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Distribution of genetic variation underlying adult migration timing in steelhead of the Columbia River basin

Erin E. Collins[1] | **John S. Hargrove**[2] | **Thomas A. Delomas**[2] | **Shawn R. Narum**[1]

[1]Columbia River Inter-Tribal Fish Commission, Hagerman, ID, USA
[2]Eagle Fish Genetics Lab, Pacific States Marine Fisheries Commission, Eagle, ID, USA

***Correspondence**
Erin E. Collins, Hagerman Genetics Lab, 3059-F National Fish Hatchery Road, Hagerman, ID 83332 USA.
Email: ecollins@critfc.org

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Abstract

Fish migrations are energetically costly, especially when moving between freshwater and saltwater, but are a viable strategy for Pacific salmon and trout (*Oncorhynchus* spp.) due to the advantageous resources available at various life stages. Anadromous steelhead (*O. mykiss*) migrate vast distances and exhibit variation for adult migration phenotypes that have a genetic basis at candidate genes known as *greb1L* and *rock1*. We examined the distribution of genetic variation at 13 candidate markers spanning *greb1L*, intergenic, and *rock1* regions versus 226 neutral markers for 113 populations (n = 9,471) of steelhead from inland and coastal lineages in the Columbia River. Patterns of population structure with neutral markers reflected genetic similarity by geographic region as demonstrated in previous studies, but candidate markers clustered populations by genetic variation associated with adult migration timing. Mature alleles for late migration had the highest frequency overall in steelhead populations throughout the Columbia River, with only 9 of 113 populations that had a higher frequency of premature alleles for early migration. While a single haplotype block was evident for the coastal lineage, we identified multiple haplotype blocks for the inland lineage. The inland lineage had one haplotype block that corresponded to candidate markers within the *greb1L* gene and immediately upstream in the intergenic region, and the second block only contained candidate markers from the intergenic region. Haplotype frequencies had similar patterns of geographic distribution as single markers, but there were distinct differences in frequency between the two haplotype blocks for the inland lineage. This may represent multiple recombination events that differed between lineages where phenotypic differences exist between freshwater entry versus arrival timing as indicated by Micheletti et al. (2018a). Redundancy analyses were used to model environmental effects on allelic frequencies of candidate markers, and significant variables were migration distance, temperature, isothermality, and annual precipitation. This study improves our understanding of the spatial distribution of genetic variation underlying adult migration timing in steelhead as well as associated environmental factors and has direct conservation and management implications.

KEYWORDS
anadromous, *greb1L*, landscape genetics, *Oncorhynchus*, population genetics

1 | INTRODUCTION

Many animals undertake long-distance migration from their natal sites to capitalize on abundant resources that may increase survival, fecundity, and fitness (Dingle & Drake, 2007). Migrations offer temporal and spatial availability of resources, along with seasonal suitability of migratory corridors and natal areas (Edwards & Richardson, 2004; Forrest & Miller-Rushing, 2010). The migration of *Oncorhynchus* spp. (Pacific salmon and trout) is a critical cultural, economic, and ecological resource throughout their native range. Conservation of salmon and steelhead is based on maintaining phenotypic and genetic variation of distinct populations, and a principal focus involves conserving adult migration timings across large drainages such as the Columbia River basin. Many populations are managed according to the degree of reproductive isolation and life-history variation. Evolutionarily significant units (ESU) of Pacific salmon and trout are defined as a distinct population segment (DPS) under the US Endangered Species Act (ESA) (Ryder, 1986; Waples, 1991) and each DPS is determined by whether it is sufficiently reproductively isolated and of evolutionary importance to the species (Waples, 1991). Since the late 1800s, wild Pacific salmon and trout have experienced a steady decline in abundance and range. The freshwater range of Pacific salmon and trout has shrunk to about 60% of the historical range (English, Peacock, & Spilsted, 2006; National Research Council, 1996). The decline has been initially attributed to overharvest, habitat degradation (logging, mining, agricultural practices), and other anthropogenic development, but modern anthropogenic activity including hydroelectric dams' disruption of migratory routes, climate change, introgression between native populations and hatchery stocks, and an ongoing decrease in suitable habitat have also contributed to decline (Chapman, 1986; Crozier et al., 2008; Meehan, 1991).

Steelhead (*O. mykiss*) may undertake long migrations (over a thousand kilometers) in early life stages and return to natal sites to spawn (Busby et al., 1996; Keefer & Caudill, 2014). Steelhead in the Columbia River basin vary by genetic lineage that has been previously characterized as either coastal or inland (Busby et al., 1996; Quinn, 2018; Utter et al., 1980). The two genetic lineages are geographically separated: The coastal lineage inhabits streams west of the Cascade Mountains and the inland lineage inhabits streams east of the Cascades (Brannon, Powell, Quinn, & Talbot, 2004; Busby et al., 1996). Out of 15 steelhead ESUs in the Columbia River basin, 11 are listed under the ESA (Waples et al., 2001): One steelhead ESU is endangered and ten are threatened (Quinn, 2018). According to the ESA, an estimated one-third of Pacific salmon and trout populations and all five DPS of steelhead in the Columbia River are listed as threatened or endangered (Gustafson et al., 2007). Steelhead have also been extirpated from the upper Snake River and Columbia River headwaters (Gustafson et al., 2007).

Populations of steelhead consist of individuals that spawn at similar times and are genetically similar at neutral genetic markers, but adult individuals within a population may display significant variation in when they enter freshwater or arrive at spawning grounds (Quinn, 2018). Steelhead spawn in the spring, but can begin adult migration as early as summer of the previous year before spawning or as late as winter/spring just before spawning (Quinn, McGinnity, & Reed, 2015). Steelhead adult migration may be characterized as bimodal in some rivers (Hess, Zendt, Matala, & Narum, 2016; Leider, Chilcote, & Loch, 1986), with adult migrations referred to as early migrating summer run (premature) or late migrating winter run (mature; Quinn et al., 2015). Steelhead that exhibit early migration enter freshwater before they are sexually mature, and then hold in freshwater for several months throughout the winter before maturing and spawning the following spring (Quinn, 2018; Quinn et al., 2015). Steelhead that exhibit late migrations as adults become sexually mature in the ocean before adult migration into freshwater only weeks to a few months before spawning at natal sites in the spring (Quinn, 2018; Quinn et al., 2015). Significantly more stream-maturing steelhead populations have been extirpated than ocean-maturing steelhead populations (Gustafson et al., 2007).

Phenotypic traits associated with migration have been demonstrated to be heritable in both juvenile and adult Pacific salmon and trout (Carlson & Seamons, 2008; Thériault, Garant, Bernatchez, & Dodson, 2007). Additionally, migration timing of adult Pacific salmon and trout has also been demonstrated to be heritable (Quinn et al., 2015; Quinn, Unwin, & Kinnison, 2000). Further, adult migration timing is associated with a genomic region of major effect in both steelhead and Chinook salmon (*O. tshawytscha*; Hess et al., 2016; Micheletti, Hess, Zendt, & Narum, 2018; Narum, Di Genova, Micheletti, & Maass, 2018; Prince et al., 2017; Thompson et al., 2019). Restriction site-associated DNA sequencing (RADseq) studies have revealed single-nucleotide polymorphisms (SNPs) within the *greb1L* gene region that are associated with adult migration timing in steelhead (Hess et al., 2016; Prince et al., 2017). Additional whole-genome resequencing approaches have revealed further SNPs associated with adult migration timing and expanded the genomic region of discovered SNPs to three more candidate genes (*rock1*, *mib1*, *abhd3*, and intergenic region between *greb1L* and *rock1*; Micheletti, Hess, et al., 2018). While this genomic region of major effect may have direct conservation applications such as refining conservation units and fisheries harvest (Waples & Lindley, 2018), further understanding is needed including inheritance patterns and linkage relationships among candidate markers, and the influence of landscape characters on the distribution and frequency of candidate markers.

The *greb1L* gene is broadly present and conserved in vertebrates and the function is believed to be similar to *greb1*, which has been shown to modulate estrogen receptors and augment the role of estrogen receptor-mediated gene expression in humans (Mohammed et al., 2013). Markers shown to have nonconservative and nonsynonymous mutations by Micheletti, Hess, et al. (2018) indicate that this genetic region is under selection and the markers in the intergenic region, upstream of *greb1L*, associated with adult migration timing could be promoters or enhancers and regulate expression of *greb1L* (Kilpinen et al., 2013). Recent studies suggest that *greb1L* plays a role in early and late adult migration phenotypes in steelhead and Chinook salmon (Hess et al., 2016; Micheletti, Hess, et al., 2018; Narum et al., 2018; Prince et al., 2017; Thompson et al., 2019). Adult migration to spawning grounds is intrinsically linked to sexual development and maturation in Pacific salmon and trout, and these processes have been attributed to *greb1L* in chum salmon (*Oncorhynchus keta*) and other species (Choi, Kim, Shin, & Choi, 2014; Ghosh, Thompson, & Weigel, 2000; Pellegrini et al., 2012; Rae et al., 2006).

In this study, we examined the distribution of genetic variation for the candidate genomic region associated with adult migration timing in steelhead to better inform conservation and management decisions across the Columbia River basin. To supplement and improve upon findings of previous studies, we were able to expand the number of candidate markers associated with adult migration timing, the number of individuals sampled, and escalate sampling coverage across the Columbia River basin (Hess et al., 2016; Micheletti, Hess, et al., 2018; Prince et al., 2017). We used 13 candidate markers spanning *greb1L*, *rock1*, and the intergenic region to test combinations of markers and identify the haplotypes most representative of adult migration timing phenotypes across a large number of steelhead populations. Four of the candidate markers were previously identified with RADseq and pooled sequencing methods (Hess et al., 2016; Micheletti, Hess, et al., 2018), and nine additional candidate markers were developed from SNPs identified with pooled sequencing methods (Table 1). Sample collections were distributed across the Columbia River basin, allowing for comparisons of candidate allelic and haplotypic frequencies for adult migration timing in a variety of steelhead habitats to better understand the spatial distribution of genetic variation underlying adult steelhead migration timing. Finally, we use landscape genetic analyses to expand upon the evaluation of environmental drivers of genetic variation identified by Micheletti, Matala, Matala, and Narum (2018) for these candidate markers and for an expansion of collection sites. To distinguish between adult migration timing phenotypes and associated genetic variation, we use the terminology of "early" and "late" to refer to adult migration phenotypes and “premature” and “mature” to refer to genetic variation (alleles, genotypes, or haplotypes).

2 | METHODS

2.1 | Sample collection

Natural-origin steelhead were collected from populations of both the inland and coastal lineages across multiple years from 1996 to 2018. Samples were collected with a variety of methods, such as electrofishing, smolt traps, and weirs. Nonlethal fin tissue samples and biologically relevant metrics were collected from both smolts and returning adults (Table S1). Steelhead were collected from locations distributed throughout the Columbia River basin with sample sizes and coordinates for each collection provided in Figure 1 and Table S1.

2.2 | Molecular methods

DNA was extracted from tissue in accordance with a Chelex 100 method (Sigma-Aldrich, St Louis, MO) from a total of 9,471 steelhead representing 113 collection sites and the sample size ranged between 16 and 589 steelhead from each collection (Table S1). All specimens were genotyped with genotyping-in-thousand by sequencing method (GTseq) as described in detail in Campbell, Harmon, and Narum (2015). Briefly, our study followed standard GTseq methods that involved two rounds of PCR to first amplify targeted SNPs and then add dual barcodes to enable each individual sample to be identified. After the dual barcoding step, the concentration of each sample was normalized and then pooled into a single tube as a “library” of samples for sequencing. Multiple libraries were prepared with ~1,000 samples per library, and between 3 and 5 libraries were sequenced on an Illumina NextSeq 550 instrument prior to genotyping with scripts from Campbell et al. (2015). All samples and loci with ≥10% missing genotypes were removed from further analyses for quality control purposes. Over the period that these individuals were genotyped, various genetic marker panel updates occurred, resulting in slight variances of the mix of putatively neutral and adaptive markers available (Table 1; Tables S1, S2). Samples were genotyped with GTseq panels ranging from 368 to 390 SNPs, and genotype data were retained when >90% loci successfully genotyped and had an estimated <0.5% genotyping error based on replicate genotyping.

2.3 | Statistical analyses

2.3.1 | Population structure and genetic lineages

Putatively neutral markers were assessed using a combination of multivariate methods to detect underlying population structure, which we expected to coincide with coastal and inland lineages described in previous studies (Blankenship et al., 2011; Matala, Ackerman, Campbell, & Narum, 2014; Micheletti, Matala, et al., 2018). All neutral markers were mapped to their physical location on the *O. mykiss* genome assembly available in NCBI (accession number GCF_002163495.1), and multiple markers were found on all chromosomes with physical distance ranging from 194 KB to 39 MB. All markers had physical distance greater than 194 KB which would be greater than expected linkage decay in this species and thus were not expected to be in linkage disequilibrium. This expectation was tested with pairwise LD estimates in GenePop for a representative

TABLE 1 Adult steelhead migration timing-associated candidate marker information

Order ID	SNP	Chr	Position	Gene	Forward primer	Reverse primer	Probe	Orientation
1	Omy28_11607954	28	11,607,954	*greb1L*	TGACACTGATCACAATGGTGAAAT	TAAACTGGAAGGAGAGAGCAAAAT	TGTGGGCTGC**[A/G]**AACATACTCA	+
2	Omy_RAD52458-17	28	11,609,794	*greb1L*	ACGTGTCCCTGAGGATGGTA	AGCTCTAGGTCTGGGTCCTG	ATGGCCC**[C/A]**[CT]AAGAACCC	-
3	Omy_GREB1_05	28	11,618,027	*greb1L*	TGGGCAGATATGGAAGAACGG	ACCTTCTAAATGGCCTCTGTGT	CGGTGGCTC**[T/G]**C	+
4	Omy28_11625241	28	11,625,241	*greb1L*	CAACATTTAGGGAGAGGTTGCTAT	ATCATCAAGTTTGCCTACGACAC	CCTCCTCCCT**[A/G]**TGGTTGTCTC	+
5	Omy28_11632591	28	11,632,591	*greb1L*	GTAGAGGCCAAAGGCTTGAG	TGCTCTTATTACCTTCCAGACTCC	TGAGAA**[G/A]**AACACAGAGG	+
6	Omy_GREB1_09	28	11,641,623	*greb1L*	CCAGTGGCAACCTCAGGTAG	GACTCCAGTCACCCAAGTCA	TCAA**[T/G]**GGAGA	+
7	Omy28_11658853	28	11,658,853	intergenic	CAACATATGACCACTCGAAAACTC	ATTAATCACACCGTGAGACTCCTC	TGGTACAGAC**[A/C]**CGCACTAGCA	+
8	Omy28_11667578	28	11,667,578	intergenic	ACAGTAAACCCATTCAGGCATAGT	TTATCCTCTCAATCCACATCAAGA	GTATTGATCC**[T/C]**GTGGGAGACA	+
9	Omy_RAD47080–54	28	11,667,915	intergenic	TCAAAACCTGCAGGACTTGGA	TGGTTATATCTACAGTACAGTTCGT	TGCAAG**[A/G]**CTTAAAACGA	+
10	Omy28_11671116	28	11,671,116	intergenic	AATTTCCCCAAATTTGAAACTCTT	GTGTACATTGTCAGGCAGAAACAT	CTGGTGAGAA**[C/T]**AGGAATTACC	+
11	Omy28_11676622	28	11,676,622	intergenic	CGAATGCACTGTAGCTCATTCTAA	GCAGTAGAATGTCTCGCAAATACA	ACATGTCATT**[T/G]**ATTGTTATCT	+
12	Omy28_11683204	28	11,683,204	intergenic	CAAGAAAGAAACAGATGTTGTCCA	TTGTGACTCAAATCTGCAACCTAT	ATGTAAAAAA**[G/T]**GGCAGAAAA	+
13	Omy28_11773194	28	11,773,194	*rock1*	AGTTTGACACCCCTGTACTAGAGC	GTCTAACAAGCTCTGGGTGATTTA	GCAATTTTTT**[T/A]**AAATTACCGC	+

Note: The "Order ID" column corresponds to the SNP order, according to the physical position within the genome assembly. SNP names, chromosome number, position, gene, primers, probes, and orientation are also listed and are based on the genome assembly NCBI accession GCF_002163495.1. The premature allele is indicated in the probe column with an underline.

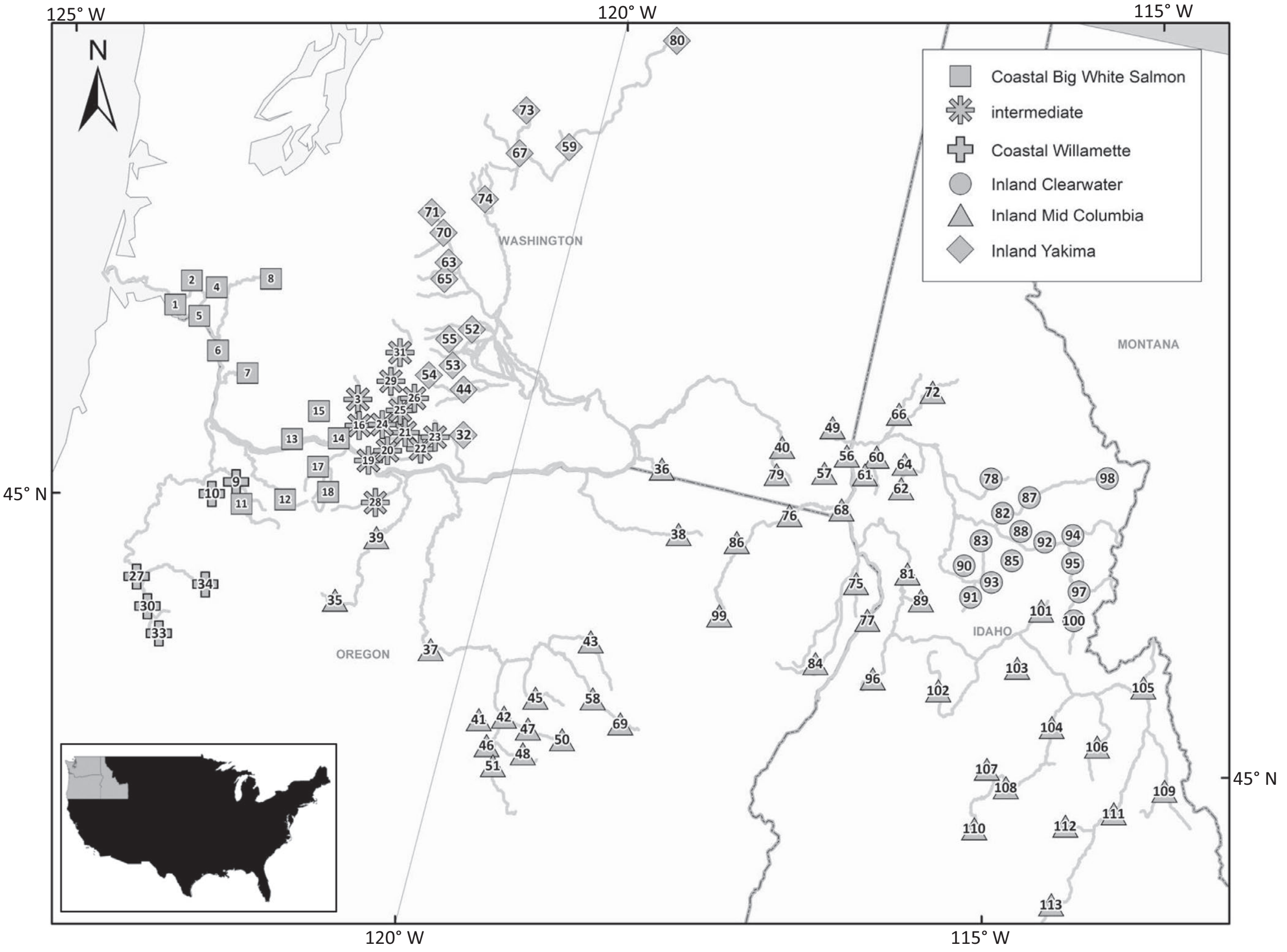


FIGURE 1 Steelhead collection sites numbered according to Table 2

subsample of 25 collections. In cases where markers were consistently significant for LD tests in multiple populations, one in each significant pair was removed leaving a total of 226 markers for all subsequent analyses with neutral markers.

A principal component analysis (PCA) was plotted for all populations based on allele frequencies of putatively neutral markers determined to be without linkage disequilibrium (LD). A discriminant analysis of principal components (DAPC) was conducted with the R package adegenet 2.1.0 to assign probability of individual membership to genetic groups (*K*) (Jombart, 2008; Jombart & Ahmed, 2011). The DAPC recovers maximum genetic variation between groups, while minimizing genetic variation within groups (Jombart, 2008; Jombart & Ahmed, 2011). The adegenet package was used to identify clusters with successive *K*-means and ran for 25 instances for $K = 1$ through $K = 10$. The Bayesian information criterion (BIC) was averaged and scaled by the standard deviation for each *K* value. The most appropriate number of genetic groups was determined with the greatest ΔK value as described in Evanno, Regnaut, and Goudet (2005). The LEA 2.0 R package was used to estimate population structure through sparse non-negative matrix factorization (Frichot & François, 2015).

The distribution of genetic variation underlying adult migration timing in steelhead across the landscape was described by genotype frequencies. We examined 13 markers occurring on chromosome 28 within the *greb1L*, *rock1*, and intergenic region between *greb1L* and *rock1* that were previously shown to be strongly associated with adult migration timing (Hess et al., 2016; Micheletti, Hess, et al., 2018; Table 1). Initially, the two most significant SNPs were retained from a previous RAD study (Hess et al., 2016), and the remaining 11 SNPs with the strongest association with adult migration timing from the whole-genome resequencing conducted by Micheletti, Hess, et al. (2018). To reduce ascertainment bias, we examined genetic variation in this candidate region from several populations of *O. mykiss* in the region to design primers (Table 1). Premature, mature, and heterozygote genotypes for adult migration timing were established based on genotype association from previous studies (Hess et al., 2016; Micheletti, Hess, et al., 2018), as well as using a reference collection of Skamania Hatchery steelhead, which is a hatchery strain intensively selected for early adult migration and cultured since 1956 with steelhead from the Washougal and Klickitat Rivers (Chilcote, Leider, & Loch, 1986; Crawford, 1979). Premature, mature, and heterozygote adult migration timing genotype proportions were assessed across all collection locations. A PCA of allele frequencies of adaptive markers was also conducted for all collection locations to assess genetic groupings based on adult migration timing.

2.3.2 | Haplotype blocks and frequencies

We assessed linkage disequilibrium (LD) within the 13 candidate markers to identify haplotype blocks that would be informative for estimating frequencies of adult migration types. Haplotype blocks within the 13 candidate markers were defined with solid spine LD analysis in the Java Runtime Environment software, Haploview 4.2, across all collection locations (Barrett, Fry, Maller, & Daly, 2005). A solid spine of LD was extended across a haploblock if D', or a normalization of the coefficient of LD, exceeded 0.74. The same markers were assessed for LD in individuals from coastal and inland lineages (as delineated by DAPC) separately. The effect of population structure on the LD of the markers was corrected in the analysis with the LDcorSV 1.3.2 R package (Mangin et al., 2012; Table 2). Variation of genotype proportions was also evaluated with various groupings of the candidate markers.

2.3.3 | Environmental influence on adaptation

Redundancy analyses (RDAs) were conducted for all Columbia River basin collections to model the degree to which the variation in environmental variables explained the variation in allele frequencies of candidate markers included in the haplotype blocks (Borcard, Legendre, & Drapeau, 1992; Kierepka & Latch, 2015). Redundancy analysis was performed on two sets of collections, all populations and each lineage (coastal versus inland), using the R package Vegan 2.5-6 (Oksanen et al., 2019). We selected environmental variables for RDAs at collection sites in this study based on the variables significantly associated with adaptive genetic variation in a previous study (Micheletti, Matala, et al., 2018; Table 3; Table S3). When two highly correlated (>0.75 pairwise correlation; Asuero, Sayago, & Gonzalez, 2006) environmental variables were identified, one was removed from further analyses and the variable kept was determined from biological relevance to salmonids according to previous studies (Hecht, Matala, Hess, & Narum, 2015; Micheletti, Matala, et al., 2018; Olsen et al., 2011). One-way analysis of variance (ANOVA) with Tukey's range test (Tukey, 1949) identified significant variability in salmonid habitat. Environmental variables were analyzed with the "envfit" PCA function of the vegan R package. The ANOVA test and PCA together determined significant environmental variables within and among *O. mykiss* habitats measured in this study. The final RDAs were run with significant environmental variables retained from permutation tests with 1,000 permutations ($\alpha = 0.05$). Frequency of alleles in the haplotype block associated with adult migration timing was correlated with environmental variables with RDA constraint scores. Constraint scores indicated the degree of correlation and whether there was a positive or negative relationship between environmental variables and allelic frequencies.

3 | RESULTS

After aligning markers in common for all samples and accounting for LD, 226 neutral markers (Table S2; Hess et al., 2016) and up to 13 candidate markers from chromosome 28 (Table 1) were included for further analyses. A total of 9,471 individuals from 113 populations met inclusion criteria (>90% loci successfully genotyped and had an estimated <0.5% genotyping error based on replicate genotyping) and were included in this study.

3.1 | Population structure and genetic lineages

Population structure as visualized by PCA of allelic frequencies of neutral markers indicated genetic divergence by geographic locations (Figure 2). The DAPC with neutral markers assigned steelhead to two clusters ($K = 2$): 25 putative coastal collections grouped into one cluster and 90 putative inland collections grouped into the second cluster (Figure 3). Additionally, DAPC and ΔK exposed hierarchical structure with a smaller peak at $K = 6$ (Figure 3). The hierarchical population structure includes well-known population structure within the coastal and inland regions (Blankenship et al., 2011; Matala et al., 2014; Micheletti, Matala, et al., 2018), and admixture coefficient analyses were plotted for $K = 6$ with LEA to visualize the genetic mixing within finer-scale geographic groupings (Figure 4). These finer-scale geographic groupings are also represented by shapes in Figure 2. Most coastal collections, except for Indian Creek, exhibited nonoverlapping allele frequencies relative to all inland collections. The Klickitat River which is located between coastal and inland populations formed a cluster intermediate of the two population types. Inland collections from the Yakima and Clearwater rivers clustered distinctly from others in study (Figure 2).

A second PCA was produced using candidate markers and separated individuals by the proportion of premature and mature adult migration genotypes with markers (2, 3, 6, 9) to incorporate as many collection sites as possible (Figure 5). In contrast to results with neutral markers that separated individuals by sample location and population structure, the PCA with adaptive markers separated individuals by adult migration timing genotypes.

3.2 | Haplotype blocks and frequencies

Candidate markers were analyzed for all sampling locations in Haploview with solid spine, and this resulted in two haploblocks, one with markers 1–7 and another with markers 8–13 (Figure 6a). One haplotype block contained all markers within *greb1L* and another included all or the majority of markers located within the intergenic region upstream of *greb1L* and *rock1*. There was one marker located within *rock1*, but it did not demonstrate as strong of LD as other markers included in the second haplotype block. The intergenic haplotype block, containing markers 8–12, maintained high LD in both inland and coastal collections.

TABLE 2 Linkage disequilibrium R^2 values corrected for population structure for all steelhead collection sites and coastal and inland sites separately

All locations	1	2	3	4	5	6	7	8	9	10	11	12
1	–	–	–	–	–	–	–	–	–	–	–	–
2	31	–	–	–	–	–	–	–	–	–	–	–
3	20	85	–	–	–	–	–	–	–	–	–	–
4	10	7	9	–	–	–	–	–	–	–	–	–
5	34	70	52	9	–	–	–	–	–	–	–	–
6	28	5	6	71	5	–	–	–	–	–	–	–
7	28	2	4	72	5	97	–	–	–	–	–	–
8	0	6	9	8	3	2	2	–	–	–	–	–
9	0	21	23	6	3	3	2	94	–	–	–	–
10	1	3	4	4	1	6	6	63	64	–	–	–
11	0	9	11	3	3	2	2	81	83	65	–	–
12	0	7	10	7	3	2	2	94	95	64	83	–
13	8	2	3	3	4	3	3	30	29	24	29	29
Coastal	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	–	–	–	–	–	–	–	–	–	–	–	–
2	70	–	–	–	–	–	–	–	–	–	–	–
3	42	72	–	–	–	–	–	–	–	–	–	–
4	8	7	10	–	–	–	–	–	–	–	–	–
5	50	68	45	5	–	–	–	–	–	–	–	–
6	20	13	17	80	3	–	–	–	–	–	–	–
7	20	4	5	83	3	99	–	–	–	–	–	–
8	3	8	10	53	4	29	30	–	–	–	–	–
9	2	16	20	37	3	48	21	81	–	–	–	–
10	19	5	5	39	3	54	54	17	25	–	–	–
11	5	12	11	16	5	10	10	57	66	25	–	–
12	3	8	11	38	4	21	22	77	85	24	63	–
13	7	5	7	11	8	8	8	9	7	2	4	7
Inland	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	–	–	–	–	–	–	–	–	–	–	–	–
2	24	–	–	–	–	–	–	–	–	–	–	–
3	19	93	–	–	–	–	–	–	–	–	–	–
4	0	3	0	–	–	–	–	–	–	–	–	–
5	27	67	56	5	–	–	–	–	–	–	–	–
6	7	1	1	47	0	–	–	–	–	–	–	–
7	7	0	0	55	0	92	–	–	–	–	–	–
8	1	7	6	0	3	1	1	–	–	–	–	–
9	1	27	26	0	4	0	1	98		–	–	–
10	0	1	1	0	0	0	0	76	75	–	–	–
11	1	5	7	0	2	1	1	92	91	76	–	–
12	0	9	7	0	5	1	1	96	96	73	89	–
13	2	3	4	0	5	0	0	48	47	41	52	47

Note: Column and row numbers indicate the SNP order ID from Table 1.

Haplotype blocks were examined separately for coastal (Figure 6b) and inland (Figure 6c) lineages. In the coastal lineage, high LD was retained at markers 1–12 relative to the inland lineage. Elevated LD in the coastal lineage markers resulted in one haplotype block, spanning markers 1–12 (Figure 6b). Marker 13 was not retained in the coastal lineage haplotype block and is the only marker from the *rock1* gene.

The solid spine analysis revealed three haplotype blocks in the inland lineage, which were split between markers 2 and 3 and markers 7 and 8 (Figure 6c). Additionally, minor allele frequencies (MAFs)

TABLE 3 Notation, descriptions, units, resolution, variable class, source, and whether the variable was retained in the model are listed for all environmental variables assessed with the RDA models

Notation	Description	Unit	Res. (m)	Class	Source	Retained in model
mig_dist	Migration Distance	km	30	Topography	USGS	Y
elev_mean	Elevation	m	30	Topography	USGS	N
wtemp	Water Temp	°C	30	Temperature	NorWeST	Y
hli	Heat Load Index	hli	30	Temperature	ESRI	N
B1_meanT	Annual Mean Temp	°C	1,000	Temperature	WorldClim	Y
B2_meantrange	Mean Diurnal Range	°C	1,000	Temperature	WorldClim	N
B3_isotherm	Isothermality	°C	1,000	Temperature	WorldClim	Y
B4_tseason	Temp Seasonality	°C	1,000	Temperature	WorldClim	N
B5_maxtwarmmon	Max Temp Warmest Month	°C	1,000	Temperature	WorldClim	Y
B6_mintcoldmon	Min Temp Coldest Month	°C	1,000	Temperature	WorldClim	N
B7_trange	Temp Annual Range	°C	1,000	Temperature	WorldClim	N
B8_meantwetq	Mean Temp Wettest Quarter	°C	1,000	Temperature	WorldClim	N
B9_meantdryq	Mean Temp Driest Quarter	°C	1,000	Temperature	WorldClim	N
B10_meantwarmq	Mean Temp Warmest Quarter	°C	1,000	Temperature	WorldClim	N
B11_meantcoldq	Mean Temp Coldest Quarter	°C	1,000	Temperature	WorldClim	Y
B12_Prec	Annual Precip	mm	1,000	Precipitation	WorldClim	Y
B13_precwetmon	Precip Wettest Month	mm	1,000	Precipitation	WorldClim	Y
B14_precdrymon	Precip Driest Month	mm	1,000	Precipitation	WorldClim	N
B15_precseason	Precip Seasonality	mm	1,000	Precipitation	WorldClim	N
B16_precwetq	Precip Wettest Quarter	mm	1,000	Precipitation	WorldClim	N
B17_precdryq	Precip Driest Quarter	mm	1,000	Precipitation	WorldClim	N
B18_precwarmq	Precip Warmest Quarter	mm	1,000	Precipitation	WorldClim	N
B19_preccoldq	Precip Coldest Quarter	mm	1,000	Precipitation	WorldClim	N

were lower for all inland markers except for candidate markers 8–12 (Figure 7; Table S4). Variation in LD occurred among markers 1–7 and was weaker than in the coastal lineage (Figure 6b,c). The haplotype block split between markers 7 and 8 observed in the inland lineage was the same position as the split in all collections (Figure 6a), indicating the split for all collections was influenced by the inland collections. Further, a greater divergence between average MAF values can be observed between markers 7 and 8 of the inland collections than in the coastal collections (Figure 7).

Subsequent LD analyses were applied to the inland lineage samples, and variation was observed in the resulting haplotype blocks between analyses. The LD analyses done in addition to solid spine were conducted with confidence intervals (0.95 upper, 0.7 lower; Gabriel et al., 2002) and the four gamete rule, which assumes recombination when all four possible haplotypes are detected at frequencies exceeding 0.01 (frequency > 0.02–0.03; Wang, Akey, Zhang, Chakraborty, & Jin, 2002). The differing results between analyses were the inclusion or exclusion of markers 1 and 13 and the split between markers 5 and 6 or between markers 7 and 8. The difference in the location of where haplotype blocks were split could be influenced by fixed alleles at markers 4, 6, and 7 in some collections (Table S4). All Snake River haplotype block analyses were limited to markers 2, 3, 6, and 9 because these markers were developed earlier than the rest and were the only markers available when samples were collected in this basin. This resulted in limited data availability (4 instead of 13 candidate markers) for the farthest inland collections. Haploview linkage analysis comparing lineages was done both with and without the

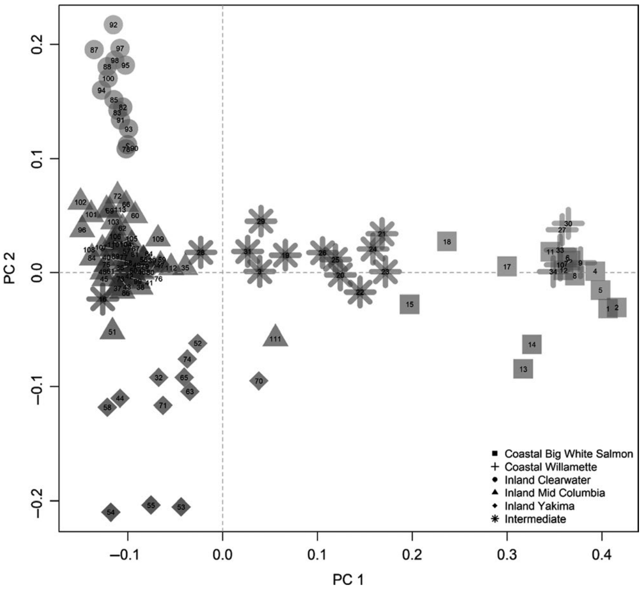


FIGURE 2 Neutral marker PCA plot for all steelhead populations. See Table 2 for collection names. Shapes indicate the geographic region of the population

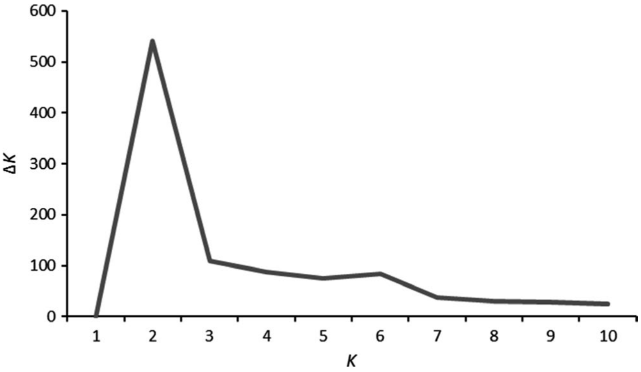


FIGURE 3 Delta K results based on DAPC Bayesian information criterion (BIC) values averaged over 25 iterations and divided by the standard deviation for K values 1–10

individuals that were only genotyped at 4 of the 13 markers and both analyses yielded the same results.

We examined six different combinations of markers to ascertain which sets of markers produce similar genotype frequencies. Genotype frequencies of marker combinations were evaluated to determine whether all markers are necessary to detect the genotypes associated with adult migration timing. The marker combinations included a single marker (9), three markers (2, 3, 6), four markers (2, 3, 6, 9), five markers (8–12), six markers (2–7), and 11 markers (2–12). This allowed for comparison across marker groups to determine whether frequencies across different marker combinations were similar. In general, all six combinations of marker groups provided similar haplotype frequencies with differences in associated haplotypes only differing by 1%–7% (Figure 8). The groups with the most similar genotype frequencies were marker 9 alone and markers 8–12; markers 2, 3, and 6 and markers 2–7 were similar; and markers 2, 3, 6, and 9 and markers 2–12 also had similar average genotype frequencies (Figure 8).

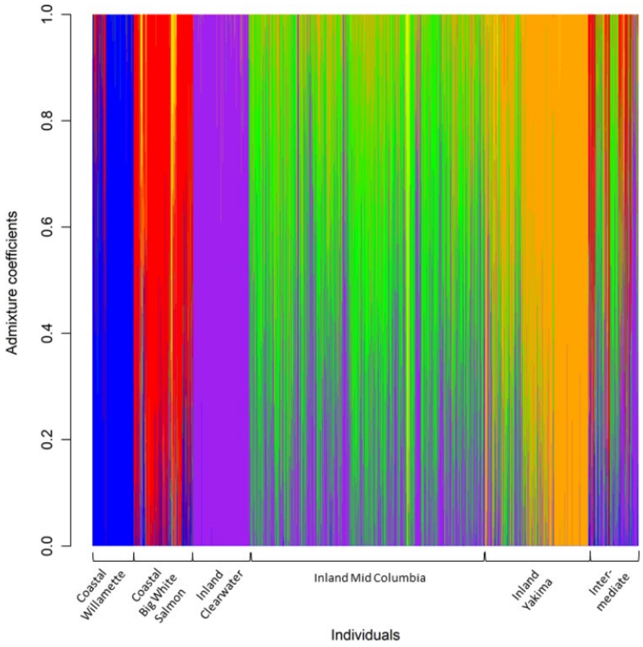


FIGURE 4 Admixture coefficients for each individual based on sparse non-negative matrix factorization least-squares optimizations to estimate hierarchical population structure at $K = 6$ for steelhead collections

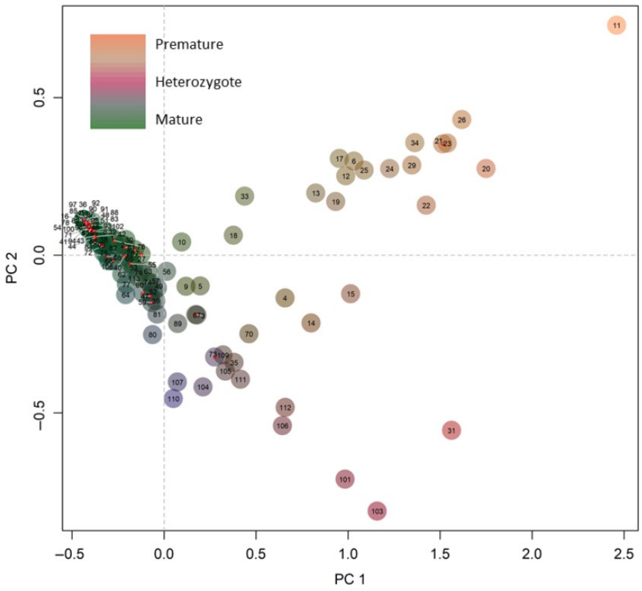


FIGURE 5 PCA of candidate markers (2, 3, 6, 9) for all steelhead populations. Populations are color-coded by genotype (premature, mature, heterozygous) combinations of the candidate markers. See Table 2 for collection names. Four markers were included in the analysis and thus represent a range of genotype combinations shown in various shades

The mature genotype was predominant throughout much of the range in the Columbia River; however, many populations west of the Cascade Mountains and in the Salmon River have greater

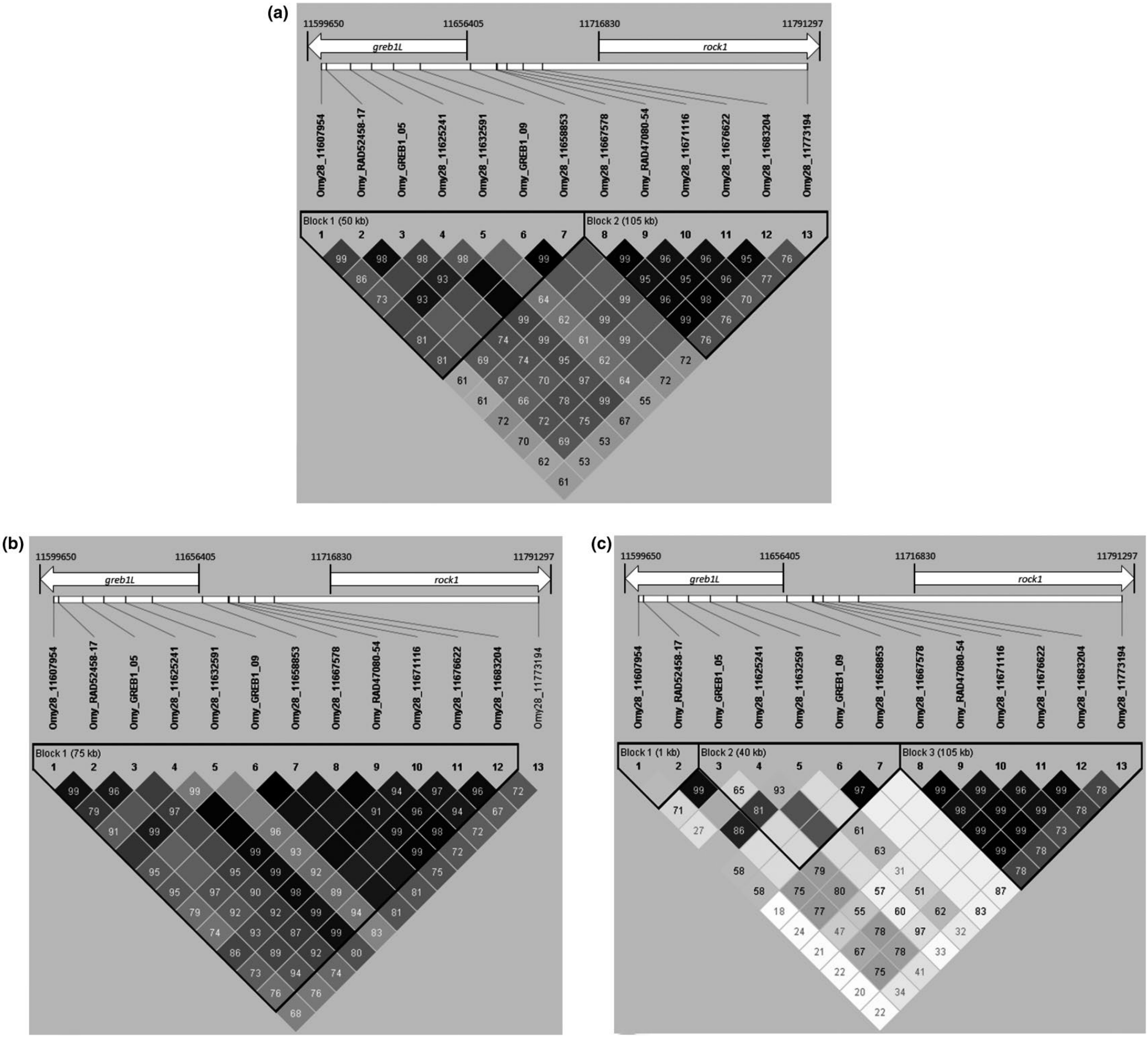


FIGURE 6 Linkage relationships for 13 candidate markers in Haploview for (a) all steelhead populations, (b) coastal populations, and (c) inland populations

proportions of the premature genotype than other collections (Figure 9a,b). However, only 9 of the 113 populations had a higher frequency of premature alleles for early adult migration. To evaluate haplotype frequencies for a single haplotype block in as many locations as possible, we further scrutinized haplotypes for markers 2, 3, and 6 across the landscape and found five unique haplotypes (Figure 9a). Haplotype frequencies for collections (Figure 9a) showed similar patterns of geographic distribution as the genotype frequencies (Figure 9b), but with improved resolution for heterozygous haplotypes that were within a single haplotype block underlying *greb1L*. According to results of overall haplotype frequency (Figure 9a), the recombinant haplotype 4 is present more frequently than the premature haplotype 5. Additionally, there is a distinct separation of recombinant haplotypes between coastal (haplotypes 2 and 3) and inland (haplotype 4) collections (Figure 9a).

3.3 | Environmental influence on adaptation

To model impacts of significant environmental variables on allelic frequencies of adult migration timing-associated markers, RDAs were done for all Columbia River basin collections and then separately for coastal and inland lineage collections. The length of the arrow from the RDA represents the magnitude of the correlation of the environmental variable in the model, and the direction of the arrow represents whether the relationship to the variable is positive or negative for a given population (Figure 10a–c). Significant environmental variables retained in the RDA for all collections were adult migration distance, minimum temperature of the warmest month, 20-year average August water temperature, annual mean temperature, isothermality, and annual precipitation (Figure 10a). Annual precipitation had the greatest effect when all collections

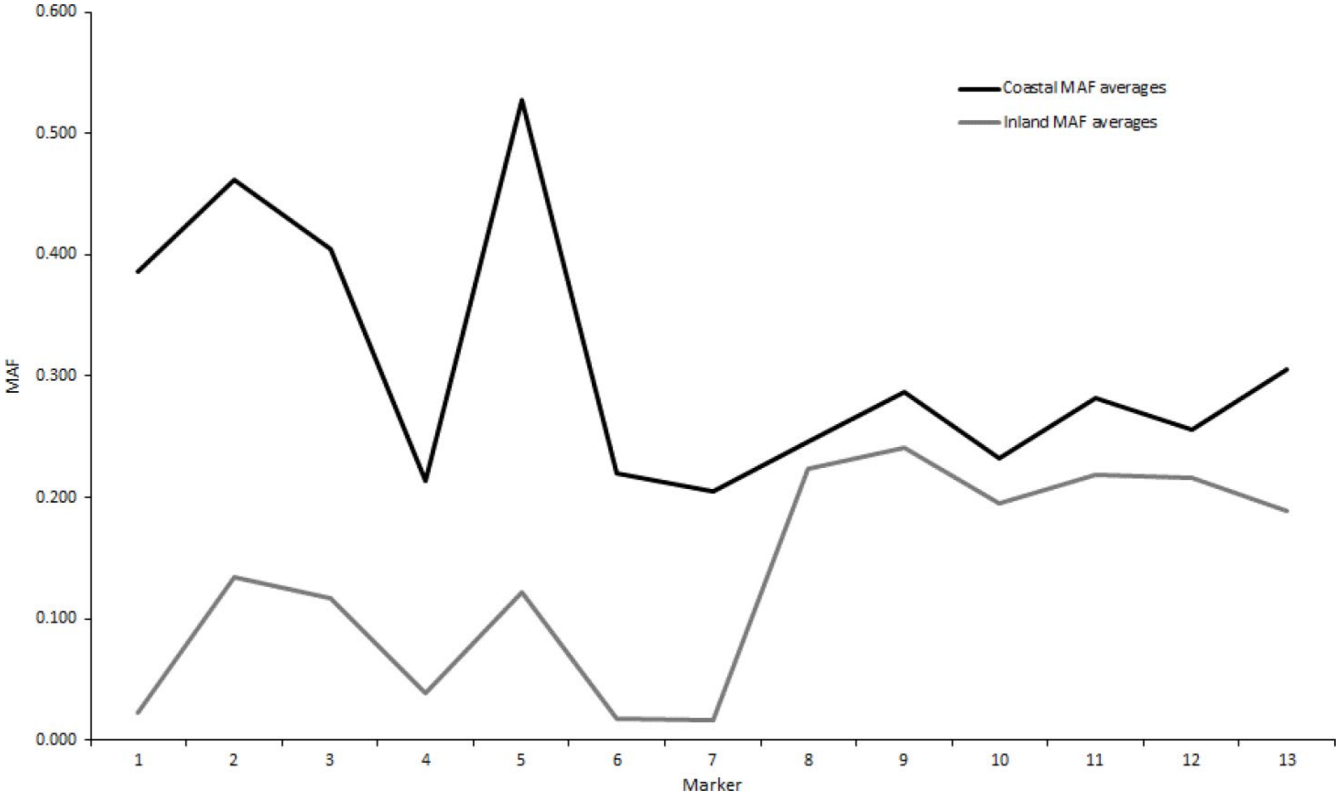
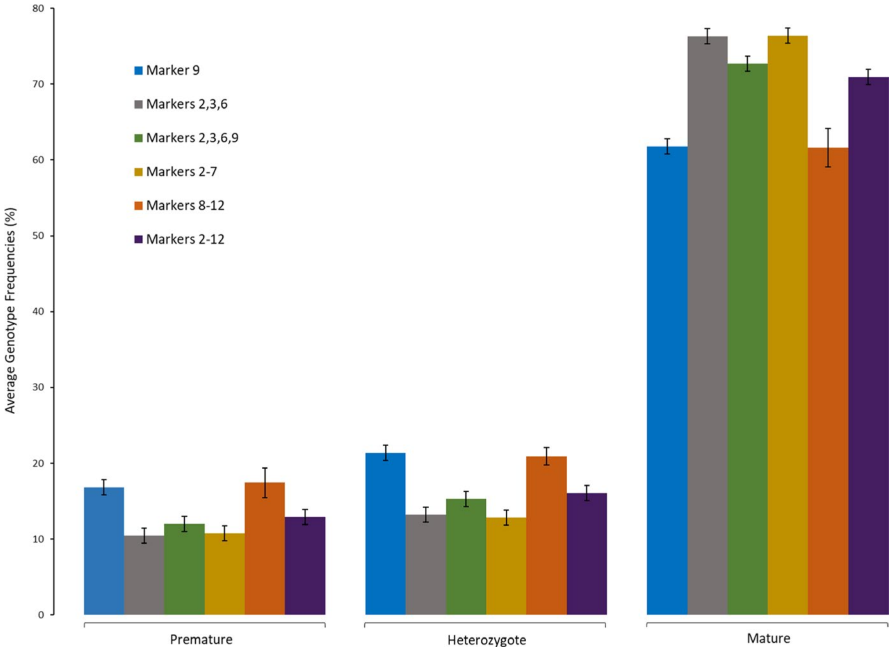


FIGURE 7 Minor allele frequency (MAF) for 13 candidate markers for each of the two major lineages of steelhead in the Columbia River. Coastal collection averages are represented by the black line, and inland collection averages are represented by the grayline

FIGURE 8 Comparison of average genotype frequencies (all steelhead populations) for different sets of candidate markers. The six sets of markers include the following: a single marker (9 as in Micheletti, Hess, et al., 2018); three markers from the same *greb1L* haplotype block (2,3,6); four markers available for all collections (2, 3, 6, 9); five markers from the intergenic haplotype block (8–12); six markers from the *greb1L* haplotype block (2–7); and 11 markers excluding one from each of the distal ends of the candidate genomic region. Error bars represent standard error



were analyzed together (Figure 10a). Environmental variables retained in the coastal lineage RDA were average temperature of the coldest quarter and precipitation of the wettest month (Figure 10b). Environmental variables retained in the interior lineage RDA were 20-year average August water temperature and minimum temperature of the warmest month (Figure 10c). To compare genotypes to the environmental variables, we graphed each significant variable against the premature allele frequency for markers (2, 3, 6, 9) at each collection site (Figure S1). The relationships between genotypes and significant environmental variables were not robust for these data, but were significant for maximum temperature of the warmest month, annual precipitation, and migration distance (Figure S1).

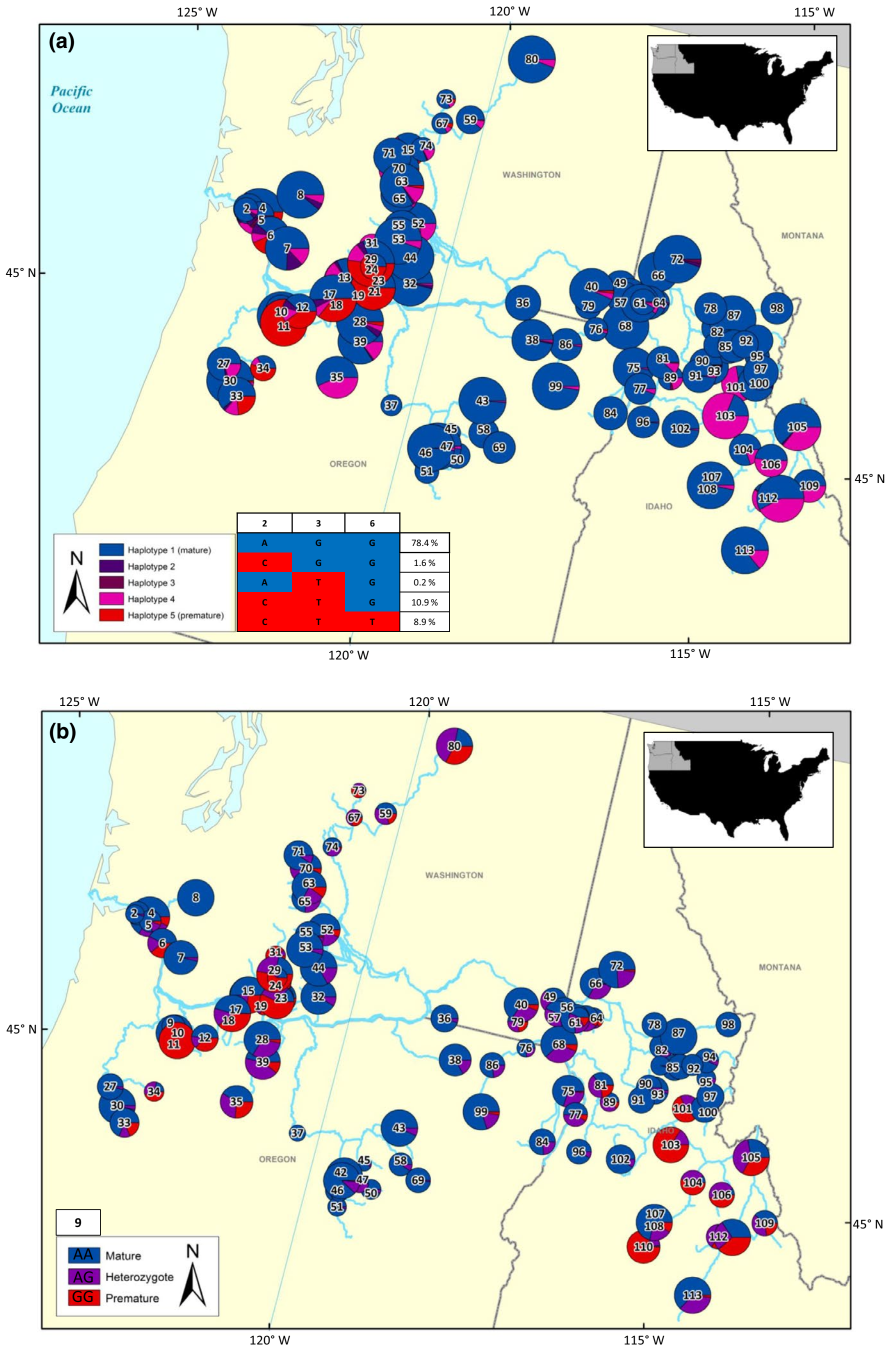


FIGURE 9 (a, b) Maps of haplotype and genotype proportions for all steelhead collection locations. Pie chart size corresponds to population size, except populations that exceeded 100 individuals were reduced to 100 with the same haplotype proportions to keep the circles on the map as visible as possible. See Table 2 for collection names and exact genotype proportions. The first map (a) demonstrates the proportions of individuals at each collection location with the five unique haplotypes from markers 2, 3, and 6. These 3 markers were evaluated to include as many populations as possible, while excluding marker 9 due to a greater association with haplotype block 2. The haplotypes representative of the heterozygote genotype are depicted as a gradient corresponding to the number of markers that match either fixed genotype. The percentage of individuals with each haplotype is reported in the table. The completely blue haplotype matches the mature genotype and is the most frequent, while the completely red haplotype matches the premature genotype and is the third most frequent. The haplotypes with a mixture of blue and red represent the different possible heterozygote genotypes. The second map (b) incorporates only candidate marker 9 (Omy_RAD47080-54), as it was in a different linkage block than the other three markers

4 | DISCUSSION

This study provides further insight into the spatial distribution of genetic variation underlying adult migration timing in a broad range of steelhead populations. Genetic relationships were characterized for neutral markers for 113 populations, supporting previous findings of population structure and demonstrated strong differences between major lineages (Blankenship et al., 2011; Matala et al., 2014; Micheletti, Matala, et al., 2018). Further, we determined linkage blocks for 13 candidate markers associated with adult migration timing and different recombinant haplotypes were found to be predominant in coastal versus inland lineages. Environmental drivers of candidate variation revealed the importance of temperature and precipitation to selection on variation for adult migration in this system. Overall, this study provides extensive geographic variation for candidate markers associated with adult migration timing that is expected to be important for conservation applications in this species (Waples & Lindley, 2018).

4.1 | Population structure and genetic lineages

Patterns of genetic variation among steelhead populations were highly distinct between neutral and candidate markers. Neutral structure was consistent with previous studies with various marker types that largely correspond to geographic population structure and

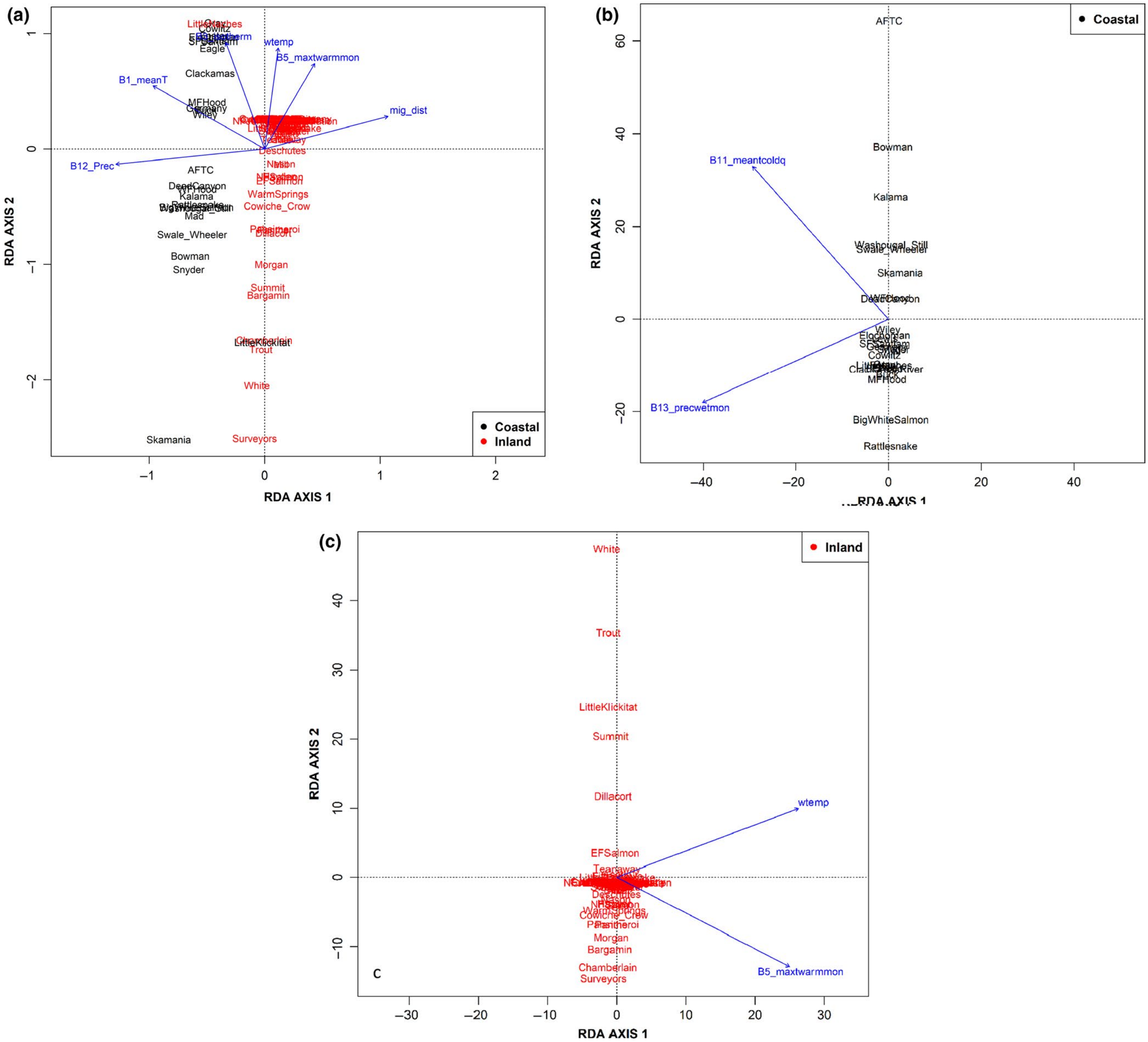


FIGURE 10 (a–c) RDA of all steelhead collections in Columbia River basin to model the degree to which the variation in environmental variables explains the variation in allele frequencies for candidate markers for all collections in the *greb1L* haplotype block (2, 3, 6). The populations are represented by text and colored black or red in accordance with their lineage determined by DAPC in adegenet. The arrows spatially denote a significant influence of environmental variables, and the length of the arrow indicates the extent of the effect. Environmental variables retained were migration distance, minimum temperature warmest month, August water temperature over a 20-year average, annual mean temperature, isothermality, and annual precipitation. Coastal populations (b) and inland populations (c) were analyzed separately. Environmental variables retained in RDA of coastal populations were mean temperature coldest quarter and precipitation of the wettest month. Environmental variables retained in RDA of inland lineage populations were August water temperature over a 20-year average and minimum temperature of the warmest month

significant heterogeneity in environmental conditions (Blankenship et al., 2011; Matala et al., 2014; Micheletti, Matala, et al., 2018). For example, steelhead in the Clearwater River have consistently shown a distinct genetic signal from others in the Snake River basin regardless of marker type (Campbell et al., 2012; Matala et al., 2014; Micheletti, Matala, et al., 2018; Narum et al., 2008). Additionally, the neutral markers provided further resolution than previous studies for the inland lineage, especially for populations in the Yakima River drainage that were distinct from the rest of the populations in the middle Columbia River. The distinct neutral patterns in the Clearwater and Yakima River drainages were likely due to different levels of genetic influence from hatchery programs (Blankenship et al., 2011). Current steelhead populations in the Yakima River are natural origin, but have been influenced by prior hatchery programs, such as introgression from Skamania and Wells stocks (Freudenthal, Lind, Visser, & Mees, 2005; Howell et al., 1985). Large stretches of the Clearwater River basin, including the Selway and Lochsa Rivers, are managed exclusively for wild fish (Campbell et al., 2012; Nielsen, Byrne, Graziano, & Kozfkay, 2009). The intermediate status of the Klickitat River collections was evident in neutral PCA clusters which are consistent with the previous studies (Micheletti, Matala, et al., 2018). This intermediate signal was also observed in two other populations, Fifteenmile Creek and Mill Creek, which may indicate gene flow with steelhead in the Klickitat River or admixture.

In contrast to geographical patterns observed at neutral loci, the candidate PCA divided collections by their predominant adult migration timing. The Skamania stock was a useful reference for the extreme extent of fixed genetic variation for premature alleles as this Skamania stock is well known for early adult migration and represents the majority of the early returning adult steelhead each migration year (Hess et al., 2016). The development of the Skamania stock started in the 1950s and included intentional selection for early returning fish so that smolts could be released within a year rather than the typical two-year smolt age of wild fish (Crawford, 1979). At the other end of the spectrum, the mature genotype was predominant in most collections, while the heterozygote collections were dispersed across the basin, but with divergent ratios of haplotypes between coastal and inland lineages. The presence of genetic variation for premature alleles in the inland lineage suggests that some populations of steelhead (i.e., those in the Salmon River drainage) may exhibit phenotypic variation for early and late adult arrival timing to spawning grounds as shown by Micheletti, Matala, et al. (2018).

4.2 | Haplotype blocks and frequencies

Haplotype blocks of markers with the greatest association with one another and with the adult migration timing phenotype improve ability to evaluate genetic variation associated with adult migration timing across the landscape. In addition to LD assessments, we evaluated differences between average genotype frequencies with fewer candidate markers. Marker 9 had the most similar average genotype frequencies to markers 8–12 for all genotypes, and markers 8–12 had the greatest LD in all collections. This finding suggests that marker 9 could be useful under circumstances of limited genotyping abilities. This same marker was also helpful at distinguishing patterns in steelhead arrival timing to spawning grounds as shown previously (Micheletti, Matala, et al., 2018). However, it is still beneficial to assess collections with entire haplotype blocks when possible, to generate numerous haplotype combinations instead of only three genotypes gained from a single marker.

In this study, we assessed the spatial distribution of candidate haplotype frequencies because selective pressures on adult steelhead migration are disparate across the heterogeneous landscape. The coastal lineage contained steelhead maturing both in the ocean and streams, whereas inland lineage steelhead only matured in streams. Initial studies (Hess et al., 2016; Prince et al., 2017; Thompson et al., 2019) identified and associated *greb1L* genotypes with adult freshwater entry, while Micheletti, Hess, et al. (2018) revealed a greater *greb1L* association with arrival timing to spawning grounds. We also detected more than one genotype present in inland collections, further supporting an association with arrival timing to spawning grounds because if the association was only with freshwater entry, all inland steelhead with early freshwater entry would be expected to maintain the same premature genotype. Our study incorporated more collections and more candidate markers associated with adult migration timing than previous studies, which allowed us to determine haplotypes to describe the spatial pattern of mature and premature genotypes across the Columbia River basin in greater detail. Coastal collections exhibited greater genetic diversity at candidate markers, but greater influence of premature alleles from Skamania stocks (Chilcote et al., 1986; Kostow, Marshall, & Phelps, 2003; Reisenbichler, McIntyre, Solazzi, & Landino, 1992). In the inland lineage, the mature genotype was detected at high frequency despite all inland steelhead maturing in freshwater, supporting findings by Micheletti, Hess, et al. (2018). Variation in the second haplotype block, which includes markers in the intergenic region, indicates that inland populations retain genetic variation that may allow for variable timing in arrival to spawning grounds. Additionally, the distinct separation of recombinant haplotypes between coastal (haplotypes 2 and 3) and inland (haplotype 4) collections (Figure 9a) further supports multiple recombination events within the inland lineage where phenotypic timings between freshwater entry and arrival timing differ (Micheletti, Hess, et al., 2018). However, further studies are needed that dissect arrival phenotypes and the association at candidate markers at *greb1L* and *rock1*.

4.3 | Environmental influence on adaptation

We observed significant association between multiple environmental variables and candidate markers when examined across lineages, which was expected given that environmental conditions vary significantly across the Columbia River basin landscape. We found adult migration distances, temperature variables, and precipitation variables had the strongest association with adaptation for all collections which was consistent with previous landscape genomics analyses (Micheletti, Matala, et al., 2018). In this model, the direction of the relationship with the collection sites was not the same for each site. Significant relationships between environmental variables and candidate allele frequencies suggest that these may be environmental drivers leading to local adaptation among populations. Adult migration distance traveled between the Pacific Ocean and spawning sites ranged from 60 to 1,400 km, presenting a vast difference between coastal and inland lineages of salmonids in energetic allocation before spawning (Hecht et al., 2015; Olsen et al., 2011). However, adult migration distance was not significantly associated with candidate markers for either lineage when analyzed separately. This result suggests that variation at candidate markers is not highly distinct among populations within each of the two lineages. Significant association of temperature with candidate markers was not surprising since fish rely on environmental temperatures to regulate body temperatures and trigger migratory behavior (Jonsson, 1991; Sykes, Johnson, & Shrimpton, 2009), and extreme temperatures can inhibit cardiac and metabolic proficiencies (Chen, Farrell, Matala, Hoffman, & Narum, 2018). Further, genetic disparities in thermal tolerance when encountering temperature barriers have been found to contribute to local adaptation in salmonids (Eliason et al., 2011; Muñoz, Farrell, Heath, & Neff, 2015; Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013). Finally, the significance of precipitation with variation at candidate markers is expected to be important since precipitation conditions can impact survival and selection on genes associated with thermal tolerance when stream flows are low (Heath, Busch, Kelly, & Atagi, 2002) and water temperatures are elevated (Narum et al., 2013). In contrast, when precipitation is high and stream flows are powerful, conditions may become energetically costly for migrating steelhead, but also provide cues for adult migration to spawning grounds (Keefer & Caudill, 2014; Keefer et al., 2018). Significantly associated environmental variables within each lineage were more limited than across lineages of steelhead and largely reflected regional differences in precipitation within the coastal lineage and temperature within the inland lineage.

From a management perspective, accounting for the distribution of genetic variation underlying adult migration run timing has direct conservation implications as described in detail by Waples and Lindley (2018). Early migrating fish spend less time feeding in the nutrient-rich ocean, resulting in less opportunities for growth and potential for decreased reproductive success. Further, more time in freshwater systems exposes early migrators to thermal stress, disease, and greater risk of impacts of climate change and selective fisheries (Quinn et al., 2015). Thus, adult steelhead with this early migration pattern have increased odds of extirpation and may require greater conservation efforts (Prince et al., 2017). Previous findings (Micheletti, Hess, et al., 2018) were bolstered by this study that indicates greater genetic diversity at candidate genes for inland collections than previously understood. Effective conservation efforts to maintain or increase genetic variation underlying adult migration timing are expected to provide broader life-history diversity for populations to endure stochastic environments. Thus, the maintenance of genetic diversity associated with adult migration timing across the Columbia River basin may be a key to promote resilient steelhead populations that are able to recover from anthropogenic impacts.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

Erin E Collins: Formal analysis (equal); Visualization (equal); Writing-original draft (equal); Writing-review & editing (equal). **John Hargrove:** Writing-review & editing (equal). **Thomas A Delomas:** Writing-review & editing (equal). **Shawn Narum:** Conceptualization (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing-review & editing (equal).

DATA AVAILABILITY STATEMENT

Genotype data are available in Dryad at https://doi.org/10.5061/dryad.jh9w0vt80.

ORCID

Erin E. Collins https://orcid.org/0000-0002-6097-8479
Thomas A. Delomas https://orcid.org/0000-0001-5154-759X
Shawn R. Narum https://orcid.org/0000-0002-7470-7485

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Table A1. Map identifiers, collection names, lineages, sample sizes, latitudes, longitudes, genotype proportions, year, and number of SNPs for all steelhead sample collections. The numbers in parentheses after the number of SNPs are the number of candidate markers sequenced. Lineages were determined with DAPC with markers (2,3,6,9).

Map ID	Population	Lineage	Sample size (n)	Latitude	Longitude	Premature genotype (%)	Heterozygote genotype (%)	Mature genotype (%)	Year collected	Number of SNPs	Life Stage
1	Gray River	Coastal	26	46.21	-123.36	0.00	11.54	88.46	2006	390 (13)	Adult
2	Elochoman River	Coastal	41	46.23	-123.33	1.83	12.20	85.98	2004-2006	390 (13)	Adult
3	Mill Creek	Intermediate	180	46.19	-123.18	9.17	29.44	61.39	2005	390 (13)	Both
4	Abernathy FTC	Coastal	90	46.23	-123.15	22.78	34.44	42.78	2001-2002	390 (13)	Adult
5	Germany Creek	Coastal	36	46.19	-123.12	13.89	19.44	66.67	2005	390 (13)	Juvenile
6	Kalama River	Coastal	64	46.03	-122.87	39.06	24.61	36.33	2005	379 (4)	Adult
7	Lewis River	Coastal	87	45.96	-122.56	2.30	15.80	81.90	2005	390 (13)	Adult
8	Cowlitz River	Coastal	120	46.50	-122.59	0.63	7.29	92.08	2005	390 (13)	Adult
9	Eagle Creek	Coastal	41	45.35	-122.38	9.15	23.17	67.68	2005, 2006	390 (13)	Both
10	Clackamas River	Coastal	194	45.30	-122.35	8.63	21.13	70.23	2006, 2010	390 (13)	Both
11	Skamania Stock	Coastal	301	45.24	-122.28	100.00	0.00	0.00	2006	390 (13)	Adult
12	Washougal & Still Creeks	Coastal	54	45.39	-122.10	40.74	18.52	40.74	2006, 2010	390 (13)	Both
13	Buck Creek	Coastal	72	45.80	-121.53	20.14	49.31	30.56	2016, 2018	390 (13)	Both
14	Rattlesnake Creek	Coastal	102	45.80	-121.48	32.84	26.23	40.93	2005, 2018	390 (13)	Both
15	Big White Salmon	Coastal	55	47.22	-121.10	40.45	26.36	33.18	2006, 2016	390 (13)	Both
16	Indian Creek	Intermediate	22	45.80	-121.48	0.00	0.00	100.00	2007	390 (13)	Juvenile
17	WF Hood River	Coastal	186	45.60	-121.63	38.44	20.16	41.40	2008, 2011-2015	390 (13)	Adult
18	MF Hood River	Coastal	42	45.58	-121.63	13.10	32.14	54.76	2000	379 (4)	Adult
19	Dillacort Creek	Intermediate	24	45.74	-121.22	27.08	43.75	29.17	2011	390 (13)	Juvenile
20	Snyder Creek	Coastal	24	45.82	-121.16	60.42	34.38	5.21	2017	379 (4), 390 (13)	Both
21	Swale & Wheeler Creeks	Coastal	89	45.81	-121.07	50.56	35.96	13.48	2005, 2011, 2017	390 (13)	Both
22	Little Klickitat River	Intermediate	70	45.84	-121.06	50.36	32.50	17.14	2005	390 (13)	Both
23	Bowman Creek	Coastal	82	45.85	-121.04	52.44	33.84	13.72	2005	390 (13)	Both
24	Dead Canyon Creek	Coastal	31	45.94	-121.14	40.32	37.10	22.58	2005	390 (13)	Adult
25	Summit Creek	Intermediate	52	45.99	-121.13	35.10	37.02	27.88	2005	390 (13)	Adult
26	White Creek	Intermediate	30	46.01	-121.15	60.00	23.33	16.67	2005	390 (13)	Adult
27	SF Santiam River	Coastal	51	44.69	-123.01	2.45	18.14	79.41	2010	390 (13)	Adult
28	Fifteenmile Creek	Intermediate	121	45.45	-121.12	1.65	16.74	81.61	2012, 2013	390 (13)	Adult
29	Trout Creek	Intermediate	122	46.04	-121.20	51.64	22.54	25.82	2005, 2007	390 (13)	Adult
30	Foster	Coastal	346	44.54	-122.84	1.66	13.44	84.90	2017	379 (4)	Adult
31	Surveyors Creek	Intermediate	30	46.20	-121.25	68.33	15.83	15.83	2005	390 (13)	Adult
32	Quartz Creek	Inland	93	45.95	-120.51	0.00	4.84	95.16	2008, 2012	390 (13)	Both

Table A1. Continued.

Map ID	Population	Lineage	Sample size (n)	Latitude	Longitude	Premature genotype (%)	Heterozygote genotype (%)	Mature genotype (%)	Year collected	Number of SNPs	Life Stage
33	Wiley Creek	Coastal	64	44.42	-122.67	20.70	19.92	59.38	2010	390 (13)	Adult
34	Mad Creek	Coastal	29	44.75	-122.40	43.97	37.93	18.10	2010	390 (13)	Juvenile
35	Warm Springs River	Inland	80	44.86	-121.24	17.19	30.31	52.50	2008-2009	390 (13)	Adult
36	Touchet River	Inland	56	46.03	-118.68	0.00	1.34	98.66	2010	390 (13)	Adult
37	Bridge Creek	Inland	20	44.73	-120.31	0.00	0.00	100.00	2005	390 (13)	Juvenile
38	Iskuulpa Creek	Inland	77	45.70	-118.40	0.00	6.82	93.18	2011	390 (13)	Both
39	Deschutes River	Inland	92	45.26	-121.03	7.07	18.21	74.73	2011	390 (13)	Adult
40	Tucannon River	Inland	89	46.31	-117.66	1.40	15.17	83.43	2010, 2011, 2013	379 (4)	Adult
41	SF John Day River	Inland	272	44.42	-119.54	0.37	4.69	94.94	2019	358 (13)	Adult
42	MS John Day River	Inland	420	44.46	-119.44	0.24	6.19	93.57	2011, 2013, 2019	358 (13), 390 (13)	Both
43	NF John Day River & Desolation Creek	Inland	162	45.00	-118.94	0.46	2.31	97.22	2007, 2014-2016	358 (13), 390 (13)	Both
44	Satus Creek	Inland	393	46.19	-120.61	0.13	5.15	94.72	2017	379 (4)	Adult
45	Fox Creek	Inland	16	44.62	-119.29	0.00	1.56	98.44	2005	390 (13)	Juvenile
46	Black Canyon Creek	Inland	43	44.33	-119.57	0.00	3.49	96.51	2005, 2007	390 (13)	Juvenile
47	Belshaw Creek	Inland	25	44.44	-119.29	0.00	30.00	70.00	2005, 2007	390 (13)	Juvenile
48	Murderer's Creek	Inland	24	44.32	-119.53	0.00	0.00	100.00	2007	390 (13)	Both
49	Alpowa Creek	Inland	49	46.41	-117.22	6.63	19.90	73.47	2010	379 (4)	Adult
50	Beech Creek	Inland	30	44.41	-119.12	0.00	1.67	98.33	1996, 2005	390 (13)	Juvenile
51	Deer Creek	Inland	26	44.19	-119.51	0.00	0.96	99.04	2007	390 (13)	Juvenile
52	Ahtanum Creek	Inland	79	46.53	-120.68	2.53	25.32	72.15	2014	390 (13)	Both
53	Agency Creek	Inland	28	46.34	-120.87	2.68	16.07	81.25	2017	379 (4)	Adult
54	Toppenish Creek	Inland	589	46.32	-120.87	0.08	2.80	97.11	2009	390 (13)	Both
55	Simcoe Creek	Inland	62	46.45	-120.86	2.02	16.13	81.85	2017	379 (4)	Adult
56	Asotin Creek	Inland	57	46.34	-117.04	7.02	21.49	71.49	2010	379 (4)	Adult
57	George Creek	Inland	51	46.30	-117.12	3.92	22.55	73.53	2010	379 (4)	Adult
58	Camp Creek	Inland	40	44.69	-118.80	0.00	3.75	96.25	2005, 2007	390 (13)	Juvenile
59	Entiat River	Inland	36	47.66	-120.24	7.64	15.97	76.39	2006	379 (4), 390 (13)	Juvenile
60	Webb Creek	Inland	30	46.31	-116.81	4.17	19.17	76.67	2013	379 (4)	Adult
61	Sweetwater Creek	Inland	64	46.29	-116.86	2.34	12.89	84.77	2013	379 (4)	Adult
62	Mission Creek	Inland	50	46.32	-116.71	2.50	13.00	84.50	2013	379 (4)	Adult
63	Nile Creek	Inland	88	46.86	-121.05	5.40	16.76	77.84	2005, 2008, 2012	390 (13)	Both
64	Lapwai Creek	Inland	32	46.33	-116.60	3.91	13.28	82.81	2013	379 (4)	Adult

Table A1. Continued.

Map ID	Population	Lineage	Sample size (n)	Latitude	Longitude	Premature genotype (%)	Heterozygote genotype (%)	Mature genotype (%)	Year collected	Number of SNPs	Life Stage
65	Little Rattlesnake Creek	Inland	64	46.76	-121.05	1.17	17.19	81.64	2005, 2012	390 (13)	Both
66	Little Bear & Big Bear Creeks	Inland	73	46.64	-116.68	0.34	10.62	89.04	2012	379 (4)	Adult
67	Icicle Creek	Inland	20	47.56	-120.67	7.50	35.00	57.50	2007	390 (13)	Adult
68	Joseph Creek	Inland	100	46.04	-117.00	1.50	9.50	89.00	2011-2012	390 (13)	Adult
69	Clear & Granite Creeks	Inland	46	44.59	-118.51	0.00	0.54	99.46	2000, 2005, 2015	390 (13)	Juvenile
70	Cowiche & Crow Creeks	Inland	73	47.02	-121.17	15.07	38.36	46.58	2012	390 (13)	Both
71	Little Naches River	Inland	63	47.11	-121.32	0.00	2.38	97.62	2008, 2012	390 (13)	Both
72	Potlatch River	Inland	102	46.80	-116.42	0.49	8.33	91.18	2010, 2016	379 (4)	Adult
73	Nason Creek	Inland	16	47.80	-120.72	21.88	15.63	62.50	2006	390 (13)	Juvenile
74	Teanaway River	Inland	26	47.26	-120.88	3.85	20.19	75.96	2007, 2008	390 (13)	Adult
75	Lightning Creek	Inland	76	45.66	-116.73	0.33	9.21	90.46	2001	390 (13)	Both
76	Wenaha River	Inland	25	45.95	-117.45	0.00	13.00	87.00	2010	390 (13)	Juvenile
77	Sheep Creek	Inland	44	45.47	-116.56	2.27	15.91	81.82	2014	379 (4)	Adult
78	Lolo Creek	Inland	46	46.39	-115.72	0.00	0.00	100.00	2012	379 (4)	Adult
79	Crooked Creek	Inland	31	46.15	-117.65	5.65	14.52	79.84	2010	379 (4)	Juvenile
80	Omak Creek	Inland	112	48.40	-119.50	9.15	15.63	75.22	2010	390 (13)	Adult
81	White Bird Creek	Inland	47	45.77	-116.29	6.91	20.21	72.87	2014	379 (4)	Adult
82	Canyon Creek	Inland	44	46.22	-115.56	0.00	13.07	86.93	2004	379 (4)	Adult
83	Ohara Creek	Inland	28	46.05	-115.52	0.00	0.89	99.11	2013	379 (4)	Adult
84	Gumboot & Mahogany Creeks	Inland	51	45.17	-116.93	0.00	5.88	94.12	2011-2013	379 (4)	Adult
85	Gedney Creek	Inland	51	46.06	-115.31	0.00	0.00	100.00	2004	379 (4)	Adult
86	Grande Ronde River	Inland	47	45.73	-117.86	0.00	7.98	92.02	2011-2014	390 (13)	Adult
87	Fish Creek	Inland	331	46.33	-115.35	0.00	0.45	99.55	2016	379 (4)	Adult
88	Selway River	Inland	47	46.07	-115.24	0.00	1.60	98.40	2008	379 (4)	Adult
89	Slate Creek	Inland	27	45.64	-116.12	3.70	34.26	62.04	2013	379 (4)	Adult
90	Newsome Creek	Inland	48	45.84	-115.62	0.00	3.65	96.35	2012	379 (4)	Adult
91	Tenmile Creek	Inland	51	45.72	-115.68	0.00	1.96	98.04	2013	379 (4)	Adult
92	ThreeLinks Creek	Inland	34	46.11	-115.07	0.00	0.00	100.00	2012	379 (4)	Adult
93	Crooked River	Inland	56	45.82	-115.53	0.00	2.23	97.77	2013	379 (4)	Adult
94	Moose Creek	Inland	44	46.17	-114.89	0.00	1.70	98.30	2012	379 (4)	Adult
95	Bear Creek	Inland	27	46.02	-114.84	0.00	4.63	95.37	2012	379 (4)	Adult
96	Boulder Creek	Inland	46	45.15	-116.40	0.00	3.26	96.74	2014	379 (4)	Adult

Table A1. Continued.

Map ID	Population	Lineage	Sample size (n)	Latitude	Longitude	Premature genotype (%)	Heterozygote genotype (%)	Mature genotype (%)	Year collected	Number of SNPs	Life Stage
97	White Cap Creek	Inland	55	45.87	-114.73	0.00	0.00	100.00	2008	379 (4)	Adult
98	Crooked Fork Lochsa River	Inland	46	46.53	-114.68	0.00	0.00	100.00	2000	379 (4)	Adult
99	Catherine Creek	Inland	269	45.31	-117.87	0.84	6.69	92.47	2012-2014	390 (13)	Adult
100	Little Clearwater River	Inland	57	45.75	-114.77	0.00	4.39	95.61	2008	379 (4)	Adult
101	Bargamin Creek	Inland	56	45.72	-115.03	41.96	29.46	28.57	2015	379 (4)	Adult
102	Secesh River	Inland	62	45.17	-115.80	0.00	4.03	95.97	2011	379 (4)	Adult
103	Chamberlain Creek	Inland	96	45.38	-115.14	52.34	22.66	25.00	2016	379 (4)	Adult
104	Big Creek	Inland	46	45.09	-114.73	15.76	24.46	59.78	2011	379 (4)	Both
105	NF Salmon River	Inland	217	44.12	-114.43	16.24	29.95	53.80	2016	379 (4)	Adult
106	Panther Creek	Inland	48	45.03	-114.30	27.08	32.81	40.10	2013	379 (4)	Adult
107	Pistol Creek	Inland	37	44.72	-115.15	18.24	10.14	71.62	2012	379 (4)	Adult
108	Rapid River	Inland	317	44.68	-115.15	2.52	8.68	88.80	2012	379 (4)	Adult
109	Hayden Creek	Inland	49	44.86	-113.63	13.78	32.65	53.57	2017	379 (4)	Adult
110	Bear Valley Creek	Inland	82	4.45	-115.23	21.95	2.74	75.30	2011	379 (4)	Adult
111	Pahsimeroi River	Inland	113	44.68	-114.04	25.22	17.92	56.86	2016	379 (4)	Adult
112	Morgan Creek	Inland	46	44.67	-114.23	29.35	27.72	42.93	2012	379 (4)	Adult
113	EF Salmon River	Inland	109	44.12	-114.43	1.83	20.18	77.98	2009, 2010, 2016	379 (4)	Adult

Table A2. Steelhead neutral marker names, chromosomes, positions, primers, probe, and orientation of the SNP based on the genome assembly NCBI accession GCF_002163495.1.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
OMS00078	1	10499333	GAGGGAAGCAGCCATAAACAGAATA	GTCTCACTATGGTCCATATCTGTGTAGA	TCACATGCAT[A,G]AGAGTG	+
Omy_gadd45-332	1	12240053	AGAGAAGACTCACTGCTGTTTGC	AAATCAGTTCCCACGCTATGCT	TTGCTCC[A,G]AAATGG	+
Omy_107031-704	1	18131799	GGCTTTCGGATACTGAGCAACAA	TGAACTCACTGTTGGTATGGACTAGA	TGGACATGATT[G,A]CATAGAC	-
Omy_metA-161	1	24257340	CGCATGCACCAGTTGTAAGAAAG	AGTGCCACCAGCGATAAGAAAA	CAAGTAAGTGGTT[A,C]TATTCT	-
OMS00070	1	55279296	CGTTCCTGCGGGACAGT	GTTTCTCTCACGTCCACAGATCT	AAATACGG[A,G]AATGCAG	+
OMS00003	1	59464348	GTGCCACTGATGAGGATGAGATCA	GTAATAAAGCCCTTTTGTGAGGAAAAACTAAT	TACTGTCG[A,C]CATTTTA	+
Omy_gdh-271	1	63738413	AGGTCAGTCTACTTACAGTATAAAGCAGT	GTCATGTCAACAGAGTAACATAATAAATCTGC	TCACCCTGAA[G,A]TGTAGAC	-
Omy_cyp17-153	1	64509889	GCCCTCCAAGTTCCAAGTGAAAA	CAGGTCATTGATGAAACGTCAGAAC	ATACCTGAGT[G,A]TCATCG	+
OMGH1PROM1-SNP1	1	70466474	TCAAACTGCATTTGATGGAAACAAACAT	AGGACAATTCTAAGTGACCTCAAACTG	TAGTGT[T,A]CACTGACTTCA	-
OMS00008	1	77384035	CCCTTTAAGGAGGATTTTAAATATGTGAGATAGAA	GGATACAGCGTTTTGGAATGAAACT	TCAAATATCCATAAT[T,A]ATATC	+
Omy_stat3-273	1	79898098	CAGACCTCCTCTATCTCCCTATGAG	ACCTCCTTTAAATTGTGCCCAAGAA	[C,T]CAGTTTG	-
Omy_G3PD_2-371	2	6083946	GCAGGTAAGGTACACCATAGAGACA	CTCCCCCTGCCTTACCAAAC	AGACATGTG[G,T]ATTGGCA	-
Omy_98683-165	2	8555140	GCCATTGCCAGAGAATTTGGTTAA	AACACACGCACCATCTTAAAGC	CCAGATACA[T,G]ATTTGT	+
OMS00156	2	12397197	GAGCAGAACACATAGAGGAAAGACT	GTAATCACCCTCTTAGCCTGTATGG	TGTGTCCTGC[T,A]GTAACA	+
Omy_114587-480	2	16204563	CAGATTACGTTATTACGTTTGGGAAATTTTTAAGT	GTGAAAGAGTGGGAAATATAATTATAAGGTCAGA	CCTGTCCA[A,C]AATTGT	-
OMS00138	2	22318448	TCGGACCACATGAGCAGTTC	GTTCAACAGGTGCCCACAC	CTAACAATAACCA[A,C]AGACTG	-
Omy_NaKATPa3-50	2	23893157	GTTGAGCGTGTTATGGGAAAAGAG	TTGCATCGGCTTTCTGAAAACC	TCTGTTTCC[T,G]TTCTTT	+
Omy_u09-53.469	2	48859883	ACAGCCTGAGCGTTTGCA	GGAAACTGGGAGAGATCAAAGGA	TTGCAGCCCTT[A,G]TTGTG	+
Omy_metB-138	2	53792050	TCTGTCCCTGACGCTATAAAAACG	GAAGTATTTCAGCTTAATTTCACTGTTGAGTT	TTCGCCAAAG[A,T]GAAAT	-
Omy_lpl-220	2	60022813	TGACAATCACTGAGCAACTGAACTC	GTCCAGTCTTGCTTCAACTCATTCT	AGT[G,C]ACAGTCA	-
OMS00096	3	29109394	CATGAGAATGGATCAGTCTCCACAA	GATGAAATCTGAATGTGTTGACACTACAG	AAAGAGGAAG[A,C]GTCTCG	+
Omy_112301-202	3	37590554	GTAAACCCTGCCCACATAATTAGGT	CTGAGACACTGCTCCAAGGT	AATGCGAAG[A,C]CAAACT	+
Omy_u09-54-311	3	42920502	GTGGCTCCCCAGGAACAAG	AAGTTTCATGTCACATTCCAGTTACCT	TGGTAATTATTCAACA[G,A]ATCAGT	+
Omy_RAD58835-15	3	53735338	GTCTGCTAAGGTCCTGCAGG	GCCGACCATGAGAGACCTG	ATAGCT[G,T]CTGGGACCCA	+
Omy_u07-79-166	3	57523676	CCCGCTATATTATTTGATCACCCTTGA	ATTTAAATCCATTTCTAAAAATAAGCAAACCTAACCA	CTTGGGAATA[C,A]CCCAGCC	-
Omy_117815-81	3	67894257	CTGCTTTATGCACACCACATTGT	GCTCTTTCTGGAGAACAAGGTACTG	CTATACGGA[G,A]ACCAGC	-
Omy_118654-91	3	75458069	CAGCGTAGACCGTTTCCTCATTAT	GCGCCGATGAGCAGCTT	CAGCTTGTC[T,C]TGCCGC	-
Omy_aromat-280	4	3391469	CTCCATTGATTCATGCCGAACATT	GGAGAGGTCAAACATAGCCTGGTA	TCTTGC[A,G]AACTCC	-
Omy_105075-162	4	7635334	GGAGAAGGACAAGGACATTGGTAAT	AAAGCAGACCACACCATACTTCTC	CTTTCTCTCCT[A,C]CTTTCC	+
Omy_myoD-178	4	10520793	GGTCAAATATTTCATTTACGATTACACTTAGGC	TGGCAAAGCTGTCATTCCTTCTAAT	TTTTATGAGATAT[A,C]ATTTCC	-
Omy_128923-433	4	16436234	CTATGTCCTTGGCAGAAGTCTACA	ACGTTTCTTTGGGCTGAGACTTATT	CATTTTCATTC[A,G]CTGTTTT	+
Omy_130524-160	4	28889024	CGAAGGTAGCGATTGGTCGTT	TGTCTGTTCTGCTGTGTGCTT	ATGGCTT[G,C]ATCCTCA	-
OMS00079	4	35250473	GTAACATTATGAATCTATCAGTTTCCCTAGCT	ACCTGCAACGTTAGAGCTGTTTATT	CTACTTTTCACAGT[A,G]ACACAG	+
Omy_117286-374	4	52310165	TGATGTGTTGTTCCTCATGGCTTA	CTGTGCATTTATTCTTGTGATGCTAGG	TCCTCATCATAC[T,A]CTATGG	-
OMS00087	4	52511964	GCAAATTTCACCCTTAACGTGGTTT	GATTTGATGTGTGTGTATTACCTCCTCTA	GTTAAA[A,G]CTGACAAAGTGT	+
OMS00111	4	63031581	CATGCGGACCTGCATAGCT	GCTTAGCCATTGACAGAGCATATCA	AACCAGACT[A,G]CCATTC	+
OMS00116	5	11341754	GCCTTTCTCCCATATCACATTCGA	AAACGCATCTTACACTGTGTTGTG	TTTACATTTTCAAT[A,T]TTCTG	+
Omy_110362-585	5	14307279	GCAGCCAAGATGAACGAAAACTTC	CCGGCCTGGGTCTCAATG	CACCGCC[C,T]TGCCCGT	+
Omy_bcAKala-380rd	5	53469295	TTGCTCTCTTCTGGTTGCCTTA	CTTCAGGAGAAAGCGCTACTGT	CATAC[C,T]CATCCTATGTCAG	-
Omy_u09-61.043	5	58840810	TAGTCACATCCATAGTAATACTTCC	TGTTCAGAAGCAGAAAACCAATCTCT	CTTGGTCC[T,A]TTTTCA	-
Omy_109525-403	5	84224892	CCTCATTCTCATTGGTGAGTTGTCT	TGTAAGATCTGACCACATGAGTATAACCA	CCTACACCTCTTTT[T,C]TCCACA	-
Omy_105714-265	6	7594847	CCACTCAGTGCAAGCATGGA	GCTTTCAATCCTTGGCTCCAATATC	TGTTGTTTGAG[G,A]TTCAG	-

Table A2. Continued.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
Omy_107285-69	6	9809156	GCCCTTGTGACAATGCACTGTTATA	AGGTCTAGACAGTGTGCCATTTG	ACGTTACTTTT[G,C]ACCTTGT	-
OMS00013	6	10325271	GCCTTTGTTCTCCTTGGTGGTTA	AGAAAAGTGTGGACTGAGGTTGAG	CTTTTCCCT[T,C]GCTACTC	+
Omy_b9-164	6	17599731	GCACAGAACACAGCCAATATTAACA	GCCTTGACTCTCCCTTCATGAC	CCTACAACTTGATCTA[T,-]CGTG	-
Omy_vamp5-303	6	33625138	CTGCTTCCCAATTCAGTATCGTCTT	AGGCTGAAGCATTTCTGAGTATGAA	TGGCCGTAG[-,TAG]TTGGTCA	-
Omy_star-206	6	36624863	CGTGTGCCAGCCCTTCT	GACCACTGAGATCATTGCTGTGA	TTTGGCAC[T,C]ATATCT	+
Omy_IL1b-163	6	42313129	GGAACAACAGGATTAAGCCTACTCT	CCTAAAGGCCTAGGAAACTAAACTTCA	CTGAGGTCATA[A,C]AAATA	+
Omy_cd28-130	7	19036977	CACAACTCCACAGAGACAGTGA	GAGGACAAAACTGACCGTATGGT	CTGTTC[A,G]TTCACCC	-
Omy_131460-646	7	36600038	GTGAAAAGGAATGGAGGAGTACAGT	TGCTAGGACAGGAAGATCATTTGTG	AAAGCAGAATTT[G,A]TTACTG	+
Omy_pad-196	7	44470619	CAAACAACCACAGTAGTCCTCCAAT	GCTTTTCACCCTTTTGTAAATTAAGCCAAA	AAGACAAAGGT[G,A]TAATACC	+
OMS00064	7	45227750	GTGGATATGTAGTTCGATGGAACAGT	TTTACAACAATCTTCTTTTAATAAAAATATAGCCACTTAT	CAGGCAACATTTTAT[A,C]TAACTA	-
OMS00154	7	56234573	GATGTTGGCTGGAGGTGTAGT	TGGGAACACTTTGCCTACCC	AGGGCTTC[T,A]GATTGA	-
Omy_sys1-188	7	56428776	CTTAAATGGTGCTGGTTGCTGTATT	AGTGATATCTTAGTGGGTCGAGGAAA	AAACATGTAC[G,T]ACCTGTC	+
Omy_arp-630	7	57239751	CTGCACAACTTGTTTCCTGCTATT	ACCAAGTGTCCCTGTAAGCC	CCGCTC[C,T]GTCTGCT	-
Omy_97077-73	7	61875465	GTGTAAACAAAATGACTCTGGGATTCAG	AGAAGTGGCAATGGTGTGAAGTAT	TGGTGCAATAG[A,T]AATA	-
OMS00057	7	67908135	GAGAAAGGGAGCATGAGACAGA	GTTGGGCTCCGGTACGAT	CTCCACAG[A,C]ACCTTG	+
Omy_105105-448	7	68303584	CAATTTGCAAGCAGGGAAAGGTTAT	GTGATGGGCTGCAATTGCTT	AAGGAGAAT[G,A]CATAATC	+
OMS00132	8	9451350	GTTTATGACTCCATTGCCGAAATGATT	ACGCGACCTGCAATTCATCAATA	AGCAGTCCTC[T,A]GTGTGG	-
Omy_hsp90BA-193	8	10667361	GGAATCGATGACGACGAAGTGATC	TTCCTCCATGCGTGATGCA	CCTCCGC[G,A]CCTGC	-
OMS00153	8	16303316	ACTTTGCACCATAGGCTTGACAT	TGATAAGGATGATCAAAAAGCTGAAGTATGTA	CAAAATGT[A,C]ATTTTCC	+
Omy_hsf2-146	8	17470364	CCAACAATTGCAGCCTCATCTTAAT	GGAGCAGAAAAAGGATTGGACCTT	ATAATCTA[C,A][T,C]A	-
Omy_srp09-37	8	21426823	TAGTTGTATTAACTCTTCTTTGAGTCTAGA	TCATTCCAGCTCCGTTCTCTTC	TTGTGCTATTGAC[G,A]CCACAG	+
OMS00151	8	23949230	CTAACGTCTTCCCAATGATATTTCACAAGATA	ACCGTGGAAATACAATTTTTTATGCCAAT	ATGACCT[T,C]GATAATC	+
OMS00179	8	25539930	GTCATAACAAAATCAGGGCTTTCCAA	TGGGAGATTTGGGCTGCTTTAAA	CCTCTTCTCTT[T,G]TCTCAT	+
Omy_120255-332	8	29102820	GCTAGCTAACATTGAAGGGTGGAAT	GGCTACAGGGACTTTACAATGGG	ACTATGCCA[T,A]GAAGTTA	+
Omy_104519-624	8	42764123	CGTGTGAGTTTGCGGTAAAGAC	TGACGAGTCCGTCTTATCATCCT	AGCAGGATAC[A,G]TCCGACT	+
Omy_108007-193	8	43671991	GTGAATACCACCCAGGCTTGT	GTCCCTTCCCCAGTTTCACTTAATT	TTTTCTCCC[T,C]ACTTAAC	-
Omy_nkef-241	8	69427037	AGTGTCATTGATGTCGGCCTATTTT	AAACGAATGTCCACCTCAGATGTT	CTTCTGTAT[C,A]ATTTTTG	+
Omy_97865-196	8	82513344	TCCAGACTTCTGGTTTGTTCCATT	CCAGCCCCTATATTCACAATTAAGTGT	ATTAATT[A,G]ACAAGCT	-
Omy_rbm4b-203	9	12485117	CTGAAATTTGATGAATGGAAGCTGCA	CGTATTCAAGTCGATATACAGTCACGAT	ACGTTATTATG[A,-]AAAAGGATGT	+
Omy_112820-82	9	21861264	CCTTTCCTTTTGCATTTCCTCTACTTATTTATTT	AAATGAACTCACGTTGACCTCTGA	CGCCGC[C,T]AAGTTA	-
OMS00103	9	38335692	GAGATCACTGTAGGATTGGCTGTTT	CCTCAGAGCAGCTCACAATGGCATC	CCACAGTAATT[T,A]TTTTTT	+
OMS00056	9	43771325	TCAGGAAGTAAACTGAAAATTCCAATGTATGA	CCCCAACCATGCTTGTTATTGAAC	CTTGACC[A,G]AATAGCA	+
OMS00175	9	50381707	TTGCGATATGGGACTGTATACATTTATTCC	ACTACCTCCAGTTAAAATAGTGTGGGAAA	ATCACTAGTTCA[A,G]ATACAA	-
Omy_tlr5-205	9	60510483	GAGCGTATCTGGTATGGTAACAACA	CTCCAGCAGCTTTAGAGAGTTTACA	CAGTAATATTTC[A,T]GTGCCCG	-
Omy_114315-438	9	64564508	CCTCACCGATCTAGTCAACTTCATC	AGGAGGCTGAGGGAGATTCTAG	TTATGGGCTTA[A,C]GGGTC	-
Omy_gluR-79	10	7508221	GACTGTCTATAGCTATTCTTCTCAAACTGT	AGAAACTACCATTGTGATTAACAGATAGAAAATACAT	CAAGTATTTTGC[G,A]TAGGAAT	+
Omy_109894-185	10	10852282	GGGAGGAATTGGAATGACAGATTAAC	CGGTGTCATTATGGTTGTCATTGTG	CTCCCTG[A,G]TCCCCC	-
M09AAE.082	10	32872704	CTATGTGCAGTGCCCTTCTCA	GGCTTACAAGTATGCATGACTAGCT	AGGTTGTTTTACA[A,C]ATTTAA	+
OMS00095	10	38704654	CTCCAATGGCTGTCAACAATTAAATATAAGAC	GTGTGCTGGTCTCTTCTTTTATTCTCA	AGGCAACTATATAT[T,A]TTTTT	+
Omy_RAD26080-69	10	40486618	TGTGGGACAGCACATACTCC	CCAGGACACCAGTGGAGAAG	ATTAGTA[G,A]CATCATCGAG	+
Omy_ftzf1-217	10	44060919	ACAGGGATGGGCAACTTTGTT	GGATGACCCACGTGACACT	TGACGAGTTC[T,A]GATTT	-
Omy_p53-262	10	46190822	CCCCAACATCCAGTATACAGTTTCA	CCCAAATTGGCAATTTTAATAGGATTCAGA	AAGTAGTATGG[A,T]GCTCTAT	-

Table A2. Continued.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
Omy_tlr3-377	10	56447078	GTCGCTCCGGGTGCTT	GGCCCAAACACTTCCTTCCT	CGTGATTAG[G,A]TTCTTC	+
OMS00106	10	59855697	CGTGTAGCATTCTTGAGGAAGCTT	TTTCCAACAGATGCCAGAATCCT	TGATGG[A,C]AACTTTC	+
OMS00030	10	60401405	CCTCGTGACTACAGAGCTATACAAC	GATCTGATCGGTCGGGAGAGA	ATGAGGGTCCCT[A,C]TACAGG	+
Omy_UT16_2-173	10	61496264	ATTGACTCATTATCACCTTAGTTGTAGCTTCA	GCAGCTACTTGCTGTATCACATGTTTGT	ACAGTCAA[C,T]AAGGGACTTAA	+
Omy_RAD36848-7	10	63650747	CGAGGACGTTCATAGGGAGC	TCGATAAGTCCACCAGCTGG	TGCAGG[G,A]ACACCACCCT	+
Omy_g12-82	11	14764807	GATCAATTCGATCGCTCATGAAACTT	CTTCTCTCGTTCTCATTGTGTCTCA	AAACTCTC[A,G]GGATTAG	+
Omy_BAMBI4.238	11	24411085	CATGATGAGGAGGACCAAGATGAG	AGGTGTGGTTCAGGGCAG	ACCGC[A,G]ATCACCG	-
Omy_MYC_2	11	33841260	CGGTTGCAGAACTCTCATGTTTG	CACGCCATGTCTTAACTTGCATTA	CATAGACTTTTTG[A,G]CCTTAT	+
Omy_BAC-F5.284	11	42789302	CCTCATTTACTGTAGGACCATGCA	ACAACGCCAACAACTTTCTCTTG	CAGTAGG[G,A]CGGCAAG	+
Omy_cox1-221	11	47052310	CACTGAACTGTAAGCCATTGTGATT	GCAACATGGGAATGATTCATAAATGCA	CGGTAAGACCATT[A,T]AAA	-
OMS00120	11	51013177	GGCAGAAGAGGAGAGAGATATGATTG	CCTCAAATACCTCTGACATTGAAGGTT	CGCCCAC[T,C]AAAAC	+
Omy_102867-443	11	55934332	CATTTGTTTAATTTGATTTGGCACAACTTCA	CCCTAGTTCTGTAACACAAGACGTAA	TGGGTACAT[A,C]ATTTTT	+
Omy_oxct-85	11	68405894	CGTCACTGAAACATTACTGTAACATCCA	CATCATCACGCTGTTGGTTTCTTAA	CATCGCT[T,A]ATTTATGC	-
Omy_117540-259	12	5079371	GGCAGGTTAACACAGTCATCTACTATAAA	CAGCATGTTGCTTTAATCCTTCACA	TGTCACTTCAA[A,C]GTTTG	+
Omy_110201-359	12	28727952	GGTAAGGCCTGTCTGACTATTTTGA	AGAGGTCAATGGATGCCAGTTT	TTGGCTATTGAAATT[A,C]TACATT	-
OMS00074	12	31104727	CCTGTTTATTCATCTAAACCAGTTCTTTAAAAT	AACTTAATTTAGCAAACAAATGTCTGAACAGAA	AAACAAAACA[A,C]ATGTTCC	-
Omy_hsc715-80	12	40136309	CCGGTCTACCCTATAGCTGTTG	AGTCAGTCAATTAGTGGTTTGAAATACTATCA	AACTGTATTTG[G,T]GAAAAT	-
OMS00077	12	48861950	AATACCATCTTGAGCTCATTAGTAATTATTCAA	CCAGACTTTACACACTCTTGACTGA	CCGGTG[G,C]TGAAGTT	-
Omy_gh-475	12	62308122	AAGTTACCAGAATTTTGCAAACTCAACT	CCATATTTTGAGGTGTAGCTTTACCCT	CTGAAACTCATG[G,A]TATACA	-
OMS00149	12	65894619	GGCATCATTGTTCTTGCTCTGTTTA	CCTGGGAGGGTTTATATCGGAGTAT	GCTAAA[T,G]GCACAG	
Omy_hsp70aPro-329	12	66828836	TGCGTATTATTGTTTTTCAAGGACTTTCAAA	TGAATATTTTCAAATACATGCCAATTCTTTCCAA	CATTCCAATAT[T,C]CAACTAT	+
OMS00112	12	68382081	TGGCAGCAAAAGGGATGCA	TCCTGAGCAACCAGTCAACATT	CGGTTTCAAGT[T,A]TACTTGT	+
Omy_111666-301	12	70990844	GGGTGAAAAGAGTGGGACATTTACA	GTCAATTTCAAGGCACCAGACAAT	AGTATAACACAGT[A,T]AGACAAT	-
Omy_118175-396	13	20282478	AGGCTTCACACACACATGCA	GACGCGCAACCTCTAGATTATACTT	CTCTTGCAGACAT[A,T]CCCGTA	+
Omy_129870-756	13	22915161	TCGTTATTTTGCCTCGCGGTA	TCCCATGAAGATGTATACATGTTTTGTGA	CAGGTATTTC[G,A]TGAAATG	+
Omy_113490-159	13	26494831	CATAGTACATTTACAGATAATGTTTTAAAGTGCATGT	CGAGATACCAAAATGCCACAGTTACAT	CATCTGTTTT[G,A]GTTTAGC	-
Omy_nach-200	13	30001796	CTCATGAAAAACGGGAGAGCAAAG	CAGCGGCTCTTCAGTAGTCT	CTGACAGAG[T,A]CACAAC	+
OMS00180	13	32462775	GCGCCGAATGGCATTAGG	CACATTGCTGTCGTTTAGTTTGACT	CTAAAAGTGC[A,C]TTAAGCC	-
Omy_110064-419	13	36272850	GTGCAAGGGACCTAGCTAATCC	TCTGAACTGACACTGAAGAACAAAGAA	ACGTTAGCTTTT[A,C]ATTTC	+
Omy_g1-103	13	39287232	AGTCGTGACAATGAGAAACAGTGTT	CTCAGCAAAAAAGAAACGTCCCTTT	CTTTTACA[A,G]TGAAGATC	+
Omy_IL6-320	14	7102407	CGACTGATCTCCTGCAGACATG	CTTGTTCCTCGTTGTCTTCCTTCTA	ATAGGAGAGA[G,A]GACAACA	+
OMS00089	14	13150534	GCACCATTTGAATAAAAAATCTGCTTTGT	GCAACCCAATTCAATATTAAGCACATGAT	AATCCCAAA[T,C]AAGAAC	-
Omy_116733-349	14	18498042	GAAATGGACATGCCTACAAATTGCT	GATGTGATCAGTTTAGGCAAGGC	AGAGAATCTGATA[G,A]TATTTC	+
Omy_ntl-27	14	22071034	GGTGTGTTACTGTAGTTGTGTCCTT	TGTGTAGCTAGTGATCCTGATTGTCT	CAGACAAGAGTAC[C,T]CCAAGAC	-
Omy_txnip-343	14	24435825	CCTTCAAACTAACGCATCATAGACATG	GGTCACTTGGCTAATCCCCTTAT	AACTGAAG[A,G]GATCTG	+
Omy_UBA3b	14	28552649	GCCACTCAATGCATGTGTTTTCTAG	CAGCTAGCTTAAGTGGGATGCAA	AGATAACGC[T,A]AACTATT	-
Omy_mcsf-268	14	48758470	CCAGCATTCGTTCCCATTTCC	CTTTTAATGTAGATTATATTCTTCTGTAGCCACTATGG	AAATAA[T,C]AGATAAACCCT	+
Omy_101554-306	14	53660117	GCCTGTATTTCTCCTGTATGTGCAT	TCAACTTTTGCAAACTTTTTTATTCTTTGTCATTT	TGCTTCTCAC[A,G]TTTTTA	-
Omy_RAD47444-53	14	56051917	GTCGTCTGGAGGAGCTGAAG	GGGTGACGTTTTCCTTCAGC	GGCGAG[C,T]TTGGCCCAAA	-
OMS00072	14	59971780	GTGGGAGAGCTCGTCTATGG	ACAACAGGTCATTGGATGTGATCAG	AAGGTCCATG[T,C]ATCTC	-
Omy_redd1-410	14	69044121	GTACTCCCACTAACATACAGTAGACTCA	GGCACCATTGTGTTTTAGGATGTAG	AATATCCTGCAAG[G,A]AAT	-
Omy_nxt2-273	14	71969784	CTTTAGAAAAGCCAAGGTATATTTTAACATACTTCT	CTGCTGCCCTCTAATGGTAAGATAG	AAGGCA[C,T]	-

Table A2. Continued.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
Omy_110689-148	14	72350872	GTGTGTGGCAGAGAACTAACTGAT	GGTTAAGACATTAACATAACACTGGACTCT	ATGAACACAT[T,G]ATTTATC	+
Omy_Ogo4-212	15	12595806	TGAAAGGTTTTATGCAGGTTATTTTCT	GTGTGTGTTAAATAAGCATTTGATGA	ATTTGATGAG[A,G]CATCTT	-
Omy_hus1-52	15	13042222	CTTGCCGGAGGGTAGCT	CCACAACTTCTCAAATGAATGGAATGT	CCCATCCCT[C,T]CTCCTGG	+
Omy_111084-526	15	17300850	CACCACACCAAGCAACTATTTCATT	ACCCAACTACTGTCCCATTTTTCAT	CAGTGAAAT[T,G]TATTTTT	-
Omy_111383-51	15	21239768	CACGCGCAATCTCTCGTTTTAC	TCTTTAGGCAACAAGCGTGTCA	AGCAAG[C,T]GCACTAGGT	-
Omy_carban1-264	15	21454064	GCAAAGCCTCATCTTCAATCATTTGT	GCAAAACACAAGTCAGGAATCACTTA	ATTAATATTGCTAATAACAC[C,T]AAG	+
Omy_96222-125	15	24041111	GTAAGGAACTAATTGGCGCAACATT	CAGTTTGTCTAACACCCAGGCATAT	CAACTGT[A,G]GCTAATT	-
Omy_RAD52812-28	15	28623729	AGGAGTCCTGTCCCATGTCA	GCTTAAGGCTGTGGTATGTGG	CAACCT[C,G]TATTCCACAT	-
OMS00061	15	31231975	AAGTGGAGGCTGACCTGTTG	GCTGATGGCACCTGACAGTTAATT	TGCCATTT[A,G]CAGACTT	-
Omy_nips-299	15	39788784	GACAGGATAGGAACGGTTTCTCAAT	ATCAGAAGTTTAATTCAATATGTACACGATCCT	CTGGATTTCAC[A,-]GTAATAC	-
Omy_LDHB-1_i2	15	44094440	ACGCACACTTATCCTTGACAATGTT	ACTGTGACAACAAATTCGGTGACA	TGGGCA[G,A]TCATTCA	-
OMS00143	15	59233015	GGAGGCACGCCCCAAA	TTTGTTAAAATAGAGCCCTTAGTGGGTTT	CCTGATCCAGA[A,G]TCTAGA	-
Omy_97954-618	16	11433435	GCTCTGCTTCCTCGGCAAATA	CACAATTGGTTTTTGCACAAAAGTAAAGTATT	CAACGCTTACC[G,A]GTGTGT	-
Omy_117370-400	16	14853962	TGCAAACACAGAGGAAAGGGATTT	GGCTTATTTGTTCCGTACTTGCATT	AACTCCAA[T,C]GAATTAA	+
Omy_RAD88122-32	16	16334074	TCAGTGGATGGAGTGTCCCT	GGTCTTTGGCCTTGTTGCTG	GCTGTGGA[G,A]ATCATCCG	+
OMS00041	16	18279174	GATTCTGTTCCATCCTCTTTCTGTCA	AAACATAAAAAAGGGCATGAAGGTGTC	CACTCTATGC[C,G]TGCCCT	-
OMS00119	16	27506018	AGCGGCAGTTGTGTTAATGAGA	CTTCCTAAAGCCTGACAGTCTGT	CACACAGC[T,A]GCCTGT	+
Omy_anp-17	16	30728525	GGTAATGCCACATGCGGTAAATT	GGCGAAATCTGAAAATGTGCTGTTA	CTCATTGGTATA[G,T]TAACC	-
OMS00018	16	46432462	AGAGTACATGTGTGGCTGCAA	GTCATAAATCAACACAATTATCTTCTTCACAGAA	CCACATAATT[A,C]ATAATTC	+
OMS00134	16	47743637	GAAACTGAAATGATCCCATCGTGTT	GCTAGCATAACAGCATTGCCATAT	TAGCTGCAG[T,C]ATATTA	-
OMS00006	16	63247944	TCCACGTAGGACATAGTTTGAGCTA	TGTGGTGTCATGTTTGCCCTAC	CTTACAAAT[A,G]CAAAATT	-
Omy_103705-558	17	7065986	CTCCAATCGCAAATACCCAGACT	CGCAGGAGACGGATGCC	ACTTACCCAG[A,G]GTGAGAG	-
OMS00128	17	14488587	ATGAAAGAACTCCCAGACACGTATTTT	ACATTTTAACACAGTAACACTAATACACACCA	ACTCTCAGAATT[A,C]ATTATG	+
Omy_101832-195	17	17015658	TGGCTCTGGACCTGTTGAGA	CGTCACAGCTATTTTAGGCGTAGT	TAGTCTTTCAGAG[T,G]AGTATG	-
Omy_RAD45104-18	17	20693754	TGGTGCTTCAGTGCTGTCAA	AGAGTGAAAACTGTGTGCGG	CAAGAC[A,G]CCGCACACAG	+
Omy_101993-189	17	21491290	ACAAAACACAGTGGAATTACAATTAACGTT	GGAAGTTAAATTTCGCTTCGTCAGAA	TGATTTGCAGC[T,A]TGTCAA	-
Omy_114976-223	17	41462973	GACAAACAGCACTTCATTGCAGTAA	GTTGCTCCAGCACCAGGT	CCGATGG[A,C]ACAATC	-
Omy_u09-56.119	17	41690956	CCAAGGTGGACCCACCAG	GCTGAGTTTATAGGTCAGTCATTATACATATTGA	TGAGCTGAA[A,G]CAGAGCA	-
Omy_ca050-64	17	48651793	GTCATACAGAACTGTTTTGTTGTGTCAA	ACCTTGAATTGGTTCCTAATGCTATTGT	CAGTTTGAAGA[A,C]TATACTC	+
Omy_RAD58213-70	17	58266227	CCTGATGGGTGCTCTTCTCTC	AAACAGCATCATTATCCATAGTGTT	TTTTTT[T,A][A,T]AAATATACT	-
Omy_U11_2b-154	17	59466696	GGGAAGCAGAAAAACTGGAAGTT	CCCTCTGTGGGCTTGATATTCA	TGATACTTTTCAG[A,G]TTGTAAC	-
Omy_sast-264	18	28252083	GAAGTAGGGTTTGTTGACCATGTGA	TGGATTCCATTTTAGGCTGTAATACATCTT	CTAGCCAATG[C,T]GTCTAA	+
Omy_RAD43612-42	18	29118777	GTGGAGAGGGATTTTGGGGG	TGACAGGACAAACACAAGCCA	AAATGTG[T,C]ATTTGTGTA	+
Omy_128996-481	18	30802101	CTCATCCACACTGTACAGTACAAGT	CATGCCTTCGTCTCATCAATAACAC	CAAACC[T,G]CAACCAC	+
OMS00121	18	34232991	GGAAGGAGGTCCAGTGTGAGT	AAAATATGCAACACCACTAAAACTGGAAAA	CAGCGTG[A,G]TAAATT	-
OMS00127	18	36268423	CACCTTTCTCTCTCTCTCCATCTCA	AGTGTGCTACACAACCTTAAAAAATATATATCTATT	ACACACCC[A,C]AATGTA	+
OMS00118	18	42212299	GCTTATTTAGAGTGCATGCCAGATG	TGGAACCAATGGGACAGTCCTA	GCGGGG[T,G]GTGCACATT	-
Omy_RAD7210-8	18	42558654	ACACCACACTCCACAAAGCA	GCGCCTTGGTCTCCTTCATA	TGCAGGA[C,A]TTGCTTTGT	-
M09AAD.076	18	53717512	ACTGTTACCACTCTCTCATCAACCT	GGGTCCAGGAGGTTTTTAAACAACAT	CCAACC[A,G]CTGGTGAA	-
Omy_BAMBI2.312	19	6114061	CGAGCTCATGTCCGAAACTCAT	TTTGACAGCCTCAACTTCTAGGG	CCGAAAGTT[C,A]AACTTT	-
Omy_Il-1b_.028	19	10329530	ACTGTCTGGCTAGAGCACATTG	ATCTTCTACCACCGCACTGTTTTAA	TGAGGCA[A,G]CTTTTGT	+
OMS00092	19	12518551	TCTCCAGGTGTATCTTGAGAAGGT	AGGGTTCACACAGGGAAGATATCAT	AGCTGAGAA[T,G]AGGTTC	-

Table A2. Continued.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
OMS00017	19	19111591	ATTAAGTTCATACAAAAGTTCATCATAAATATTTTCCTTT	GGAGAACAAAGGGAAAGAGAAGACA	CCTCGG[T,C]GCTGTAG	-
OMS00105	19	20264968	ACATTTGAAGTCAGTATGGGTGTTGAG	GAACCTCACCACAGTACTAAATGCA	CTGCTATTCA[A,C]ATTGCT	+
OMS00133	19	23702093	GACCACTTCACTCATTCCTCCTTTT	TCCGGTTTACACACTTCATGCA	CGCCTCCATCT[T,C]TGTGGT	+
Omy_rapd-167	19	27362424	CCCAACATGCTCTATTGCAGCTA	AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT	AAACAATCCC[C,A]CCCAAA	-
Omy_128693-455	19	32890059	GCCTGCAGGAGAAGGTAGAGTTA	GAAATGGAATGGACCCCAATCCT	CTCA[A,G]CTGATACCC	+
M09AAC.055	19	53855167	GTCTCCGACGTGTGGCT	TGGAACGAACCTGAGAACATAAGG	ACCTCCAC[G,A]CTGTCC	+
OMY1011SNP	19	54446225	AGGCTGGTTTGGGATTCACTG	CGCCAAACACTAACTCTCTGTCT	CTTTACCTC[G,T]AAGACAAT	+
Omy_RAD29700-18	20	1673196	AATGGAATTGGCCCCAACCC	TCTCCATTGTGTGTAATCATGGT	ACAATT[C,A]AAATGATTTA	-
OMS00039	20	4800495	GTCAGTACTGTGTGTGTCTGTGT	CCATCTACATTGTCAGCAGTGTGA	GT[A,G]CGTGTCTCTGACC	+
OMS00114	21	11035495	GGATGATGCTGTGAGTCGAGAAG	ACCTTCGCCACCCATGTTTTATT	AAACGTTTCAC[A,C]TGCACC	+
Omy_99300-202	21	14697073	CAGTTTGACCCGATGGTGTGA	GATTATGGCGTGGCCTTTTGG	TCAGGCATG[A,T]GAGAAA	+
Omy_cin-172	21	21602618	CGCATGGGACAGGTGTGT	GAGAAAGCCTGTAGAACCATGTCT	CGCTCACC[G,A]TGGTTAC	-
Omy_vatf-406	21	23806220	TTGCTTCATTTTGTCATAACCTTGGG	TGCATGCTCTGACAAATGTTACACT	ATGACT[A,G]TCCACA	-
Omy_LDHB-2_e5	21	24129907	TGCTAGGTGAGTCAGAGGTACATATT	GACTGGAAGGCCACCCATAAG	CCTGTC[A,G]AC	-
Omy_zg57-91	21	32766740	CACTCATACACTCACTCACAAAGGA	AGCAGATAAGCCTTGTGAGTGAATCTT	CACAGACT[G,T]CACAGCC	+
Omy_b1-266	21	41255773	TCATGTGAACTTTAATTGACTAGGAAGTCG	GATATGAAAATATCTGAAGAGTTATATTTGGGAAATTGAC	TCTATAAACAA[C,A]ATTTTTC	-
Omy_1004	21	41944218	GAGAATCGGAGCTAATCTTAGTTATTGTGA	CACTTTATTGAGCTACATGGCAAATCTG	ATGTGATG[T,A]TTTTTGC	-
Omy_102505-102	22	7759328	CTGCAAACTGACATGGTAGCAAAA	TGCTTGCTTTTTAAAAACAATCTCCCA	CAGGATG[T,C]TTTTGC	-
OMS00173	22	9105891	TGGAAGTAGCTACTTAACAGGAAATGG	AACACGTGTGCTTGTTTTGTCAA	ATTAGCTTGTGT[A,G]TGAACT	-
Omy_Ots249-227	22	18917430	CTATCTATCTATCTATCTATCTATCTATCTATCTATCTACTTACTGAGA	CCCCTAGATTAAACCTGTCCAGTCT	CCTCTGA[G,A]AACTAC	+
OMS00058	22	19922139	GTGACATTTGGAGCCACTGC	GCTAGGAGACAGAGGGTGAAAG	CACTTTG[T,C]ACCCCTC	+
Omy_IL17-185	22	27226063	CCACCACACTCTGCAGCTT	TTGACGGGAATCCGAGACTTC	AAGAATCTCAC[C,T]TGCCCAT	-
Omy_107806-34	23	10095418	TCTTTGTCCATGCACATTGATATT	AGCACATTTAGTTAGCAGTGATGGA	ATTGGATGTCA[G,A]TGTCATT	-
Omy_RAD48799-69	23	21581080	GCTGAGCCACCTACACACAG	GTCTAACACTCGCAGCAGGT	CATCCT[A,G]GAATAGAAGT	-
Omy_187760-385	23	23659737	CGGCTATTCTCGCGTAAAAGCT	AAATGCAACCAGAAACGGAATGTC	CTTATCCAAAAT[T,A]ATTGTGC	+
OMS00024	23	31418339	CACATACAACCATCACCCTTCCTAA	AGCATTGAGCGAAATTACCAAGAGT	AA[A,C]CCCAATTTTAC	+
OMS00048	23	37125668	GGAAGAGCTGGAGAACAACGT	TGCAGTTGACAGAGGCTTTCTTT	AGCTAAACTC[A,G]GCAAAA	-
Omy_e1-147	23	38313338	GCACTGACTGTTACCAGGAAAGAG	GTACTGCAGTGTTGAGGCTATATCA	CCATCCTGAAT[C,A]TGATTAA	-
Omy_109243-222	24	7009687	ATGTGCACCTCTTAAATTGTAAGTAAAATGT	ACCCTATATTCAGTGGCAAGATTGC	TTCATTAAAT[T,G]GACTTTTT	+
OMS00101	24	10475451	GCGTGTCGTGGGTCAGTTAAATA	GTGCAATCCAACCTATTAGTAGATATGCT	CTAGTAGCCTTA[T,C]AGAAAG	+
Omy_inos-97	24	24547687	GATGGACAGGGTCCTCTTCAC	CCTGTAGATAAAACATGGTACCAGGTC	CCTTTCTTGAT[G,T]GTATCC	+
OMS00052	24	28174967	TGCGTTTTTCATCCCAATCATTCAC	GGCATCAGGCTCTTCTTCCT	CCTTTTG[A,C]GAATAAT	-
Omy_107336-170	25	30017455	GCCCTCTCACTCATGACATCAAC	GCTCCAGCCACTCGCA	ACTCCTG[G,C]GTGCAGAA	-
OMS00174	25	47325393	TGACTAACTATGCAGCCTGAAAGG	GGGATACTCTTGTAATAAACTGTTGGTTAGTA	CAAGAACAGGA[T,G]AAATGT	-
OMS00071	25	48248474	CCGGAGTGACCTCACATTTGG	GCATCGTACAGTTCACCTACCT	TTGTTTGAGC[T,C]TTTTCT	+
Omy_ppie-232	25	54990925	CTGTTTTAGATTAGAATGTTTTTGGTCAGGT	CTGAACATAGGCTTTCATTTCAGACAT	AAATAGC[G,A]GAGAAAAT	+
OMS00002	25	82366395	TTTGATTTGATTTGTATCTGCTTCTT	CCAACATGCCTCACACAAAA	TGTTT[T,G]GCAGCGCT	+
Omy_cd59-206	26	8028322	CGATTGGCCCAGATGTTTCCAT	GCTCCGTTGCATAGGTGACT	CAACAATC[G,A]AAGGTAAAT	-
Omy_ada10-71	26	18315420	TCTTTGAGCGACAAAGTCCTTGT	ACCCACACATGAACGCAAAAG	CTTCCTGC[G,A]TCCAA	+
Omy_aspAT-123	26	19289582	GCCCATTTCACTGATGCTGTGA	AGGAGACCACTCCAAAGAGAACT	TTCCT[A,G]GGCAGTCAG	-
OMS00014	27	6297526	CTTACACACAAGGGCTTCATTCTG	GATGTCTCTGGGTGGTTGTCA	TTGATGAATT[A,G]AACTTC	+
OMS00015	27	23730945	TCAGACCCTATTTTTGGCACAAGT	GTCTAACTGATCCCACTTCTGCAT	CAAGTCACACTT[T,A]TAATGAA	-

Table A2. Continued.

Locus	Chr	Position	Forward primer	Reverse primer	Probe	Orientation
Omy_hsp47-86	27	27944494	CACATTAAGCACTCCCAGGGA	TTGCAAAGGCCAAACAGCATT	CAGGAGTGTA[A,T]ATGTTT	-
OMS00090	28	4245997	AGGGCACAACACCACTCTAAATT	TCGAAAAGCAACATCTGTCTCAGT	AACCAC[A,G]CAAGATT	-
OMS00129	28	22531125	GGAGATGATGAAATAAAAATTGAGGAAAAGATGA	TGTCTGGTGAATTATCGCAAATAACCA	TTGAACAACAA[G,C]AAAAA	+
Omy_97660-230	28	35742946	TCAGTTATGTGTAATCTCATTACCTCTCCAA	AACAGAAAAGGTCTCAATGTATTTTTTGCA	ACGTAACTTGTA[G,C]CGTTTT	-
Omy_impa1-55	28	36390509	CGCTGAGAGGATTGTCAA	TTTTCTTTGTTCAGTCTTCTGTCTCTG	CGAGATGATGC[G,A]TCTACA	-
Omy_BAC-B4-324	29	14528635	CGTACTTTTCTTTTACAAAATTAAGTGGAGGAT	GCCTAATATTGGCCTAATGTCCTTCA	CATTG[C,A]CAAATACG	-
Omy_OmyP9-180	29	15673414	CTGGATGTGTAGTATCGGTGGAAAA	CACTGGGCACCTCTGATCTC	CTGTAGTAGTCC[C,G]CATTGT	+
OMS00164	29	17249654	CAGAGGAGAGGAGAGCAAAATACTT	ACAACCTACTCATTGAAACTCATTGGA	CAGATTCAATT[A,C]AATTTA	+
Omy_crb-106	29	30701374	GCTCAAAAAGATTCTGCCAAATTCACA	ATTACAATGAAAGTACTTGAGTGTTTATGCAAA	TTGCAATG[C,A]GTCTTT	+
Omy_sSOD-1	29	39802221	GCCGGACCCCACTTCAA	CAGACTAACCGAACAGCATCAGTGG	CCACAAC[A,C]AGACCC	+

Table A3. Environmental data collected for all locations throughout the Columbia River.

Population	Migration Distance	Eleva -tion	Water Temp	Heat Load Index	Annual Mean Temp	Mean Diurnal Range	Isother -mality	Temp Season -ality	Min Temp Warmest Month	Min Temp Coldest Month	Temp Annual Range	Mean Temp Wettest Quarter	Mean Temp Driest Quarter	Mean Temp Warmest Quarter	Mean Temp Coldest Quarter	Annual Precip	Precip Wettest Month	Precip Driest Month	Precip Season -ality	Precip Wettest Quarter	Precip Driest Quarter	Precip Warmest Quarter	Precip Coldest Quarter
Abernathy FTC	88.21	15	14.74	0.850	95	104	43	4798	236	-3	239	40	156	158	37	1775	279	29	60	823	136	148	758
Agency Creek	737.13	53	15.53	0.831	73	126	38	6988	262	-63	325	-10	161	164	-14	570	102	10	67	289	46	48	267
Ahtanum Creek	730.83	125	18.09	0.826	80	134	39	7336	276	-65	341	-8	172	175	-12	351	60	7	60	171	32	37	158
Alpowa Creek	720.74	6	17.37	0.840	100	127	37	7259	301	-38	339	15	192	195	10	441	51	17	29	151	62	75	138
Asotin Creek	741.84	80	21.16	0.783	104	126	36	7379	306	-35	341	17	197	201	12	429	47	18	26	140	65	78	127
Bargamin Creek	1087.47	0	11.56	0.834	19	165	42	7491	240	-151	391	-66	112	117	-75	649	76	32	23	205	114	133	198
Bear Creek	1155.09	2	15.53	0.823	49	157	40	7614	271	-113	384	84	142	149	-47	501	58	28	22	156	99	117	131
Bear Valley Creek	969.82	0	14.16	0.791	3	173	42	7922	231	-180	411	-86	100	105	-99	570	72	26	29	201	92	106	196
Beech Creek	1294.64	117	16.67	0.844	78	164	44	6912	297	-75	372	-1	165	171	-7	346	40	14	27	114	54	66	100
Belshaw Creek	724.47	158	19.65	0.822	74	163	44	6808	290	-78	368	-4	160	165	-11	361	42	15	27	121	55	67	107
Big Creek	706.51	76	17.15	0.833	30	170	41	8012	259	-151	410	-64	127	132	-74	481	52	28	20	143	94	111	137
Big White Salmon	704.08	2	17.47	0.839	93	122	39	6603	275	-35	310	15	179	179	10	863	160	9	74	451	47	47	424
Black Canyon Creek	278.11	0	15.57	0.860	64	164	44	6891	280	-91	371	-16	151	155	-22	357	43	15	29	125	55	66	109
Boulder Creek	987.66	0	11.22	0.831	38	156	40	7664	258	-128	386	-49	132	137	-60	670	87	22	34	242	88	106	234
Bowman Creek	318.13	15	15.11	0.822	84	129	39	6900	275	-51	326	2	174	174	-3	596	111	8	73	314	39	39	292
Bridge Creek	766.81	144	19.46	0.806	95	152	44	6667	299	-46	345	17	180	184	13	320	40	12	30	111	46	56	95
Buck Creek	560.89	0	12.39	0.821	94	118	39	6307	268	-28	296	21	176	176	15	1219	220	11	73	624	63	63	590
Camp Creek	275.65	13	17.4	0.847	42	156	42	6839	252	-113	365	-36	128	131	-45	539	74	19	36	207	73	86	188
Canyon Creek	901.47	0	13.6	0.831	55	150	40	7374	270	-99	369	-31	146	152	-38	767	89	31	27	249	115	134	235
Catherine Creek	1002.73	156	18.77	0.831	83	141	40	7144	291	-59	350	0	173	177	-7	445	51	16	28	147	63	79	134
Chamberlain Creek	1112.57	199	10.26	0.847	21	170	42	7635	245	-155	400	-67	114	120	-76	617	73	31	23	197	108	127	190
Clackamas River	235.47	71	16.37	0.836	106	106	42	5081	255	5	250	48	171	172	44	1665	256	25	56	738	133	134	684
Clear_Granite Creek	796.13	113	15.25	0.847	44	163	42	7144	261	-120	381	-39	132	137	-48	500	70	18	37	194	69	82	177
Cowiche/ Crow Creek	802.07	31	11.82	0.869	42	85	32	6224	194	-68	262	-28	122	124	-33	1815	303	31	61	861	145	156	800
Cowlitz River	190.81	120	14.45	0.834	97	110	43	4995	246	-5	251	40	162	162	36	1557	247	28	58	719	133	138	651
Crooked Creek	889.43	150	7.13	0.856	46	135	39	6886	248	-95	343	-33	134	136	-40	691	90	21	38	264	85	99	246
Crooked Fork Lochsa River	1000.68	21	14.14	0.777	35	149	39	7521	253	-121	374	66	-15	134	-58	477	56	29	21	148	102	119	121
Crooked River	945.39	174	15.67	0.841	32	161	41	7375	249	-135	384	-52	124	129	-61	747	90	32	26	243	116	138	235
Dead Canyon Creek	334.94	129	15.74	0.831	84	129	39	6900	275	-51	326	2	174	174	-3	596	111	8	73	314	39	39	292
Deer Creek	725.64	76	13.81	0.828	64	164	44	6891	280	-91	371	-16	151	155	-22	357	43	15	29	125	55	66	109
Deschutes River	625.17	80	17.62	0.833	97	142	42	6820	297	-41	338	16	183	186	12	389	61	8	53	175	38	40	161
Dillacort Creek	293.25	35	15.31	0.837	92	125	39	6836	279	-41	320	11	180	180	6	643	121	7	74	339	38	38	317
EF Salmon River	289.76	162	12.75	0.822	87	118	40	6192	261	-32	293	16	168	168	11	1271	223	13	69	632	73	73	598
Eagle Creek	1395.98	1	11.81	0.845	6	169	41	8162	232	-179	411	-85	72	111	-98	445	50	26	21	138	87	102	136
Elochoman River	768.76	87	14.82	0.803	92	102	43	4671	228	-4	232	40	152	154	36	2038	324	32	61	948	147	164	882
Entiat River	65.84	175	16.67	0.841	77	122	33	8365	281	-82	363	-24	179	183	-31	377	64	10	55	177	41	47	160
Fifteenmile Creek	352.92	99	17.79	0.825	89	138	41	6782	287	-47	334	9	175	178	4	428	70	8	57	198	39	40	184
Fish Creek	926.57	0	18.01	0.830	52	153	40	7441	270	-105	375	-34	144	150	-41	707	81	30	25	225	111	130	212
Foster	378.57	97	17.1	0.840	113	118	44	5108	273	6	267	54	180	180	51	1145	179	14	60	523	80	80	486
Fox Creek	700.56	240	15.63	0.830	55	158	44	6637	265	-94	359	-20	139	143	-27	435	54	17	30	154	64	76	136
Gedney Creek	916.8	7	15.68	0.850	45	157	41	7492	265	-117	382	-42	138	143	-49	650	73	31	23	200	111	130	189
George Creek	749.81	0	16.75	0.842	91	128	37	7262	293	-48	341	6	183	186	1	469	51	19	25	150	71	85	137

Table A3. Continued.

Population	Migration Distance	Eleva -tion	Water Temp	Heat Load Index	Annual Mean Temp	Mean Diurnal Range	Isother -mality	Temp Season -ality	Min Temp Warmest Month	Min Temp Coldest Month	Temp Annual Range	Mean Temp Wettest Quarter	Mean Temp Driest Quarter	Mean Temp Warmest Quarter	Mean Temp Coldest Quarter	Annual Precip	Precip Wettest Month	Precip Driest Month	Precip Season -ality	Precip Wettest Quarter	Precip Driest Quarter	Precip Warmest Quarter	Precip Coldest Quarter
Germany Creek	89.37	70	16.1	0.809	95	104	43	4798	236	-3	239	40	156	158	37	1775	279	29	60	823	136	148	758
Gumboot and Mahogany Creeks	909.59	37	11.11	0.822	37	150	40	7469	251	-123	374	-49	130	133	-59	586	70	22	29	200	86	105	190
Hayden Creek	1274.09	64	12.69	0.831	38	166	39	8495	270	-148	418	123	-52	147	-72	271	43	10	44	109	38	97	40
Icicle Creek	788.09	0	14.82	0.824	40	108	34	7031	219	-92	311	-41	131	131	-47	1142	199	22	63	563	98	98	519
Indian Creek	787.65	2	16.3	0.839	93	122	39	6603	275	-35	310	15	179	179	10	863	160	9	74	451	47	47	424
Iskuulpa Creek	278.22	92	18.59	0.803	93	130	38	7071	297	-43	340	11	183	187	6	574	77	13	44	227	58	67	208
Joseph Creek	688.28	129	21.14	0.865	82	134	38	7225	286	-61	347	-3	173	176	-9	500	54	21	25	159	77	93	145
Kalama River	113.62	23	16.35	0.814	106	108	43	5002	252	4	248	48	169	171	44	1398	221	24	58	638	116	122	586
Lapwai Creek	779.07	210	16.66	0.840	82	128	37	7199	284	-57	341	-2	173	177	-7	601	65	25	26	192	91	106	178
Lewis River	167.08	72	13.83	0.811	101	109	42	5100	253	-2	255	43	167	168	39	1835	295	27	59	845	142	148	785
Lightning Creek	1009.37	10	16.27	0.894	56	145	39	7345	266	-97	363	-29	148	151	-37	555	59	23	24	170	88	107	157
Little Clear-water River	838.14	0	13.82	0.805	34	165	41	7633	257	-136	393	68	127	133	-63	528	58	30	21	158	104	122	145
Little Klickitat River	316.53	8	18.01	0.824	84	129	39	6900	275	-51	326	2	174	174	-3	596	111	8	73	314	39	39	292
Little Naches River	820.39	36	11.2	0.855	42	85	32	6224	194	-68	262	-28	122	124	-33	1815	303	31	61	861	145	156	800
Little Rattlesnake Creek	780.46	43	10.1	0.838	44	99	34	6444	208	-75	283	-29	127	129	-34	1282	217	22	63	623	104	107	578
Little and Big Bear Creeks	782.58	130	18.12	0.817	90	128	37	7239	292	-49	341	4	181	184	-1	578	67	22	30	200	80	94	185
Lolo Creek	880.85	163	12.61	0.835	54	145	39	7316	267	-96	363	-30	145	151	-38	874	108	31	32	304	119	136	290
Mad Creek	82.72	120	15.17	0.838	88	106	42	5063	239	-10	249	33	154	156	28	2182	351	26	61	1003	148	151	930
MF Hood River	289.64	0	12.75	0.816	87	118	40	6192	261	-32	293	16	168	168	11	1271	223	13	69	632	73	73	598
Mill Creek	413.79	0	14.79	0.835	92	102	43	4671	228	-4	232	40	152	154	36	2038	324	32	61	948	147	164	882
Mission Creek	773.55	91	17.4	0.811	67	130	38	7082	268	-73	341	-15	157	160	-21	592	63	25	25	184	92	109	169
Moose Creek	959.78	0	13.56	0.839	41	155	40	7540	261	-119	380	74	135	140	-53	513	59	29	22	158	103	120	135
Morgan Creek	1321.36	0	13.04	0.829	17	169	40	8182	246	-167	413	55	-36	122	-88	381	47	23	22	120	80	107	91
MS John Day River	578.87	175	19.91	0.838	77	164	44	6743	292	-73	365	-1	162	167	-6	353	42	14	29	121	52	65	106
Murderer's Creek	708.94	14	18.46	0.832	64	164	44	6891	280	-91	371	-16	151	155	-22	357	43	15	29	125	55	66	109
Nason Creek	937.05	0	15.93	0.851	59	122	37	7245	248	-81	329	-27	152	152	-33	1005	186	17	71	527	76	76	485
Newsome Creek	829.15	36	14.67	0.849	36	158	41	7340	251	-128	379	-48	127	132	-56	756	90	32	26	243	118	140	234
NF John Day River / Desolation Creek	275.65	44	17.89	0.864	58	157	43	6706	268	-93	361	-20	142	146	-27	484	62	17	34	179	65	78	160
NF Salmon River	1188.66	0	14.09	0.832	41	168	40	8193	271	-140	411	82	-45	146	-65	345	48	18	31	122	64	105	69
Nile Creek	778.31	78	12.37	0.860	50	101	35	6521	215	-72	287	-25	134	135	-30	1222	209	21	63	595	99	103	552
Ohara Creek	904.65	0	14.42	0.841	58	152	40	7414	275	-98	373	-28	150	156	-35	737	82	31	25	231	113	134	216
Omak Creek	899.14	65	17.98	0.848	87	130	34	8705	296	-84	380	-19	191	197	-27	312	43	14	31	114	48	59	107
Pahsimeroi River	1298.26	3	13.79	0.857	46	168	39	8603	281	-143	424	131	-44	156	-67	279	40	13	32	101	49	89	55
Panther Creek	1190.06	89	12.48	0.836	22	170	41	8093	251	-160	411	60	-29	126	-83	405	50	25	22	130	86	111	96
Pistol Creek	1243.41	153	14.2	0.802	21	174	42	8028	251	-162	413	-72	118	124	-83	520	61	26	24	171	91	106	165
Potlatch River	820.78	118	18.22	0.828	67	133	38	7172	271	-75	346	-17	157	161	-23	810	106	27	36	302	104	117	289
Quartz Creek	1249.41	44	13.21	0.815	78	123	37	7018	268	-57	325	-4	168	170	-9	453	77	9	61	220	41	43	199
Rapid River	402.09	0	14.75	0.830	21	174	42	8028	251	-162	413	-72	118	124	-83	520	61	26	24	171	91	106	165
Rattlesnake Creek	277.84	2	17.47	0.839	93	122	39	6603	275	-35	310	15	179	179	10	863	160	9	74	451	47	47	424
Satus Creek	698.95	74	15.86	0.821	90	134	38	7452	289	-56	345	0	183	187	-4	312	54	6	59	152	30	32	139
Secesh River	1104.87	159	10.67	0.839	27	166	42	7479	250	-141	391	-57	120	125	-67	679	86	26	30	235	100	118	225
Selway River	929.33	0	9.02	0.875	45	157	41	7492	265	-117	382	-42	138	143	-49	650	73	31	23	200	111	130	189

Table A3. Continued.

Population	Migration Distance	Eleva -tion	Water Temp	Heat Load Index	Annual Mean Temp	Mean Diurnal Range	Isother -mality	Temp Season -ality	Min Temp Warmest Month	Min Temp Coldest Month	Temp Annual Range	Mean Temp Wettest Quarter	Mean Temp Driest Quarter	Mean Temp Warmest Quarter	Mean Temp Coldest Quarter	Annual Precip	Precip Wettest Month	Precip Driest Month	Precip Season -ality	Precip Wettest Quarter	Precip Driest Quarter	Precip Warmest Quarter	Precip Coldest Quarter
SF John Day River	288.41	0	18.71	0.848	77	164	44	6743	292	-73	365	-1	162	167	-6	353	42	14	29	121	52	65	106
SF Santiam River	350.85	82	18.19	0.831	112	120	44	5167	275	6	269	53	179	180	50	1112	183	12	65	528	69	69	499
Sheep Creek	877.09	0	20.97	0.833	54	147	39	7462	267	-101	368	-31	148	152	-40	593	67	23	27	191	88	108	180
Simcoe Creek	740.6	95	15.23	0.828	73	126	38	6988	262	-63	325	-10	161	164	-14	570	102	10	67	289	46	48	267
Skamania Stock	235.47	71	16.37	0.836	106	106	42	5081	255	5	250	48	171	172	44	1665	256	25	56	738	133	134	684
Slate Creek	930.78	187	14.62	0.786	33	154	41	7199	245	-126	371	-48	123	127	-57	719	84	29	27	231	109	131	220
Snyder Creek	307.89	0	17.29	0.854	87	131	39	6863	280	-49	329	5	176	176	0	526	97	7	71	272	36	36	253
Summit Creek	343.41	24	13.85	0.851	84	129	39	6900	275	-51	326	2	174	174	-3	596	111	8	73	314	39	39	292
Surveyors Creek	379.86	0	10.96	0.825	55	107	36	6403	226	-65	291	-18	139	139	-23	1276	222	18	67	638	89	89	595
Swale and Wheeler Creeks	315.45	176	18.22	0.823	87	131	39	6863	280	-49	329	5	176	176	0	526	97	7	71	272	36	36	253
Sweetwater Creek	771.11	166	15.26	0.840	92	127	37	7310	294	-47	341	7	185	188	2	476	51	21	24	145	76	90	132
Teanaway River	835.03	119	14.05	0.849	64	117	36	7029	246	-72	318	-19	154	154	-24	873	154	16	65	434	71	71	399
Tenmile Creek	939.09	153	11.21	0.887	31	158	41	7260	246	-132	378	-51	122	127	-59	753	89	31	26	243	116	138	233
ThreeLinks Creek	940.49	0	14.92	0.828	45	158	41	7544	266	-118	384	79	138	144	-50	578	63	30	21	172	106	125	162
Toppenish Creek	737.48	7	15.53	0.854	66	120	38	6864	250	-65	315	-14	156	156	-19	673	120	11	67	342	53	53	316
Touchet River	528.4	85	21.88	0.827	115	131	37	7518	316	-30	346	25	208	214	20	290	39	8	39	113	34	38	101
Trout Creek	353.21	121	15.26	0.831	76	123	38	6755	262	-54	316	-4	164	164	-9	888	164	11	73	466	55	55	436
Tucannon River	673.9	240	14.59	0.820	47	131	38	6861	246	-91	337	-32	135	136	-38	692	91	21	38	266	84	97	248
Warm Springs River	900.24	40	16.15	0.786	83	143	43	6387	278	-48	326	9	165	168	5	572	87	12	52	255	57	60	233
Washougal River Still Creek	474.42	0	12.24	0.858	66	100	39	5426	220	-35	255	8	137	139	3	2170	354	26	62	1013	145	153	956
Webb Creek	259.96	28	16.83	0.863	67	130	38	7082	268	-73	341	-15	157	160	-21	592	63	25	25	184	92	109	169
Wenaha River	769.73	171	15.91	0.830	72	143	40	7128	281	-73	354	-12	161	165	-18	546	64	20	30	191	77	90	175
WF Hood River	286.09	48	13.17	0.803	87	118	40	6192	261	-32	293	16	168	168	11	1271	223	13	69	632	73	73	598
White Creek	347.21	223	14.5	0.806	72	122	38	6786	258	-59	317	-8	161	161	-13	740	136	10	72	386	50	50	359
White Bird Creek	852.52	0	16.23	0.866	84	138	38	7366	292	-62	354	115	177	182	-7	557	68	26	26	184	94	114	131
White Cap Creek	996.51	129	12.45	0.830	44	161	41	7610	266	-122	388	79	137	143	-53	494	57	29	21	153	100	118	130
Wiley Creek	407.68	67	12.93	0.820	106	119	44	5025	267	2	265	49	173	173	45	1435	227	18	59	653	103	103	595

Table A4. Minor allele frequency (MAF) for all steelhead collections genotyped at all 13 markers. The table includes average MAF for all collections and by lineage. Fixed alleles are outlined in red and averaged MAF for markers 8-12 are highlighted gray.

MAF Summary	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
All collection averages	0.190	0.318	0.287	0.156	0.323	0.145	0.134	0.281	0.314	0.259	0.292	0.280	0.253
Coastal collection averages	0.386	0.461	0.405	0.213	0.527	0.220	0.206	0.246	0.286	0.233	0.281	0.256	0.306
Inland collection averages	0.022	0.134	0.117	0.038	0.122	0.017	0.017	0.223	0.241	0.196	0.218	0.217	0.188

MAF coastal collections	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Abernathy FTC	0.661	0.733	0.494	0.122	0.800	0.111	0.111	0.206	0.261	0.172	0.361	0.217	0.172
Buck Creek	0.354	0.486	0.465	0.361	0.500	0.354	0.354	0.472	0.486	0.389	0.493	0.486	0.403
Clackamas River	0.139	0.307	0.278	0.046	0.198	0.075	0.044	0.052	0.108	0.046	0.067	0.054	0.098
Cowlitz River	0.104	0.108	0.063	0.000	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071
Eagle Creek	0.354	0.415	0.341	0.000	0.451	0.000	0.000	0.049	0.073	0.012	0.098	0.061	0.171
Elochoman River	0.171	0.171	0.122	0.000	0.463	0.000	0.000	0.000	0.024	0.037	0.037	0.024	0.195
Germany Creek	0.375	0.417	0.361	0.028	0.569	0.028	0.028	0.111	0.139	0.097	0.181	0.153	0.139
Gray River	0.135	0.135	0.077	0.000	0.519	0.000	0.000	0.019	0.019	0.000	0.038	0.019	0.077
Lewis River	0.253	0.253	0.138	0.000	0.305	0.000	0.000	0.000	0.017	0.029	0.029	0.017	0.132
Mad Creek	0.621	0.672	0.672	0.586	0.672	0.552	0.552	0.569	0.621	0.672	0.603	0.534	0.603
Rattlesnake Creek	0.348	0.667	0.662	0.172	0.784	0.123	0.113	0.328	0.387	0.137	0.358	0.387	0.534
Skamania Stock	0.990	1.000	1.000	1.000	1.000	1.000	1.000	0.993	1.000	1.000	1.000	0.997	0.910
Washougal_Still Creek	0.593	0.620	0.537	0.398	0.676	0.398	0.398	0.407	0.444	0.417	0.435	0.417	0.463
WF Hood River	0.323	0.554	0.497	0.253	0.366	0.425	0.250	0.234	0.465	0.242	0.255	0.234	0.288
Wiley Creek	0.375	0.383	0.359	0.234	0.414	0.234	0.234	0.242	0.250	0.242	0.266	0.242	0.328

Table A4. Continued.

MAF inland collections	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Ahtanum Creek	0.006	0.184	0.184	0.000	0.203	0.000	0.000	0.222	0.241	0.114	0.222	0.209	0.165
Belshaw Creek	0.000	0.300	0.000	0.300	0.300	0.000	0.000	0.280	0.300	0.000	0.000	0.300	0.000
Black Canyon Creek	0.000	0.023	0.012	0.000	0.035	0.000	0.000	0.035	0.035	0.035	0.023	0.047	0.070
Camp Creek	0.000	0.013	0.013	0.000	0.013	0.000	0.000	0.038	0.050	0.050	0.050	0.050	0.075
Catherine Creek	0.007	0.030	0.026	0.004	0.046	0.000	0.002	0.108	0.112	0.099	0.110	0.108	0.117
Crooked Creek	0.048	0.013	0.013	0.013	0.013	0.013	0.013	0.083	0.096	0.088	0.092	0.075	0.100
Deschutes River	0.027	0.163	0.158	0.005	0.136	0.005	0.005	0.299	0.321	0.315	0.321	0.288	0.277
Entiat River	0.028	0.111	0.111	0.014	0.111	0.014	0.014	0.361	0.389	0.333	0.389	0.361	0.319
Fifteenmile Creek	0.066	0.116	0.070	0.029	0.165	0.029	0.029	0.169	0.186	0.174	0.169	0.153	0.227
Grande Ronde River	0.000	0.021	0.021	0.000	0.043	0.000	0.000	0.106	0.117	0.106	0.106	0.138	0.138
Icicle Creek	0.075	0.175	0.175	0.075	0.175	0.075	0.075	0.525	0.575	0.475	0.575	0.500	0.500
Iskuulpa_Desolation	0.006	0.032	0.019	0.000	0.045	0.000	0.000	0.065	0.084	0.058	0.071	0.071	0.091
MS John Day River	0.005	0.022	0.019	0.002	0.024	0.000	0.000	0.071	0.073	0.066	0.065	0.069	0.088
NF John Day River	0.003	0.013	0.013	0.000	0.036	0.000	0.000	0.039	0.042	0.032	0.039	0.042	0.058
SF John Day River	0.004	0.018	0.018	0.000	0.018	0.000	0.000	0.068	0.072	0.063	0.068	0.066	0.066
Lightning Creek	0.000	0.020	0.020	0.000	0.020	0.000	0.000	0.158	0.158	0.158	0.158	0.158	0.118
Little Rattlesnake	0.008	0.133	0.117	0.016	0.125	0.008	0.008	0.125	0.133	0.047	0.125	0.125	0.125
Nason Creek	0.063	0.188	0.188	0.094	0.188	0.063	0.063	0.719	0.750	0.719	0.750	0.656	0.500
Nile Creek	0.051	0.165	0.148	0.040	0.165	0.023	0.023	0.193	0.216	0.091	0.182	0.193	0.142
Omak Creek	0.013	0.067	0.058	0.000	0.071	0.000	0.000	0.518	0.554	0.513	0.540	0.460	0.491
Quartz Creek	0.011	0.038	0.016	0.022	0.038	0.000	0.000	0.043	0.043	0.016	0.022	0.043	0.097
Surveyors Creek	0.017	0.950	0.950	0.367	0.633	0.200	0.183	0.950	0.950	0.950	0.917	0.833	0.650
Teanaway River	0.058	0.192	0.173	0.000	0.192	0.000	0.000	0.192	0.192	0.096	0.135	0.173	0.115
Toppenish Creek	0.004	0.018	0.017	0.000	0.014	0.000	0.000	0.013	0.025	0.007	0.014	0.012	0.014
Warm Springs River	0.044	0.431	0.438	0.000	0.300	0.000	0.000	0.363	0.425	0.425	0.419	0.381	0.256
Wenaha River	0.040	0.060	0.060	0.020	0.060	0.020	0.020	0.060	0.120	0.060	0.120	0.120	0.100

Figure A1. Relationship between candidate allele frequency (markers 2,3,6,9) and each environmental variable that was significant in RDA results from 113 steelhead collections in the Columbia River. The geographic region of each population is represented by shapes.

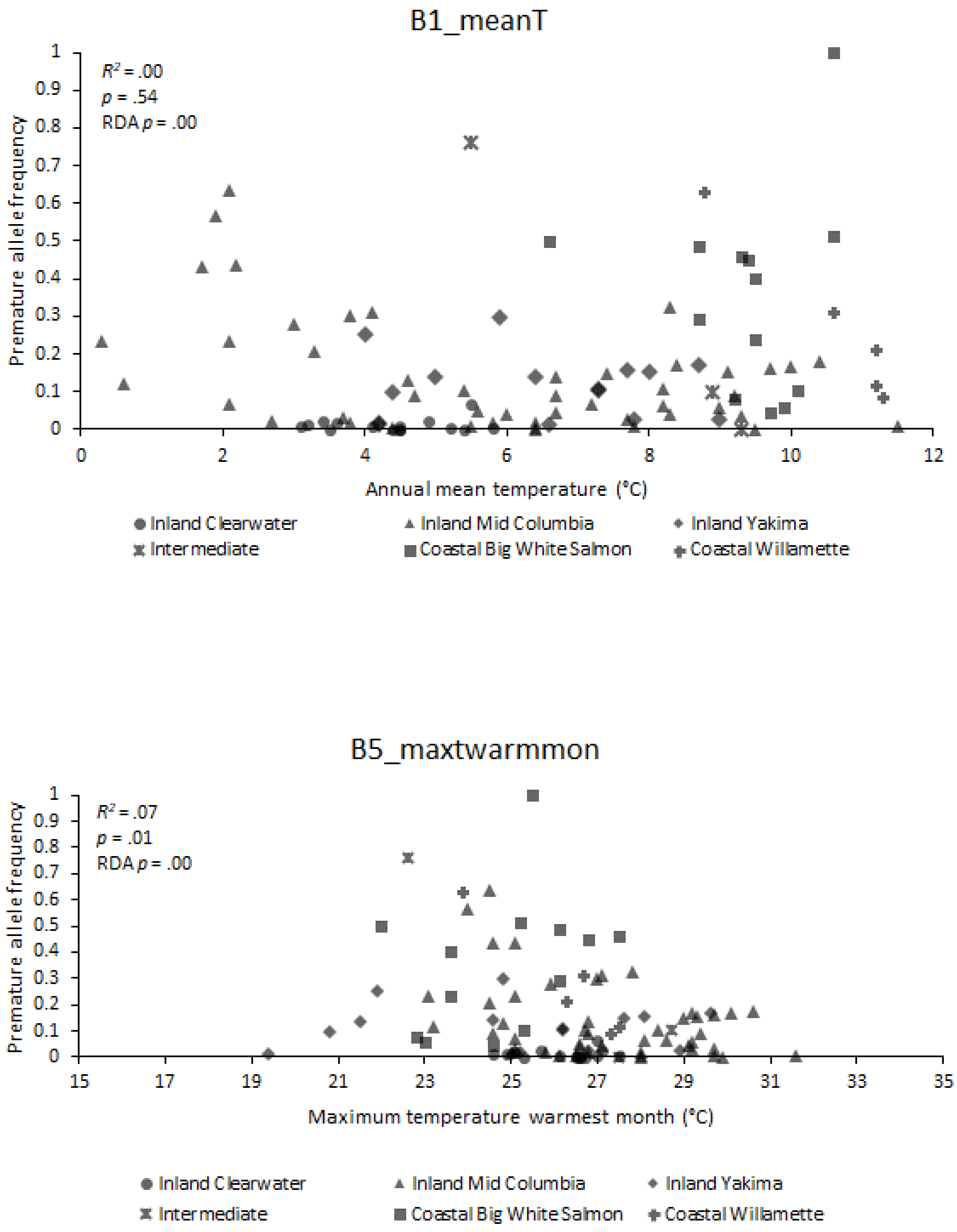
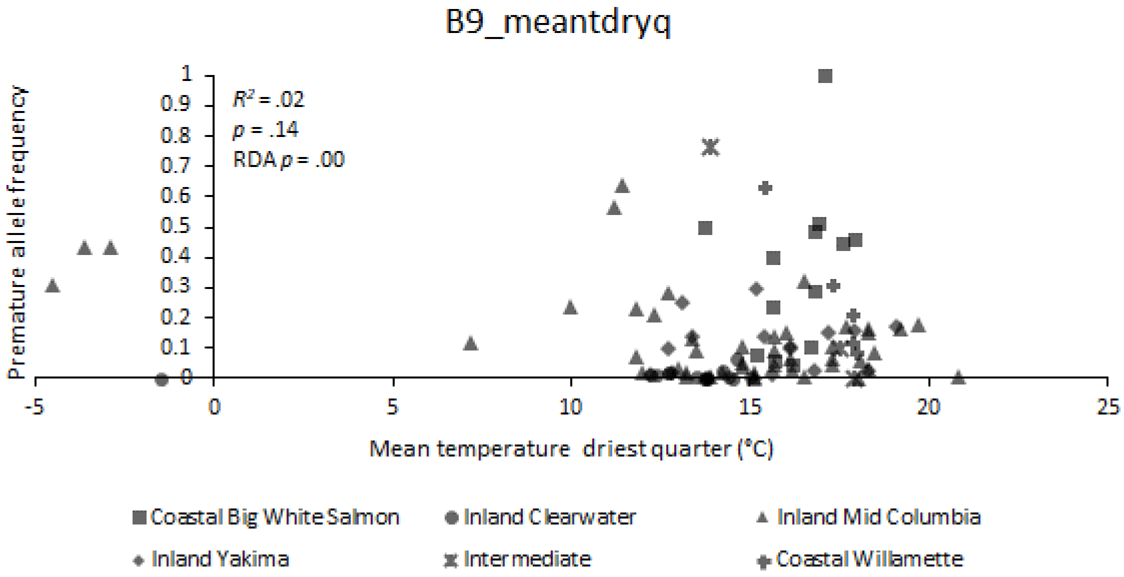


Figure A1. Continued.



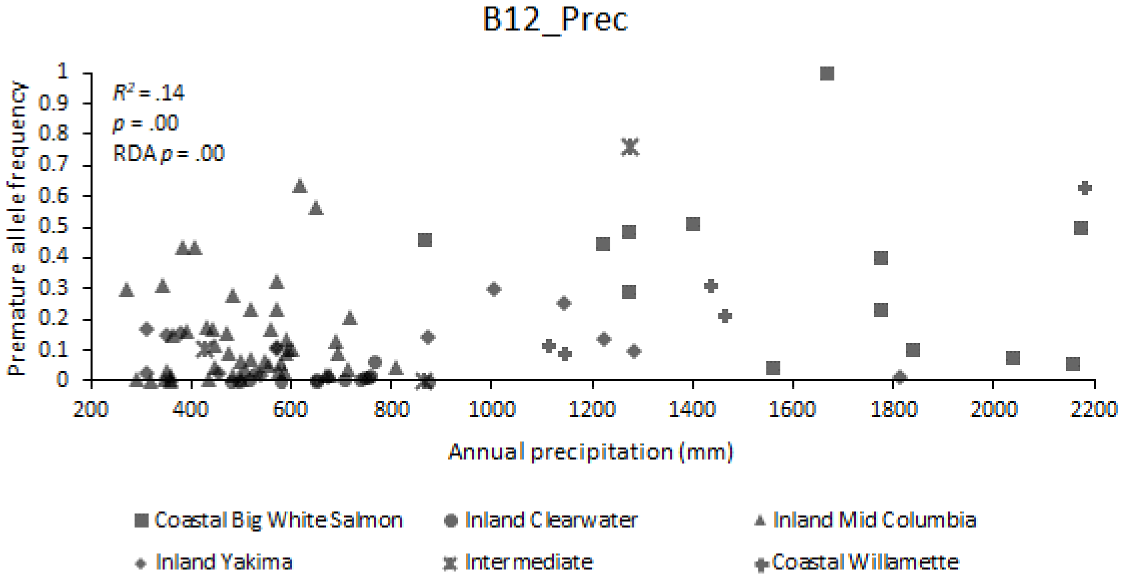


Figure A1. Continued.