Transactions of the American Fisheries Society 140:829–842, 2011
American Fisheries Society 2011
ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2011.588094

SPECIAL SECTION: GENETIC ADAPTATION

A Microsatellite Genome Screen Identifies Chromosomal Regions under Differential Selection in Steelhead and Rainbow Trout

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Abstract

Novel genetic resources now make it possible to directly assess the effects of natural selection on specific regions of the salmonid genome. Natural selection on specific genetic loci will also affect the variation in linked flanking regions through "hitchhiking" effects, causing greater differentiation between populations than would be expected from purely neutral processes. Here we identify candidate genomic regions for the effects of differential natural selection in two closely related populations of steelhead–rainbow trout *Oncorhynchus mykiss* separated for the last century following anthropogenic introduction above a barrier waterfall in a coastal California stream. A set of 110 expressed sequence tag (EST)-linked and 188 anonymous microsatellite loci, most of which are mapped, were chosen to provide distributed coverage of all linkage groups in the *O. mykiss* genome. A genome screen was then performed to identify genomic regions under divergent selection. Multiple complementary statistical methods were used to detect outlier loci, and loci identified by more than one method were considered strong candidates for genomic regions affected by recent natural selection and genomic adaptation to the environment above the waterfall. We identified six strongly supported outlier loci, including one linked to an EST and two located in a genomic region that contains quantitative trait loci associated with egg development, spawning time, and other life history variation. Identification of regions in the genome that are potentially under selection provides valuable information about the genetic basis of selection in novel habitats and the genetic architecture of salmonid life history variation.

The life history variation of salmonid fishes has been the focus of many theoretical studies of heritable traits related to fitness (Hutchings and Morris 1985; Hutchings 1993; Thorpe et al. 1998). One strategy exhibited by many salmonid species is anadromy, in which juveniles are born and initially grow in freshwater before undergoing a suite of physiological changes (known as smoltification) that typically culminate in migration to the ocean. Anadromous fish then spend a few months to several years in the ocean, where they experience rapid growth, before returning to freshwater to spawn. An alternative life history strategy in some salmonid species is residency; fish born in rivers, streams, or lakes do not migrate to sea, but instead inhabit freshwater for their entire lives.

Numerous studies have examined the relationships among life history forms in salmonid species (e.g., brook trout *Salvelinus fontinalis*: Curry 2005; brown trout *Salmo trutta*: Charles et al. 2006). In the species *Oncorhynchus mykiss*, anadromous individuals are known as steelhead while residents are called rainbow (or redband) trout. A population may contain fish expressing both of these phenotypes, as well as a full range of intermediate life history strategies (Shapovalov and Taft 1954; Wilson 1997). In many cases, there is ongoing reproduction between individuals with different life history phenotypes, and their progeny may develop a range of strategies depending on their physiological responses to the environmental characteristics of the stream (e.g., Olsen et al. 2006). Conversely, when

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Received August 24, 2010; accepted November 3, 2010

there is allopatric separation between individuals with different phenotypes, such as that caused by a barrier dam or waterfall, differentiation can occur that reduces reproduction between the two groups if they come into secondary contact (Pearse et al. 2009).

Rapid adaptation in salmonid species following introduction into new habitats has been reported in previous studies (e.g., Hendry et al. 2000; Hendry 2001; Unwin et al. 2003). Populations derived from anadromous steelhead transplanted to above-barrier habitat will experience strong selection because only those offspring that adopt a completely resident life history will remain above the barrier. Thus, the strong selection imposed by this shift from anadromy to residency in the above-barrier population is expected to lead to rapid adaptation in specific genomic regions related to migratory behavior and the associated physiological changes. Nonetheless, anadromy may persist in the above-barrier population for many generations through phenotypic plasticity and negative genetic correlations with other traits (Thrower et al. 2004). In other fish species, translocation of wild fish to habitat above waterfall barriers has been shown to result in strong natural selection and consequent rapid adaptation (Reznick et al. 1990, 1997; Stockwell and Weeks 1999).

The identification of specific genetic responses to natural selection in different environments is of great interest for understanding population divergence and ultimately speciation. Heritable phenotypic variation is the basis for selection on introduced populations in novel habitats, and differential selection will affect genomic regions linked to specific adaptive traits (Cavalli-Sforza 1966; Storz 2005; Bonin et al. 2006). Methods for the identification of gene regions responsible for adaptation or speciation in novel habitats include the analysis of quantitative trait loci (QTL; Mauricio 2001; Slate 2005), admixture mapping (Rieseberg et al. 1999), and neutrality tests (Hudson et al. 1987; Tajima 1989; McDonald and Kreitman 1991; Fu 1996). These methods have generally been limited to model organisms with extensive genomic information and species with short generation times that can be bred and raised in controlled environments. However, in *O. mykiss*, several studies have used QTL mapping on established families or clonal lines to identify the genes underlying phenotypic variation in salmonid life history, including spawn timing, circadian/circannual rhythms (O'Malley et al. 2003; Leder et al. 2006), body weight (O'Malley et al. 2003), egg development rate (Nichols et al. 2007), early sexual maturation (Haidle et al. 2008), upper thermal tolerance (Jackson et al. 1998; Perry et al. 2001, 2005; Coulibaly et al. 2006), and smoltification (Nichols et al. 2008). However, owing to the highly domesticated origin of the rainbow trout families or clonal lines used for QTL mapping, the generality of these results to natural populations remains unclear.

In recent years, genomic resources, including extensive marker libraries and linkage maps, have accumulated for some nonmodel organisms. Many of these marker loci, which include microsatellites and single-nucleotide polymorphisms, have been developed from genes known to be expressed (expressed sequence tags [ESTs]; Bouck and Vision 2006). In addition, linkage maps have been developed that provide information about the relative locations of each marker locus along the chromosome. The rapid processing of multiple genetic markers in many individuals is now possible through the use of automation, high-throughput analytical instrumentation, and high-density expression and genotyping microarrays (Gunderson et al. 2005). Together, these advances now allow biologists to directly investigate the genomic effects of selection and local adaptation in natural populations.

When selection increases the frequency of a beneficial variant of a specific gene, this "selective sweep" will affect neighboring regions owing to linkage between loci. As a result, neutral marker loci in chromosomal regions linked to the selected gene will also display altered allele frequencies, a phenomenon known as "genetic hitchhiking" (Maynard-Smith and Haigh 1974; Schlötterer 2003). Hitchhiking is one of a number of related phenomena whereby selection on one locus affects the population genetic patterns in others (Barton 2000). Another such effect is background selection, in which purifying selection removes nondeleterious alleles at linked loci (Charlesworth et al. 1993). These effects provide the basis for population-based genome screens (Storz 2005), in which a large number of marker loci are evaluated to identify statistical outliers that are inferred to be the result of selection on other, nearby genes.

The genome screen approach allows the identification of chromosomal regions under selection in wild populations without any a priori information about the selected genes. Genome screens have recently been performed in a variety of nonmodel organisms, including larch budmoths *Zeiraphera diniana* (Emelianov et al. 2004), walking stick insects *Timema cristinae* (Nosil et al. 2008), periwinkle snails *Littorina saxatilis* (Wilding et al. 2001), frogs *Rana temporaria* (Bonin et al. 2006), and whitefish *Coregonus clupeaformis* (Campbell and Bernatchez 2004). These studies have focused on populations with notable phenotypic variation in contrasting environments (Scotti-Saintagne et al. 2004; Oetjen and Reusch 2007) as well as populations that inhabit a physical selective gradient resulting in continuous phenotypic variants rather than two distinct phenotypes (Vasemägi et al. 2005; Bonin et al. 2006; Jump et al. 2006). In the present study, we used a genome screen approach to test for population-based signals of natural selection in two closely related subpopulations of steelhead–rainbow trout in Scott Creek, California, that have been subjected to different environmental conditions and selective constraints over the past century.

METHODS

Study populations and sampling.—Scott Creek is a small coastal stream in central California that supports both steelhead and coho salmon *O. kisutch*. One of the three main tributaries is blocked at the upstream end by Big Creek Falls, a waterfall that is approximately 20 m high and impassable by

upstream-migrating fish. Previous work has shown that the resident trout population above Big Creek Falls was established by anthropogenic transfer from the below-falls population circa 1910 and is now marginally genetically distinct from the source population (Pearse et al. 2009). Thus, this population has recently adapted to an entirely resident life history and the fish that remain above the falls have been subject to strong selection not to descend the falls.

Resident rainbow trout ($n = 24$) were sampled by hook and line from approximately 1.7 km of isolated fluvial habitat above Big Creek Falls in July and August 2004. Adult steelhead ($n = 24$) were trapped at a resistance board weir approximately 6 km below Big Creek Falls as they returned to spawn in Scott Creek in March 2004. Fish were handled and sampled according to the protocol of Hayes et al. (2004), with fin clips subsequently being dried on filter paper in a dessicator until DNA extraction. DNA was isolated using DNeasy 96 tissue extraction kits on a BioRobot 3000 (Qiagen), following the manufacturer's recommendations.

Microsatellite markers.—Altogether, 363 microsatellite loci (including loci with di-, tri-, tetra-, and pentanucleotide repeat motifs) were screened for amplification and variability in the sampled populations. These loci can be divided into two basic groups. First, 214 previously mapped loci were chosen based on map location in order to cover all known linkage groups and space loci as evenly as possible within linkage groups (Nichols et al. 2003; Danzmann et al. 2005; Rexroad et al. 2008). To distinguish these loci from the EST-linked ones described below, they are referred to here as anonymous loci. The relative locations of mapped loci are based on their position on the *O. mykiss* linkage map of Rexroad et al. (2008) and use the chromosome names (e.g., Omy3 and Omy7) established by Phillips et al. (2006). Comparison of linkage map names with the physical chromosomal map can be found in Phillips et al. (2006), Guyomard et al. (2006; see the table of supplementary data available in the online version of this article), and Rexroad et al. (2008; supplementary data). An additional 27 unmapped microsatellite markers were included because of prior use in our laboratory (Deiner et al. 2007; Pearse et al. 2007, 2009; Clemento et al. 2009).

The second group of microsatellite loci consisted of 122 loci developed from ESTs and subsequently included on the linkage map (Coulibaly et al. 2005; Rexroad et al. 2005, 2008). Because these loci are embedded within expressed genes, we hypothesized that a larger proportion would show strong signals of natural selection than would microsatellite markers not known to be located within expressed genes.

PCR, genotyping, and standard population genetic analysis.—With the exception of the 27 unmapped loci, which were amplified using standard laboratory polymerase chain reaction (PCR) protocols with 5′ dye-labeled forward primers (Deiner et al. 2007; Pearse et al. 2007; Clemento et al. 2009), all microsatellite loci were amplified using the three-primer PCR protocol described by Schuelke (2000), which uses a universal M13 primer to incorporate a fluorescent dye into PCR products, eliminating the need for specific, labeled primers for each locus. The method uses a locus-specific forward primer with an M13 "tail" (5′-CAC GAC GTT GTA AAA CGA C-3′) added to the 5′ end, a locus-specific reverse primer, and a fluorescently-labeled (FAM, VIC, NED, PET) M13 primer. All PCRs were carried out in 15-μL volumes containing 4 μL of 20:1 diluted template DNA, 6.66 μL autoclaved distilled H_2O, 1.5 μL 10× PCR buffer, 1.2 μL 25 mM $MgCl_2$, 0.6 μL deoxynucleotide triphosphates, 0.04 μL Amplitaq DNA polymerase (Applied Biosystems), 0.1 μL forward primer, 0.45 μL reverse primer, and 0.45 μL M13 fluorescent dye-labeled primer.

The PCR thermal cycling profile was chosen to accommodate the broad range in annealing temperatures across loci and was comprised of two distinct amplification phases. The first phase began with denaturation at 95°C for 3 min and then 95°C for 30 s, 48–57°C for 30 s, and 72°C for 45 s repeated nine times with a 1°C drop in annealing temperature each cycle. The second amplification phase consisted of 89°C for 30 s, 48°C for 30 s, and 72°C for 45 s repeated 20 times, followed by a final extension at 72°C for 5 min. The resulting PCR products were electrophoresed on an Applied Biosystems 3730 capillary DNA sequencer following the manufacturer's protocols. Data collection and analysis of fragment sizes were conducted using GeneMapper version 4.0 (Applied Biosystems) software. Loci were considered for further analysis if at least half of the individuals ($n = 12$) from each population successfully produced genotypes.

Hardy–Weinberg equilibrium for each locus was evaluated with the Markov chain–Monte Carlo approximation of an exact test, as described by Guo and Thompson (1992) and implemented in GenePop 3.4 (Raymond and Rousset 1995). The method of Cockerham and Weir (1977), also as implemented in GenePop 3.4, was used to estimate the significance of allelic associations (i.e., linkage disequilibrium [LD]) between all pairs of loci. Owing to the large number of pairwise tests in this analysis, LD is reported as the proportion of marker pairs with significant tests from all pairwise comparisons.

Detection of outlier loci.—We used three complementary approaches to distinguish loci linked to regions under selection from those in regions that conform to neutral expectations. Two of the methods compare estimates of population divergence based on allele frequencies, while the third method is based on differences in heterozygosity between the two populations. Concordant identification of an outlier locus by all three approaches was considered the strongest evidence that a marker locus is located in a region under selection.

The first method, fdist2 (Beaumont and Nichols 1996; Beaumont and Balding 2004), uses Cockerham and Weir's (1993) β to estimate F_{ST} between a pair of populations and produces a simulated distribution of heterozygosity and F_{ST} with which observed values are compared to identify potential outliers. Parameters for fdist2 were as follows: 100 simulated demes, 2 populations sampled from each deme, 48 individuals

in each sample, a stepwise model of mutation, and 0.026 "trimmed" F_{ST}. The trimmed F_{ST} value removes the 5% of the loci with the highest F_{ST} values, thus providing a more accurate average F_{ST} value for neutrally evolving loci and a conservative approach to the identification of putative adaptive loci (Beaumont and Nichols 1996; Bonin et al. 2007). In each analysis, 50,000 loci were simulated to approximate the trimmed F_{ST} value from the empirical data set. Candidate loci were identified as those lying above the 95% confidence envelope. Although loci that fall below the 95% confidence interval are potentially indicative of balancing selection, their values could not be differentiated from zero in our data set and were not considered further.

The second method, DetSel 1.0, is based on a similar approach to outlier detection (Vitalis et al. 2001, 2003). DetSel produces an expected joint distribution (confidence envelope) of divergence values using coalescent simulations and population-specific parameter estimates for all observed loci based on divergence under random genetic drift. Loci that fall outside this expected confidence envelope are inferred to have been influenced by selection. Parameter values were selected to achieve a distribution of alleles similar to the observed data, as recommended by Vitalis et al. (2001). We applied four mutation rate (μ) values (0.01, 0.05, 0.005, 0.0005) for the infinite-alleles model that encompass the range of μ values estimated from *O. mykiss* microsatellite loci (4×10^{-3} to 5×10^{-4}; Martínez et al., unpublished data). Ancestral population size before the split (N_e) was set to 250, 500, and 1,000, and population size before the split was set to 500 individuals. The time since the assumed bottleneck was set to 30, 75, and 150 generations, and the number of generations since the population split was set to 40. These value ranges best represent the empirical data set based on our prior knowledge of the population (Anderson and Slatkin 2007; Pearse et al. 2009) and provide a conservative estimate of the distribution of neutrally evolving loci. The distribution was constructed using 50,000 simulated markers, with number of alleles being similar to the observed data. Loci were not considered outliers unless they were consistently outside of the confidence interval over multiple analyses.

The third method for detection of outlier loci is based on the expectation of reduced genetic diversity at a locus in a population that has undergone a selective sweep. This class of methods was described by Schlötterer (2002, 2003) and detects evidence for selection at a particular locus by considering the natural logarithm of the ratio of either allele size or expected heterozygosity (gene diversity) between the two populations (e.g., $\ln(\mathrm{RH}) = \ln({H_E}^{\mathrm{above}}/{H_E}^{\mathrm{below}})$, where RH is the ratio of expected heterozygosities in the two populations and H_E is expected heterozygosity).

Although there is no standard method to estimate the expected distribution of ln(RH) values under neutrality, previous studies have shown that simulated values for ln(RH) and its allele-size based analog ln(RV) (Schlötterer 2003) are normally distributed under neutral conditions (Kauer et al. 2002; Schlötterer 2002; Kauer et al. 2003). Therefore, under neutrality and after standardization (mean = 0, SD = 1), the ln(RH) values of 95% of loci are expected to fall between the values –1.96 and +1.96, and outliers are identified manually from this distribution. The ln(RH) method is expected to be more sensitive than the ln(RV) method because variance in heterozygosity achieves equilibrium values faster than variance in allele size (Kimmel et al. 1998). In our data, the values from the ln(RH) analysis were not significantly different from a data set of 500 normally distributed values (Kolmogorov–Smirnov test; $P = 0.33$), and a comparison of the observed ln(RH) values with a simulated data set of 1,000 normally distributed values showed no significant difference in the means of the two data sets (t-test; $P = 0.94$). We therefore used the ratio of gene diversities to detect loci with significant differences between the above- and below-barrier population samples. Loci that were monomorphic in either population were excluded from the analysis.

Importantly, unlike the other methods, the ln(RH) ratio is not based on simple genetic differentiation and can provide an indication of the direction of selection, since an outlier locus can have either a positive or negative value depending on which population has higher genetic diversity. Here, a negative ln(RH) value indicates that the marker had higher gene diversity in the below-barrier population, whereas a positive value indicates higher gene diversity in the above-barrier population. If reduced genetic diversity is indicative of a recent selective sweep, this information can be used to infer whether recent selection pressure is higher above or below the barrier waterfall. Alternatively, a selective sweep may temporarily increase diversity if a positively selected allele is linked to a marker allele that was initially present at low frequency.

RESULTS

Population Genetic Analysis

Two hundred ninety-eight microsatellite loci were included in the final analysis (82% success overall); 188 of these were anonymous and 110 were EST-linked (supplementary data). The average number of individuals genotyped per locus across both populations was 43.9, representing more than 91% success (above barrier: mean = 21.5, range = 13–24; below barrier: mean = 22.4, range = 12–24; supplementary data). Mean observed (H_O) and expected heterozygosity (H_E) and allelic richness (A_R) were all higher in the below-barrier population (H_O: 0.64 versus 0.67 [not significant]; H_E: 0.67 versus 0.71 [$P \leq 0.01$]; A_R: 5.6 versus 6.6 [$P \leq 0.001$]), consistent with the expectation of founder effects and a smaller effective population size in the above-barrier population. All but five loci (*OMM1587*, *OMM1748*, *OMM5195*, *OMM5243*, and *OMY11DIAS*) were polymorphic in the above-barrier population, with H_E ranging from 0.043 (*Ssa85*) to 0.944 (*OMM1097*). In the below-barrier population, all but three loci (*OMM1748*, *OMM5020*, and *OMM5243*) were polymorphic, with H_E ranging from 0.043 (*OMM1587*) to 0.949 (*OMM1056*). Allelic richness ranged from

TABLE 1. Proportions of loci in linkage disequilibrium at different levels of probability, by recombinational distance and location above or below a barrier to upstream passage.

	All loci			Loci 5–20 cM apart			Loci <5 cM apart		
P-value	Above	Below	Combined	Above	Below	Combined	Above	Below	Combined
<0.05	9	3	6	17	4	10	25	9	19
<0.01	3	0.9	2	8	0.6	6	15	6	15
<0.001	0.6	0.1	0.4	4	0	2	8	2	8
Bonferroni corrected	0.07	0.04	0.08	0.9	0	0.9	1	1	1

1 (monomorphic) to 13.5 (*OMM1097*) in the above-barrier sample and from 1 to 14.7 (*OMY1008UW*) below the waterfall. Two loci that were monomorphic in all samples (*OMM1748* and *OMM5243*) were excluded from further analysis.

Tests for deviation from Hardy–Weinberg equilibrium found 11 and 13 loci with significant deviations in the above- and below-barrier populations, respectively, after Bonferroni correction for multiple tests ($\alpha = 0.05$, $P \leq 0.00008$; supplementary data). Tests for linkage disequilibrium among all pairs of loci yielded 4,014 (9%) significant ($P \leq 0.05$) tests in the above-barrier population, 1,518 (3%) significant tests in the below-barrier population, and 2,602 (6%) significant pairwise tests in both populations combined. When Bonferroni correction was applied to maintain an experimentwide α of 0.05 ($P \leq 1.1 \times 10^{-6}$), the numbers of significant tests were reduced to 32 (0.07%), 19 (0.04%), and 36 (0.08%), respectively (Table 1). The larger number of significant tests in the above-barrier sample is probably due to a combination of family structure, small effective population size, and founder effects.

The overall genetic differentiation between the two populations for all 298 loci was estimated to be 0.034 using the F_{ST} estimator (Θ) of Weir and Cockerham (1984) and ranged from –0.026 to +0.36 for single loci, with a mean, single-locus pairwise F_{ST} of 0.032 (Figure 1). The overall differentiation of 0.034 is somewhat higher than the value (0.018) found by Pearse et al. (2009) for these same populations with 18 microsatellite loci.

Genomic Distribution of Loci

The loci in the final data set were located on all 29 linkage groups of the approximately 2,900 centimorgan (cM) linkage map of Rexroad et al. (2008), corresponding to the 28 autosomes and the sex chromosome of the *O. mykiss* karyotype (Phillips et al. 2006; Rexroad, personal communication). Of the 298 loci, 264 (89%) are mapped, with an average of 9 loci per linkage group (range, 4–16). The average distance between markers (marker density) for the area sampled ranged from 3.8 to 16.3 cM/marker per linkage group, with an average density of 8.8 cM/marker (range, 6.9–31.1) across all linkage groups, or an average density of 11.7 cM/marker when considering the telomeric regions beyond the last sampled marker.

Loci known to be located in close physical proximity are expected to have a higher incidence of LD than loci on separate linkage groups. In our data, 85 pairs of loci were within 5 cM of each other on the Rexroad et al. (2008) map, and a much higher proportion of these locus pairs showed evidence of LD in the above, below, and combined samples (Table 1). Similarly, the proportion of significant tests was greater for pairs of loci separated by 5–20 cM than for loci assigned to different linkage groups (Table 1). However, map location did not perfectly predict linkage among pairs of loci, and of the 18 pairs of loci that mapped to identical chromosomal locations only 10 had significant LD (supplementary data).

Detection of Outlier Loci

Altogether, 14 loci were identified by fdist2 as significant outliers (Figure 2; Table 2) and 4 of these were EST-linked (*OMM5008*, *OMM5011*, *OMM5229*, and *OMM3090_MHC1*). All 14 outliers were mapped, with three pairs assigned to the same linkage group and one pair (*OMM1009* and *BHMS426*) mapped to an identical position on chromosome Omy5 (linkage group 8; Table 2).

The DetSel method identified 29 significant outlier loci, including 13 EST-linked and 16 anonymous loci (Table 2). Twenty-seven of the 29 outliers were on the linkage map, including the pair of loci with the identical map position (*OMM1009* and *BHMS426*; Table 2) identified by fdist2 as outliers.

The ln(RH) method identified 15 outlier markers. The marker *OMM1120* was nearly significant (ln(RH) = –1.91; Figure 3) but was counted as an outlier with the ln(RH) detection method because it was also identified as a significant outlier with both the fdist2 and DetSel methods (Table 2). Six of the 15 outliers (*OMM5011*, *OMM5136*, *OMM3024_MHC2*, *OMM5109*, *OMM5124*, and *BX076085*) were EST-linked (Table 2) while 9 were anonymous. Although the below-barrier population had a higher mean gene diversity, the distribution of ln(RH) values was not significantly skewed (χ^2 test; $P = 0.64$). Of the 15 outlier markers, there were 6 with positive ln(RH) values (indicating higher gene diversity above the barrier) and 9 with negative ln(RH) values (indicating higher gene diversity in the below-barrier population) (Figure 3).

Concordance of Tests for Selection

A total of 18 EST-linked and 19 anonymous microsatellite loci, mapped to 19 of the 30 *O. mykiss* linkage groups,

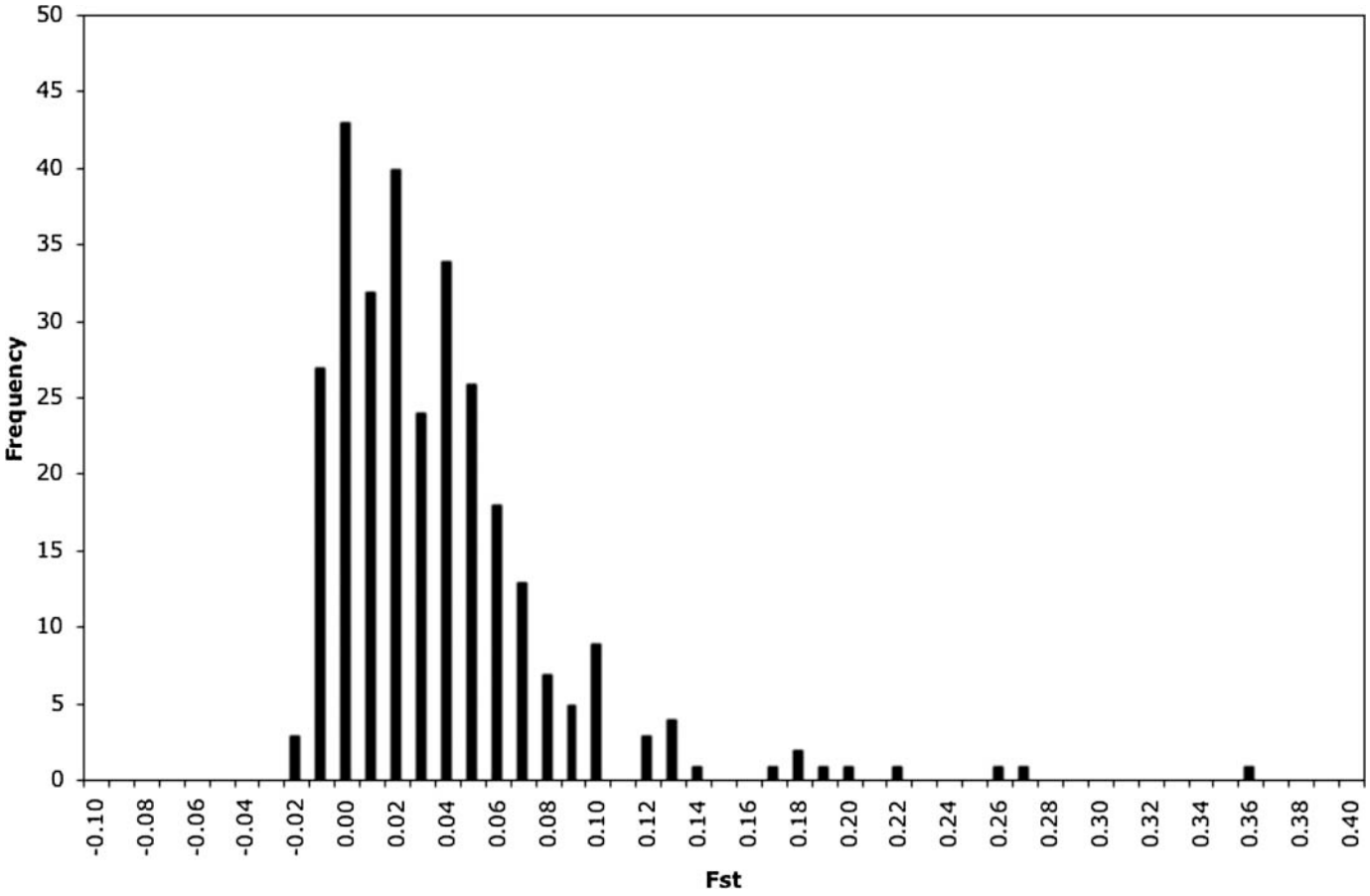

FIGURE 1. Distribution of pairwise F_{ST} values between above-barrier and below-barrier populations for all microsatellite loci included in the final analysis.

were identified as outliers by at least one detection method (Table 2). Since each method identifies outlier loci based on different evolutionary assumptions and signals in the data, we consider concordant identification by multiple methods to provide the best indication of genomic regions under recent natural selection. Six loci were identified by all three methods ($6/37 = 16.2\%$) as deviating significantly from neutral expectations, while 9 loci were identified by two methods ($9/37 = 24.3\%$), and 22 were identified by only one method ($22/37 = 59.5\%$) (Table 2).

DISCUSSION

We describe one of the first genome screens for the species *O. mykiss* that attempts to detect the genomic signatures of natural selection by comparing two populations of recent common origin but now inhabiting different environments with different presumptive selective regimes. Natural selection on specific alleles will generally only affect the region of the genome linked to the selected locus, while genetic drift, gene flow, inbreeding, and other population-level processes will influence all regions of the genome similarly (Cavalli-Sforza 1966). Genomic regions targeted by natural selection can therefore be identified by their altered patterns of population genetic variation (relative to neutral loci) between pairs of populations influenced by different selective pressures (Beaumont and Nichols 1996; Vitalis et al. 2001; Beaumont and Balding 2004). The strength of the signature of selection on linked marker loci is influenced by the intensity of selection, the distance of the marker loci from the selected locus, the initial frequencies of the selected and marker alleles, and the mutation rate (Lewontin and Krakauer 1973; Maynard-Smith and Haigh 1974; Kaplan et al. 1989; Stephan et al. 1992; Wiehe and Stephan 1993; Wiehe 1998).

In the present study, 298 microsatellite loci, chosen to represent most of the *O. mykiss* genome, were analyzed to identify genomic regions under differential selection in a pair of populations that are experiencing different selection pressures on life history variation. The elevated differentiation detected at many of these marker loci provides evidence of the effects of non-neutral evolution on genomic regions linked to those markers. We hypothesize that these markers are linked to genes under divergent natural selection, presumably owing to the different environmental constraints imposed on the above- and below-barrier populations, and particularly the strong selection for a purely resident life history above the barrier waterfall. These two populations have been isolated for approximately 100 years,

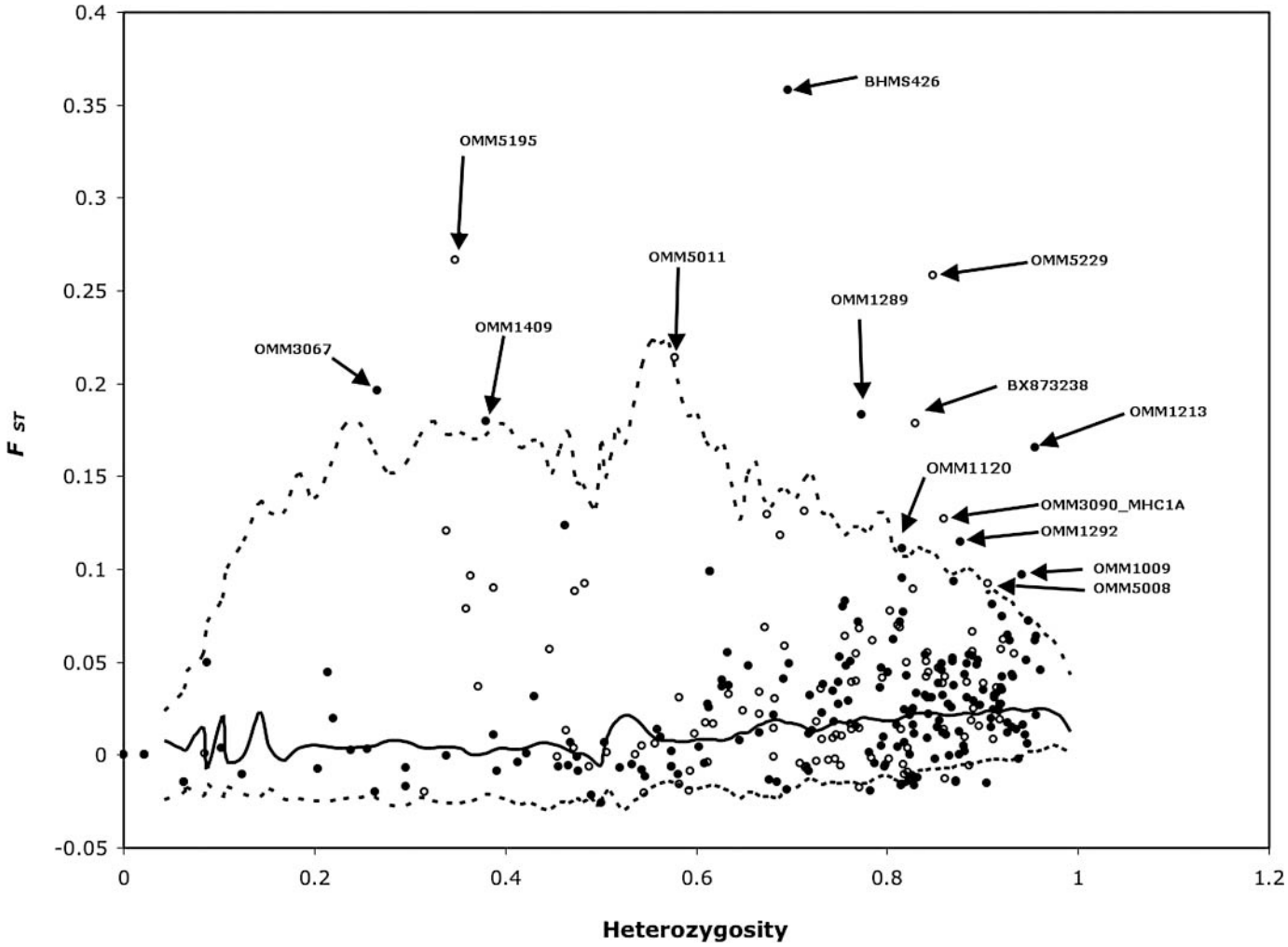

FIGURE 2. Plot of pairwise F_{ST} values versus heterozygosity between the above-barrier and below-barrier populations for EST-linked (open circles) and anonymous microsatellite loci (solid circles). The upper and lower dashed lines designate the 95% confidence intervals defined by fdist2, and the solid line shows the median values produced by the simulations.

following anthropogenic transplantation of juvenile steelhead above a barrier waterfall in the Scott Creek watershed (Pearse et al. 2009). The significantly reduced gene diversity and allelic richness found in the above-barrier population is consistent with its being founded from the below-barrier population, in spite of a relatively large estimated founding size (estimated $N_E = 421$; Anderson and Slatkin 2007).

Concordance of Detection Methods

The identification of false positive tests is important when employing large numbers of loci (Excoffier et al. 2009). Both fdist2 and ln(RH) identified the approximate number of loci (15) expected to fall outside the confidence intervals by chance alone, while DetSel identified twice as many, as has been seen previously (Vasemägi et al. 2005; Bonin et al. 2006; Oetjen and Reusch 2007; Tsumura et al. 2007). Thus, DetSel is either more effective in identifying real outliers or has more false positives than the other methods. DetSel uses a population divergence model that may be sensitive to parameter estimates in the simulation of the allele frequency distributions in the founding and ancestral populations, including mutation rate and time since the assumed population split (Vitalis et al. 2001). In our analysis, alterations in the parameter values did not influence the significance of the identified outliers as long as the simulated allelic distributions were similar to the observed values.

DetSel and fdist2 estimate differentiation among loci using F_{ST} and attempt to identify loci where recent selection has changed the frequency of alleles in one or both populations (Beaumont and Nichols 1996; Vitalis et al. 2001). A similar method that reduces false positives by accounting for hierarchical population structure found results identical to those of our analysis using fdist2 (Excoffier et al. 2009; data not shown). In contrast, ln(RH) differentiates outlier loci based on the relative gene diversity in the two populations, identifying genomic

TABLE 2. Outlier loci identified by fdist2, DetSel, and ln(RH). Loci linked to known ESTs are denoted by asterisks.

Marker	F_{ST}	fdist2	DetSel	ln(RH)	Chromosome	Linkage group	Position (cM)
OMM1120	0.111	X	X	X	10	20	80.5
*OMM5011**	0.214	X	X	X	23	30	24.2
OMM1289	0.183	X	X	X	21	15	32.4
OMM3067	0.196	X	X	X	19	14	39.3
OMM1409	0.180	X	X	X	15	7	93.4
OMM1009	0.097	X	X	X	5	8	76.7
BHMS426	0.358	X	X		5	8	76.7
*OMM3090**[a]	0.127	X	X		18	16	62.7
OMM1213	0.166	X	X		6	10	74.4
*OMM5229**	0.258	X	X		Sex	1	48.3
*OMM5195**	0.267	X	X		10	20	104.4
*BX873238**	0.179	X	X		18	16	110.2
OMM1292	0.115	X	X		22	5	26.4
*OMM5136**	0.092		X	X	Sex	1	35.6
*OMM3024**[a]	0.096		X	X	17	29	0
*OMM5008**	0.092	X			12	9	30.1
*OMM5166**	0.090		X				
*OMM5117**	0.121		X				
*OMM5014**	0.057		X		9	21	61.9
*CA361411**	0.130		X		22	5	45.4
*OMM5038**	0.079		X		20	17	43
*CA054538**	0.118		X		6	10	51.9
OMM1756	0.099		X		24	26	9.6
OMM1000	0.044		X		Sex	1	21.1
*OMM5060**	0.131		X		5	8	60.5
BX310634	0.095		X		9	21	106.4
OMM1386	0.020		X		22	5	19.8
OMM3097	0.124		X		11	19	51.6
OMM1231	0.099		X		1	6	96.8
OMM1581	0.048		X		11	19	22.8
OMM1036	0.037			X	21	15	52.4
OKI23	0.048			X			
*OMM5109**	0.045			X	3	31	120.4
*OMM5124**	0.047			X	18	16	76
OMM1762	0.025			X	28	13	5.3
*BX076085**	0.062			X	Sex	1	35.6
OMM1241	0.074			X	19	14	29

[a]*OMM3090* and *OMM3024* are linked to the MHC1 and MHC2 genes, respectively.

regions where hitchhiking or background selection has reduced the variation in one of the populations. This difference in the underlying measure of differentiation examined may identify loci with different selection processes affecting their variation. For example, the locus with the highest pairwise F_{ST} value, *BHMS426*, was a strongly significant outlier with fdist2 and DetSel but not with ln(RH) because the H_E values were similar in both populations even though the allelic frequencies were different. Concordance between multiple detection methods to identify candidates for further investigation is likely to reduce false positives, but it is possible that some loci identified by a single detection method are also true outliers. In addition, the ln(RH) method indicates which population displays reduced heterozygosity relative to the other. Novel selection pressures are expected to be stronger on a population in a new environment than on one remaining in established habitat. Here, the average of the unstandardized ln(RH) values was negative, which is consistent with the expectation of selection acting in the novel above-barrier environment (though these deviations were not significant).

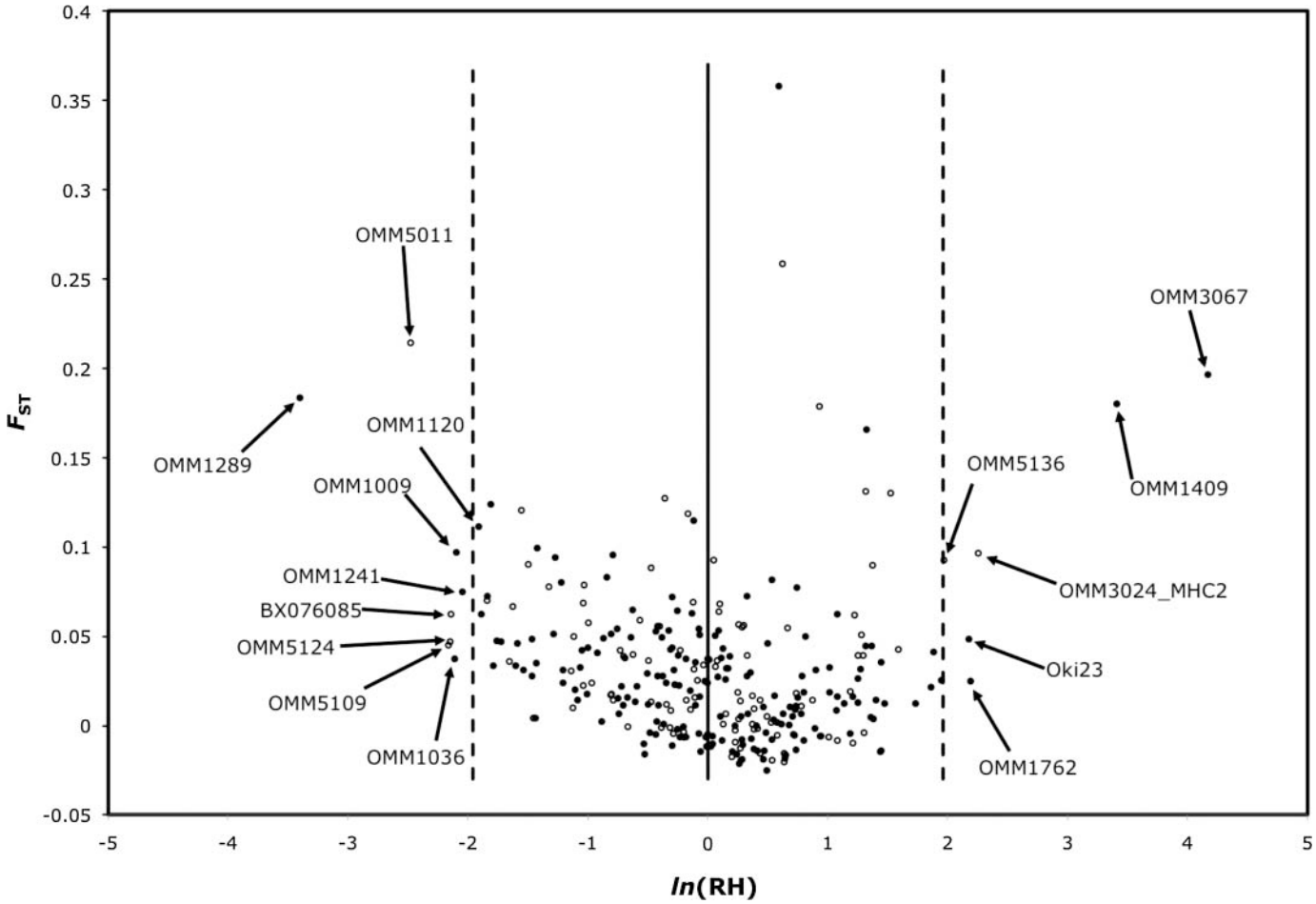

FIGURE 3. Plot of pairwise F_{ST} values versus standardized ln(RH) for EST-linked (open circles) and anonymous microsatellite loci (solid circles). The dashed lines designate the 95% confidence intervals (–1.96, +1.96), and the solid line represents the mean value of the standardized empirical data set.

EST-Linked versus Anonymous Microsatellite Loci

Previous studies have identified a greater proportion of outlier loci among EST-linked microsatellite loci than loci not known to be tightly linked to expressed genes (Li et al. 2004; Scotti-Saintagne et al. 2004; Vasemägi et al. 2005). Similarly, in our data a greater proportion of EST-linked loci (16.4% [18 of 110]) than of anonymous loci (10.1% [19 of 188]) were identified as outliers with at least one method, but this difference was not statistically significant. However, because some loci were identified by more than one method, overall, 18 (48.6%) of the 37 total identified outlier loci were EST-linked, which is also not a significant deviation from the expectation of 13.7 (110/298 = 36.9% of 37; binomial probability of 18 or more EST-linked loci if there is no difference; $P < 0.01$). It is important to note, however, that the genomic regions in which anonymous microsatellite loci are found may nonetheless be in close proximity to expressed genes under the influence of selection. Therefore, when conducting a broad-scale genome screen for signatures of selection it is more important to include loci that best cover the genome than to choose markers on the basis of their known proximity to expressed genes.

Linkage Disequilibrium among Loci

As expected, LD between locus pairs separated by less than 5 cM was much higher than that in the data set overall, and LD between pairs of loci separated by 5–20 cM was lower than that between those separated by less than 5 cM but still greater than that between all pairs of loci (Table 2). Although some studies have detected LD extending just a few kilobases from the selected region, others have suggested that linkage disequilibrium can extend 100 kilobases or more (Collins et al. 1999; Dunning et al. 2000; Taillon-Miller et al. 2000; Reich et al. 2001; Flint-Garcia et al. 2003; Scotti-Saintagne et al. 2004; Storz 2005). Linkage disequilibrium was higher in the above-barrier population, most likely as a result of smaller effective population size and family effects. Of the 37 outlier loci, there were two pairs (*OMM1009–BHMS426* and *OMM5136–BX076085*) in which both loci mapped to

identical positions on the Rexroad et al. (2008) linkage map (Table 2). In the first pair, both loci had F_{ST} values in the highest 10% and *BHMS426* had the highest pairwise F_{ST} value (0.36) of all loci in the data set (supplementary data). These two loci were in strong LD in the above-barrier, below-barrier, and combined population tests. The other pair of loci, *OMM5136–BX076085*, was not in significant LD. No other pairs of outlier loci were closely linked on the Rexroad et al. (2008) map, and the proportion of pairs of outlier loci in LD was similar to the proportion of all loci in LD in our data set. Indeed, a substantial number of loci assigned to identical locations on the Rexroad et al. (2008) map were not in LD here. The minimum resolution of the Rexroad et al. (2008) map is 0.3 cM, so loci that map to the same location could still be hundreds of kilobases apart. Other studies have detected evidence for LD and selection effects between loci up to 2 cM apart (e.g., Scotti-Saintagne et al. 2004), and our results suggest that such effects can occur at least up to that same distance in *O. mykiss*.

Associations between Outlier Loci and Known Genes and QTL

Leder et al. (2006) determined the associations between six candidate genes and a previously identified set of QTL for spawning date in rainbow trout. They found that the *Clock* gene, which is known to be involved in the regulation of daily and seasonal rhythms in invertebrates and vertebrates, mapped to the same region of linkage group 8 (chromosome Omy5; Phillips et al. 2006) as a major QTL for spawning time (O'Malley et al. 2003; Leder et al. 2006). Similarly, two studies using an independent mapping family identified major multitrait QTL associated with egg development and differentiation between resident and anadromous *O. mykiss*, respectively, in this same region on chromosome Omy5 (Nichols et al. 2007; 2008). Two of the most strongly supported outlier loci (*OMM1009* and *BHMS426*) from the present study map to this exact same region of Omy5. A third outlier locus, *OMM5060*, is also located on Omy5 and was in significant LD, with *BHMS426* in the above-barrier and combined populations tests despite its being more than 15 cM away from the other two outliers. Together, these results provide strong evidence that this genomic region, and most likely the *Clock* gene, is an important contributor to heritable life history and physiological variation in *O. mykiss*.

The *Clock* genes are responsible for the regulation of other genes that require daily rhythmic expression (Leder et al. 2006). Regulatory gene involvement in circadian and circannual timing is not well understood, and can include complex epigenetic effects (Whitmore et al. 1998; Dunlap 1999; Falcon 1999; Stanewsky 2003). In salmonids, seasonal timing and life history decisions, such as migration, maturation, and smoltification, are strongly influenced by specific environmental cues and food availability. Steelhead growth and reproduction are highly dependent on seasonal cycles in the marine environment, whereas those of rainbow trout are influenced entirely by freshwater environmental factors, and this difference may lead to distinct developmental pathways. The identification here of the *Clock* gene region as under recent natural selection, along with the previous implication of this region in the heritable variation of associated physiological traits, supports the hypothesis that *Clock* is involved in adaptive divergence between resident and anadromous *O. mykiss*.

Nichols et al. (2008) also located a QTL region for 10 different nonindependent phenotypic traits, including an overall binary "smoltification" trait, on linkage group 20 (= chromosome Omy10). Although Nichols et al. (2003) and Rexroad et al. (2008) report somewhat different linkage relationships among the markers on this chromosome, it is clear that *OMM1120* (which we identified as an outlier with all three methods) and *OMM5195* (identified as an outlier with two methods) are tightly linked to this genomic region. The QTL on chromosomes Omy5 and Omy10 were the primary ones identified by Nichols et al. (2008), so our detection of multiple outlier loci in each region strongly supports the hypothesis that genes in these regions are important contributors to the heritable life history variation in *O. mykiss*. Interestingly, two of the outlier loci discussed above, *OMM5060* and *OMM5195*, were in strong LD ($P < 0.0001$) in both the below-barrier and combined tests in spite of the fact that they mapped to different chromosomes (Omy5 and Omy10, respectively) and, as noted above, *OMM5060* was also in LD with one of the markers (*BHMS426*) from the *Clock* gene region.

The results of present study are also concordant with those of several other studies that have identified genes or genomic regions involved in the life history variation of *O. mykiss*. Haidle et al. (2008) found major QTL for early maturation (a trait associated with the resident life history) on four linkage groups, one of which corresponds to chromosome Omy5, which contains the *Clock* gene region mentioned above. Also, in a study of mixed resident and anadromous *O. mykiss* populations in Russia and Alaska, the sex-linked locus *OMM5136* showed significant genetic differentiation between samples of resident and anadromous individuals (M. McPhee, University of Alaska Fairbanks, personal communication) despite a lack of differentiation at suites of 10–13 nuclear microsatellite loci (Olsen et al. 2006; McPhee et al. 2007). We identified this locus as an outlier with both DetSel and ln(RH), and three other outliers (*Omm1000*, *OMM5229*, and *BX076085*) were also located on the sex chromosome, supporting the hypothesis that genes differentially expressed or selected in males and females, such as those associated with early maturation and residency, are likely to be located on sex-linked chromosomal regions.

Summary

This study exemplifies the utility of genome screens for the identification of regions presumably affected by recent natural selection when prior knowledge about candidate genes or family relationships within a population is lacking. However, this identification is only the first step in our understanding of phenotypic differentiation. The outlier loci identified here had elevated differentiation between just two subpopulations, while

many genome screens have examined multiple populations in different environments (Vasemägi et al. 2005; Aguilar 2006; Bonin et al. 2006; Tsumura et al. 2007). For example, Vasemägi et al. (2005) used a genome screen approach to identify EST-linked microsatellites potentially under selection among eight populations of Atlantic salmon *Salmo salar* across a gradient of salinity. Future research with the outlier loci identified in Scott Creek will include extending the evaluation of selection signals to additional *O. mykiss* populations and to markers that map to the same genomic regions. Ultimately, a genome sequence may allow us to identify genes under selection using a candidate gene approach to evaluate specific nucleotide polymorphisms (Kohn et al. 2000; Wootton et al. 2002; Turner et al. 2005).

Quantitative trait locus mapping is an alternative approach to identification of genes involved in the genetic architecture of life history variation in steelhead–rainbow trout. This approach involves the creation of mapping families in which the trait(s) of interest segregate. This is extremely difficult for traits that exhibit extensive phenotypic plasticity, such as migratory and reproductive behavior and their associated developmental pathways. Further complicating its application in natural populations of *O. mykiss* are 3–4-year generation times and high mortality during the marine life history phase. However, research on a natural population of red deer *Cervus elaphus* that has been studied for several decades has successfully used molecular markers to reconstruct pedigrees among wild individuals and then applied these pedigrees to heritability and QTL analysis (Pemberton 2008). Ultimately, a combined approach that uses complementary methods to identify genomic regions and then the specific genes that influence life history and ecological traits will probably prove the most productive in natural populations (Rogers and Bernatchez 2005, 2007).

Bridging the gap between a genome screen or QTL-identified genomic region and the identification of nucleotide polymorphisms resulting in selectively advantageous genotypes is an arduous process, even with model organisms (Erickson et al. 2004; Flint and Mott 2001). However, the rapid acquisition of whole genome sequences for nonmodel organisms is becoming more common, and an *O. mykiss* genome sequence will greatly improve our ability to identify the specific genes involved in life history variation in steelhead–rainbow trout populations.

ACKNOWLEDGMENTS

Members of the Southwest Fisheries Science Center Molecular Ecology and Genetic Analysis Team (A. Abadía-Cardoso, E. C. Anderson, G. Charrier, A. J. Clemento, E. Gilbert-Horvath, and H.A. Starks) provided critical contributions to this study. We thank M. Bond, C. Hanson, and S. Hayes for sample collection as part of an ongoing ecological study in Scott Creek and R. Waples for stimulating discussions. C. Rexroad graciously provided map information prior to publication. All samples were collected with permission from landowners, including California Polytechnic University's Swanton Pacific Ranch, Lockheed Martin Corporation, and Big Creek Lumber and were approved by the University of California–Santa Cruz's Animal Use Committee according to National Institutes of Health guidelines under NMFS ESA Section 10 permit 1112. Funding for this research was provided by the National Marine Fisheries Service and the California Department of Fish and Game, as well as through a genome scholar fellowship awarded to A.M. by the Society for the Advancement of Chicanos and Native Americans in Science.

REFERENCES

Aguilar, A. 2006. Analysis of F_{ST} outliers at allozyme loci in Pacific salmon: implications for natural selection. Environmental Biology of Fishes 76:329–339.

Anderson, E. C., and M. Slatkin. 2007. Estimation of the number of individuals founding colonized populations. Evolution 61:972–983.

Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity 90:281–288.

Barton, N. H. 2000. "Genetic hitchhiking." Philosophical Transactions of the Royal Society of London B 355:1553–1562.

Beaumont, M. A., and D. J. Balding. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology 13:969–980.

Beaumont, M. A., and R. A. Nichols. 1996. Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London B 263:1619–1626.

Bonin, A., F. Nicole, F. Pompanon, C. Miaud, and P. Taberlet. 2007. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conservation Biology 21:697–708.

Bonin, A., P. Taberlet, C. Miaud, and F. Pompanon. 2006. Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). Molecular Biology and Evolution 23:773–783.

Bouck, A., and T. Vision. 2006. The molecular ecologist's guide to expressed sequence tags. Molecular Ecology 16:907–924.

Campbell, D., and L. Bernatchez. 2004. Genomic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. Molecular Biology and Evolution 21:945–956.

Cavalli-Sforza, L. L. 1966. Population structure and human evolution. Proceedings of the Royal Society of London B 164:362–379.

Charles, K., J.-M. Roussel, J.-M. Lebel, J.-L. Bagliniere, and D. Ombredane. 2006. Genetic differentiation between anadromous and freshwater resident brown trout (*Salmo trutta* L.): insights obtained from stable isotope analysis. Ecology of Freshwater Fish 15:255–263.

Charlesworth, B., M. T. Morgan, and D. Charlesworth. 1993. The effect of deleterious mutations on neutral molecular variation. Genetics 134:1289–1303.

Clemento, A. J., E. C. Anderson, D. Boughton, D. Girman, and J. C. Garza. 2009. Population genetic structure and ancestry of *Oncorhynchus mykiss* populations above and below dams in southcentral California. Conservation Genetics 10:1321–1336.

Cockerham, C. C., and B. S. Weir. 1977. Digenic descent measures for finite populations. Genetical Research 30:121–147.

Cockerham, C. C., and B. S. Weir. 1993. Estimation of gene flow from *F*-statistics. Evolution 47:855–863.

Collins, A., C. Lonjou, and N. E. Morton. 1999. Genetic epidemiology of single-nucleotide polymorphisms. Proceedings of the National Academy of Sciences of the USA 96:15173–15177.

Coulibaly, I., R. G. Danzmann, Y. Palti, R. Vallejo, S. A. Gahr, J. Yao, and C. E. Rexroad. 2006. Mapping of genes in a region associated with upper temperature tolerance in rainbow trout. Animal Genetics 37:595–607.

Coulibaly, I., K. Gharbi, R. G. Danzmann, J. Yao, and C. E. Rexroad III. 2005. Characterization and comparison of microsatellites derived from repeat-enriched libraries and expressed sequence tags. Animal Genetics 36:309–315.

Curry, R. A. 2005. Assessing the reproductive contributions of sympatric anadromous and freshwater-resident brook trout. Journal of Fish Biology 66:741–757.

Danzmann, R. G., M. Cairney, W. S. Davidson, M. M. Ferguson, K. Gharbi, R. Guyomard, L. Holm, E. Leder, N. Okamoto, A. Ozaki, C. E. Rexroad III, T. Sakamoto, J. B. Taggert, and R. Woram. 2005. A comparative analysis of the rainbow trout genome with 2 other species of fish (Arctic charr and Atlantic salmon) within the tetraploid derivative Salmonidae family (subfamily: Salmoninae). Genome 48:1037–1051.

Deiner, K., J. C. Garza, R. Coey, and D. J. Girman. 2007. Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and manmade barriers in the Russian River, California. Conservation Genetics 8:437–454.

Dunlap, J. C. 1999. Molecular bases for circadian clocks. Cell 96:271–290.

Dunning, A. M., F. Durocher, C. S. Healey, M. D. Teare, S. E. McBride, F. Carlomagno, C. F. Xu, E. Dawson, S. Rhodes, S. Ueda, E. Lai, R. N. Luben, E. J. Van Rensburg, A. Mannerma, V. Kataja, G. Rennart, I. Dunham, I. Purvis, D. Easton, and B. A. Ponder. 2000. The extent of linkage disequilibrium in four populations with distinct demographic histories. American Journal of Human Genetics 67:1544–1554.

Emelianov, I., F. Marec, and J. Mallet. 2004. Genomic evidence for divergence with gene flow in host races of the larch budmoth. Proceedings of the Royal Society of London B 271:97–105.

Erickson, D. L., C. B. Fenster, H. K. Stenoien, and D. Price. 2004. Quantitative trait locus analyses and the study of evolutionary process. Molecular Ecology 13:2505–2522.

Excoffier, L., T. Hofer, and M. Foll. 2009. Detecting loci under selection in a hierarchically structured population. Heredity 103:285–298.

Falcon, J. 1999. Cellular circadian clocks in the pineal. Progress in Neurobiology (Oxford) 58:121–162.

Flint, J., and R. Mott. 2001. Finding the molecular basis of quantitative traits: successes and pitfalls. Nature Reviews Genetics 2:437–445.

Flint-Garcia, S. A., J. M. Thornsberry, and E. S. Buckler IV. 2003. Structure of linkage disequilibrium in plants. Annual Review of Plant Biology 54:357–374.

Fu, Y. X. 1996. New statistical tests of neutrality for DNA samples from a population. Genetics 143:557–570.

Gunderson, K. L., F. J. Steemers, G. Lee, L. G. Mendoza, and M. S. Chee. 2005. A genomewide scalable SNP genotyping assay using microarray technology. Nature Genetics 37:549–554.

Guo, S. W., and E. A. Thompson. 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. Biometrics 48:361–372.

Guyomard, R., S. Mauger, K. Tabet-Canale, S. Martineau, C. Genet, F. Krieg, and E. Quillet. 2006. A type I and type II microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) with presumptive coverage of all chromosome arms. BMC Genomics 7:302.

Haidle, L., J. E. Janssen, K. Gharbi, H. K. Moghadam, M. M. Fuguson, and R. G. Danzmann. 2008. Determination of quantitative trait loci (QTL) for early maturation in rainbow trout (*Oncorhynchus mykiss*). Marine Biotechnology 10:579–592.

Hayes, S. A., M. H. Bond, C. V. Hanson, and R. B. MacFarlane. 2004. Interactions between endangered wild and hatchery salmonids: can the pitfalls of artificial propagation be avoided in small coastal streams? Journal of Fish Biology 65:1–21.

Hendry, A. P., J. K. Wenberg, P. Bentzen, E. Z. Volk, and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science (Washington, D.C.) 290:516–518.

Hendry, A. P. 2001. Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. Genetica 112-113:515–534.

Hudson, R. R., M. Kreitman, and M. Aguadé. 1987. A test of neutral molecular evolution based on nucleotide data. Genetics 116:153–159.

Hutchings, J. A. 1993. Adaptive life histories affected by age-specific survival and growth rate. Ecology 74:673–684.

Hutchings, J. A., and D. W. Morris. 1985. The influence of phylogeny, size, and behaviour on patterns of covariation in salmonid fishes. Oikos 45:118–124.

Jackson, T. R., M. M. Furguson, R. G. Danzmann, A. G. Fishback, P. E. Ihssen, M. O'Connell, and T. J. Crease. 1998. Identification of two QTL influencing upper temperature tolerance in three rainbow trout (*Oncorhynchus mykiss*) half-sib families. Heredity 80:143–151.

Jump, A. S., J. M. Hunt, J. A. Martínez-Izquierdo, and J. Peñuelas. 2006. Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. Molecular Ecology 15:3469–3480.

Kaplan, N. L., R. R. Hudson, and C. H. Langley. 1989. The "hitchhiking effect" revisited. Genetics 123:887–899.

Kauer, M. O., D. Dieringer, and C. Schlötterer. 2003. A microsatellite variability screen for positive selection associated with "out of Africa" habitat expansion of *Drosophila melanogaster*. Genetics 165:1137–1148.

Kauer, M. O., B. Zangerl, D. Dieringer, and C. Schlötterer. 2002. Chromosomal patterns of microsatellite variability contrast sharply in African and non-African populations of *Drosophila melanogaster*. Genetics 160:247–256.

Kimmel, M., R. Chakraborty, J. P. King, M. Bamshad, W. S. Watkins, and L. B. Jorde. 1998. Signatures of population expansion in microsatellite repeat data. Genetics 148:1921–1930.

Kohn, M. H., H. J. Pelz, and R. K. Wayne. 2000. Natural selection mapping of the warfarin-resistance gene. Proceedings of the National Academy of Sciences of the USA 97:7911–7915.

Leder, E. H., R. G. Danzmann, and M. M. Ferguson. 2006. The candidate gene, *clock*, localizes to a strong spawning time quantitative trait locus region in rainbow trout. Journal of Heredity 97:74–80.

Lewontin, R. C., and J. Krakauer. 1973. Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics 74:175–195.

Li, Y. C., A. B. Korol, T. Fahima, and E. Nevo. 2004. Microsatellites within genes: structure, function, and evolution. Molecular Biology and Evolution 21:991–1007.

Mauricio, R. 2001. Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. Nature Review Genetics 2:370–381.

Maynard-Smith, J., and J. Haigh. 1974. The hitchhiking effect of a favourable gene. Genetical Research 23:23–35.

McConnell, S. K., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Canadian Journal of Fisheries and Aquatic Sciences 52:1863–1872.

McDonald, J. H., and M. Kreitman. 1991. Adaptive protein evolution at the *Adh* locus in *Drosophila*. Nature (London) 351:652–654.

McPhee, M. V., F. Utter, J. A. Stanford, K. V. Kuzishchin, K. A. Savvaitova, D. S. Pavlov, and F. W. Allendorf. 2007. Population structure and partial anadromy in *Oncorhynchus mykiss* from Kamchatka: relevance for conservation strategies around the Pacific Rim. Ecology of Freshwater Fish 16:539–547.

Morris, D. B., K. R. Richard, and J. M Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic studies of salmonids. Canadian Journal of Fisheries and Aquatic Sciences 53:120–126.

Nichols, K. M., K. W. Broman, K. Sundin, J. M. Young, P. A. Wheeler, and G. H. Thorgaard. 2007. Quantitative trait loci × maternal cytoplasmic environment interaction for development rate in *Oncorhynchus mykiss*. Genetics 175:335–347.

Nichols, K. M., A. F. Edo, P. A. Wheeler, and G. H. Thorgaard. 2008. The genetic basis of smoltification-related traits in *Oncorhynchus mykiss*. Genetics 179:1559–1575.

Nichols, K. M., W. P. Young, R. G. Danzmann, B. D. Robinson, C. Rexroad, M. Noakes, R. B. Phillips, P. Bentzen, I. Spies, K. Knudsen, F. W. Allendorf, B. M. Cunningham, J. Brunelli, H. Zhang, S. Ristow, R. Drew, K. H. Brown, P. A. Wheeler, and G. H. Thorgaard. 2003. A consolidated linkage map for rainbow trout (*Oncorhynchus mykiss*). Animal Genetics 34:102–115.

Nosil, P., S. P. Egan, and D. J. Funk. 2008. Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. Evolution 62:316–336.

Oetjen, K., and T. B. H. Reusch. 2007. Genome scans detect consistent divergent selection among subtidal vs. intertidal populations of the marine angiosperm *Zostera marina*. Molecular Ecology 16:5156–5167.

Olsen, J. B., P. Bentzen, and J. E. Seeb. 1998. Characterization of seven microsatellite loci derived from pink salmon. Molecular Ecology 7:1087–1089.

Olsen, J. B., K. Wuttig, D. Fleming, E. J. Kretschmer, and J. K. Wenburg. 2006. Evidence of partial anadromy and resident-form dispersal bias on a fine scale in populations of *Oncorhynchus mykiss*. Conservation Genetics 7:613–619.

O'Malley, K. G., T. Sakamoto, R. G. Danzmann, and M. M. Ferguson. 2003. Quantitative trait loci for spawning data and body weight in rainbow trout: testing for conserved effects across ancestrally duplicated chromosomes. Journal of Heredity 94:273–284.

Pearse, D. E., C. J. Donohoe, and J. C. Garza. 2007. Population genetics of steelhead (*Oncorhynchus mykiss*) in the Klamath River. Environmental Biology of Fishes 80:377–387.

Pearse, D. E., S. A. Hayes, E. C. Anderson, M. H. Bond, C. V. Hansen, R. B. MacFarlane, and J. C. Garza. 2009. Over the falls? Rapid evolution of ecotypic differentiation in steelhead/rainbow trout (*Oncorhynchus mykiss*). Journal of Heredity 100:515–525.

Pemberton, J. M. 2008. Wild pedigrees: the way forward. Proceedings of the Royal Society of London B 275:613–621.

Perry, G. M. L., R. G. Danzmann, M. M. Ferguson, and J. P. Gibson. 2001. Quantitative trait loci for upper thermal tolerance in outbred strains of rainbow trout (*Oncorhynchus mykiss*). Heredity 86:333–341.

Perry, G. M. L., M. M. Ferguson, T. Sakamoto, and R. G. Danzmann. 2005. Sex-linked quantitative trait loci for thermotolerance and length in the rainbow trout. Heredity 96:97–107.

Phillips, R. B., K. M. Nichols, J. J. DeKoning, M. R. Morasch, K. A. Keatley, C. Rexroad III, S. A. Gahr, R. G. Danzmann, R. E. Drew, and G. H. Thorgaard. 2006. Assignment of rainbow trout linkage groups to specific chromosomes. Genetics 174:1661–1670.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Reich, D. E., M. Cargill, S. Bolk, J. Ireland, P. C. Sabeti, D. J. Richter, T. Lavery, R. Kouyoumjian, S. F. Farhadian, R. Ward, and E. S. Lander. 2001. Linkage disequilibrium in the human genome. Nature (London) 411:199–204.

Rexroad, C. E., Y. Palti, S. A. Gahr, and R. L. Vallejo. 2008. A second-generation genetic map for rainbow trout (*Oncorhynchus mykiss*). BMC Genetics 9:74.

Rexroad, C. E., M. F. Rodriguez, I. Coulibaly, K. Gharbi, R. G. Danzmann, J. DeKoning, R. Phillips, and Y. Palti. 2005. Comparative mapping of expressed sequence tags containing microsatellites in rainbow trout (*Oncorhynchus mykiss*). BMC Genomics 6:54.

Reznick, D., H. Bryga, and J. A. Endler. 1990. Experimentally induced life history evolution in a natural population. Nature (London) 346:357–359.

Reznick, D., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science (Washington, D.C.) 275:1934–1937.

Rieseberg, L. H., J. Whitton, and K. Gardner. 1999. Hybrid zones and the genetic architecture of a barrier to gene flow between two sunflower species. Genetics 152:713–727.

Rogers, S. M., and L. Bernatchez. 2005. Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (*Coregonus clupeaformis)*. Molecular Ecology 14:351–361.

Rogers, S. M., and L. Bernatchez. 2007. The genetic architecture of ecological speciation and the association with signatures of selection in natural lake whitefish (*Coregonus* sp. Salmonidae) species pairs. Molecular Biology and Evolution 24:1423–1438.

Schlötterer, C. 2002. A microsatellite-based multilocus screen for the identification of local selective sweeps. Genetics 160:753–763.

Schlötterer, C. 2003. Hitchhiking mapping: functional genomics from the population genetics perspective. Trends in Genetics 19:32–38.

Schuelke, M. 2000. An economic method for the use of fluorescent labeling of PCR fragments. Nature Biotechnology 18:233–234.

Scotti-Saintagne, C., S. Mariette, I. Porth, P. G. Goicoechea, T. Barreneche, C. Bodénès, K. Burg, and A. Kremer. 2004. Genome scanning for interspecific differentiation between two closely related oak species (*Quercus rubus* L. and *Q. petraea* [Matt.] Liebl.). Genetics 168:1615–1626.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Canadian Journal of Fisheries and Aquatic Sciences 53:833–841.

Shapovalov, L., and A. C. Taft. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*). California Department of Fish and Game, Fish Bulletin 98.

Slate, J. 2005. Quantitative trait locus mapping in natural populations: progress, caveats, and future directions. Molecular Ecology 14:363–379.

Small, M. P., T. D. Beacham, R. E. Withler, and R. J. Nelson. 1998. Discrimination of coho salmon (*Oncorhynchus kisutch*) populations within the Fraser River, British Columbia, using microsatellite DNA markers. Molecular Ecology 7:141–155.

Stanewsky, R. 2003. Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. Journal of Neurobiology 54:111–147.

Stephan, W., T. Wiehe, and M. W. Lenz. 1992. The effect of strongly selected substitutions on neutral polymorphism: analytical results based on diffusion theory. Theoretical Population Biology 41:237–254.

Stockwell, C. A., and S. C. Weeks. 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). Animal Conservation 2:103–110.

Storz, J. F. 2005. Using genome scans of DNA polymorphism to infer adaptive population divergence. Molecular Ecology 14:671–688.

Taillon-Miller, P., I. Bauer-Sardiña, N. L. Saccone, J. Putzel, T. Laitinen, A. Cao, J. Kere, G. Pilia, J. P. Rice, and P.-Y. Kwok. 2000. Juxtaposed regions of extensive and minimal linkage disequilibrium in human *Xq25* and *Xq28*. Nature Genetics 25:324–328.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Thorpe, J. E., M. Mangel, N. B. Metcalfe, and F. A. Huntingford. 1998. Modeling the proximate basis of salmonid life history variation with application to Atlantic salmon, *Salmo salar* L. Evolutionary Ecology 12:581–599.

Thrower, F. P., J. J. Hard, and J. E. Joyce. 2004. Genetic architecture of growth and early life history transitions in anadromous and derived freshwater populations of steelhead. Journal of Fish Biology 65(Supplement A):286–307.

Tsumura, Y., T. Kado, T. Takahashi, N. Tani, T. Ujino-Ihara, and H. Iwata. 2007. Genome scan to detect genetic structure and adaptive genes of natural populations of *Cryptomeria japonica*. Genetics 176:2393–2403.

Turner, T. L., M. W. Hahn, and S. V. Nuzhdin. 2005. Genomic islands of speciation in *Anopheles gambiae*. PLoS Biology 3:1572–1578.

Unwin, M. J., M. T. Kinnison, N. C. Boustead, and T. P. Quinn. 2003. Genetic control over survival in Pacific salmon (*Oncorhynchus* spp.): experimental evidence between and within populations of New Zealand Chinook salmon (*O. tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 60:1–11.

Vasemägi, A., J. Nilsson, and C. R. Primmer. 2005. Expressed sequence tag-linked microsatellites as a source of gene-associated polymorphisms for detecting signatures of divergent selection in Atlantic salmon (*Salmo salar* L.). Molecular Biology and Evolution 22:1067–1076.

Vitalis, R., K. Dawson, and P. Boursot. 2001. Interpretation of variation across marker loci as evidence of selection. Genetics 158:1811–1823.

Vitalis, R., K. Dawson, P. Boursot, and K. Belkhir. 2003. DetSel 1.0: a computer program to detect markers responding to selection. Journal of Heredity 94:429–431.

Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370.

Whitmore, D., N. S. Foulkes, U. Strahle, and P. Sassone-Crosi. 1998. Zebrafish clock rhythmic expression reveals independent peripheral circadian oscillators. Nature Neuroscience 1:701–707.

Wiehe, T. 1998. The effect of selective sweeps on the variance of the allele distribution of a linked multiallele locus: hitchhiking of microsatellites. Theoretical Population Biology 53:272–283.

Wiehe, T., and W. Stephan. 1993. Analysis of a genetic hitchhiking model and its application to DNA polymorphism data from *Drosophila melanogaster*. Molecular Biology and Evolution 10:842–854.

Wilding, C. S., R. K. Butlin, and J. Grahame. 2001. Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. Journal of Evolutionary Biology 14:611–619.

Williamson, K. S., J. F. Cordes, and B. P. May. 2002. Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and cross-species amplification in other salmonids. Molecular Ecology Notes 2:17–19.

Wilson, M. F. 1997. Variation in salmonid life histories: patterns and perspectives. USDA (U.S. Department of Agriculture) research paper PNW-RP-498. Available: fs.fed.us/pnw/pubs/pnw_rp498.pdf. (August 2010).

Wootton, J. C., X. Feng, M. T. Ferdig, R. A. Cooper, J. Mu, D. I. Baruch, A. J. Magill, and X. Z. Su. 2002. Genetic diversity and chloroquine selective sweeps in *Plasmodium falciparum*. Nature (London) 418:320–323.

Supplementary data available at http://dx.doi.org/10.1080/00028487.2011.588094