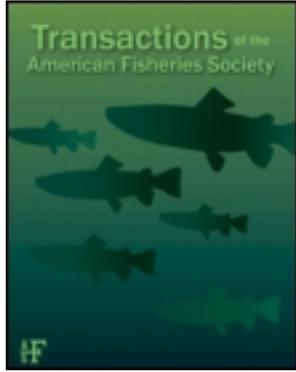


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Major Lineages and Metapopulations in Columbia River *Oncorhynchus mykiss* Are Structured by Dynamic Landscape Features and Environments

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SPECIAL SECTION: GENETIC ADAPTATION

Major Lineages and Metapopulations in Columbia River *Oncorhynchus mykiss* Are Structured by Dynamic Landscape Features and Environments

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Abstract

It is widely recognized that genetic diversity within species is shaped by dynamic habitats. The quantitative and molecular genetic patterns observed are the result of demographics, mutation, migration, and adaptation. The populations of rainbow trout *Oncorhynchus mykiss* in the Columbia River basin (including both resident and anadromous forms and various subspecies) present a special challenge to understanding the relative roles of those factors. Standardized microsatellite data were compiled for 226 collections (15,658 individuals) from throughout the Columbia and Snake River basins to evaluate the genetic patterns of structure and adaptation. The data were primarily from fish of the anadromous life history form, and we used a population grouping procedure based on principal components and hierarchical *k*-means clustering to cluster populations into eight aggregates or groups with similar allele frequencies. These aggregates approximated geographic regions, and the two largest principal components corresponded to ancestral lineages of Sacramento redband trout *O. m. stonei*, coastal rainbow trout *O. m. irideus*, and interior Columbia River redband trout *O. m. gairdneri*. Genetic data were partitioned among primary aggregates (lower Columbia, middle–upper Columbia, and Snake rivers), and the magnitude of genetic divergence relative to genetic diversity was analyzed (per locus) to test for evidence of selection and subsequent signals of adaptation. Two loci showed higher divergence than expected by chance (i.e., positive selection); however, both of these loci were on the fringe of the 99% confidence level and are potential false positives. Genetic patterns were also significantly correlated with certain environmental and habitat parameters (e.g., precipitation), but the extent to which those correlations are causal as opposed to effectual remains unclear. Despite the remaining questions, these data provide a foundation for more detailed investigations of harvest, admixture, and introgression between hatchery- and natural-origin fish and differences in reproductive success among individuals as well as monitoring trends in productivity.

The genus *Oncorhynchus* contains a diverse assemblage of species inhabiting freshwater and marine habitats of western North America and eastern Asia. The two major North American evolutionary lineages are Pacific salmon and the cutthroat and rainbow trouts (Stearns and Hendry 2004). Behnke (1992) presented a general model for the evolution of rainbow trout *O. mykiss*. During the Pleistocene Epoch a series of specialized populations formed in the interiors of large river systems, and there are thought to be four taxonomic groups within rainbow trout: the Sacramento redband trout *O. mykiss stonei*, the Klamath Lake redband trout *O. mykiss newberrii*, the Columbia River redband trout *O. mykiss gairdneri*, and the more ubiquitous coastal rainbow trout *O. mykiss irideus* (Behnke 1992; Currens et al. 2009). In this paper we will follow the naming convention of Behnke (1992) and focus on the genetic and ecological characteristics of the Columbia River redband and coastal rainbow trout present in the Columbia and Snake River basins.

Rainbow trout exhibit differences in migration behavior, ocean-going (i.e., anadromous) individuals being referred to as steelhead and nonmigratory (nonanadromous) individuals as resident rainbow trout or redband trout; however, anadromy as an evolutionary character appears to have little value for inferring historical relationships (McDowall 1997). While the

genetic architecture of the anadromous phenotype is complex (Nichols et al. 2008), anadromy may have arisen multiple times in the evolutionary record (Stearley and Smith 1993; Oakley and Phillips 1999), and instances of populations losing or gaining anadromy have been reported (Unwin et al. 2000; Quinn et al. 2001; Riva-Rossi et al. 2007). Additionally, anadromous forms may produce nonanadromous offspring and vice versa (Zimmerman and Reeves 2000; Thrower et al. 2004). At least three *O. mykiss* subspecies are currently present in the Columbia and Snake River systems (Sacramento redband, coastal rainbow, and Columbia River redband trout), although their geographic distributions are quite different (Busby et al. 1996; Scott and Gill 2008). Individuals that migrate may do so over a broad time period, such that stream-maturing “summer steelhead” often return to freshwater in April–March (peaking in July) and ocean-maturing “winter steelhead” commonly return in October–July (peaking in April) (Scott and Gill 2008). Winter-run steelhead predominate in the lower Columbia River west of the Cascade Mountain crest, and with few exceptions naturally reproducing steelhead that occur in the interior Columbia River basin are summer run, as earlier migration timing is required to traverse the long distances to spawning habitat (Busby et al. 1996). Genetic evidence has supported a biogeographic boundary at the Cascade crest, which presumably demarcates the eastward

extent of coastal rainbow trout and the transition to predominantly Columbia River redband trout (Allendorf 1975; Busby et al. 1996; Currens et al. 2009). Additionally, the transition from coastal to inland *O. mykiss* at the Cascade crest corresponds to the boundary between the lower Columbia River evolutionary significant unit (ESU) and middle Columbia River ESU. All five ESUs of *O. mykiss* from the Columbia River basin (lower Columbia River, middle Columbia River, upper Columbia River, upper Willamette River, and Snake River) are listed as threatened or endangered under the Endangered Species Act (Busby et al. 1996; USOF 2006).

The Columbia River basin has been a dynamic landscape, with freshwater habitats being shaped by glacial, volcanic, and tectonic forces (Orr and Orr 2006). As recently as 14,000 years ago, lobes of the Cordilleran ice sheet occupied northern Washington and Idaho, creating large glacial lakes (Waite and Thorson 1983). The phenotypic and genotypic diversity observed for *O. mykiss* endemic to the Columbia River basin may have evolved through chance extinctions and subsequent recolonizations as well as periods of localized isolation (Busby et al. 1996; Currens et al. 2009). Additionally, interactions among ancestral diversity and dynamic environmental processes have probably contributed to life history diversification (Storfer et al. 2007) and provided a mechanism for adaptation (Funk et al. 2005; Narum et al. 2008). Landscape features have been shown to shape population structure (Hanotte et al. 2002; Narum et al. 2008), but studies of this nature are not common for *Oncorhynchus* species. Recent anthropogenic alterations of habitat and artificial propagation efforts (Busby et al. 1996) compound the diversifying pressure of the natural landscape. Issues like barriers to movement and the introduction of nonnative stocks of *O. mykiss* have influenced the genetic affinities observed currently and may be altering fitness potential by changing behavioral, physiological, and genomic characteristics optimized for local habitats.

An extensive genetic data set (in terms of both geographic scope and density) based on microsatellite loci is presented for *O. mykiss* collections from throughout the Columbia River basin. These data represent the consolidation of regional genetic data sets from a consortium of Pacific Northwest genetics laboratories (Washington Department of Fish and Wildlife, Idaho Department of Fish and Game, Columbia River Inter-Tribal Fish Commission, the National Oceanic and Atmospheric Administration's Northwest Fisheries Science Center, and the U.S. Fish and Wildlife Service's Abernathy Fish Technology Center) that were standardized following the Stevan Phelps Allele Nomenclature (SPAN) convention (Stephenson et al. 2009). Our primary objective was to generate a comprehensive survey of the genetic variation among the steelhead present in the system in the context of historical contingency and adaptation. We described the influence of ancestral lineage, geography, and artificial propagation on genetic diversity. We also compared the observed population structure with that expected under neutrality and explored correlations between population structure and

environmental variables (precipitation, temperature, and elevation). These efforts will provide a foundation for further study of evolutionary issues such as life history diversity, gene flow dynamics between ecotypes, adaptation, and the role of historical and contemporary landscape processes in shaping molecular and quantitative genetic diversity. The data may also assist with regulatory issues such as the impacts of harvest, hatchery interactions, recovery planning, and trends in productivity among conservation units. The perspective we seek is especially relevant to extinction risk conservation priorities under various climate change scenarios.

METHODS

Tissue Collection

Our combined efforts collected samples of *O. mykiss* from 226 locations in the Columbia and Snake River basins (Figure 1). Although these collections predominantly targeted the anadromous life history form of *O. mykiss*, resident rainbow trout are included in the data set. Additionally, collections of basin-transferred nonnative Sacramento redband trout (i.e., the McCloud River stock) and "early-winter" steelhead derived from Puget Sound's Chamber's Creek coastal stock are also represented. Overall, 15,658 individuals were sampled (see Table A.1 in the appendix), with 18 collections of hatchery origin and 200 of natural origin (8 collections were of unknown origin). With respect to the distribution of adult run timing, these collections were partitioned into early winter (1 collection), late winter (1), winter (32), summer (142), nonmigratory redband trout (3), and unknown run type (47). The collections in Table A.1 are organized regionally following the ESU designations presented by Busby et al. (1996): southwest Washington (number 3), lower Columbia River (4), upper Willamette River (5), middle Columbia River (13), upper Columbia River (14), and Snake River (15). Additionally, more refined regional identifiers were used in association with the genetic clustering analysis, with coding as follows: Columbia Lower Winter (1), Columbia Lower Summer (2), Willamette Winter (3), Willamette Summer (4), Columbia Middle Summer (5), Deschutes Summer (6), Columbia Upper Summer (7), Snake Summer (8), Grande Ronde Summer (9), Imnaha Summer (10), Clearwater Summer (11), and Salmon Summer (12). Collection methods varied across laboratories, but collections typically consisted of operculum punches or fin tissue that were either stored in ethanol or air-dried.

Laboratory Analysis

DNA was extracted from stored tissue by a representative laboratory using a silica-based membrane procedure (e.g., Nucleospin 96 [Macherey-Nagel] or DNAEasy [Qiagen]) following the manufacturer's standard protocol. Polymerase chain reaction (PCR) amplification was performed using 13 fluorescently end-labeled microsatellite marker loci, namely, *Ogo4* (Olsen et al. 1998), *Oke4* (Buchholz et al. 1999), *Oki23* (Smith et al.

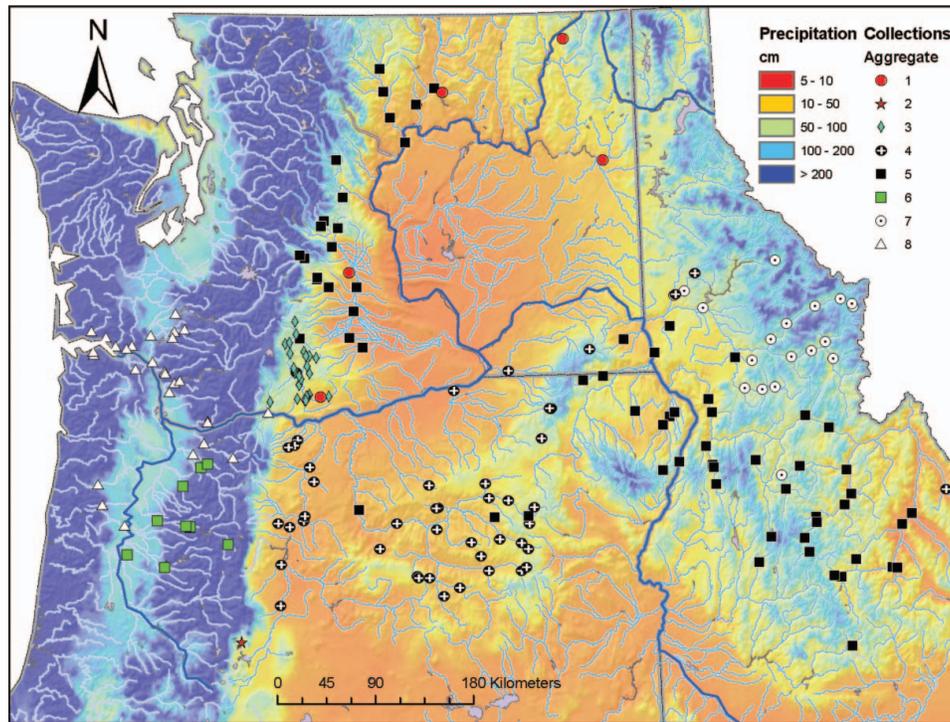


FIGURE 1. Map of the locations at which *Oncorhynchus mykiss* samples were collected, incorporating aggregates derived from the k -means procedure and mean annual precipitation.

1998), *Omy1001* and *Omy1011* (Spies et al. 2005), *Omy7* (K. Gharbi, French National Institute for Agriculture Research, unpublished), *One14* (Scribner et al. 1996), *Ots100* (Nelson and Beacham 1999), *Ots3* and *Ots4* (Banks et al. 1999), *Ssa289* (McConnell et al. 1995), and *Ssa407* and *Ssa408* (Cairney et al. 2000). The PCR reaction protocols varied among laboratories (Stephenson et al. 2009). Standardized microsatellite data were produced by all laboratories following the SPAN convention.

Genetic Data Analysis

Within-locus and within-population genetic diversity.—MICRO-CHECKER (Van Oosterhout et al. 2004) was used to determine whether the excess homozygosity observed per locus was consistent with the presence of null alleles or genotyping errors (e.g., large-allele dropout). Some collections consisted of temporally replicated samples from the same site and/or geographically proximate sites, so it was necessary to determine whether sample consolidation could occur. To this end, allele frequencies were compared within and among collection sites by the simulated Fisher's exact test implemented in FSTAT version 2.9.3.2 (Goudet 1995). Alleles were randomized between samples (i.e., by the genic test) and the significance level was adjusted for multiple comparisons using the Bonferroni correction ($\alpha = 0.05$; Rice 1989). Genetic diversity was quantified for each collection following Nei's (1987) unbiased gene diversity formula (H_S), Hedrick's (1983) formula for observed

heterozygosity (H_o), and Kalinowski's (2004) unbiased rarefaction method for allelic richness.

For each locus and collection, GENEPOP 4.3 (Raymond and Rousset 1995) was used to assess Hardy–Weinberg equilibrium, where deviations from the neutral expectation of random associations among alleles (F_{IS}) were calculated using a simulated Fisher's exact test. The acceptance zone for the null hypothesis was generated using Markov chain–Monte Carlo (MCMC) simulation (Raymond and Rousset 1995). If the null hypothesis of random association among genotypes was rejected, a second test was performed to explicitly test the alternatives (i.e., heterozygote excess or deficit; Raymond and Rousset 1995). The significance levels for all tests of Hardy–Weinberg expectation were adjusted for multiple comparisons using the Bonferroni correction ($\alpha = 0.05$; Rice 1989). Calculation of linkage (gametic-phase) disequilibrium followed Weir (1979), where statistical significance was assessed by a permutation procedure for each collection by comparing the proportion of MCMC realizations of the pairwise linkage disequilibrium value to the observed value (Belkhir et al. 2001). Results are reported as the proportions of pairwise combinations that were significant at $\alpha = 0.05$ (P -values were adjusted using the Bonferroni correction).

To test for past bottleneck events (population reductions up to 500 generations ago), we used the software program AGARST (eric.harley@uct.ac.za) to calculate a mean M -ratio and its variance for each collection. The M -ratio is the ratio of the number of alleles present at a locus (k) to the range of allele size in

base pairs (r) for the same locus (Garza and Williamson 2001). During population declines, k decreases faster than r , resulting in gaps in the size distribution of alleles and a corresponding reduction in the M -ratio. Four loci (*Oke4*, *Omy7*, *Omy1001*, and *Ssa289*) were dropped from these calculations because they exhibited 1 base pair mutation, which violates the assumptions of the model. To determine the significance of the estimated M -ratios, we also ran the software program Critical_M.exe to calculate a critical value (M_{CRIT}) below which a past bottleneck event is inferred. The M_{CRIT} estimates were calculated using three values: theta, the percentage of one-step mutations, and the mean size of non-stepwise mutations. For the last two parameters, we used the recommended values of 0.1% and 3.5, respectively (Garza and Williamson 2001). For theta, which was calculated from the equation

$$\text{Theta} = 4 \times \text{effective population size} \times \text{mutation rate},$$

we used a mutation rate of 0.0005 (Estoup and Angers 1998) and a prebottleneck effective population size (N_e) of 5,000 (theta = 10). Because the chosen effective population size affects theta, we explored a range of values between 500 and 5,000. For example, setting theta to 1 (corresponding to an N_e of 500) yielded an M_{CRIT} ranging from 0.88 to 0.77. Increasing theta to 10 (corresponding to an N_e of 5,000) yielded an M_{CRIT} of 0.81–0.72. We adopted the more conservative M_{CRIT} value of 0.72, and collections that exhibited lower M -ratios were considered to provide evidence of past bottleneck events. Based on recommendations from Garza and Williamson (2001), M -ratios estimated for collections with sample sizes less than 25 were not included as part of the M -ratio summary descriptions.

Among-population genetic differentiation.—Some tests of the distribution of genetic diversity may be influenced by the inclusion of demographically separate populations; therefore, STRUCTURE 2.2 (Pritchard et al. 2000) was used to identify high-level genetic affinities. The genetic partition identified by STRUCTURE was applied to subsequent neutrality tests and multivariate multiple regression (see below). STRUCTURE sorts individuals (or portions of individuals if they appear to have mixed ancestry) into a number of hypothetical genetic clusters or populations (K) to minimize Hardy–Weinberg disequilibrium and linkage disequilibrium in the clusters or populations. The program calculates a likelihood value for the number of clusters or populations given the data, with the highest likelihood value among the number of K s tested indicating the number of genetically identifiable clusters in the data set. We included the entire greater Columbia River basin data set in one analysis and set the number of clusters or possible populations at 1–9. For simplicity, the collections from the southwest Washington ESU were combined with those from the lower Columbia ESU for this analysis. Further, since the Goldendale and Spokane hatcheries were physically located in the upper Columbia River, we included these collections in the middle and upper Columbia River region despite their stock origin being nonnative Sacra-

mento redband trout (Crawford 1979; Busack and Gall 1980; Busby et al. 1996). As Goldendale and Spokane hatchery *O. mykiss* have been released in the basin, by including them in the data set we hypothesized that introgression is detectable due to their ancestral differences.

We ran five iterations of K -values 1 through 9 with 100,000 burn-in runs and 400,000 iterations and averaged the estimated log likelihoods ($\log_e[\text{Pr}\{K\}]$) to determine the most likely value of K . We calculated ΔK using the method described by Evanno et al. (2005), with $K = 2$ being the most likely (data not shown). Subsequently, 40 iterations were run at $K = 2$, and the 10 iterations with the highest $\log_e[\text{Pr}\{K\}]$ value were averaged using the full search algorithm in CLUMPP (Jakobsson and Rosenberg 2007), which finds the configuration having the highest pairwise similarity (H') to align cluster membership. After examining 512 possible run configurations and K clusters, the best configuration ($H' = 0.998$) was used in CLUMPP to average individual fish Q -values (membership per cluster) across the ten STRUCTURE iterations. Setting $K = 2$ corresponded to a division within the data set between coastal and inland subspecies of *O. mykiss* (data not shown). Therefore, Q -values above 50% represented the assignment of each individual to either the coastal or the inland lineage.

We constructed a consensus neighbor-joining dendrogram by using bootstrap resampling of loci (1,000 iterations) and clustering based on chord distances (Cavalli-Sforza and Edwards 1967) among collections. The computer programs Populations 1.2.30 (Langella 1999) and FigTree were used for bootstrap consensus clustering and creation of the dendrogram, respectively. To facilitate interpretation of geographic relationships, we collapsed branches that contained only collections from a given region. For reasons explained in Results, the collapsing of branches was done irrespective of the bootstrap support for those individual branches.

Principal component and k-means clustering analysis.—We used a population aggregation procedure based on principal components and k -means clustering to cluster collections into groups with similar allele frequencies. A more detailed description and validation of this procedure is forthcoming (Warheit, unpublished); we summarize the method as follows: First, we reduced the number of collections from 226 to 220, eliminating those with sample sizes less than 10. Second, we performed a principal components analysis (PCA) on a matrix of standardized-collection allele frequencies (220 collections by 301 alleles). Standardized allele frequencies have means equal to zero and unit standard deviations, and their use in a PCA is equivalent to conducting the PCA with a correlation rather than a covariance matrix (see Jombart et al. 2009 for a brief summary of the history of PCA in genetic analyses and Manly 1986 for a simple description of PCA methods and terminology). Since each collection is represented by a sample, the allele frequencies for each collection depend on the individuals sampled within that population. It is desirable to construct a set of principal components that is stable under conditions of changing

collection allele frequencies, similar to what would result from adding individuals to a collection or sampling repeatedly from each collection. To obtain this set of stable components, we bootstrapped the original genotypic data by randomly selecting, with replacement, individuals from each collection while maintaining the original sample size per collection. We then repeated the PCA procedure described above, generating a new set of allele coefficients (i.e., eigenvectors). We repeated this bootstrap resampling procedure 500 times, generating 500 sets of allele coefficients for 301 principal components. The bootstrapped principal components differed from each other and from the original PCA as a function of the individuals included in the analysis, and therefore by changes in the collection allele frequencies. To measure principal component stability, we calculated means and standard deviations for the allele coefficients from all 301 components across all 500 bootstrap runs. An allele coefficient for a component was considered stable if its 95% confidence interval, calculated as the absolute value of its mean minus 1.96 times its standard deviation, did not include zero. A principal component was considered stable if at least one of its allele coefficients was stable. This process reduced the number of components from 301 to 11 (principal components 1 through 11).

Collections with similar allele frequencies tend to cluster near each other when principal components are plotted, and principal components are often viewed as bivariate plots, showing principal components 1 and 2. Instead of arbitrarily limiting our analysis to these two dimensions, we aggregated collections based on their clustering across 11 dimensions defined by the stable principal components 1–11. To objectively define clusters, we used k -means clustering, varying k from 1 to some large number that was less than the total number of populations (in this case 25). K -means clustering is a well-established clustering method (e.g., MacQueen 1967, Dillon and Goldstein 1984; Morrison 1990; and see Jombart et al. 2010 for an application of PCA and k -means clustering similar to that presented here) that produces clusters of collections that minimize within-cluster distances summed across all clusters. Initially, k centroids were randomly chosen and each collection was assigned to a cluster such that collections-to-centroid distances were minimized. This initial step assigned each collection to a cluster, and based on these clusters a new set of centroids were calculated, shifting each centroid from an initial random position to a new position that was calculated directly from the collections within the cluster. Since the centroids had shifted, collection assignments may have shifted, so new collections-to-centroid distances were calculated. This process was repeated until the centroids were stable, the cluster composition converged, and the sum of within-cluster distances did not change. Since this method started with a randomly selected set of centroids, the analysis was replicated 500 times for each value of k , where each replicate started with a different set of centroid positions and cluster assignments were selected with the lowest total point-to-cluster centroid distance.

To determine which of the $k = 1$ –25 clusters provided the best fit for the data, we evaluated each set of clusters by calculat-

ing a silhouette score (Rousseeuw 1987) for each collection and value of k . A collection's silhouette score compared its average within-cluster distance (i.e., the average of the distances between that collection and each of the other collections within its cluster) with the smallest of the between-cluster distances (i.e., the average distance between the collection and all of the collections within a cluster to which it is not a member, repeated for all other clusters; the smallest between-cluster distance is usually with the collection's neighboring cluster). Silhouette scores range from -1 to $+1$, with 1 indicating that the collection is tightly clustered with the collections within its assigned cluster, values less than 0 indicating that the collection is closer to its neighboring cluster than to its assigned cluster, and 0 indicating that the collection falls between its assigned cluster and its neighboring cluster. We calculated the mean silhouette score for each value of k and selected the k with the highest mean.

Aggregates of collections can be hierarchical (i.e., groups within groups). To determine the hierarchical structure of our k groups, for each of the k clusters defined above we conducted a separate k -means clustering analysis, limiting each analysis to the collections within that cluster. We repeated this procedure for each subsequent cluster until any further subdivision of the cluster would result in at least one group with few collections (here, 10 populations). In other words, we hierarchically clustered collections until we obtained the maximum number of clusters, each composed of more than 10 collections. Choosing a threshold value of 10 collections was arbitrary; however, if we used the next level in the hierarchy for each aggregate, we would have had a total of 64 aggregates, with more than half the clusters consisting of a single collection (data not shown). While further description of the k -means clustering method will be presented in a forthcoming article, the final sets of clusters presented here were aggregates of collections with similar allele frequencies, as defined within principal component space. We performed this collection aggregation procedure using a program written by Warheit in MatLab (version 2010a; The MathWorks).

Neutrality tests.—Since genetic variation at markers linked to genes may be affected by natural selection and thus deviate from neutral expectations, it is possible to identify loci under selection by outlier patterns (i.e., Luikart et al. 2003). Outlier tests compare the estimated genetic differentiation (F_{ST}) of each locus given its allelic diversity (heterozygosity) with that expected under neutrality. Selection that is divergent (i.e., positive) is expected to result in a larger genetic distance among collections than expected relative to neutral markers, and conversely, balancing selection will result in a lower genetic distance than expected. The null hypothesis of neutrality is rejected if a locus has an unlikely F_{ST} value given the range of F_{ST} and H_T expected under the neutral model. Patterns of deviation from neutral expectations among the 13 microsatellite loci were investigated with the outlier approach of Beaumont and Nichols (1996) as implemented in LOSITAN (Antao et al. 2008). Simulations were run to independently generate a distribution of F_{ST} based on 50,000 replicates for 13 microsatellites under a neutral

stepwise mutation model. The simulation results were then plotted to represent the median and 99% quantiles. Loci with F_{ST} values lying above or below these quantiles were outliers potentially under directional or balancing selection, respectively. Simulations were done iteratively to avoid an upward bias in quantile ranges (potentially resulting in a type I error for balancing selection) by removing outlier loci above the 99% quantiles in the initial runs as implemented in LOSITAN. Collections were split into one of two lineages, coastal or inland, for two separate runs in LOSITAN. Since inclusion of demographically distinct populations can greatly influence outlier tests (Excoffier et al. 2009), only collections with a mean membership of 95% or more to either the coastal or inland lineage (as determined with STRUCTURE; see above) were included in the outlier tests. With these criteria, 37 collections comprised the coastal set and 76 collections were included in the inland set.

Landscape features and population structure.—DISTLM forward (McArdle and Anderson 2001) was used to perform a multivariate multiple regression on the basis of a pairwise F_{ST} distance measure and sets of predictor variables with permutation tests. This analysis has been increasingly used for landscape genetic applications (Geffen et al. 2004; Carmichael et al. 2007; Olsen et al. 2010a). The forward-selection procedure fits individual environmental predictor variables or sets of predictor variables sequentially in the linear model. In our case, we used the following predictor variables: the Euclidean distance derived from latitude and longitude in decimal degrees, elevation (m), precipitation (total annual accumulation [cm]), annual daily means of minimum and maximum temperature ($^{\circ}$ C), and lineage (based on average individual Q -values from the STRUCTURE analysis greater than 50% to either the coastal or inland lineage). Elevation was determined from a U.S. Geological Survey 10-m digital elevation model using the National Elevation Data Set and Global Elevation Data (<http://www.latlontoelevation.com/>). Temperature and precipitation measurements were generated using PRISM (parameter–elevation regressions on independent slopes model) of the Oregon Climate Service (<http://www.prism.oregonstate.edu/>). Annual average maximum and minimum air temperatures were simulated at an 800-m cell resolution from a model based on climate normals from a 30-year period (1971–2000) in PRISM.

Individual estimates of population ancestry (Q -values) were calculated using a Bayesian analysis implemented in STRUCTURE (see above).

Marginal tests were performed to estimate the proportion of the total sum of squares explained by fitting each variable individually, ignoring other variables. We used 9,999 random permutations of the raw data (pairwise F_{ST} matrix) for the marginal tests. For conditional tests, the program uses permutations of residuals under a reduced model (Anderson 2003). Conditional tests were performed using a stepwise forward-selection procedure that identifies the most informative predictor variables sequentially while holding constant the variables already selected. We analyzed some of the predictor variables as sets, as

demonstrated by Anderson et al. (2004). Latitude and longitude made up our “distance” variable set, “lineage” was comprised by ancestry (Q -values) to either coastal and inland groups, and minimum and maximum temperatures were analyzed together as “temperature.” Elevation and precipitation were each analyzed as individual variables.

RESULTS

Microsatellite Diversity within Populations

Using statistical tests of allele frequency distributions, 336 collections (data not shown) were consolidated into the 226-collection data set that was analyzed (Table A.1). By definition, statistically significant differences in allele frequencies were observed for the 226 collections analyzed and no trends in genetic data artifacts were observed using MICRO-CHECKER (Van Oosterhout et al. 2004; data not shown). Collections were categorized based on the aggregate to which they were assigned (Table A.1) following the clustering analysis described above. Where informative, some genetic diversity metrics were also summarized over aggregates. Allelic diversity was high, with an average unbiased gene diversity of 0.77 calculated over all collections (range, 0.74–0.81). Mean allelic richness was 8.98 (range, 7.62 [aggregate 1] to 9.96 [aggregate 8]). The single-locus genotype frequencies for 219 of the 226 collections were consistent with Hardy–Weinberg expectations. In contrast, significant associations among alleles between different loci (linkage disequilibrium) were commonly observed, consistent with differentiation at the population, brood year, or family level.

M -ratio estimates varied widely across the study collections (0.51–0.86; Table A.1). Of the 192 collections that met the $n = 25$ sample size criterion, 82 (42.9%) showed evidence of past bottleneck events. Hatchery collections ($n = 18$) exhibited significantly lower average M -ratios than wild collections ($n = 166$) (0.67 versus 0.73; $t = -3.38$, $df = 182$, $P = 0.0012$). Winter-run collections ($n = 27$) exhibited significantly lower average M -ratios than summer-run collections ($n = 124$) (0.70 versus 0.74; $t = 2.62$, $df = 149$, $P = 0.0097$). Collections in the upper Columbia River ($n = 14$) exhibited significantly higher average M -ratios than collections in the lower Columbia River ($n = 24$) (0.78 versus 0.70; $t = -4.08$, $df = 36$, $P = 0.0002$). Collections in the Snake River basin ($n = 64$) also exhibited significantly higher average M -ratios than collections in the lower Columbia River (0.74 versus 0.70, $t = -3.66$, $df = 86$, $P = 0.0004$). When M -ratios were averaged over collections within aggregations, aggregates 4, 5, and 7 had values above the 0.72 threshold, which signified no statistical evidence of a population bottleneck (Table A.1).

Among-Population Genetic Differentiation

Based on STRUCTURE (see above) with $K = 2$ as the most likely number of identifiable clusters in the full data set—which differed from the principal component and k -means analysis below—the data partitioned into genetic clusters associated with

coastal and inland lineages as previously described by allozyme data and ecological criteria (Allendorf 1975; Busby et al. 1996). In general, steelhead from tributaries originating west of the Cascade crest occupied the genetic cluster identified as the coastal cluster (i.e., coastal rainbow trout *O. m. irideus*) and steelhead from tributaries originating east of the Cascade crest occupied the inland cluster (i.e., Columbia River redband trout *O. m. gairdneri*); however, some deviations from this pattern (data not shown) were apparent. For example, the Hood River collection (lower Columbia River ESU) was estimated to have an average inland ancestry (over all individuals) of 23%. In contrast, the Klickitat River collection (middle Columbia River ESU) showed 56% coastal ancestry averaged over all individuals from the basin. Additionally, the coastal cluster membership observed for Goldendale and Spokane hatchery collections, despite their physical location in the interior Columbia River basin, corroborated the out-of-basin (i.e., Sacramento redband trout *O. m. stonei*) origin of their broodstocks.

There was general concordance between the bootstrap consensus clustering of genetic distances and that of geographic regions (Figure 2). While the bootstrap values for a majority of the dendrogram nodes were small, suggesting little if any statistical support for topology, inspection of those branches indicated that there was broad concordance between geography and the relative relationships among collections within the dendrogram. Where clusters contained only populations from a single region, branches were collapsed (irrespective of bootstrap value) to facilitate presentation and interpretation of the clustering results in the context of geography (the full tree is available in the online version of this article). For example, the Clearwater River and upper Columbia River collections (regions 7 and 11, respectively) were distinct, with each forming a single major cluster that contained nearly all of the populations in that region. In contrast, the middle Columbia River summer populations (region 5) formed multiple distinct clusters.

Principal Component and *k*-Means Clustering Analysis

The first iteration of the *k*-means clustering analysis produced a maximum mean silhouette score at $k = 6$. For convenience, final cluster memberships are used to label each aggregate within this first iteration of the procedure. The initial six clusters corresponded to aggregates 1–3, 6, 8, and a combined 4, 5, and 7 (Figure 1; Table A.1). Aggregates 1 ($n = 6$) and 2 ($n = 1$) were composed of less than 10 populations; however, these aggregates were basal in the hierarchy and cannot be clustered with any other aggregate. Therefore, 1 and 2 remained distinct aggregates following the initial and secondary rounds of clustering despite being composed of few (or 1) collections.

During the second round of *k*-means clustering, aggregates 1–3, 6, and 8 could not be further subdivided such that no aggregate within these larger aggregates would be composed of less than 10 collections. Therefore, these five aggregates remained intact. However, the aggregate composed of 4, 5, and 7 could be further subdivided into two aggregates, composed of 4 and 5 as a single aggregate and 7. The third round of *k*-means

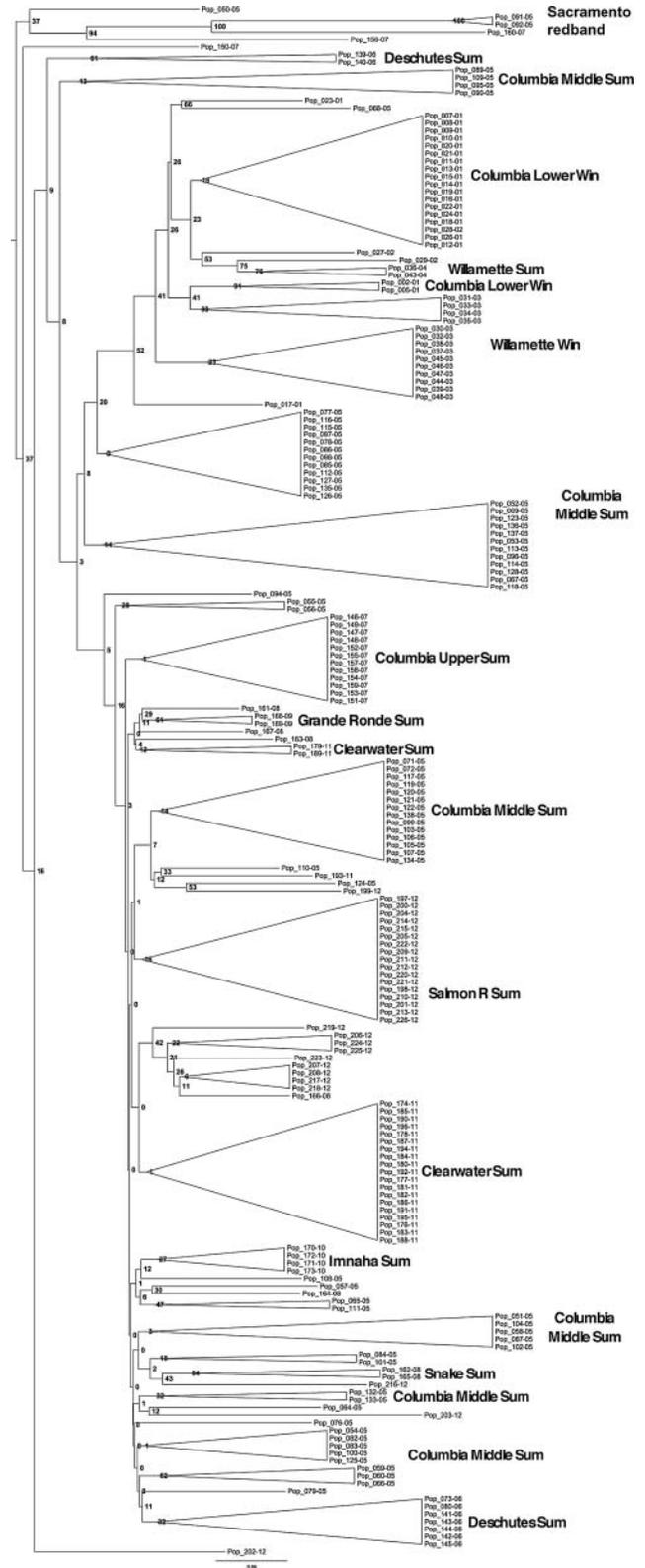


FIGURE 2. Consensus neighbor-joining dendrogram for *Oncorhynchus mykiss* populations. Clustering was based on chord distances among collections, and consensus was generated from 1,000 iterations of bootstrap resampling over loci. Branches that contained only collections from a given region were collapsed. [The full tree is available in the online version of this article.]

clustering did not subdivide aggregate 7 but did subdivide the combined 4 and 5 into distinct aggregates. Therefore, the hierarchical k -means procedure produced a total of eight aggregates, with aggregates 4 and 5 being more similar to each other than to aggregate 7 and aggregates 4, 5, and 7 being more similar to each other than to any other aggregate.

Following the hierarchical clustering described above, aggregate 1 was comprised of the Goldendale and Spokane Hatchery collections (whose origin is Sacramento redband trout), one collection from the middle Columbia River ESU (Umtanum), and two collections from the upper Columbia River ESU (Omak and Phalon Lake) (Figure 1; Table A.1). Aggregate 2 was comprised solely of the Crane Prairie collection. Thirty-two steelhead collections represented aggregate 3, with 29 from the middle Columbia River ESU, 1 from the lower Columbia River ESU (Clackamas River Hatchery), 1 from the upper Willamette River ESU (South Santiam Hatchery), and 1 from Skamania Hatchery (Figure 1; Table A.1). Aggregate 4 was composed of 47 collections, 42 from the middle Columbia River ESU, 1 from the upper Columbia River ESU (Okanogan River), and 4 from the Snake River ESU. Seventy-three collections comprised aggregate 5, 19 collections from the middle Columbia River ESU, 11 from the upper Columbia River ESU, and 43 from the Snake River ESU. Aggregate 6 was composed of 4 collections from the lower Columbia River ESU and 9 from the upper Willamette River ESU. Aggregate 7 was composed of 19 collections from the Snake River ESU. Twenty-nine collections comprised aggregate 8, 7 from the southwest Washington ESU, 19 from the lower Columbia River ESU, and 3 from the upper Willamette River ESU (Figure 1; Table A.1).

Neutrality Tests and Landscape Genetics

An F_{ST} outlier approach was used to explicitly test whether genetic loci deviated from neutral expectations. Simulations from LOSITAN indicated that two markers were putative outliers for divergent positive selection. For the analysis regarding coastal-origin collections, locus *Ssa407* was more divergent than expected by chance, lying above the upper 99% quantile (Figure 3). Regarding the inland collections partition, locus *Ots3M* was a statistically significant outlier, lying above the upper 99% quantile (Figure 3). Beyond tests for selection at individual loci, landscape genetics analyses were performed to investigate whether aspects of the spatial distribution of genetic diversity could be explained by environmental variables. DISTLM revealed that all five factors explained a significant portion of the variation in the genetic distance matrix ($P = 0.0001$), with lineage accounting for the most variance in both marginal (non-conditional) and conditional tests. Because correlations were high among some variables, such as elevation and minimum temperature ($r = -0.93$; Table 1), only conditional tests are reported. Conditional tests using forward selection showed that lineage, distance, and precipitation (in order of informativeness; Table 2) were the top-ranked predictor variables and each added significant proportions ($P = 0.0001$) of explained vari-

ation (cumulative proportion for the three variables = 33.3%). The stepwise addition of temperature to the model was not significant ($P = 0.064$), but further inclusion of elevation showed that the full model with all five variables was significant ($P = 0.0005$) and explained 36.3% of the variation.

DISCUSSION

In this study we evaluated the genetic structure of *Oncorhynchus mykiss* across the Columbia and Snake River basins using genetic data derived from 15,658 individuals (226 collections) and 13 standardized microsatellite loci (SPAN; Stephenson et al. 2009). Across this broad geographic area, ancestral lineage and the restriction of gene flow due to geographic separation were the dominant factors shaping genetic diversity. Genetic characteristics were probably also influenced by artificial propagation, genetic introgression of nonnative trout into native Columbia River redband trout populations, and the environment.

The k -means clustering analysis detected aggregates that flanked both sides of the Cascade crest (Figure 1), a known biogeographic break (Busby et al. 1996). A transition from coastal winter- and summer-run steelhead (i.e., coastal rainbow trout) west of the Cascade crest to Columbia River redband trout in the interior Columbia and Snake River basins was first suggested by Allendorf (1975), and genetic differences between the regions have been observed by others (Busby et al. 1996; Currens et al. 2009). Huzyk and Tsuyuki (1974) also observed a distinction between coastal and inland populations of *O. mykiss* in the Fraser River. We plotted the first and second principal components from the analysis using standardized-collection allele frequencies (Figure 4). Principal component 1 accounted for 9.4% of the total genetic variance and was situated along an axis from the interior of the basin toward the lower Columbia River. This result suggested that aggregates 6 and 8 correspond to coastal rainbow trout and aggregates 4, 5 and 7 to Columbia River redband trout. Aggregates 4, 5 and 7 were grouped together after the first round of k -means clustering. The disposition of aggregate 2 (Crane Prairie) and aggregate 3 (Big White Salmon and Klickitat rivers) was unclear, as these collections formed distinct clusters but appeared to be intermediate between coastal and inland *O. mykiss* (Figure 4). Additionally, it appears that the Klickitat River is a contact point between coastal rainbow trout and Columbia River redband trout that may be creating complex population dynamics and differential reproductive success of the lineages and life history strategies. Waples et al. (2004) provides a parallel example of the Cascade crest boundary's influence on life history diversity for Columbia River Chinook salmon *Oncorhynchus tshawytscha*. In the lower Columbia River, fall-run "Tule" Chinook salmon arrive sexually mature and predominate over a genetically similar spring run. In contrast, the interior Columbia and Snake River basins are occupied by genetically diverse earlier-returning and later-maturing summer and spring Chinook salmon, along with fall-run groups. The influence of geographic distance on genetic differentiation was also observed

TABLE 1. Correlation matrix for environmental variables included in landscape-genetic analyses for 226 populations of *O. mykiss* from the Columbia River. Temperature includes both the maximum (T_{\max}) and minimum (T_{\min}) mean annual daily means; distance includes latitude and longitude.

Variable	Elevation	Precipitation	Temperature		Distance		Lineage
			T_{\max}	T_{\min}	Latitude	Longitude	
Elevation	1.00						
Precipitation	-0.38	1.00					
T_{\max}	-0.78	-0.01	1.00				
T_{\min}	-0.93	0.42	0.77	1.00			
Latitude	-0.45	0.01	0.12	0.27	1.00		
Longitude	0.70	-0.49	-0.40	-0.69	-0.18	1.00	
Lineage	-0.56	0.64	0.24	0.58	0.06	-0.66	1.00

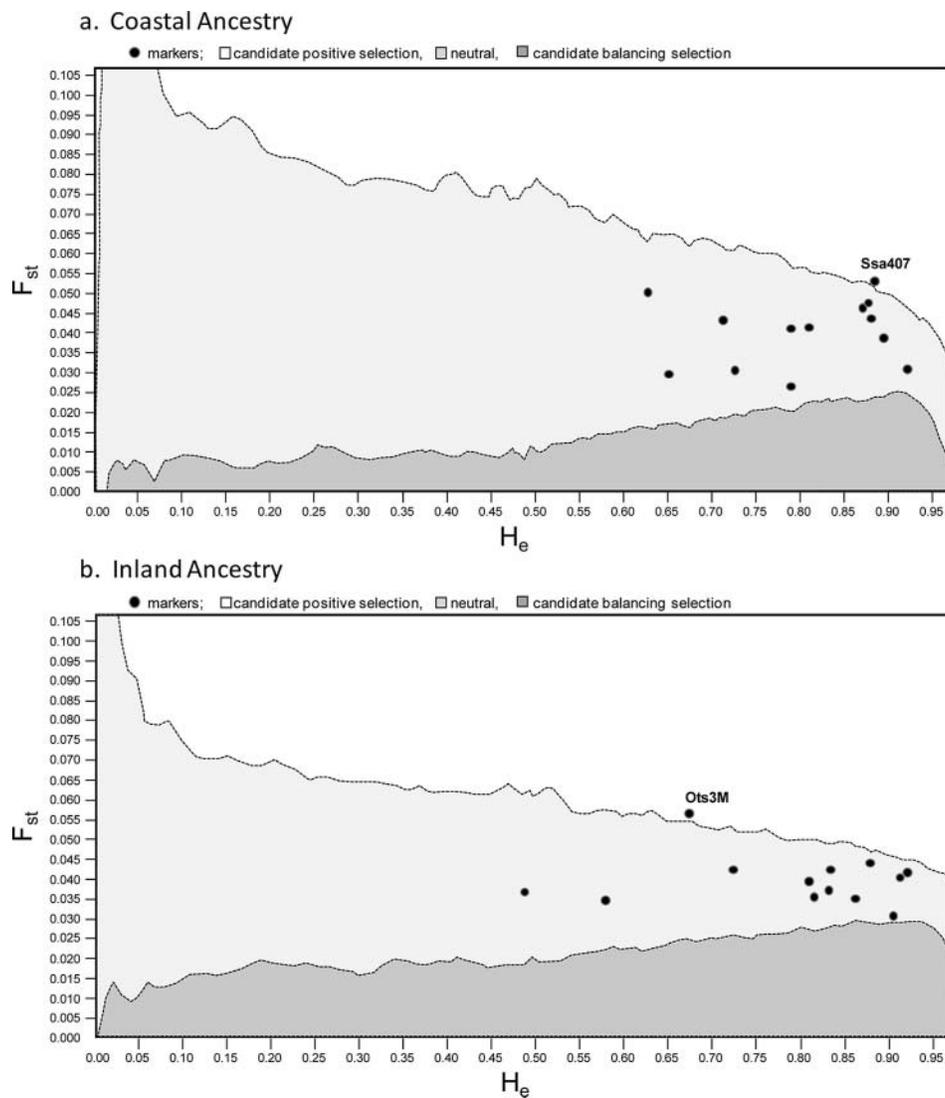


FIGURE 3. F_{ST} outlier simulation following Beaumont and Nichols (1996); H_e = expected heterozygosity.

TABLE 2. DISTLM results for both sequential-inclusion, forward-selection conditional analyses.

Variable	<i>F</i>	<i>P</i>	Proportion	Cumulative proportion
Lineage	75.61	0.0001	0.2524	0.2524
Distance	8.76	0.0001	0.0547	0.3071
Precipitation	8.73	0.0001	0.0263	0.3334
Temperature	2.00	0.0640	0.0119	0.3454
Elevation	5.96	0.0005	0.0174	0.3628

in these data, where the *k*-means analysis generated genetic clusters that were spatially coherent (Figure 1). Concordance between geographic and genetic distance was also observed in the neighbor-joining clustering of Cavalli-Sforza and Edwards (CSE) distances, although the relative relationships among collections were less compelling using the CSE distances than the *k*-means analysis because the partially distinct terminal groups on the dendrogram were poorly resolved (Figure 2).

Artificial propagation of steelhead in Washington State increased through the latter half of the 20th century, and current annual releases into the Columbia River basin are approximately 9 million smolts (Busby et al. 1996; United States v. Oregon 2008). In general, large-scale artificial production facilities in the Columbia River basin have operated to support harvest as opposed to maintaining diversity (Scott and Gill 2008). Stock transfers among hatcheries were commonplace,

and widespread releases of smolts from large production facilities have occurred throughout the Columbia and Snake River systems. For example, Wells Hatchery, located on the upper Columbia River and having a broodstock origin of mixed upper Columbia and Snake River descent, has released smolts into the middle and upper Columbia and Snake rivers (Howell et al. 1985). Lyons Ferry Hatchery, located on the Snake River and having a broodstock predominantly of Snake and Willowa River origin, has released smolts into the Snake and Walla Walla River systems (Delarm and Smith 1990). While artificial production facilities could have a variety of direct and indirect genetic effects (Utter 1998), homogenization of populations is a concern given the scale of the production in the Columbia River basin coupled with the downward trend in natural steelhead abundance. We observed a distinct genetic cluster composed primarily of collections from the Deschutes and John Day rivers (aggregate 4), where hatchery programs do not operate (Busby et al. 1996; United States v. Oregon 2008; Figure 1). Conversely, aggregate 5 showed genetic affinities across a wide geographic area, the area subjected to the stock transfers referred to above. These results suggest that gene flow among regions facilitated by artificial propagation has influenced genetic distinctiveness in the interior of the Columbia River basin. Yet, it should be pointed out that the middle Columbia River summer collections clustered in multiple areas of the dendrogram (Figure 2), suggesting that there is population-genetic structure that is not captured by the *k*-means analysis presented here.

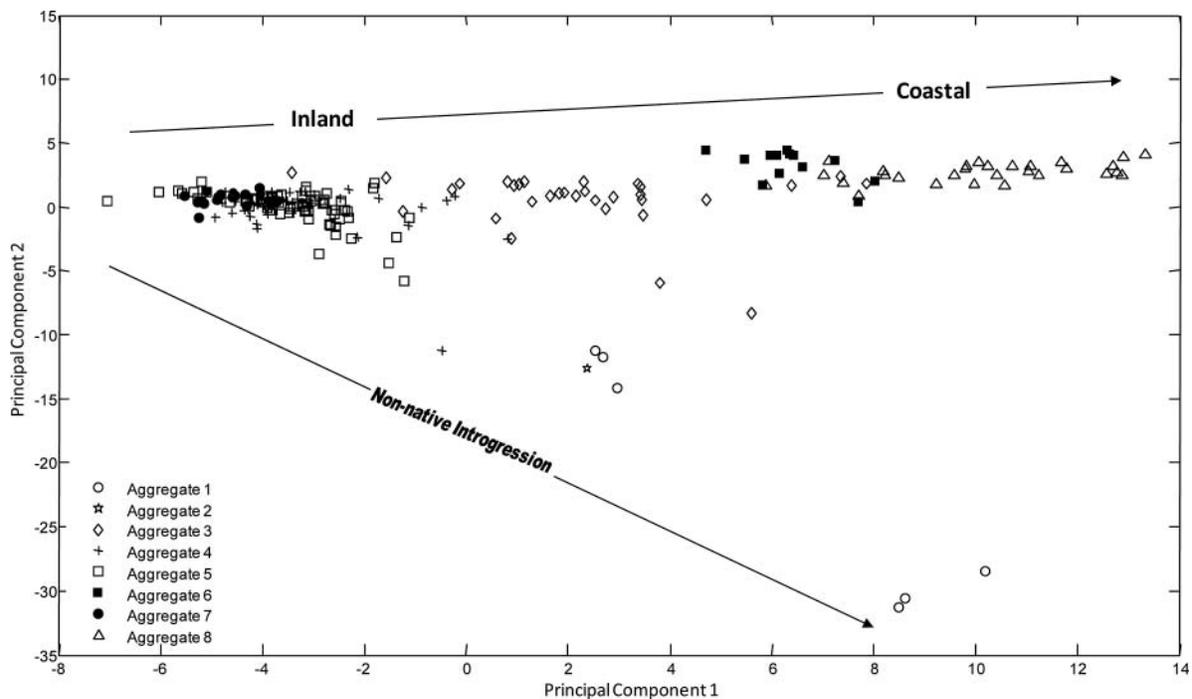


FIGURE 4. Plot of principal components 1 and 2 from an analysis of collection allele frequencies. Principal component (PC) 1 corresponds to inland (Columbia River redband trout) versus coastal subspecies (coastal rainbow trout) of *O. mykiss* and PC 2 to Sacramento River redband trout. The aggregates correspond to those shown in Figure 1 and Table A.1 and are composed of collections with similar allele frequencies.

Extinction–recolonization events and natural rates of gene flow could have also contributed to the genetic similarities among the collections from the Columbia and Snake River systems. While the dynamic geologic and environmental processes of the Columbia River basin may have led to lower overall species diversity (Smith et al. 2002), the evolutionary complexity of *O. mykiss* appears intact where the species still occurs (Currans et al. 2009). Currans et al. (2009) observed that the diversity of *O. mykiss* has persisted by the species exploiting and adapting to varying habitats rather than being limited by environmental instability. Regarding gene flow, there is scant demographic information available pertaining to natural straying rates and the magnitude of gene flow among steelhead populations in the Columbia River basin. Yet philopatry—the return of anadromous adults to the locations of their birth (Groot and Margolis 1991; Behnke 1992)—and adaptation to local environments (Taylor 1991) are notable characteristics of salmonids. Acting as diversifying forces across the Columbia and Snake River basins, these characteristics would tend to increase population differentiation as opposed to homogenizing genetic diversity.

Genetic introgression from nonnative trout into native Columbia River redband trout is also a concern associated with artificial propagation, as hatchery programs composed of Sacramento River redband trout operate in the Columbia River basin (Crawford 1979). *K*-means clustering analysis showed that aggregate 1 consisted of Sacramento River redband trout collections from Spokane and Goldendale hatcheries and geographically disparate collections from Omak Creek, Phalon Lake, and the Umtanum River (Figure 1; Table A.1). Additionally, other steelhead collections not contained within aggregate 1 were associated with the second principal component (Figure 4), suggesting that gene flow from Sacramento River redband trout occurred sporadically within the basin. Campton and Johnston (1985) postulated that recent anthropogenic hybridization had occurred for *O. mykiss* in the Yakima River. Utter (1998) questioned the hypothesis of Campton and Johnston (1985) given their limited data and suggested that the observations in the Yakima River could be explained by a more ancient introgression between coastal and inland *O. mykiss*. Our results support the conclusion of Campton and Johnston (1985), although the occurrence of introgression in the Yakima Basin appeared quite limited geographically. The collection from Phalon Lake is also known to have been introgressed with nonnative trout (Small et al. 2007).

We also investigated whether other factors, such as geographic separation among collections and environmental variables, have shaped genetic diversity. To test for evidence of selection on loci, we partitioned the genetic data among coastal and inland lineages and estimated the magnitude of genetic divergence relative to genetic diversity using an F_{ST} outlier approach. Two loci showed higher divergence than expected by chance (i.e., position selection), with *Ssa407* appearing as an outlier in the coastal lineage and *Ots3M* as an outlier in the inland lineage

(Figure 3). Yet both of these loci were on the fringe of the 99% confidence level and are potential false positives. Beyond investigation of selection on individual genetic loci, we performed landscape-genetic analyses to evaluate the effect of environmental variables on the spatial distribution of genetic diversity. The results from our DISTLM multivariate model corroborated those from the *k*-means clustering analysis, indicating that the genetic structure of *O. mykiss* in the Columbia River basin was primarily influenced by an ancient separation of coastal and interior lineages (i.e., Currans et al. 2009). Yet contemporary patterns of gene flow were also important in shaping genetic diversity, as geographic distance contributed significantly to the DISTLM model (e.g., Beacham et al. 1999). Among-population genetic analyses also supported the correlation between geographic distance and genetic affinity, with aggregates forming from the lower Columbia River, upper Willamette River, the Oregon (Deschutes and John Day) and Washington (Klickitat) sides of the Columbia River just east of the Cascade crest, the interior Columbia River basin, and the Clearwater River (Figures 1, 2). Of the climate-related environmental variables tested using the multivariate model DISTLM, precipitation accounted for the highest proportion of genetic variation. Previous studies have demonstrated that environmental variables may contribute to genetic structure in salmonids (Castric et al. 2001; Dionne et al. 2008; Narum et al. 2008). Additionally, our results suggest that precipitation acts as a proxy for habitat characteristics important in the local adaptation of steelhead populations, a result observed for other salmonids (Olsen et al. 2010b). Yet conclusions about adaptation cannot be based on systematic patterns of genetic variation (Allendorf and Utter 1979 and references therein). Further evaluations of adaptation using markers from functional genes or gene expression techniques will be required to confirm that selection is occurring among *O. mykiss* at local scales.

Conservation Implications

Many issues regarding the sound stewardship of *O. mykiss* populations are made difficult by the complex migratory patterns, reproductive biology, and behavior of the species. The fiduciary responsibilities of the regulatory system are further complicated by difficulties in gathering information about *O. mykiss* in the wild. The SPAN microsatellite loci reference genetic data set reported here provides a means of assessing *O. mykiss* populations in greater detail. Harvest impacts can be determined rapidly on a refined regional scale. Artificial propagation efforts for harvest augmentation or the supplementation of natural populations carry the potential for both risks and benefits. The reported reference data set can enhance the investigation of these potential effects by facilitating the estimation of admixture and introgression between hatchery- and natural-origin fish. Tremendous information gains will also be made in the area of monitoring trends in productivity through the use of new analytical tools that link juvenile to adult data and quantify differences in reproductive success among individuals. As *O.*

mykiss individuals are difficult to monitor in the wild, information regarding population status and trends is ambiguous at best. Yet genetic methodologies will allow the identification of individuals to their populations of origin. This technical advance will enable more reliable run reconstruction estimates of specific stocks obtained from mixed samples at downstream structures (e.g., weirs or dams) using genetic stock identification methods. By coupling genetic information with more traditional methods of monitoring general abundance, the tremendous genetic and life history diversity of *O. mykiss* may be effectively documented. Understanding and conserving the adaptive diversity of *O. mykiss* is paramount for the persistence of the species given the future scenarios of continuing habitat alteration and climate variability.

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Appendix: Summary Statistics for Sample Collections

TABLE A.1. *Oncorhynchus mykiss* collections used for genetic analysis and summary of within-collection genetic diversity results. Collections are ordered alphabetically within aggregate by collection label. Runs are summer (Sum), winter (Win), and unknown (Unk). Origins are hatchery (H), wild (W), and unknown (Unk). The ESU designations are those of Busby et al. (1996). Aggregate (AGG) represents the group into which each collection was placed by the *k*-means procedure. CON is an alphanumeric label used to identify collections on the dendrogram (Figure 2), with a 3-digit code for the collection followed by a 2-digit regional identifier (01 = Columbia Lower Winter, 02 = Columbia Lower Summer, 03 = Willamette Winter, 04 = Willamette Summer, 05 = Columbia Middle Summer, 06 = Deschutes Summer, 07 = Columbia Upper Summer, 08 = Snake Summer, 09 = Grande Ronde Summer, 10 = Imnaha Summer, 11 = Clearwater Summer, and 12 = Salmon Summer); *N* is the number of sampled individuals. The summary statistics shown are gene diversity (GD), observed heterozygosity (HZ), the mean number of alleles per locus (*A*), allelic richness (AR), the Hardy–Weinburg equilibrium *P*-value (HWE), the proportion of pairwise locus combinations showing significant ($\alpha = 0.05$) correlation of alleles across loci (LD), and *M*-ratio estimate. The abbreviation na = not applicable.

Collection ^a	Run	Origin	ESU	AGG	CON	<i>N</i>	GD	HZ	<i>A</i>	AR ^b	HWE	LD	<i>M</i> -ratio
Goldendale Hatchery	Unk	H	N/A	1	Pop_091–05	91	0.71	0.69	6.54	5.91	0.98	0.01	0.55
Goldendale Hatchery	Sum	H	N/A	1	Pop_092–05	113	0.7	0.71	6.54	5.81	1	0.01	0.54
Omak Creek	Unk	W	14	1	Pop_156–07	44	0.75	0.77	9.38	10.01	0.98	0.13	0.64
Phalon Lake Hatchery	Unk	W	14	1	Pop_150–07	74	0.82	0.83	12.23	10.56	0.96	0.19	0.76
Spokane Hatchery	Unk	H	N/A	1	Pop_160–07	82	0.69	0.69	6.31	5.84	0.98	0	0.54
Umtanum River	Sum	W	13	1		19	0.83	0.83	9.46		0.67	0.03	0.68
Mean by aggregate						71	0.75	0.75	8.41	7.62	na	na	0.62
Crane Prairie	Sum	W	13	2	Pop_139–06	45	0.81	0.79	10.92	10.13	0.28	0	0.67
Mean by aggregate						45	0.81	0.79	10.92	10.1	na	na	0.67
Big White Salmon River	Unk	W	13	3	Pop_050–05	81	0.83	0.77	12.77	11.16	0	0.12	0.76
Bowman Creek	Sum	W	13	3	Pop_077–05	47	0.82	0.82	11.31	10.48	0.55	0.05	0.7
Bowman Creek	Sum	W	13	3	Pop_078–05	47	0.81	0.8	11.54	10.50	0.63	0.08	0.77
Brush Creek	Unk	W	13	3	Pop_052–05	29	0.77	0.75	7.69	7.69	0.56	0.1	0.6
Brush Creek	Unk	W	13	3	Pop_053–05	48	0.46	0.47	3.08	2.93	0.99	0.14	0.51
Clackamas River Hatchery	Sum	H	4	3	Pop_036–04	48	0.76	0.79	8.54	9.96	1	0.12	0.66
Dead Canyon Creek	Unk	W	13	3	Pop_085–05	54	0.81	0.8	12.31	10.96	0.82	0.03	0.78
Dead Canyon Creek	Sum	W	13	3	Pop_086–05	36	0.82	0.82	10.69	10.42	0.59	0.04	0.67

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TABLE A.1. Continued.

Collection ^a	Run	Origin	ESU	AGG	CON	<i>N</i>	GD	HZ	<i>A</i>	AR ^b	HWE	LD	<i>M</i> -ratio
Diamond Fork	Sum	W	13	3	Pop_089–05	47	0.64	0.63	6.54	6.20	0.22	0.03	0.62
Fish Lake Stream	Sum	W	13	3	Pop_090–05	20	0.8	0.81	9.38		0.3	0.01	0.71
Klickitat River	Sum	W	13	3	Pop_095–05	48	0.76	0.75	10.15	12.22	0.88	0.04	0.7
Little Klickitat River	Sum	W	13	3	Pop_097–05	48	0.8	0.79	11.77	10.63	0.38	0	0.8
Little Klickitat River	Sum	Unk	13	3	Pop_098–05	36	0.82	0.8	10.77	10.42	0.3	0.01	0.74
Little Klickitat River	Sum	W	13	3	Pop_096–05	77	0.71	0.69	10.38	8.66	0.5	0.03	0.71
Piscoe Creek	Sum	W	13	3	Pop_109–05	47	0.71	0.7	8.85	8.28	0.85	0.05	0.65
Skamania Hatchery	Sum	H	N/A	3	Pop_029–02	87	0.76	0.77	8.77	7.52	1	0.08	0.69
Snyder Creek	Sum	W	13	3	Pop_118–05	44	0.73	0.76	7.77	7.46	0.96	0.27	0.62
Snyder Creek	Unk	W	13	3	Pop_067–05	55	0.78	0.79	9.46	8.73	0.95	0.03	0.65
South Santiam Hatchery	Sum	H	5	3	Pop_043–04	47	0.77	0.78	9.08	8.44	0.93	0.01	0.72
Summit Creek	Sum	W	13	3	Pop_112–05	47	0.81	0.8	11.46	10.44	0.72	0.04	0.74
Summit Creek	Sum	W	13	3	Pop_113–05	43	0.64	0.64	6.46	6.09	0.86	0	0.61
Surveyors Creek	Sum	W	13	3	Pop_114–05	41	0.66	0.65	7.54	7.18	0.69	0.17	0.57
Swale Creek	Unk	W	13	3	Pop_115–05	54	0.81	0.77	11.08	9.79	0.13	0.21	0.75
Swale Creek	Sum	W	13	3	Pop_116–05	44	0.78	0.78	9.54	9.04	0.84	0.36	0.7
Tepee Creek	Unk	W	13	3	Pop_069–05	46	0.75	0.77	8.69	7.99	0.83	0.03	0.67
Tepee Creek	Sum	W	13	3	Pop_123–05	50	0.76	0.78	9.77	8.76	0.99	0.09	0.68
Trout Creek	Sum	W	13	3	Pop_126–05	48	0.8	0.83	11	10.20	1	0.04	0.7
Trout Creek	Sum	Unk	13	3	Pop_127–05	78	0.8	0.79	12.23	10.37	0.75	0.15	0.8
Trout Creek	Sum	W	13	3	Pop_128–05	48	0.65	0.65	6.92	6.33	0.94	0.05	0.62
White Creek	Sum	W	13	3	Pop_136–05	45	0.71	0.74	6.08	8.72	0.99	0.17	0.57
White Creek	Sum	W	13	3	Pop_137–05	50	0.77	0.73	9.46	10.76	0.09	0.06	0.69
White Creek	Sum	W	13	3	Pop_135–05	94	0.81	0.8	13.15	5.85	0.72	0.01	0.86
Mean by aggregate						51	0.75	0.75	9.51	8.85	na	na	0.69
Bakeoven Creek	Sum	W	13	4	Pop_073–06	97	0.78	0.76	12.38	10.11	0.52	0.05	0.8
Baldy Creek	Sum	W	13	4		14	0.75	0.75	7		0.7	0.05	0.64
Beech Creek	Unk	W	13	4	Pop_076–05	26	0.79	0.78	9.15	9.15	0.87	0	0.74
Bennetts River	Sum	W	13	4	Pop_142–06	39	0.76	0.74	11.23	10.52	0.33	0.01	0.77
Black Canyon Creek	Unk	W	13	4	Pop_051–05	20	0.79	0.8	8.69		0.92	0.03	0.71
Bridge Creek	Sum	W	13	4	Pop_079–05	48	0.77	0.76	10.69	9.80	0.39	0.04	0.72
Buckhollow Creek	Sum	W	13	4	Pop_080–06	84	0.79	0.8	12.46	10.56	0.66	0.08	0.77
Camp Creek–Columbia	Sum	W	13	4	Pop_082–05	44	0.79	0.79	9.92	9.39	0.66	0.03	0.78
Camus Creek	Sum	W	13	4		14	0.78	0.78	7.38		0.96	0.01	0.62
Canyon Creek–Columbia	Unk	W	13	4	Pop_054–05	30	0.8	0.8	9.77	9.77	0.89	0.04	0.72
Clear Creek	Sum	W	13	4	Pop_084–05	23	0.73	0.74	7.92	8.89	0.99	0.04	0.74
Clear Creek	Unk	W	13	4	Pop_083–05	38	0.78	0.8	9.15	7.92	0.68	0.06	0.72
Deer Creek	Sum	W	13	4	Pop_087–05	20	0.75	0.76	7.31		0.78	0.06	0.65
Deschutes River	Sum	W	13	4	Pop_143–06	182	0.71	0.68	12.77	9.55	0.74	0.14	0.8
Desolation Creek	Sum	W	13	4		16	0.8	0.77	8.77		0.8	0.01	0.72
E.F. Potlatch River	Sum	W	15	4	Pop_183–11	41	0.77	0.78	9.46	8.87	0.9	0.01	0.77
Eight Mile Creek	Unk	Unk	13	4	Pop_055–05	41	0.8	0.81	11.08	10.43	0.99	0.04	0.8
Fifteen Mile Creek	Unk	Unk	13	4	Pop_056–05	32	0.8	0.76	10.85	10.69	0.34	0.04	0.76
Fox Creek	Unk	W	13	4	Pop_057–05	25	0.78	0.78	8.62		0.73	0	0.71
Klickitat River	Sum	W	13	4	Pop_094–05	34	0.82	0.79	12.62	9.40	0.35	0	0.82
Lemhi River	Sum	W	15	4	Pop_202–12	50	0.83	0.79	11.23	10.43	0.21	0.03	0.74

TABLE A.1. Continued.

Collection ^a	Run	Origin	ESU	AGG	CON	<i>N</i>	GD	HZ	A	AR ^b	HWE	LD	<i>M</i> -ratio
Little Bear Creek	Sum	W	15	4	Pop_188–11	94	0.77	0.78	11.15	9.29	0.95	0.12	0.8
M.F. John Day River	Sum	W	13	4	Pop_100–05	59	0.79	0.78	11.62	10.37	0.75	0.05	0.76
M.F. John Day River	Sum	W	13	4	Pop_101–05	32	0.77	0.81	8.62	8.53	0.99	0.14	0.63
Meacham Creek	Unk	W	13	4	Pop_059–05	88	0.78	0.77	12.08	10.13	0.78	0.03	0.76
Murderer's Creek	Sum	W	13	4	Pop_102–05	23	0.72	0.71	8.15	8.15	0.81	0.01	0.72
N.F. John Day River	Sum	W	13	4	Pop_104–05	20	0.78	0.76	9.38		0.73	0.01	0.76
N.F. Umatilla River	Unk	W	13	4	Pop_060–05	23	0.77	0.78	9.08	9.08	0.5	0	0.66
Okanogan River	Sum	W	14	4	Pop_153–07	85	0.76	0.76	11.85	9.44	0.95	0.42	0.79
Ramsey Creek	Unk	W	13	4		17	0.79	0.8	8.77		0.99	0.04	0.72
Reynolds Creek	Unk	W	13	4		15	0.78	0.81	7.69		0.97	0.03	0.61
Rock Creek	Sum	W	13	4	Pop_064–05	25	0.79	0.8	10.15		0.43	0.04	0.73
Rock Creek	Unk	W	13	4		15	0.78	0.76	6.77		0.99	0.03	0.62
Rudio Creek	Unk	W	13	4	Pop_065–05	29	0.77	0.8	8.23	8.23	0.75	0.04	0.73
S.F. Umatilla River	Unk	W	13	4	Pop_066–05	34	0.77	0.78	9.85	9.66	0.99	0.08	0.65
Service Creek	Sum	W	13	4	Pop_111–05	45	0.78	0.75	10.23	9.46	0.13	0.1	0.7
Shitike Creek	Sum	H	13	4	Pop_144–06	452	0.75	0.72	15.23	10.08	0.2	0.4	0.81
Touchet River	Sum	W	13	4	Pop_125–05	50	0.78	0.77	10.15	9.35	0.64	0.04	0.75
Trail Creek	Unk	W	13	4		18	0.72	0.71	7.31		0.98	0.04	0.66
Tucannon River	Sum	W	15	4	Pop_167–08	75	0.79	0.78	11.85	10.09	0.88	0.06	0.79
Tumalo Creek	Sum	W	13	4	Pop_140–06	51	0.8	0.76	11.08	9.91	0.03	0	0.78
Umatilla River	Unk	W	13	4		18	0.79	0.78	8.92		0.5	0	0.67
Umatilla River	Sum	W	13	4		13	0.79	0.82	7.31		0.91	0.03	0.63
Wall Creek	Sum	W	13	4	Pop_132–05	24	0.77	0.79	8	8.00	0.95	0.09	0.66
Wall Creek	Unk	W	13	4	Pop_133–05	27	0.8	0.82	9	9.00	1	0.01	0.68
Warm Springs River	Sum	W	13	4	Pop_145–06	134	0.79	0.78	13.62	10.78	0.81	0	0.86
Whychus Creek	Sum	W	13	4	Pop_141–06	46	0.66	0.66	9.08	8.14	0.97	0.03	0.77
Mean by aggregate						52	0.77	0.77	9.86	9.49	na	na	0.73
Ahtanum Creek	Unk	W	13	5	Pop_071–05	36	0.8	0.81	11.23	10.85	0.62	0.03	0.73
Ahtanum Creek	Sum	W	13	5	Pop_072–05	54	0.82	0.79	11.62	10.46	0.94	0.01	0.7
Asotin Creek	Sum	W	15	5	Pop_161–08	112	0.8	0.79	13.08	10.32	0.66	0.01	0.75
Bargamin Creek	Sum	W	15	5	Pop_197–12	90	0.78	0.77	10.46	8.94	0.6	0.12	0.74
Beech Creek	Sum	W	13			3	0.78	0.82	3.23		0.88	0.06	0.43
Belshaw Creek	Unk	W	13			6	0.69	0.67	4.15		0.43	0.03	0.59
Big Creek–Snake	Sum	W	15	5	Pop_214–12	44	0.77	0.77	9.23	8.66	0.82	0	0.74
Big Creek–Snake	Sum	W	15	5	Pop_215–12	47	0.74	0.75	7.08	6.82	0.99	0.36	0.69
Big Smoky Creek	Unk	W	15	5	Pop_162–08	54	0.72	0.71	8.92	8.26	0.75	0.01	0.76
Boulder Creek	Sum	W	15	5	Pop_198–12	47	0.76	0.77	10.08	9.42	0.93	0.03	0.77
Camas Creek	Sum	W	15	5	Pop_199–12	52	0.75	0.75	9.46	8.50	0.96	0.01	0.76
Camp Creek	Sum	W	15	5	Pop_173–10	141	0.77	0.8	11.08	9.08	1	0.27	0.73
Captain John Creek	Sum	W	15	5	Pop_163–08	57	0.78	0.77	10.46	9.43	0.86	0.09	0.73
Chamberlain Creek	Sum	W	15	5	Pop_200–12	64	0.76	0.78	10.62	9.13	0.99	0.09	0.74
Chewuch River	Sum	W	14	5	Pop_146–07	121	0.79	0.77	13.38	10.03	0.31	0.05	0.83
Cottonwood Creek	Sum	W	15	5	Pop_179–11	97	0.78	0.77	11.31	9.74	0.81	0.27	0.78
Crooked Creek	Sum	W	15	5	Pop_168–09	143	0.78	0.78	12.62	9.89	0.97	0.06	0.8
E.F. Salmon River	Sum	W	15	5	Pop_216–12	45	0.71	0.79	8.15	7.61	1	0.28	0.68
Elk Creek	Sum	W	15	5	Pop_164–08	98	0.76	0.77	9.92	8.54	0.99	0.17	0.77
Granite Creek	Sum	W	13	5		19	0.74	0.78	7.08		0.99	0.13	0.63
Gumboot Creek	Sum	W	15	5	Pop_170–10	123	0.76	0.77	10.08	8.39	0.99	0.12	0.79

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TABLE A.1. Continued.

Collection ^a	Run	Origin	ESU	AGG	CON	<i>N</i>	GD	HZ	<i>A</i>	AR ^b	HWE	LD	<i>M</i> -ratio
Hazard Creek	Sum	W	15	5	Pop_201–12	45	0.78	0.76	11.23	10.34	0.6	0	0.8
Horse Creek	Sum	W	15	5	Pop_171–10	120	0.78	0.77	11.62	9.35	0.76	0.04	0.77
Indian Creek	Unk	W	13	5	Pop_058–05	27	0.65	0.64	6	6.00	0.69	0.01	0.57
Lightning Creek	Sum	W	15	5	Pop_172–10	74	0.78	0.76	10	8.93	0.48	0.01	0.82
Little Rattlesnake Creek	Sum	W	13	5	Pop_099–05	46	0.79	0.8	10.15	9.50	0.99	0.04	0.75
Little Salmon River	Sum	W	15	5	Pop_203–12	52	0.74	0.71	8.85	8.12	0.18	0.1	0.68
Loon Creek	Sum	W	15	5	Pop_204–12	61	0.73	0.73	8.77	7.82	0.78	0.03	0.74
Lower Valley Creek	Sum	W	15	5	Pop_223–12	55	0.79	0.81	10.08	9.01	0.98	0.05	0.69
M.F. Payette River	Unk	W	15	5	Pop_165–08	45	0.72	0.72	8.69	8.07	0.8	0.03	0.66
Marsh Creek	Sum	W	15	5	Pop_205–12	82	0.73	0.74	7.77	6.96	1	0.37	0.64
Methow River	Sum	W	14	5	Pop_147–07	59	0.78	0.75	11.46	10.03	0.28	0.01	0.78
Methow River	Sum	W	14	5	Pop_152–07	262	0.8	0.79	13.69	10.06	0.81	0.04	0.81
Mission Creek	Sum	W	15	5	Pop_189–11	51	0.76	0.76	10.54	9.58	0.91	0.03	0.73
Morgan Creek	Sum	W	15	5	Pop_206–12	46	0.81	0.8	11.62	10.48	0.55	0.06	0.73
N.F. Little Naches River	Sum	W	13	5	Pop_105–05	21	0.76	0.77	8.38	8.38	0.74	0.03	0.68
Naches River	Sum	W	13	5	Pop_103–05	278	0.79	0.78	13.92	9.95	0.9	0.09	0.84
Nason Creek	Unk	W	14	5	Pop_148–07	33	0.77	0.77	8.69	8.63	0.93	0.03	0.68
Nile Creek	Sum	W	13	5	Pop_106–05	59	0.8	0.8	10.38	9.51	0.94	0.05	0.73
Okanogan River	Sum	W	14	5	Pop_154–07	119	0.81	0.77	12.85	10.08	0	0.31	0.81
Omak Creek	Sum	Unk	14	5	Pop_155–07	270	0.8	0.79	13.85	8.64	0.8	0.35	0.79
Oxbow Hatchery	Sum	H	15	5	Pop_166–08	44	0.79	0.77	9.92	9.31	0.37	0.01	0.74
Pahsimeroi River	Sum	H	15	5	Pop_207–12	87	0.79	0.79	10.54	8.93	0.94	0.05	0.75
Pahsimeroi River	Sum	W	15	5	Pop_208–12	88	0.81	0.8	12	9.84	0.57	0.03	0.79
Peshastin Creek	Unk	W	14	5	Pop_149–07	91	0.8	0.76	13	10.56	0.01	0.03	0.79
Pile Up Creek	Sum	W	13	5	Pop_107–05	20	0.76	0.83	7.92		1	0.01	0.63
Pine Creek	Unk	W	13	5	Pop_108–05	28	0.81	0.78	9.46	9.46	0.2	0.04	0.7
Pistol Creek	Sum	W	15	5	Pop_209–12	23	0.73	0.76	7.62		0.97	0.01	0.64
Rapid River	Sum	W	15	5	Pop_211–12	45	0.72	0.7	8.54	7.82	0.21	0.04	0.68
Rapid River	Sum	W	15	5	Pop_210–12	310	0.76	0.75	12.54	9.30	0.99	0.26	0.85
S.F. Salmon River	Sum	W	15	5	Pop_220–12	46	0.75	0.77	8.31	7.73	1	0.05	0.69
Satus Creek	Sum	W	13	5	Pop_110–05	158	0.76	0.76	11.38	8.84	0.93	0.01	0.84
Sawtooth Fish Hatchery	Sum	H	15	5	Pop_218–12	47	0.79	0.76	10.08	9.22	0.19	0.04	0.74
Sawtooth Fish Hatchery	Sum	W	15	5	Pop_217–12	50	0.79	0.78	10.92	9.47	0.63	0	0.76
Secesh River	Sum	W	15	5	Pop_212–12	75	0.73	0.7	8.77	7.75	0.29	0.08	0.77
Slate Creek	Sum	W	15	5	Pop_213–12	47	0.79	0.77	10.85	9.95	0.8	0.08	0.77
Squaw Creek Weir	Sum	H	15	5	Pop_219–12	47	0.79	0.77	10.23		0.52	0.05	0.7
Stolle Meadows	Sum	W	15	5	Pop_221–12	47	0.72	0.72	8.23	7.67	0.9	0.01	0.66
Sulfur Creek	Sum	W	15	5	Pop_222–12	55	0.72	0.77	8.08	7.43	1	0.05	0.7
Swauk River	Sum	W	13	5	Pop_117–05	31	0.8	0.77	9.08	9.03	0.32	0	0.74
Taneum River	Sum	W	13	5	Pop_119–05	91	0.76	0.78	11.23	9.23	1	0	0.7
Taneum River	Sum	W	13	5	Pop_120–05	24	0.78	0.74	9.23	9.23	0.29	0.03	0.66
Teanaway River	Sum	W	13	5	Pop_121–05	252	0.76	0.76	12.85	9.39	0.96	0.14	0.76
Teanaway River	Sum	W	13	5	Pop_122–05	241	0.78	0.77	12.69	9.77	1	0.22	0.78
Toppenish Creek	Sum	W	13	5	Pop_124–05	212	0.72	0.73	11.46	8.11	1	0.04	0.75
Twisp River	Sum	W	14	5	Pop_151–07	42	0.78	0.74	11.08	10.45	0.09	0	0.74
Twisp River	Sum	W	14	5	Pop_157–07	296	0.79	0.78	13.08	9.70	0.97	0.19	0.84
Upper Columbia River	Sum	W	14	5	Pop_158–07	264	0.8	0.79	14.31	10.23	0.91	0.05	0.83
W.F. Yankee Fork	Sum	W	15	5	Pop_224–12	47	0.81	0.83	10.23	9.35	1	0	0.72

TABLE A.1. Continued.

Collection ^a	Run	Origin	ESU	AGG	CON	N	GD	HZ	A	AR ^b	HWE	LD	M-ratio
W.F. Yankee Fork	Sum	W	15	5	Pop_225-12	59	0.75	0.75	9.77	8.56	0.88	0.18	0.68
Wells Hatchery	Sum	H	14	5	Pop_159-07	87	0.78	0.78	11.08	9.35	0.84	0.05	0.76
Wenaha River	Sum	W	15	5	Pop_169-09	96	0.78	0.77	11.77	9.65	0.73	0.04	0.79
West Quartz Creek	Sum	W	13	5	Pop_134-05	26	0.77	0.76	8.62		0.47	0	0.64
Whitebird Creek	Sum	W	15	5	Pop_226-12	109	0.78	0.76	11.15	9.30	0.35	0.09	0.76
Yakima River—Roza Trap	Sum	W	13	5	Pop_138-05	353	0.8	0.78	14.92	10.75	0.07	0.14	0.76
Mean by aggregate						91	0.77	0.77	10.30	9.10	na	na	0.73
Calapooia River	Win	W	5	6	Pop_037-03	33	0.68	0.68	7.85	7.73	0.8	0.03	0.66
Clackamas River	Unk	Unk	4	6	Pop_030-03	74	0.75	0.76	9	7.86	0.94	0.33	0.74
Clackamas River—N.F. Dam	Win	W	4	6	Pop_038-03	118	0.79	0.77	12.38	8.17	0.66	0.04	0.77
Eagle Creek	Unk	W	4	6	Pop_032-03	53	0.78	0.78	10.23	9.20	0.89	0.01	0.71
Little Rock Creek	Unk	W	5	6		14	0.71	0.74	6.38		0.91	0.06	0.54
Mad Creek	Unk	W	5	6		16	0.78	0.73	7.92		0.13	0.04	0.65
Marion Forks Hatchery	Win	H	5	6	Pop_044-03	38	0.72	0.75	6.85	6.65	0.99	0.05	0.59
N.F. Molalla River	Win	W	4	6	Pop_039-03	49	0.77	0.79	10.31	9.42	0.98	0.01	0.76
S. Santiam River	Win	W	5	6	Pop_047-03	77	0.73	0.73	10.08	8.35	0.93	0.03	0.71
Santiam River—Bennet Dam	Win	W	5	6	Pop_045-03	93	0.74	0.73	10.92	8.52	0.59	0.01	0.74
Santiam River—Bennet Dam	Win	W	5	6	Pop_046-03	70	0.75	0.73	10.69	9.06	0.57	0	0.7
Santiam River—Rock Creek	Unk	W	5	6		15	0.76	0.7	6.77		0.05	0.08	0.57
Wiley Creek	Win	W	5	6	Pop_048-03	36	0.76	0.8	9	8.70	1	0.06	0.68
Mean by aggregate						53	0.75	0.75	9.11	8.37	na	na	0.68
Bear Creek	Sum	W	15	7	Pop_174-11	64	0.76	0.78	8.77	7.97	1	0.33	0.68
Big Bear Creek	Sum	W	15	7		12	0.75	0.71	6.92		0.31	0.01	0.66
Canyon Creek—Snake	Sum	W	15	7	Pop_192-11	81	0.74	0.75	9.85	8.50	1	0.13	0.77
Cedar Creek	Sum	W	15	7	Pop_176-11	50	0.72	0.77	8.54	7.89	1	0.51	0.69
Clear Creek	Sum	W	15	7	Pop_193-11	45	0.75	0.74	9.54	8.95	0.5	0	0.77
Collins Creek	Unk	W	15	7	Pop_177-11	56	0.71	0.71	8.85	8.04	0.98	0.01	0.76
Colt Creek	Sum	W	15	7	Pop_178-11	58	0.71	0.72	8.31	7.48	0.99	0.18	0.71
Crooked Fork Lochsa River	Sum	W	15	7	Pop_180-11	47	0.75	0.76	8.69	8.09	0.96	0.08	0.76
Crooked River	Sum	W	15	7	Pop_181-11	185	0.74	0.72	10.31	8.50	0.92	0.24	0.81
Dworshak Hatchery	Sum	H	15	7	Pop_182-11	47	0.73	0.72	8.77	8.18	0.77	0.05	0.72
Fish Creek	Sum	W	15	7	Pop_184-11	85	0.75	0.75	10.15	8.85	0.88	0.05	0.81
Gedney Creek	Sum	W	15	7	Pop_185-11	174	0.75	0.75	11.31	8.78	0.99	0.01	0.8
John's Creek	Sum	W	15	7	Pop_186-11	40	0.76	0.75	10.08	9.55	0.87	0.03	0.77
Lake Creek	Sum	W	15	7	Pop_187-11	53	0.72	0.74	8.77	8.04	1	0.03	0.72
N.F. Moose Creek	Sum	W	15	7	Pop_190-11	98	0.75	0.74	10	8.36	0.72	0.09	0.71
O'Hara Creek	Sum	W	15	7	Pop_191-11	47	0.76	0.75	9.69	8.92	0.75	0.01	0.77
Storm Creek	Sum	W	15	7	Pop_194-11	39	0.73	0.75	8.08	7.80	0.98	0.05	0.74
Tenmile Creek	Sum	W	15	7	Pop_195-11	47	0.74	0.77	8.54	8.03	1	0.1	0.64
Three Links Creek	Sum	W	15	7	Pop_196-11	57	0.75	0.78	8.77	8.03	1	0.12	0.69
Mean by aggregate						68	0.74	0.75	9.15	8.33	na	na	0.74

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TABLE A.1. Continued.

Collection ^a	Run	Origin	ESU	AGG	CON	<i>N</i>	GD	HZ	<i>A</i>	AR ^b	HWE	LD	<i>M</i> -ratio
Alder Grove	Win	W	3			8	0.8	0.77	6.54		0.31	0	0.52
Big Creek	Win	W	3	8	Pop_005-01	43	0.8	0.79	11		0.47	0.01	0.64
Big Creek Hatchery	Win	H	3	8	Pop_002-01	48	0.78	0.76	10.54	9.57	0.44	0	0.67
Canyon Creek-Willamette	Unk	W	5	8	Pop_034-03	33	0.7	0.73	8.31	8.09	1	0.5	0.63
Carcus Creek	Win	W	3	8		15	0.76	0.74	7.31		0.53	0.03	0.58
Clatskanie River-Wilark	Win	W	3			3	0.64	0.72	3		0.98	0	0.59
Conyers Creek	Win	W	3			3	0.76	0.73	3.54		0.3	0	0.5
Coweeman River	Win	W	4	8	Pop_007-01	134	0.82	0.8	14	10.73	0.06	0.03	0.76
Cowlitz Hatchery	E-Win	H	4	8	Pop_008-01	97	0.81	0.81	12	10.64	0.96	0	0.68
Cowlitz Hatchery	L-Win	H	4	8	Pop_009-01	96	0.79	0.79	9.85	9.85	0.93	0.03	0.61
Cowlitz Hatchery	Sum	H	4	8	Pop_011-01	57	0.82	0.84	12.23	8.60	0.99	0.01	0.65
Cowlitz River-Barrier Dam	Win	W	4	8	Pop_010-01	134	0.8	0.77	11.23	9.63	0.03	0.08	0.64
Cowlitz River tributaries	Win	W	4	8	Pop_027-02	98	0.8	0.78	11.62	9.10	0.22	0.03	0.68
Eagle Creek Hatchery	Unk	Unk	4	8	Pop_031-03	58	0.78	0.8	9.31	8.65	0.98	0.06	0.67
E.F. Lewis River	Win	W	4	8	Pop_012-01	76	0.81	0.77	12.08	10.10	0.04	0.01	0.7
Elochoman River	Win	W	3	8	Pop_013-01	100	0.8	0.75	13.62	10.30	0	0.01	0.78
Germany Creek	Win	W	3	8	Pop_014-01	100	0.82	0.79	13.77	11.17	0.15	0.04	0.77
Grays River	Win	W	3	8	Pop_015-01	85	0.8	0.78	12.62	10.25	0.36	0.01	0.66
Green River	Win	W	4	8	Pop_016-01	94	0.82	0.81	13.92	10.99	0.71	0	0.71
Hood River-Powerdale	Win	W	4	8	Pop_017-01	95	0.82	0.81	14.31	11.52	0.68	0.04	0.75
Kalama River	Sum	W	4	8	Pop_028-02	253	0.81	0.8	15.23	10.74	0.6	0.04	0.76
Kalama River Trap	Win	W	4	8	Pop_018-01	41	0.8	0.79	11.23	10.72	0.65	0.03	0.65
Luckiamute River	Unk	W	5	8	Pop_033-03	31	0.68	0.71	7.46	7.42	1	0.14	0.63
Mill Creek	Win	W	3	8	Pop_019-01	95	0.81	0.79	12.85	10.39	0.33	0.13	0.68
N.F. Lewis River-Cedar Trap	Win	W	4	8	Pop_020-01	60	0.82	0.81	12.15	10.51	0.67	0.05	0.69
N.F. Lewis River-Merwin	Win	W	4	8	Pop_021-01	97	0.81	0.79	13.15	10.51	0.52	0.01	0.7
N.F. Toutle River	Win	W	4	8	Pop_022-01	99	0.8	0.78	12.31	10.29	0.52	0.08	0.73
S.F. Toutle River	Win	W	4	8	Pop_024-01	72	0.81	0.8	12.15	10.34	0.81	0.01	0.66
Sandy River-Marmot Dam	Win	W	4	8	Pop_023-01	97	0.83	0.82	13.92	11.33	0.92	0.04	0.84
Still Creek	Unk	Unk	4	8	Pop_068-05	26	0.81	0.81	9.69	9.69	0.9	0.53	0.7
Swedetown	Win	W	3			6	0.8	0.79	5.54		0.46	0	0.49
Washougal River	Win	W	4	8	Pop_026-01	71	0.8	0.79	12.38	10.14	0.38	0	0.68
Willamina Creek	Unk	W	5	8	Pop_035-03	34	0.73	0.79	7.77	7.61	1	0.08	0.59
Mean by aggregate						71	0.79	0.78	10.81	9.96	na	na	0.67

^a The abbreviations N.F., S.F., E.F., W.F., and M.F. stand for North, South, East, West, and Middle Fork, respectively.^b Populations with less than 20 samples per locus were removed from allelic richness estimates.