Over the Falls? Rapid Evolution of Ecotypic Differentiation in Steelhead/ Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

Adaptation to novel habitats and phenotypic plasticity can be counteracting forces in evolution, but both are key characteristics of the life history of steelhead/rainbow trout (*Oncorhynchus mykiss*). Anadromous steelhead reproduce in freshwater river systems and small coastal streams but grow and mature in the ocean. Resident rainbow trout, either sympatric with steelhead or isolated above barrier dams or waterfalls, represent an alternative life-history form that lives entirely in freshwater. We analyzed population genetic data from 1486 anadromous and resident *O. mykiss* from a small stream in coastal California with multiple barrier waterfalls. Based on data from 18 highly variable microsatellite loci (He = 0.68), we conclude that the resident population above one barrier, Big Creek Falls, is the result of a recent anthropogenic introduction from the anadromous population of *O. mykiss* below the falls. Furthermore, fish from this above-barrier population occasionally descend over the falls and have established a genetically differentiated below-barrier subpopulation at the base of the falls, which appears to remain reproductively isolated from their now-sympatric anadromous ancestors. These results support a hypothesis of rapid evolution of a purely resident life history in the above-barrier population in response to strong selection against downstream movement.

Key words: genetics, adaptation, genetics, life history, Oncorhynchus mykiss

Phenotypic plasticity, in which a given genotype produces alternative phenotypes in response to certain environmental conditions, is common among animals and plants living in variable environments and may play a critical role in adaptation to novel habitats (Agrawal 2001). Often, the benefits of plasticity are balanced by increased costs relative to individuals adapted to local conditions, but modeling studies have suggested that plasticity may be maintained in many environments, especially when gene flow among populations is substantial (Stearns 1989; Sultan and Spencer 2002). However, when spatial heterogeneity exists in tandem with a substantial barrier to gene flow, alternative phenotypes may be consistently favored in each habitat, leading to local adaptation if expression of the phenotype has a heritable genetic component (Reznick et al. 1997; Losos et al. 2001; Koskinen et al. 2002; Keeley et al. 2007). Secondary contact after a period of such adaptive divergence may then result in only limited hybridization and introgression due to pre and/or postzygotic barriers to reproduction between ecotypes.

Many species of salmonid fishes exhibit a range of life-history forms, with alternative physical and behavioral phenotypes expressed depending on the environmental conditions encountered. For example, large differences may exist between ecotypes in the timing and location of their development, migration, reproduction, maturation environment, and choice of spawning habitat (Burger et al. 2000). In some species, fully anadromous (ocean migratory) and resident (entirely freshwater) individuals may coexist within a single population, and the extent and pattern of reproductive isolation between them in these situations are variable (Skaala and Naevdal 1989; Taylor et al. 1996; Narum et al. 2004; Charles et al. 2005, 2006; McPhee et al. 2007; Thériault, Bernatchez, and Dodson 2007). Sympatric resident and anadromous individuals may have very different developmental patterns (Morinville and Rasmussen 2003) and gene expression profiles (Aubin-Horth et al. 2005), reflecting their dramatically different life-history trajectories. These patterns are at least partially due to phenotypic plasticity, although some aspects of life history

have been shown to have a heritable component (Hankin et al. 1993; Heath et al. 2002; Thériault, Grant, et al. 2007), and trait divergence among forms has been shown to occur very rapidly (Reznick et al. 1997; Hendry et al. 2000). However, the strength of heritable genetic differences relative to plasticity in the expression of particular genes due to environmental conditions remains unclear (Aubin-Horth and Dodson 2004). Nonetheless, sympatric populations of resident and anadromous fish are typically each others' closest relatives (Docker and Heath 2003; Narum et al. 2004; Olsen et al. 2006; Deiner et al. 2007; Pearse et al. 2007), a pattern consistent with both plasticity and the independent evolution of resident life history from the local anadromous population in each case.

Rainbow trout or steelhead, *Oncorhynchus mykiss*, is the most widespread Pacific salmonid and exhibits a diverse range of life-history strategies (Shapovalov and Taft 1954; Thorpe 2007). Resident rainbow trout live their entire lives in freshwater streams or lakes, often above natural or manmade river barriers. In contrast, after completion of a juvenile freshwater stage, anadromous steelhead undergo a complex physiological and morphological transformation, termed smoltification, and migrate to the ocean to grow and mature before returning to freshwater in one or more spawning migrations, usually to their natal stream. A wide variety of intermediate life-history patterns have been observed in *O. mykiss*, often including a period of time in a brackish estuarine environment (Shapovalov and Taft 1954; Bond 2006; Thorpe 2007). Furthermore, some offspring of anadromous parents may remain and mature in freshwater, an example of phenotypic plasticity in life-history pattern referred to as "residual" to differentiate them from individuals with true resident trout ancestry (Willson 1997). However, all forms of steelhead, residual trout, and resident rainbow trout are generally indistinguishable as juveniles.

When resident and anadromous populations are not fully sympatric, but instead are separated by a waterfall or other barrier, one-way migration over the barrier by resident fish can create a below-barrier population that is a mixture of anadromous juveniles and fish that either came over the barrier themselves or are descendents from above the falls population (e.g., Pettersson et al. 2001; Thrower, Guthrie, et al. 2004). In 2 such situations, Zimmerman and Reeves (2000, 2002) found that anadromous juveniles were more likely to be produced by anadromous mothers than by resident mothers, and vice versa, suggesting reproductive isolation even in the sympatric below-barrier population. Similarly, Thrower, Hard, and Joyce (2004) demonstrated a genetic basis and high heritability of resident versus anadromous life-history pattern in an above-barrier resident population that had been founded from below-barrier anadromous fish only 70 years earlier.

In Scott Creek, a small watershed in coastal California (Figure 1), waterfalls on each of the 3 main tributaries—Big Creek, Mill Creek, and upper Scott Creek—create complete barriers to upstream movement of fish in the absence of human-mediated transport. Historical records in land-owners' journals document the transfer of *O. mykiss*, captured in the watershed below all such barriers, into the section above one of these barriers, Big Creek Falls, in 1910, and additional transfers likely occurred before and after this date. Ongoing ecological studies (Hayes et al. 2004; Bond 2006; Bond et al. 2008) have documented patterns of reproduction, growth, and movement in the below-barrier portion of the watershed, confirming and extending Shapovalov and Taft's (1954) observation of high variability in life-history patterns in this same stream more than 50 years ago. In addition, mark–recapture tag data have documented movement of individuals over the barrier falls on Big Creek into the lower reaches of the watershed. Because individuals that migrate over the falls are effectively lost from the above-barrier population, intense selection against downstream movement in the remaining above-barrier fish is expected to drive the rapid evolution of a purely resident phenotype above the falls.

Here we analyze molecular genetic data from *O. mykiss* populations within Scott Creek to test the hypotheses that 1) resident trout in 4 above-barrier populations in the watershed are genetically divergent from the anadromous fish below, 2) fish *above* Big Creek Falls are derived primarily from the anadromous below-barrier population, 3) resident fish *below* Big Creek Falls are descended from the above-falls population, 4) few if any offspring from the above-barrier populations successfully complete anadromous migrations, and, 5) hybridization and introgression after secondary contact in the mixed population below Big Creek Falls has been limited. These analyses support the general hypothesis that life-history pattern in *O. mykiss* has a strong heritable genetic component despite the phenotypic plasticity associated with these traits.

Materials and Methods

Sampling

Juvenile and resident fish were sampled throughout the watershed with a seine net or hook and line from April 2002 to November 2005. Fish migrating downstream were also trapped with a hoop-net trap. Adult steelhead were captured throughout the watershed by divers, in the downstream migrant trap, with seine nets, and using a resistance board weir installed in the fall of 2003. Fish were handled in the field according to the methods of Hayes et al. (2004). Briefly, length and mass were recorded for each individual, and a small piece of caudal fin was clipped for DNA extraction. Fin clips were placed on filter paper and stored dry until DNA extraction.

For the present study, samples were grouped based on location relative to waterfall barriers and known individual life-history strategy (Figure 1). There is almost no overlap in the size distributions of anadromous adults (typically >35 cm) and fish that have not gone to sea (<25 cm) (Hayes et al. 2004). Thus, only fish captured below all barriers during the spawning season as large, phenotypically
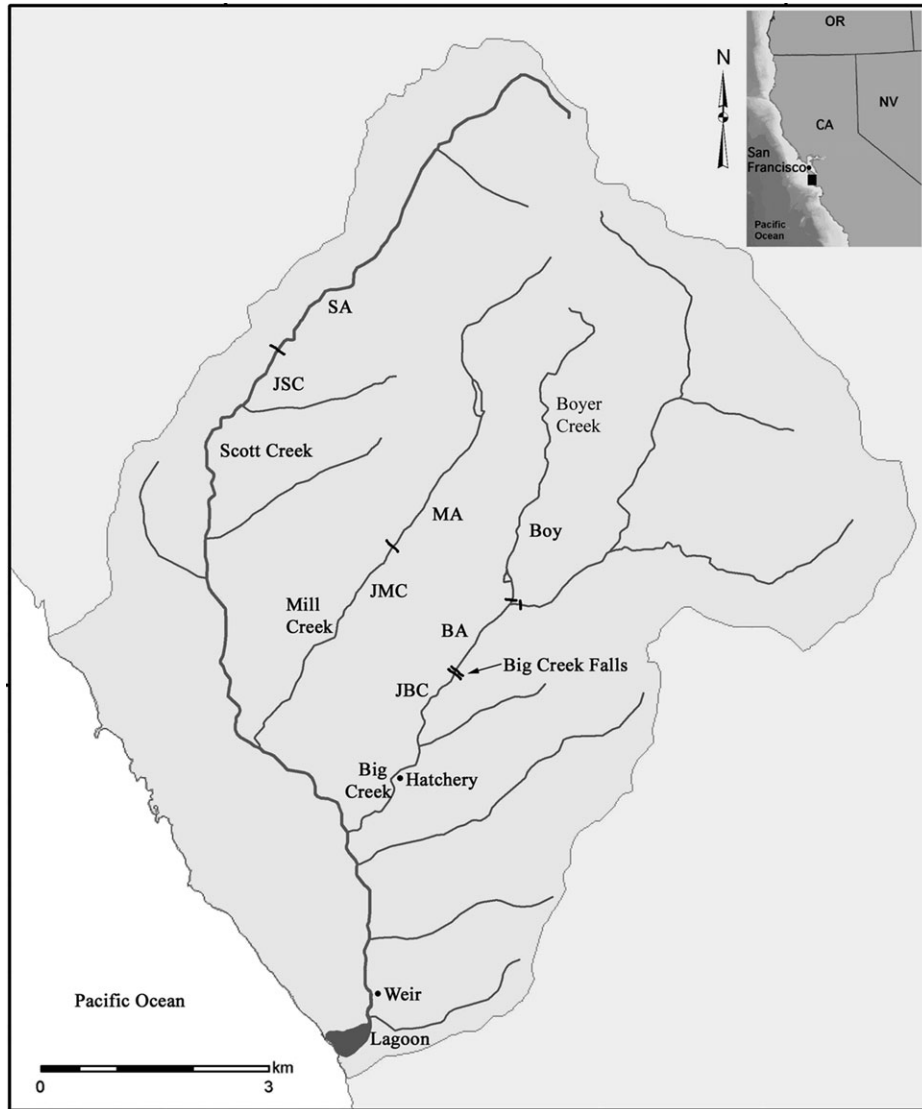
Figure 1. Map of Scott Creek basin, showing tributary steams and reservoirs. Locations of the Weir, Lagoon, Hatchery, Big Creek Falls (=), and other barrier waterfalls (–) are shown. The Big Creek Above (BA) population exists between Big Creek Falls, and the barrier falls at the confluence of Boyer and Big Creeks. The Boyer Creek population is further isolated by a small reservoir above this second falls. Inset: location in coastal California near San Francisco.

steelhead adults were defined as anadromous. All other fish captured below the barrier falls were classified as "juvenile/resident" (Table 1).

Based on the above sample groupings, 5 clearly definable groups were designated as reference populations for analysis: anadromous adults (Weir), Mill Creek above (MA), Big Creek above (BA), Scott Creek above (SA), and Boyer Creek above (Boy). Other below-barrier samples included juvenile/resident fish captured in the lagoon (LAG), fish captured in upstream tributaries but below barriers (juveniles, JSC, JMC, JBC; adults ASC, ABC), and juveniles produced from wild anadromous adults in the small hatchery operated on Big Creek (HAT). These samples were evaluated in the individual-based analyses

but were not used as reference population samples for estimation of allele frequencies or population genetic statistics (Table 1).

Genetic Data Collection

DNA was extracted from all samples 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 with dH_2O and used for polymerase chain reaction (PCR) amplification of 18 microsatellite loci previously optimized for use in *O. mykiss* (Garza et al. 2004; Pearse et al. 2007). PCR products were electrophoresed on ABI 377 sequencers (Applied Biosystems, Inc.), and microsatellite genotypes were determined

Table 1. Sample sizes (*n*) and population genetic statistics for Scott Creek *Oncorhynchus mykiss* included in this study

Population	Code	*n*	HWE[a]	LD[a]	Ar	Mean F_{ST}
Reference populations						
Anadromous adults	Weir	233	0.11	0.07	6.1	0.051
Scott Creek above	SA	36	0.00	0.01	4.8	0.096
Mill Creek above	MA	13	0.00	0.01	3.6	0.193
Big Creek above	BA	186	0.06	0.08	5.4	0.063
Boyer Creek above	Boy	17	0.00	0.00	3.7	0.116
Other below-barrier samples						
Scott Creek juv/res	JSC	43	0.00	0.02	—	—
Mill Creek juv/res	JMC	25	0.06	0.03	—	—
Big Creek juv/res	JBC	174	0.17	0.14	—	—
Hatchery juveniles	Hat	108	0.28	0.50	—	—
Lagoon juveniles	Lag	580	0.44	0.47	—	—
Anadromous, Scott creek	ASC	19	0.00	0.00	—	—
Anadromous, Big creek	ABC	41	0.00	0.02	—	—

Allelic richness (Ar) calculated based on a sample of 20 allele copies and mean pairwise F_{ST} values calculated among the 5 reference populations.

[a] Proportion of significant deviations in tests of Hardy–Weinberg (HWE) or pairwise comparisons of linkage disequilibrium (LD), after Bonferroni correction.

using Genescan 3.0 and Genotyper 2.1 software (Applied Biosystems, Inc.). All genotypes were checked independently by 2 people to ensure consistent scoring. Discrepancies between the 2 scores were resolved either by consensus, by regenotyping, or by deletion of that genotype from the data set.

Data Analysis

The computer programs GENETIX (Belkhir et al. 1996–2004), GENEPOP (Raymond and Rousset 1995), and HP_RARE (Kalinowski 2005) were used to estimate basic population genetic statistics among population and temporal samples, test for departures from Hardy–Weinberg and linkage equilibria and estimate the distribution of population genetic variation using *F*-statistics (Wright 1931). The software package PHYLIP (Felsenstein 2004) was used to calculate Cavalli-Sforza and Edwards (1967) chord distances and generate a neighbor joining phylogram of populations. Statistical support for population relationships was evaluated using 1000 bootstrap samples, and the resulting trees were visualized using TreeViewPPC (Page 1996). In these analyses, the data from Scott Creek were combined with data from representative coastal California *O. mykiss* populations (Garza et al. 2004; unpublished data).

To complement the population-based analyses described above and test hypotheses about individual genotypic composition, individual genetic analyses were conducted using both GeneClass2 (Piry et al. 2004), an allele frequency–based assignment program, and STRUCTURE (Pritchard et al. 2000), a model-based Bayesian clustering method that assigns individuals proportional ancestry

(*Q* values) to account for admixture among populations. Individual self-assignments for all individuals from the 5 defined reference populations were conducted in GeneClass2 with the leave-one-out procedure implemented to correct for bias caused by inclusion of the assigned individual in the reference data. Individuals from all other sample collections were assigned to the 5 reference populations. In all cases, the Rannala and Mountain (1997) approach was used for allele frequency estimation and only individuals assigned to a population with a probability score of >95% were reported.

To further evaluate individual ancestry, clustering analyses were conducted using the program STRUCTURE v2.2 (Pritchard et al. 2000). This method is useful for the identification of migrants and individuals of mixed ancestry without a priori designation of defined populations (Pritchard et al. 2000; Pearse and Crandall 2004). These analyses were conducted with a range of values for *k*, the number of genetic clusters, ranging from 1 to 8, with multiple runs performed for each value to evaluate consistent patterns of genetic association. Default settings were used for most parameters: burn-in and run length ranged 1000–10 000 and 10 000–100 000, respectively, and the "pop alphas" option and correlated allele frequency model were used to reflect the shared ancestry of these populations (Falush et al. 2003). Results from STRUCTURE consist of individual proportional assignments (*Q* values) to each of the *k* genetic clusters and were visualized using DISTRUCT (Rosenberg 2004).

Finally, we used the program NewHybrids (Anderson and Thompson 2002) to identify F1 and later generation hybrids between anadromous adults (Weir) and descendents of the Big Creek Above (BA) population. The Weir and BA samples were used as training populations (assigned New-Hybrids' "z" and "s" options), and NewHybrids was used to compute the posterior probability that individuals in the mixed sample immediately below the falls (JBC) represented pure anadromous (Weir), pure above-falls resident (BA), F1, F2, BxWeir, or BxBA (the 2 backcross categories). We performed 5 separate NewHybrids runs with 10 000 sweeps of burn-in and 50 000 sweeps of data collection, using unit-information priors for both Θ and π.

In order to interpret the posterior probabilities computed by NewHybrids and to evaluate the power of the data for detecting F1 individuals, we conducted multiple runs of NewHybrids on data sets containing *S* "known" F1 individuals simulated via a modified version of leave-one-out cross-validation (Efron and Gong 1983). Simulated data sets were identical to the original data set except that *S* randomly selected individuals were removed from each of the 2 training samples and combined into *S* Weir × BA pairs. A single F1 was simulated from each pair and added to the mixture sample (JBC). The randomly chosen fish from Weir and BA were excluded from further analysis to avoid biasing the inference for the simulated genotypes (Anderson et al. 2008). Four different values of *S* were used: 3, 5, 10, and 20. For each value, 50 replicate data sets were simulated, and each one was analyzed with NewHybrids

using 1000 sweeps of burn-in and 10 000 sweeps of data collection, with all other settings identical to those used in analyzing the original data set.

Results

Population Genetics of *O. mykiss* in Scott Creek

A total of 1502 individual fish were genotyped with the 18 microsatellite loci used in the study. Ten pairs of samples possessed matching genotypes at all 18 microsatellite loci. Similarly, 3 pairs of samples matched perfectly at 16 loci, with 2 failed loci in 1 of the samples. In all cases, the sampling locations, morphological data, and timing of the sampling were consistent with duplicate samples from the same individual (i.e., recaptures), and the second sample of each genotype was removed from the data set. Amplification success was high, with 4.3% missing data overall and 97.7% of the individuals successfully genotyped for at least 15 of the loci. All but 3 individuals were genotyped for at least half of the loci, and these 3 individuals were removed from the data set. These removals left a total of 1486 individuals in the final data set. We estimated the genotyping error rate using data from 22 individuals that were genotyped independently at least twice on separate gels. Across all 18 loci, a total of 5 allelic discrepancies were identified between the first and second genotyping attempts, providing an estimated discordance rate of ~1%.

Among the 5 reference populations, no pair of loci consistently displayed significant linkage disequilibrium, and across all possible pairwise combinations only 3% were significant after Bonferroni correction for multiple tests. Across loci, no consistent pattern of Hardy–Weinberg disequilibrium was observed within any reference population, but locus *Omy77* was significantly out of Hardy–Weinberg equilibrium in 2 of the 5 reference populations (BA and Weir). Among the other sample collections, 3 were significantly out of Hardy–Weinberg and linkage equilibrium for a large proportion of the surveyed loci (JBC, Hat, and Lag; Table 1).

Pairwise tests for genic (allelic) differentiation and F_{ST} were significant for all pairs of reference populations (Table 2). Mean population pairwise F_{ST} values were strongly correlated with mean allelic richness (Ar) across all loci (Table 1; $r^2 = 0.77$, $P < 0.05$), as has been found over much larger geographic scales in many anadromous populations of *O. mykiss* (Pearse et al. 2007). Tests of allelic differentiation and genetic divergence (F_{ST}) among the 3 samples of anadromous adults collected in different parts of the watershed (Weir, ABC, and ASC) were not significant. Similarly, genetic divergence between the BA and Weir samples was consistent across those years for which sufficient samples were available, and with the analysis of pooled sample years, although significant differentiation was observed among some single-year samples of the BA population, likely due to sampling effects.

Table 2. Pairwise F_{ST} values between reference populations within Scott Creek

	Weir	SA	MA	BA	Boy
Weir	—				
SA	0.047	—			
MA	0.159	0.225	—		
BA	0.018	0.060	0.172	—	
Boy	0.076	0.140	0.234	0.081	—

All values significantly different from zero at $P < 0.05$.

Influence of Out-of-Basin Stocking

Phylogenetic analyses that included populations of *O. mykiss* from throughout coastal California as well as several common hatchery strains grouped the Weir, BA, and SA populations with samples from neighboring streams, as expected based on observed patterns of isolation by distance and supporting the hypothesis of a local origin for these populations (Figure 2). These populations also showed low pairwise F_{ST} values (Table 2), consistent with intrabasin comparisons seen in other studies using these same microsatellite loci (Garza et al. 2004; Clemento et al. 2008). In contrast, Mill Creek and Boyer Creek were separated from the other *O. mykiss* in the Scott Creek watershed with significant bootstrap support and instead clustered closely with several distant populations known to have been influenced by hatchery stocking. In addition, both populations were highly differentiated when compared with the other Scott Creek populations, with Mill Creek having among the highest pairwise F_{ST} values observed with these loci for any population, including hatchery stocks (Garza et al. 2004; Clemento et al. 2008). These results reinforce both the local origin of the BA and SA populations and the suspected influence of external hatchery stocking with rainbow trout strains on the Boy and MA populations.

Distribution of Individual Genotypes among Populations

Results from the GeneClass2 assignment tests are shown in Table 3. Overall, 93% of the individuals from the reference populations received a high confidence assignment, and of these, more than 97% were correctly assigned to their population of origin. Among the below-barrier juvenile/ resident samples, 92% were assigned to 1 of the 5 reference populations with high confidence, with more than 91% of these assigned to the Weir (anadromous) population. A greater proportion of anadromous adults genetically assigned to above-barrier populations than vice versa (3.8% vs. 0.9%; Table 3), a result consistent with the potential for downstream (but not upstream) migration, but which could also be due to the greater genetic diversity present in the anadromous sample.

Following Rosenberg et al. (2001), analyses using STRUCTURE were conducted with a range of parameter values and data configurations (e.g., inclusion and exclusion of populations, equalization of sample sizes, and use of prior population information) in order to fully explore their
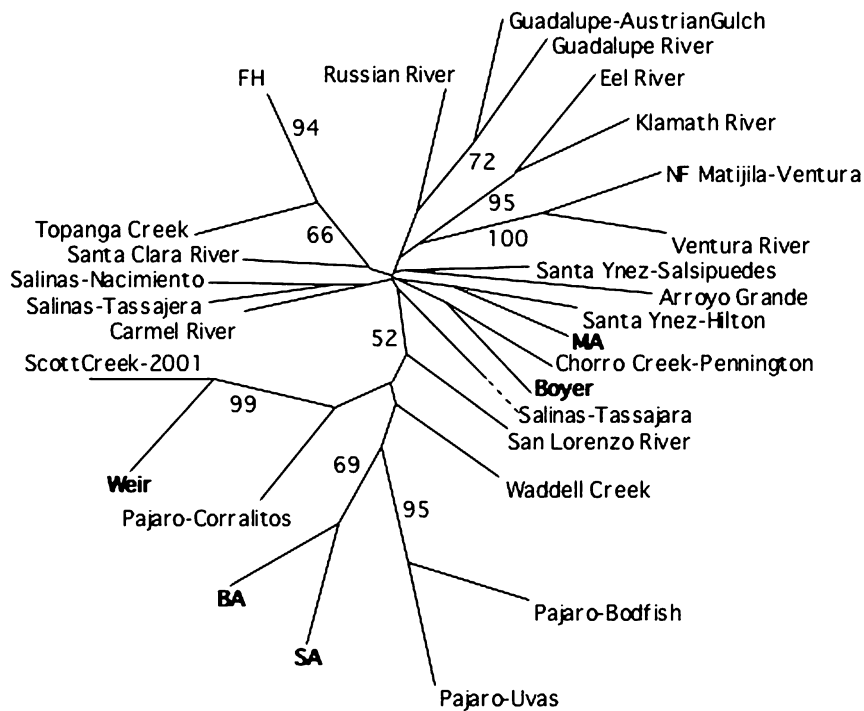
Figure 2. Neighbor joining network based on Cavalli-Sforza/Edwards chord distances, with bootstrap support from 1000 replicates shown. Populations from the Scott Creek watershed are shown in bold; all other populations are samples of juvenile *Oncorhynchus mykiss* collected in coastal California streams. Waddell Creek and the Pajaro and San Lorenzo Rivers are the 3 streams closest to Scott Creek. "FH" indicates 6 Fillmore Hatchery rainbow trout strains included separately in the analysis but combined for display.

effects. Multiple STRUCTURE runs were made for each *k* from 2 through 8, including 10 runs each at both *k* = 5 and *k* = 6. Individual genetic clustering patterns were generally consistent across values of *k*, and we focused on the biologically meaningful value of *k* = 5, representing the 5 physically separated reference populations. Variation in clustering pattern was seen in some runs, most commonly

a division of the anadromous adults (Weir) into more than one genetic cluster, presumably attributable to the presence of family structure (Rosenberg et al. 2001; Anderson and Dunham 2008). Similarly, some STRUCURE runs grouped the Mill Creek and Boyer Creek individuals into a single genetic cluster, likely due to the small size of these samples (Rosenberg et al. 2002); when sample sizes were equalized for all populations, STRUCTURE always divided Mill Creek from all other populations at *k* = 2 and partitioned Mill Creek/Boyer Creek/all other populations at *k*=3.

Individual assignments by STRUCTURE generally paralleled the assignments made by GeneClass2. Table 4 shows the distribution of maximum *Q* values (Q_{MAX}) for individuals in each of the focal populations, regardless of the specific genetic cluster assigned. This distribution shows, for example, that most individuals in 2 of the isolated above-barrier populations (MA and SA) have a high Q_{MAX}, whereas individuals in the Weir, BA, and below-barrier populations show a broader distribution of Q_{MAX}, consistent with their lower genetic differentiation (complete results from STRUCTURE are shown in Supplementary Figure S1). Fish sampled below barriers in the tributary (JSC and JMC), lagoon (Lag), and hatchery (Hat) samples were almost exclusively assigned to the anadromous (Weir) population with high population and individual *Q* values, indicating pure anadromous ancestry (data not shown). The only below-barrier sample with a substantial genetic

Table 3. Number of individuals assigned by GeneClass2 (Piry et al. 2004) to each of the 5 reference populations

Source population	Assigned population				
	Weir	SA	MA	BA	Boy
Weir	201	—	—	6	1
SA	1	33	—	—	—
MA	—	—	12	—	—
BA	1	—	—	160	2
Boy	—	—	—	—	17
JSC	41	1	—	—	—
JMC	18	—	4	—	—
JBC	96	1	—	53	2
Hat	101	—	—	—	—
Lag	508	—	1	11	1
ASC	19	—	—	—	—
ABC	31	—	—	3	—

Only individuals with assignment score >95 are reported. See text for details.

Table 4. Proportional histograms of individual Q values from STRUCTURE

	N	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
Weir	233	0.45	0.33	0.09	0.06	0.04	0.02	0.01	—	—	—
SA	36	0.31	0.36	0.19	0.11	0.03	—	—	—	—	—
MA	13	0.85	0.08	—	—	0.08	—	—	—	—	—
BA	186	0.39	0.21	0.13	0.11	0.13	0.03	—	—	—	—
Boy	17	0.71	0.29	—	—	—	—	—	—	—	—
JSC	43	0.49	0.28	0.12	0.05	0.02	0.05	—	—	—	—
JMC	25	0.48	0.32	0.08	0.08	—	0.04	—	—	—	—
JBC	131[a]	0.40	0.34	0.08	0.03	0.09	0.05	—	—	—	—

Each 0.1 incremental bin shows the proportion of individuals in each sample with a corresponding maximum Q value (Q_{MAX}) to any genetic cluster. See text for details.

[a] Only individuals sampled from the section of Big Creek immediately below the falls were included in the STRUCTURE analysis.

contribution from an above-barrier reference population was the collection immediately below Big Creek falls (JBC; Tables 3 and 4, Supplementary Figure S1b). More than 30% of these individuals were assigned with high confidence to the Big Creek above-falls (BA) population by GeneClass2 (Table 3), and this proportion increases to ~50% in the section of stream immediately below the falls. Length data for these fish also showed distinct size frequency distributions for individuals assigned to the Weir and BA populations (Figure 3) and a highly significant differences in mean and variance (84 mm, $\sigma^2 = 610$ and 125 mm, $\sigma^2 = 1681$, respectively, $P < 0.0001$). Evaluation of genetic ancestry using STRUCTURE showed that the JBC population contains a mixture of individuals with relatively pure genotypes (high Q_{MAX}; Table 4, Supplementary Figure S1b). In contrast, the other 2 below-barrier juvenile/resident

samples (JMC and JSC) contained almost exclusively anadromous genotypes (with the exception of 4 fish in JMC and one fish in JSC descended from MA and SA, respectively; Supplementary Figure S1b). Similarly, several individuals in the BA population have relatively high Q values from the upstream Boyer Creek population (Supplementary Figure S1a), although only 2 were assigned to Boyer by GeneClass2 with high confidence (Table 3).

Mixture Analysis of Fish below Big Creek Falls

All independent runs of NewHybrids converged and produced identical results. Only one individual in the 131 individuals analyzed from the JBC sample had a posterior probability >10% of being an F1 hybrid. This individual also had a posterior probability of 88.6% of being in one of the nonpure hybrid categories (F1, F2, BxWeir, BxA). Several anadromous adults from the Weir reference population, when considered as part of the mixture sample, had similarly high posterior probabilities of hybrid ancestry. Based on these results, F1 and other nonpure hybrid classes appear to be rare in this sample. However, analysis of simulated data sets confirmed that although simulated F1 individuals had higher average posterior probabilities of hybrid ancestry than individuals from the BA, Weir, and JBC samples, there was a broad range of posterior probabilities for the simulated F1s, with P(nonpure) ranging from 0.003 to 0.97 among simulated F1 hybrids. This pattern indicates that these genetic markers provide only moderate power to identify hybrids in this system due to the low genetic differentiation between the BA and Weir parental populations. Nonetheless, because the Bayesian algorithm used by NewHybrids is influenced by the inferred frequency of each genotypic class, the
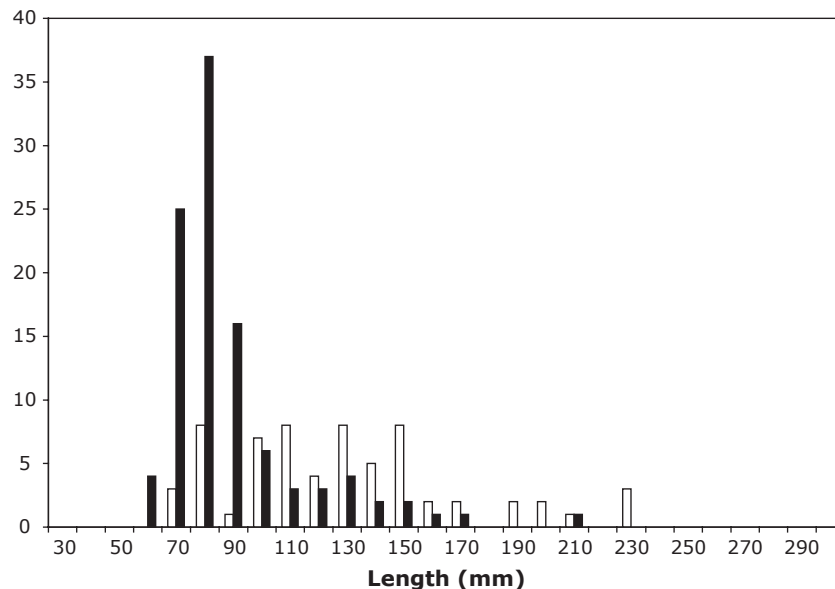
Figure 3. Size–frequency histogram of JBC juveniles sampled near the base of Big Creek Falls and genetically assigned to either the Weir population (black bars) or the BA population (open bars).

posterior probabilities assigned to hybrids are dependent on their frequency in the sample. In other words, the power to detect a signal of hybridization increases with the number of real or simulated F1s sampled. For example, the addition of 20 simulated F1s to the JBC sample led to a stronger signal of hybridization than seen with the observed data in 97.5% of simulated runs, indicating high power to detect F1 hybrids if they are common, but this power drops to 68% if hybrids are relatively rare in the sample ($n \leq 10$, ~7%). Thus, these data support the hypothesis that the resident and anadromous populations below Big Creek Falls are substantially reproductively isolated and that F1 hybrids are rare.

Discussion

Organisms introduced into novel habitats may undergo adaptive evolution to the environmental conditions encountered. Rapid heritable evolution of important life-history traits has been documented in the adaptation of invasive species after introduction into novel habitats (e.g., Phillips et al. 2006) and of fish species in response to human-induced changes in selection (fishing) pressure (Heath et al. 2003; Olsen et al. 2005; Thorpe 2007). Anadromous fish transplanted above impassable barriers will be subjected to strong natural selection to not go over the falls or undertake an anadromous migration and instead to adopt a resident life-history strategy. Such strong selection in natural settings is capable of driving both adaptive divergence and reproductive isolation in a small number of generations (Barton 2000; Reznick and Ghalambor 2005). On the other hand, antagonistic pleiotropy due to counterbalancing selection on correlated traits may maintain migratory behavior despite selection against it (Thrower, Hard, and Joyce 2004).

In the present study, patterns of genetic divergence, phylogeographic analysis, and landowner journal entries indicate that the population of *O. mykiss* above Big Creek Falls evolved in situ from anadromous individuals anthropogenically transplanted from the lower Scott Creek basin in the last century. Allelic richness in the BA population is reduced relative to the anadromous population, but a previous simulation study has shown that the initial founding event must have included a substantial number of individuals to account for the current genetic variation in this above-barrier population (Anderson and Slatkin 2007). Thus, a large and genetically diverse population of resident trout has been established in this isolated above-barrier habitat for approximately 100 years. Few if any resident-genotype fish from this population appear to successfully migrate to sea and return to Scott Creek as anadromous adults, consistent with Thrower and Hard's (2009) demonstration of greatly reduced marine survival by resident-genotype fish when compared with their anadromous counterparts and suggesting that this ability may be rapidly lost in resident populations. Furthermore, the phenotypic development of migratory smolts is greatly reduced in the

above-barrier population relative to genetically anadromous below-barrier fish raised in a common hatchery environment (unpublished data), suggesting a heritable genetic basis for these differences. Resident trout present above the other barrier falls in the Scott Creek watershed may have undergone similar adaptations, but the smaller sample sizes available from these populations and suspected stocking with divergent hatchery trout strains makes inference of resident ecotype evolution difficult.

Our data also show that ongoing downstream movement of fish over the falls has resulted in the establishment of a genetically differentiated below-barrier resident population in Big Creek. However, the distribution of resident-genotype fish below the barrier is extremely limited, and only a few individuals with an above-falls genotype were found more than 1 km below the base of the falls, indicating that this population maintains a predominantly resident phenotype. This interpretation is also supported by the length frequency data (Figure 3), which found that resident-genotype fish in this section of creek were much larger than anadromous-genotype individuals.

Multiple lines of evidence indicate that extensive introgression has not occurred between the resident and anadromous fish in the JBC population of mixed ancestry below Big Creek Falls. The observed heterozygote deficit and linkage disequilibrium (Wahlund effect; Wahlund 1928) suggest that these resident trout remain largely reproductively isolated from the sympatric anadromous steelhead with which they share recent common ancestors. This conclusion is also supported by individual assignment analyses, in which almost 90% of the JBC individuals were assigned with high confidence to either the BA or Weir reference populations (Table 3), and by the analysis using the program STRUCTURE in which the distribution of Q_{MAX} in the mixed JBC sample is similar to the parental BA and Weir populations (Table 4, Supplementary Figure S1a,b). Simulation results from NewHybrids also showed that although most parental population individuals had a relatively low probability of being not pure, simulated F1 individuals were frequently assigned as not pure with high probability. Although such classifications are subject to a high variance unless a large number of loci are used (Vähä and Primmer 2006), together these results indicate that extensive introgression has not occurred between resident and anadromous fish in the population below Big Creek Falls.

Reproductive isolation between sympatric resident and anadromous individuals may be maintained by differences in the timing of reproduction, by mate choice preferences, by hybrid inferiority and reinforcement, or by some combination of these factors (Hendry et al. 2000; Kirkpatrick 2001). Reproductive patterns of Scott Creek resident trout have not been well documented, and the timing of spawning is not known. In addition, it is important to note that residual fish (phenotypically resident fish of immediate anadromous ancestry; Willson 1997), may exist in this same habitat (Pettersson et al. 2001). If present, such residual males could provide a conduit between the resident and anadromous

genetic populations by hybridizing with resident females. Conversely, small resident males or precocious parr (Avise et al. 2002) are often seen attempting to gain fertilizations when an anadromous pair is spawning (unpublished data). Nonetheless, the present genetic analysis suggests that neither this "sneaker" strategy nor the residual male × resident female hybridization is often successful in this population.

In contrast to the high genetic differentiation and phylogenetic distinctiveness of the above-barrier populations with suspected ancestry from hatchery trout (MA and Boy), the genetic similarity between the population above Big Creek Falls and the anadromous fish below supports the hypothesis that this population was recently derived primarily or entirely from fish transported from below the barrier falls, and that it has not been strongly influenced by stocking of out-of-basin hatchery rainbow trout. In particular, the pairwise F_{ST} value between the Weir and BA populations (0.018) is at the low end of observed within-basin values for these loci, regardless of the presence of barriers (Garza et al. 2004; Clemento et al. 2008), strongly supporting a recent anthropogenic origin of the BA population. Nonetheless, although unlikely, it is possible that resident *O. mykiss* were endemic above Big Creek Falls before their anthropogenic introduction in the early 1900s, and also that a genetically divergent below-barrier resident population of *O. mykiss* existed before the formation of the allopatric above-barrier population. Similarly, due to the confounding effects of drift and human-mediated transport, the microsatellite allele frequency data cannot definitively confirm or refute the influence of vicariant or nonnative genotypes on isolated above-barrier populations (Deiner et al. 2007). Future work will include an attempt to develop more powerful genetic markers for the identification of hatchery ancestry. For example, haplotypes composed of multiple single nucleotide polymorphisms have proved useful in humans and other species for evaluation of individual ancestry and phylogenetic history (e.g., Stephens et al. 2001).

We have shown that the rainbow trout population above Big Creek Falls was derived recently from the anadromous population below the barrier and that fish in all above-barrier populations in the basin show a strong tendency toward a resident life-history strategy. Above these waterfall barriers, this tendency is presumably continuously reinforced by strong purifying selection against downstream migration. Rapid adaptation of ancestrally anadromous *O. mykiss* to the resident phenotype has been documented in *O. mykiss* in similar situations (Thrower, Guthrie, et al. 2004), but the evolutionary consequences of this selection regime and the repeatability of the response will depend on the underlying genetic architecture influencing the associated phenotype (Thrower, Hard, and Joyce 2004; Reznick and Ghalambor 2005; Thériault, Grant, et al. 2007). If resident/anadromous phenotypic differentiation is primarily a plastic trait, the threshold for expression of plasticity may itself be selected (Sultan and Spencer 2002; Aubin-Horth and Dodson 2004). In this case, the ability to exhibit the anadromous phenotype may be maintained in the above-barrier populations but with an altered threshold for expression. Alternatively, if the resident/anadromous life-history strategy is largely determined by quantitative trait loci, selection against downstream migration will favor the removal of genetic variation associated with smoltification and anadromy from above-barrier populations, reinforcing the divergence between these life-history types.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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