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[Article]

Evidence of Panmixia between Sympatric Life History Forms of Coastal Cutthroat Trout in Two Lower Columbia River Tributaries

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Abstract.—Coastal cutthroat trout *Oncorhynchus clarkii clarkii* exhibit resident and migratory life history strategies that often occur sympatrically, but the relationship between these forms within a population is poorly characterized. Through use of passive integrated transponder technology, migratory and resident coastal cutthroat trout were identified in two lower Columbia River tributaries (Abernathy Creek and the Chinook River) separated by more than 80 km. Genetic data from 17 highly variable microsatellite loci were used to ascertain the genetic population structure of these life history forms within and between streams. No distinct genetic separation was observed between the life history forms within a stream, as assessed by four different statistical approaches: permutation tests based on the genetic differentiation index F_{ST}, principal components analysis of individuals, analysis of molecular variance, and contingency tests of allele frequency heterogeneity. Genetic differences were an order of magnitude higher between stream samples ($F_{ST} > 0.03$) than between life history forms within a stream ($F_{ST} < 0.003$). The contingency test detected allele frequency differences between migratory and resident life history forms in Abernathy Creek ($P = 0.001$), but this result was influenced more by age-class structure than by reproductive isolation between life history forms. Results are consistent with a single, randomly mating population in each stream producing both migratory and resident life history forms. These data suggest that individual life history strategy in coastal cutthroat trout is predominantly determined by phenotypic plasticity rather than genotype.

There is clear evidence that populations of migratory or "sea-run" coastal cutthroat trout *Oncorhynchus clarkii clarkii* in the lower Columbia River have declined over recent decades (Hooton 1997; Williams and Nehlsen 1997; Johnson et al. 1999). This decline led to the proposed listing in 1999 of the southwest Washington/Columbia River distinct population segment of coastal cutthroat trout as "threatened" under the U.S. Endangered Species Act (NOAA and USFWS 1999). This proposed listing was subsequently withdrawn by the U.S. Fish and Wildlife Service (USFWS 2002) because of the relatively healthy total population size (all forms combined) and information suggesting the ability of above-barrier adults to produce anadromous progeny.

The life history of coastal cutthroat trout is complex. Sympatric individuals within a given watershed may

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exhibit migratory or resident life history behavior (Hall et al. 1997). Individuals exhibiting the resident life history form remain in natal streams through adulthood. Alternatively, migratory forms leave natal streams and may remain in freshwater (fluvial or adfluvial) or enter seawater (anadromous). Anadromous individuals migrate at age 2 or 3 in a protracted spring migration (Johnston 1982; Trotter 1989; Zydlewski et al. 2009). Lack of clear morphological distinctions between migratory and resident forms (Tomasson 1978; Fuss 1982) precludes field identification and raises questions about their evolutionary relationship within and among streams. This uncertainty complicates conservation and management efforts.

Genetic and behavior studies have documented fine-scale spatial structuring of coastal cutthroat trout populations among streams (Campton and Utter 1987; Wenberg and Bentzen 2001). However, the degree of reproductive isolation between migratory and resident life history forms that occur sympatrically is poorly understood. For example, differences in spawning behavior and timing of gonad maturation are two possible mechanisms that could limit gene flow between the life history forms (McMillan et al. 2007). Data from selectively neutral genetic markers, particularly microsatellite loci, provide valuable information for testing questions regarding population groupings (Waples and Gaggiotti 2006).

Studies of many species of salmonids have shown that expression of life history is influenced by genetic and environmental factors (Palm and Ryman 1999; Avise et al. 2002). However, the degree of reproductive isolation between the forms varies among species and among populations within a species (Hendry et al. 2004). For example, clear genetic differences between migratory and resident forms of sockeye salmon *O. nerka*, kokanee (lacustrine sockeye salmon; Wood 1995), and Arctic char *Salvelinus alpinus* (Jonsson and Jonsson 2001) have been found. Other studies have documented panmixia between sympatric anadromous and resident forms of brown trout *Salmo trutta* (Charles et al. 2006) and brook trout *Salvelinus fontinalis* (Theriault et al. 2007).

The goal of this study was to determine whether sympatric life history forms of coastal cutthroat trout show evidence of reproductive isolation. The study design tested for panmixia between resident and migratory forms within and between two Columbia River tributaries. Life history form was defined by using passive integrated transponder (PIT) technology to characterize individual behavioral patterns. A series of molecular markers was then used to determine whether these two life history forms could be differentiated genetically. This approach has been effectively carried out with brook trout (Boula et al. 2004), bull trout *Salvelinus confluentus* (Homel et al. 2008), and rainbow trout *O. mykiss* (Narum et al. 2004). The results were used to evaluate evolutionary mechanisms that could have resulted in the co-occurrence of coastal cutthroat trout life history forms in Columbia River tributaries. A better understanding of the evolutionary processes that have produced these two life history forms should provide valuable insights into the behavior and conservation of this diverse species.

Study Site

Coastal cutthroat trout in this study were from two tributaries of the lower Columbia River: Abernathy Creek and the Chinook River, Washington (Figure 1). Abernathy Creek is a third-order tributary with a length of approximately 17 km. The stream has a moderately high gradient, is dominated by pools and riffles, and enters the Columbia River at river kilometer (rkm) 87 (rkm 0 = mouth of the Columbia River). Although the lowermost portion (~150 m) of Abernathy Creek is tidally influenced, salinity influx is negligible. The Chinook River is a second-order tributary that is approximately 10 km in length. It is a low-gradient stream that experiences tidal influence several kilometers from its confluence with the Columbia River (rkm 6). Above tidal influence, the Chinook River is dominated by long pools with slow-moving water. The upper reaches are heavily impacted by the activity of North American beavers *Castor canadensis*. Tidal effects are moderated from historic fluctuations by the presence of tide gates at the stream mouth. Coastal cutthroat trout populations from both streams have been found to produce migratory and resident individuals, although in different proportions (Zydlewski et al. 2009).

Methods

Passive integrated transponder tagging.—From 2002 to 2003, coastal cutthroat trout were collected and tagged from Abernathy Creek ($n = 1{,}027$) and the Chinook River ($n = 754$) as described by Zydlewski et al. (2009). Briefly, coastal cutthroat trout were collected by backpack electrofishing between September 9 and October 9, 2002, and between August 26 and October 1, 2003. Samples were collected throughout the upper 10.5 km of Abernathy Creek (rkm 6.2–16.7) and the upper 3.5 km of the Chinook River (rkm 6.0–9.5). All holding habitat types (i.e., plunge pools, boulder pockets, eddies, undercut banks, and large woody debris) were sampled throughout the reaches. Captured coastal cutthroat trout were anesthetized with

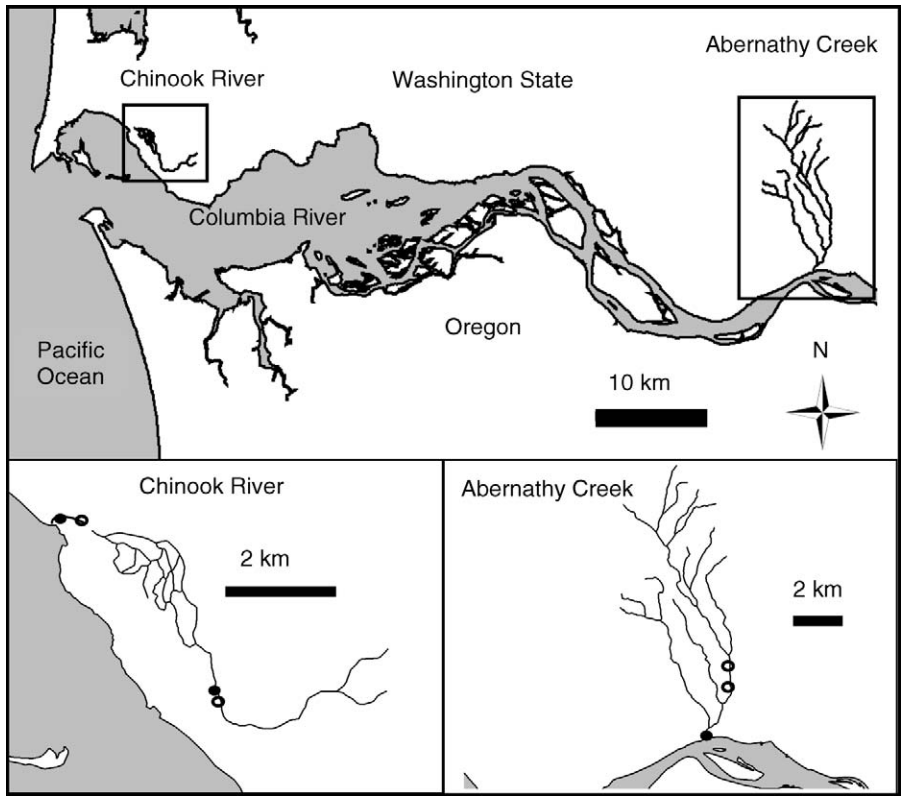

FIGURE 1.—Map of the study area in the lower Columbia River, showing the locations of Abernathy Creek (rkm 87; rkm 0 = mouth of the Columbia River) and the Chinook River (rkm 6; insets), Washington. Solid circles represent screw trap locations; open circles represent passive integrated transponder antenna array locations.

clove oil (25 mg/L) and measured for mass and fork length. Fish larger than 10 cm were tagged with a PIT tag (23 mm long, 3.84 mm in diameter, 0.6 g, 134.2 kHz, full duplex; Destron Fearing). A scale sample was taken from the left side of the fish just posterior to the distal insertion of the dorsal fin and dorsal to the lateral line. At the time of sampling, scales were placed onto adhesive scale cards for future age determination. A genetic sample was taken from each fish by clipping a piece of the ventral fin and placing it into a vial with 100% ethanol for storage. Sampled fish were allowed to recover for a minimum of 10 min and were released within 100 m of the capture location.

Stream width PIT tag interrogation.—Stream width PIT tag interrogation arrays were used to monitor downstream movement of PIT-tagged coastal cutthroat trout in both streams (Zydlewski et al. 2006, 2009) during September 2002 through June 2005. The stationary detection system monitors the entire width and depth of a stream for PIT-tagged fish, even under high water conditions, providing year-round monitoring past a single point in a stream without obstructing the path of the fish. Two antenna arrays were operated in Abernathy Creek (at rkm 3 and 4). Read efficiencies were 83–97% during the period of study (Zydlewski et al. 2006, 2009). Two interrogation arrays were also used in the Chinook River (at rkm 0 and 6). The lower array (rkm 0), which was installed at the tide gates near the mouth of the Chinook River, had a read efficiency ranging from 10% to 99%. The lower read efficiency at this array was primarily due to changing salinity (from 0 to 17‰). The upper array (rkm 6) had a read efficiency of 100% for two antennas in series.

Screw trap.—Screw traps were used to collect downstream-moving, PIT-tagged coastal cutthroat trout. Screw traps were operated at or near the mouths of both Abernathy Creek (rkm 0.5) and the Chinook River (rkm 0). Traps on Abernathy Creek were operated by the Washington Department of Fish and Wildlife from approximately April 1 to June 30, 2003–2005; traps on the Chinook River were operated by Sea Resources, Inc. (Chinook, Washington), almost continuously during 2003–2005. Fish were removed from the traps daily. All recaptured individuals were

interrogated for PIT tags and handled as described above. Screw trap data were used only to help define migratory and resident fish.

Recapture of resident individuals.—Resident individuals were recaptured by backpack electrofishing between August 26 and October 1, 2003, and between September 21 and October 7, 2004, in the same areas described above for tagging. Captured coastal cutthroat trout were anesthetized with 25-mg/L clove oil, measured for mass and length, and examined for presence of a PIT tag. The PIT tag identification code was documented from those individuals that had been previously tagged. Scales were collected from recaptured fish (as described above but from the right side) to provide a second opportunity to determine age. Fish were allowed to recover for a minimum of 10 min and were released within 100 m of the capture location.

Age determination.—Scales were pressed onto acetate and read by using a microfiche projector. Age was judged by two independent readers for verification; if the two estimates differed, a third person read the scale. If the two readers reached consensus about fish age, that age value was used. If agreement among readers was not achieved, no age was recorded for that fish. Age could not be determined for all of the samples because many scales were unreadable or regenerative, particularly scales from larger fish (as described by Cooper 1970). To reduce the potential effect of temporal changes in allele frequencies, only the fish assigned to brood years 2001 and 2002 were used in the genetic analysis.

Definition of "*migratory*" *and* "*resident*".—Migratory and resident fish were defined according to the criteria of Zydlewski et al. (2009). Individuals were defined as migratory if, subsequent to tagging, they were detected at the lowermost antenna array or screw trap. If there was no evidence of migration, then individuals were classified as resident if they were recaptured by electrofishing at age 2 or 3.

Molecular analysis.—Genomic DNA was extracted from fin tissue in a Chelex 100 (Sigma Chemical Co., St. Louis, Missouri) resin solution as described by Miller and Kapuscinski (1996). All individuals were then genotyped at 19 microsatellite loci: *Och13*, *Och15*, *Och16*, and *Och17* (Peacock et al. 2004); *Och18* (GenBank accession number DQ979814), *Och20* (DQ979815), *Och24* (DQ979818), *Och27* (DQ979819), *Och30* (DQ979822), and *Och35* (DQ979826); *One13* (Scribner et al. 1996); *OtsG253*, *OtsG401*, and *OtsG85* (Williamson et al. 2002); *Omm1220* and *Omm1231* (Rexroad and Palti 2003); *Omy1001UW* and *Omy1011UW* (Spies et al. 2005); and *Ssa407* (Cairney et al. 2000).

Polymerase chain reaction (PCR) was conducted in 15-μL volumes containing 1× polymerase buffer (10-mM Tris HCl, 50-mM KCl, 1% Triton X-100), 1.5–2.5-mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 0.5 μM of each primer, and 0.5 units of *Taq* DNA polymerase (enzyme code 2.7.7.7; IUBMB 1992). All PCRs were run at a $MgCl_2$ concentration of 2.0 mM, except for the *Och* loci (amplified at 2.5 mM) and *Omy1011*, *Omy1001*, and *Ssa407* (amplified at 1.5 mM). Temperature profiles consisted of an initial denaturing at 94°C for 30 s and then 38 cycles through the following steps: denaturing at 94°C for 30 s, annealing at 58°C for 30 s, and elongation at 72°C for 30 s; the final elongation step was extended to 8 min. The only exception to this profile was that an annealing temperature of 52°C was used for *Omy1001*. After PCR, samples were pooled for electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). Automated electrophoresis was carried out using the G5 filter set according to the manufacturer's protocols. GeneScan and Genotyper software (Applied Biosystems, Inc.) were used to determine the multilocus genotype of each fish.

Steelhead (anadromous rainbow trout) are found in both streams, creating the possibility of hybridization with coastal cutthroat trout. However, juvenile steelhead, coastal cutthroat trout, and steelhead × coastal cutthroat trout hybrids cannot be accurately identified on the basis of phenotypic information alone (Baumsteiger et al. 2005). We used the program NEWHYBRIDS to implement the Bayesian methods of Anderson and Thompson (2002) to search for steelhead or hybrids in our collection of PIT-tagged coastal cutthroat trout from Abernathy Creek and the Chinook River. The Anderson and Thompson (2002) method uses information from multilocus genotypes observed within and among fish to construct groups that maximize Hardy–Weinberg equilibrium (HWE) expectations and linkage equilibrium within groups. These genetic groups represent "purebred" steelhead and coastal cutthroat trout; individuals that are intermediate to these groups are identified as hybrids. Samples from each stream were analyzed separately. A posterior probability of more than 95% was set as the threshold for assigning a fish to the purebred coastal cutthroat trout genetic group. All fish that did not meet the coastal cutthroat trout NEWHYBRIDS threshold were eliminated from further analysis.

Statistical analysis.—Average population gene diversities (expected heterozygosity H_E; Nei 1987) and allelic richness for each locus were estimated using the program HP-Rare 1.0 (Kalinowski 2005). Allelic richness was estimated by using a rarefaction procedure to account for unequal sample sizes (Kalinowski 2004). Conformance of genotypic frequencies to HWE

was evaluated by using the methods of Guo and Thompson (1992) via the program GENEPOP (Raymond and Rousset 1995). Pairwise estimates of the genetic differentiation index F_{ST} (Weir and Cockerham 1984) and associated 95% confidence intervals generated by bootstrap sampling over all loci (Goudet et al. 1996) were calculated with the program FSTAT (Goudet 1995). Statistical significance levels for conformity to HWE and pairwise comparisons of F_{ST} were adjusted for the number of simultaneous tests by using the sequential Bonferroni correction (Rice 1989).

To determine whether partitioning of individuals into a priori migratory and resident groups was a confounding factor, we conducted a principal components analysis (PCA) of all fish based on their multilocus genotypes over all loci. The PCA plots individuals solely on the basis of the alleles each fish possesses; it does not use PIT-tag-based life history form classification or population information. A PCA plot therefore provides a multivariate representation of the genetic relationships among individuals, life history forms, and the two geographic locations examined in this study, and this representation is not influenced by life history form or population assignment. Raw data for PCA consisted of a table in which columns represented all alleles observed in the study and each row contained the observed allele states for a single fish. Allelic states for each fish were 0 if an allele was not observed, 1 if it was present on a single chromosome, and 2 if two copies were present in the homozygous state. The PRINCOMP procedure in the Statistical Analysis System version 8.1.2 (SAS Institute, Inc., Cary, North Carolina) was used to obtain the values for the first, second, and third principal components (PC1, PC2, and PC3) of the variance–correlation matrix of allele observations for all fish.

A hierarchical analysis of molecular variance (AMOVA) was used to quantify the proportion of genetic variation explained by life history form classification and by temporal changes in allele frequencies caused by sampling two brood years (2001 and 2002). An AMOVA was also used to statistically test for genetic differentiation between all coastal cutthroat trout in Abernathy Creek and the Chinook River, between migratory and resident samples within each stream, and among individuals. Hierarchical genetic structuring was analyzed with the program Arlequin 3.1 (Excoffier and Schneider 2005) by assessing the relative proportion of genetic variation explained by differences observed (1) between fish sampled from Abernathy Creek and the Chinook River; (2) between migratory and resident forms within a stream; (3) between brood years within each life history form; (4) among individuals within each life history form; and (5) among individuals within the 2001 and 2002 brood years. Negative variance components, which can sometimes occur, indicate a lack of genetic structure. Each AMOVA was carried out based on differences in allelic identity (i.e., F_{ST}), and significance values for different hierarchical levels were tested with 20,000 permutations.

To test for panmixia between any pair of samples, contingency tests of allele frequency heterogeneity were applied. Contingency tests were conducted by using the methods of Raymond and Rousset (1995) as implemented in the program GENEPOP. For each locus, the probability that the observed allele frequencies were drawn from the same population was estimated by using Markov-chain Monte Carlo methods. To provide an unbiased estimate of the probability for each randomization test, 10 batches of 10,000 replicates each were run, with 1,000 dememorization steps. For each comparison, Fisher's combined probability test (χ^2_F) was applied as a composite test over all loci. For all analyses, significance was judged at an α value of 0.05.

Results

Migrants and Residents

Between 2003 and 2005, 106 PIT-tagged coastal cutthroat trout were detected as migrating from Abernathy Creek and 330 were detected as migrating from the Chinook River. None of these fish were detected as returning within the same season. During 2003 and 2004, 93 resident fish were recaptured via electrofishing in Abernathy Creek and 39 were recaptured in the Chinook River. From this pool of fish with known histories, individuals from brood years 2001 and 2002 (as determined through scale analysis) were selected for the initial genetic analysis of each life history form in each stream. Forty-seven individuals representing the resident form and 47 individuals representing the migratory form were analyzed from Abernathy Creek, and 25 resident fish and 60 migratory fish were analyzed from the Chinook River.

Preliminary Molecular Analysis

Ninety-one percent of fish visually identified as coastal cutthroat trout when captured for PIT tagging were also identified as coastal cutthroat trout on the basis of NEWHYBRID results; 83 of 94 fish from Abernathy Creek and 80 of 85 fish from the Chinook River had a greater than 95% posterior probability of being purebred coastal cutthroat trout. The remaining 16 fish, for which posterior probabilities of being purebred coastal cutthroat trout were less than 95%, were excluded from further analysis.

Four preliminary sample groups were designated on

TABLE 1.—Genetic diversity observed at 17 microsatellite loci in resident and migratory coastal cutthroat trout of two brood years sampled from two lower Columbia River tributaries. Summary statistics are also provided for each life history form over both brood years. Sample size (N), observed heterozygosity (H_O), expected heterozygosity (H_E), average number of alleles observed at a locus (A), and a rarefaction measure of allelic richness using a sample of 44 genes (A^{44}) are reported. Number of unique alleles among the 257 alleles observed in this study are reported for each life history form within each stream. Average frequency of unique alleles observed at a locus is also reported.

Site	Life history form	Brood year	N	H_O	H_E	A	A^{44}	Unique alleles	
								Number	Average frequency
Abernathy Creek	Migratory	2001	26	0.78	0.81	11.06	—	—	—
		2002	10	0.80	0.80	6.71	—	—	—
		2001–2002	36	0.79	0.81	11.53	10.09	15	0.03
	Resident	2001	27	0.77	0.80	10.12	—	—	—
		2002	20	0.76	0.79	8.47	—	—	—
		2001–2002	47	0.77	0.79	11.35	9.53	19	0.02
Chinook River	Migratory	2001	27	0.79	0.79	9.35	—	—	—
		2002	30	0.76	0.77	9.35	—	—	—
		2001–2002	57	0.78	0.78	10.71	8.91	11	0.02
	Resident	2001	9	0.68	0.76	6.53	—	—	—
		2002	14	0.74	0.76	7.88	—	—	—
		2001–2002	23	0.72	0.76	8.53	8.45	2	0.02

the basis of source and life history designation (Table 1): Abernathy Creek migratory (AM), Abernathy Creek resident (AR), Chinook River migratory (CM), and Chinook River resident (CR). Seventeen of the 19 loci conformed to HWE expectations across all four groupings. Loci *Och13* and *Och18* both deviated from HWE in all groups (i.e., had lower-than-expected heterozygosity levels) and were excluded from further analysis.

High levels of allelic richness at the remaining 17 microsatellite loci were observed, averaging 15.11 alleles/locus (range = 8–38 alleles/locus). For each of the groups, the following levels of genetic variation were observed: AM had an H_E value of 0.81 and an A^{44} value (rarefaction measure of allelic richness using a sample of 44 genes) of 10.09; AR had an H_E value of 0.79 and an A^{44} value of 9.53; CM had an H_E of 0.78 and an A^{44} of 8.91; and CR had an H_E of 0.76 and an A^{44} of 8.45 (Table 1). Coastal cutthroat trout sampled from Abernathy Creek had the largest number of unique alleles (AM = 15; AR = 19), although the average frequency of these alleles was low (<0.03; Table 1).

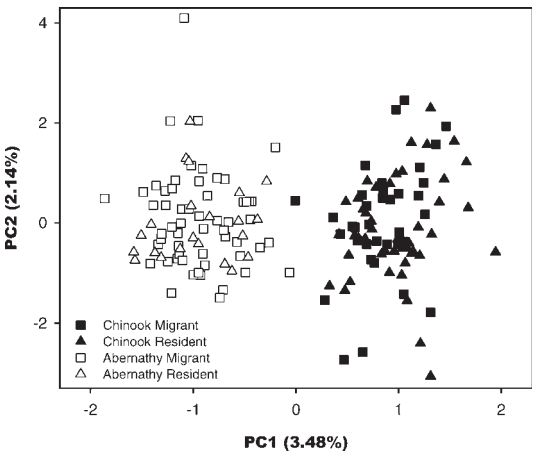

FIGURE 2.—Principal components analysis plot of individual coastal cutthroat trout from two lower Columbia River tributaries based on data from 17 microsatellite loci (squares = fish with a migratory life history; triangles = fish with a resident life history; shaded symbols = Chinook River fish; open symbols = Abernathy Creek fish). The proportion of variation attributable to each principal component (PC) is shown in parentheses.

Comparisons between Streams, Brood Years, and Life History Forms

In the PCA plot, Abernathy Creek and the Chinook River formed distinct clusters, whereas no distinction was observed between the migratory and resident life history forms within or between drainages (Figure 2). Principal component 1 clearly distinguished fish from Abernathy Creek and the Chinook River, but PC2 and PC3 did not reveal any clusters. Principal components 1 and 2 accounted for 5.6% of the total variance. Results of the AMOVA also demonstrated genetic distinction between Abernathy Creek and Chinook River coastal cutthroat trout (3.9% variation; $P < 0.001$); however, no genetic distinction was observed between the brood years or life history forms within each stream (Table 2). In fact, grouping by life history form explained the smallest amount of genetic variation in each stream. In all cases, more than 90% of the variation occurred among individuals within samples.

TABLE 2.—Hierarchical analysis of molecular variance (AMOVA) for microsatellite loci diversity observed in migratory and resident forms of coastal cutthroat trout from two lower Columbia River tributary streams (p = probability of having a more extreme variance component and Φ-statistic than the observed values by chance alone).

Variance component	Observed partition: Variance[a]	Observed partition: % total	p	Φ-statistic[a]
		Abernathy Creek		
Among forms	$\sigma_a^2 = 0.003$	0.043	0.255	$\Phi_{CT} = 0.001$
Among brood years by forms	$\sigma_b^2 = 0.037$	0.541	0.056	$\Phi_{SC} = 0.005$
Within brood years	$\sigma_c^2 = 6.793$	99.417	0.017	$\Phi_{ST} = 0.006$
		Chinook River		
Among forms	$\sigma_a^2 = -0.013$	−0.201	0.822	$\Phi_{CT} = -0.002$
Among brood years by forms	$\sigma_b^2 = 0.027$	0.405	0.126	$\Phi_{SC} = 0.004$
Within brood years	$\sigma_c^2 = 6.593$	99.796	0.177	$\Phi_{ST} = 0.002$
		Both streams		
Among streams	$\sigma_a^2 = 0.275$	3.930	<0.001	$\Phi_{CT} = 0.039$
Among forms[b] by stream	$\sigma_b^2 = 0.013$	0.184	0.147	$\Phi_{SC} = 0.002$
Within forms[b]	$\sigma_c^2 = 6.710$	95.890	<0.001	$\Phi_{ST} = 0.041$

[a] Φ and σ^2 were tested by AMOVA under random permutations of individuals using (1) brood years across forms (grouped by stream) or across streams for Φ_{CT} and σ_a^2, (2) brood years (grouped by form and stream) or forms (grouped by stream) for Φ_{SC} and σ_b^2; or (3) across brood years without regard to original brood year and form (grouped by stream) or across forms (without regard to their original form or stream) for Φ_{ST} and σ_c^2.

[b] Samples from the 2001 and 2002 brood years were grouped by life history form for each stream.

We observed F_{ST} values greater than zero only when life history forms were compared between streams (range = 0.035–0.045; Table 3). All comparisons between life history forms within each stream were not different from zero (point estimates were 0.001–0.003), indicating no evidence of genetic distinction at this level (Table 3). Allele frequencies between resident and migratory life history forms were not different at 13 of the 17 loci for Abernathy Creek and 15 of the 17 loci for the Chinook River (Figure 3). In comparison, allele frequencies were different at a minimum 15 of 17 loci for all pairwise comparisons of life history form groupings among streams (Table 3).

TABLE 3.—Test results for the genetic differentiation index (F_{ST}) calculated between paired samples of migratory (M) and resident (R) coastal cutthroat trout from Abernathy Creek (A) and the Chinook River (C), Washington. Above the diagonal are pairwise F_{ST} values; below the diagonal are the P-values from contingency tests of allele frequency heterogeneity (Fisher's combined probability test). All tests were based on data from 17 microsatellite loci. Asterisks indicate F_{ST} values that were significantly different from zero (at $P < 0.001$).

Group	AM	AR	CM	CR
AM	—	0.003	0.035*	0.036*
AR	0.001	—	0.045*	0.045*
CM	<0.001	<0.001	—	<0.001
CR	<0.001	<0.001	0.412	—

Discussion

We observed genetic distinction between coastal cutthroat trout sampled from two different streams but not between life history forms within each stream. Our results are consistent with each stream containing a single randomly mating coastal cutthroat trout population that produces both migratory and resident life history forms. Differences between coastal cutthroat trout from Abernathy Creek and the Chinook River (more than 80 km apart) are not surprising. Previous

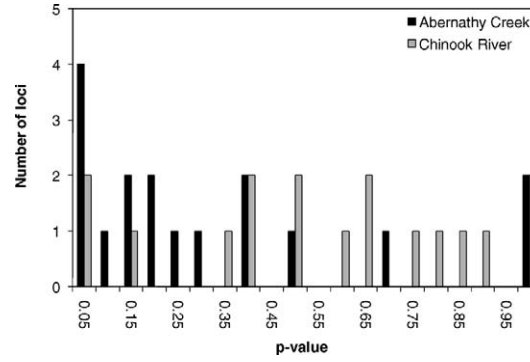

FIGURE 3.—Distribution of P-values from multiple tests of the null hypothesis of panmixia between migratory and resident forms of coastal cutthroat trout sampled from two Columbia River tributaries. Observed P-values were obtained via independent tests of allele frequencies at 17 microsatellite loci within each population.

studies have established a positive correlation between genetic distance and geographic distance on several scales (Campton and Utter 1987; Wenburg et al. 1998; Johnson et al. 1999; Blakley et al. 2000). For example, the level of genetic differentiation observed between coastal cutthroat trout from these two Columbia River tributaries ($F_{ST} = 0.04$) was comparable with levels observed among nine sea-run migratory populations in Hood Canal (average $F_{ST} = 0.03$; range = 0.013–0.115) examined by Wenburg and Bentzen (2001).

Of the tests used in this study, contingency tests of allele frequency heterogeneity were the most powerful for detecting genetic differences among units as based on microsatellite data (Waples and Gaggiotti 2006). High levels of allelic diversity observed at the large number of loci examined in this study provided us with greater than 95% power ($P < 0.05$) for rejecting the hypothesis of panmixia using the contingency table test (Ryman et al. 2006). The results of this test support panmixia of life history forms within the Chinook River but not between the streams (Table 3). In addition, the overall contingency table test indicated allele frequency differences between the AM and AR groups ($P = 0.001$; Table 3). Small artifacts such as family or age-class structure could be driving the observed differences (Waples 1998). In our case, the AMOVA results indicated that 10 times more of the genetic variation was accounted for by brood year than by life history form (Table 2). Thus, genetic differences observed within Abernathy Creek were more likely driven by temporal variation in allele frequencies between the 2001 and 2002 brood years than by differences between the life history forms.

Further evidence for panmixia between resident and migratory forms within each drainage was observed in estimates of genetic diversity. Similar estimates of allelic diversity and heterozygosity observed between life history forms within a stream are consistent with the sampling of fish from the same population (Table 1). In addition, the absence of moderate- or high-frequency unique alleles between resident and migratory life history forms within each stream is also consistent with a lack of genetic distinction between the two forms (Table 1).

Evidence from this study allows the comparison of three different evolutionary mechanisms that could produce sympatric resident and migratory forms of coastal cutthroat trout in the Columbia River. Our results are consistent with the hypothesis that each stream contains a randomly mating coastal cutthroat trout population that produces migratory and resident life history forms via the process of phenotypic plasticity (Scheiner 1993). Clustering of fish by stream rather than by life history form in the PCA plot (Figure 2) does not support the idea that these sympatric forms represent sibling species that evolved allopatrically and later invaded Abernathy Creek and the Chinook River. Alternately, if parallel evolution of life history forms had occurred within Abernathy Creek and the Chinook River during the past 10,000 years, we would expect to observe smaller genetic distances between the life history forms within a location rather than between locations. Although this pattern of genetic structure was observed, the scenario is very unlikely. The final process of adaptive radiation requires reproductive isolation between the forms (Hendry et al. 2002). Our finding of a single randomly mating population producing both life history forms runs counter to the assumption that these life history forms should be considered separate evolutionary (or taxonomic) units.

Migratory plasticity is probably favored in coastal cutthroat trout because the freshwater, estuarine, and ocean environments they occupy are highly variable. The alternating selection patterns associated with these diverse and variable environments create a fitness advantage for plastic genotypes over nonplastic genotypes. The metapopulation structure of coastal cutthroat trout populations (Wenberg and Bentzen 2001) also favors plasticity over adaptive genetic differences among populations because migration among populations increases environmental heterogeneity and favors an increase in the reaction norm of traits (Sultan and Spencer 2002). Such plasticity has been observed in Arctic char (Nordeng 1983) and steelhead (Tipping and Byrne 1996), for which a reduction in food resources influences the proportion of migrants. Bioenergetic differences demonstrated between anadromous and resident juvenile brook trout (Morinville and Rasmussen 2003) may provide insight into the proximate mechanisms influencing life history variation. In the streams studied here, faster growth rate was associated with the life history expression and the timing of migration in coastal cutthroat trout (Zydlewski et al. 2009).

Sympatric migratory and resident forms of coastal cutthroat trout in the lower Columbia River may be best described as a continuum of life history types expressed from a single population. In a study of rainbow trout, McPhee et al. (2007) suggested that considering sympatric residents and migrants as separate "evolutionary units" may not be appropriate. This may apply equally well to coastal cutthroat trout. Although alternative life history forms of coastal cutthroat trout may arise from a single population, this should not exclude the need to conserve the conditions that allow all forms to be expressed. Work with bull trout has demonstrated that loss of life history types from a population results in higher probabilities of

extirpation (Dunham and Rieman 1999). Variation in life history forms probably affords resilience in the face of environmental fluctuations.

Although environmental parameters may influence migratory behavior in coastal cutthroat trout, genetic contributions cannot be completely ruled out. Recent studies with brook trout and rainbow trout have found that migratory behavior and morphology have substantial levels of phenotypic plasticity but also display substantial heritability that has the potential to respond to selection (Keeley et al. 2006; Theriault et al. 2007). Estimates of heritability would allow the quantification of genetic and environmental contributions to the determination of life history form. Approaches such as the use of PIT telemetry in this study offer opportunities to collect information and samples from individual fish and to assign behavioral attributes once they have been determined. Used in conjunction with genetic methods, these approaches will be useful in continued efforts to better understand life history determination in coastal cutthroat trout.

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