	Both	0.0008	Y	Omy_nramp-146 [0.04/0.021/1], SH106172-332 [0.04/0.021/1]
Trinity	Both	0.0130	N	N/A
Eel	Both	0.0269	Y	SH112301-202 [0.25/0.234/0.999]

Supplemental Figure 1. Plots made by the program *Distruct* representing results of STRUCTURE analyses for the winter- and summer-run steelhead populations from the Columbia, Umpqua, Klamath, and Eel rivers, with each fish represented by a thin, vertical bar. K = number of genetic clusters.

