Isolation-by-distance and population-size history inferences from the coho salmon (*Oncorhynchus kisutch*) genome

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Running Head
coho salmon genome assembly

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Abstract
Coho salmon (*Oncorhynchus kisutch*) are a culturally and economically important species that return from multiyear ocean migrations to spawn in rivers that flow to the Northern Pacific Ocean. Southern stocks of coho salmon have significantly declined over the past quarter century, and unfortunately, conservation efforts have not reversed this trend. To assist in stock management and conservation efforts, we generated two chromosome-level genome assemblies and sequenced 24 RNA-seq libraries to better annotate the coho salmon genome assemblies. We also resequenced the genomes of 83 coho salmon across their North American range to identify nucleotide variants, characterize the broad effects of isolation-by-distance using a genome-wide association analysis approach, and understand the demographic histories of these salmon by modeling population size from genome-wide data. We observed that more than 13% of

all SNPs were associated with latitude (before multiple test correction), likely an affect of isolation-by-distance. From demographic history modeling, we estimated that the SNP latitudinal gradient likely developed as recently as 8,000 years ago. In addition, we identified four genes each harboring multiple SNPs associated with latitude; all of these SNPs were also predicted to modify the function of the gene. Three of these genes have roles in cell junction maintenance and may be involved in osmoregulation. This signifies that ocean salinity may have been a factor influencing coho salmon recolonization after the last glaciation period – generating the current pattern of variation in these three genes.

Introduction

Coho salmon have special cultural significance to the people of the First Nations in British Columbia and have traditionally been one of the highest-value Pacific salmon in the commercial and recreational fishery sectors. In 1977, a climatic regime shift in the North Pacific Ocean ushered in three decades of increasing global salmon production that culminated in 2009, when over 600 million salmon (1.1 million metric tonnes) were harvested [1]. However, this increased production of salmon masked substantial variability in regional abundances and species composition. Whereas the productivity and harvest of chum (*Oncorhynchus keta*), pink (*Oncorhynchus gorbuscha*), and sockeye salmon (*Oncorhynchus nerka*) increased throughout the North Pacific after 1977, the opposite was true for coho and Chinook salmon (*Oncorhynchus tshawytscha*). These declines became particularly acute after 1989 when marine survival for these species began a downward spiral that has yet to be reversed [1, 2]. A severe decline in the highly lucrative recreational coho salmon fishery in the Strait of Georgia saw the numbers of fish caught decline from an average of over 500,000 to less than 100,000 throughout the 1990s [3]. In 2004, the recreational catch in the Strait of Georgia was 9,500 coho salmon [4].

In British Columbia (BC), the Salmon Enhancement Program (SEP) was launched

to double salmon production with the establishment of 18 major Department of Fisheries and Oceans (DFO) operated hatchery facilities and spawning channels. Throughout a stable harvest period of the 1980s, SEP releases of coho salmon juveniles increased from 5 to over 20 million, and the proportion of hatchery salmon in the fisheries increased from 5 to 20%. A precipitous decline in coho salmon production occurred in the 1990s. The decline was even more dramatic in the inner waters of the Strait of Georgia and Puget Sound. By the end of the decade, the commercial coho salmon fishery was closed and 'marked or hatchery-only' recreational fisheries had been instituted as a wild coho salmon conservation measure in southern BC.

The concern that hatchery fish were replacing wild fish was raised and, indeed, by 1998 70% of coho salmon in the Strait of Georgia were of hatchery origin [5]. From 1998 to 2007, the survival of the Strait of Georgia coho salmon over the first four months after entering a marine environment (May-September) decreased from 15% to 1% [2]. Processes associated with the low early marine survival remains unknown, but marine climatic changes were implicated and hatchery salmon survival was even lower than for wild salmon [2]. These results led to renewed calls for improved strategies for wild and hatchery coho salmon management, and a re-evaluation of wild-hatchery interactions in the species [2]. As such, a call was made to understand genetic influences on coho salmon survival and to produce high-quality genomic resources such as a chromosome-level reference genome assembly to enable technological support in informing management practices and decision within this species.

In a large-scale coho salmon population structure analyses of coho salmon sampled from 318 localities, in 38 different regional groups in North America and Russia (representing most of the natural distribution of coho salmon), 17 microsatellite loci showed that salmon clustered geographically and regions could be delineated along a north – south gradient, with reduced variation to the north and isolated inland populations [6]. These results were refined with increased genetic markers, finding that isolation-by-distance from a main southern glacial refugia after the last ice-age could

explain most of the patterns of genetic diversity in modern coho salmon across the North American distribution [7, 8]. These last two studies were supported by the reference genome assemblies described in this study and illustrate how important such resources are for understanding the basic biology of a species.

With this in mind, the goals of this study were to expand upon our basic understanding of the coho salmon genome and help build upon the knowledge of the already excellent framework of population structure mentioned above. Our method to do this was to construct a high quality, annotated reference genome assembly and by building a comprehensive inventory of genetic variation (SNPs) from a wide geographical distribution. From the complete SNP dataset, we were then able to expand upon what was known about isolation-by-distance and demographic history of coho salmon. RNA-seq data for various tissues were also generated to facilitate genome annotation by the NCBI.

Materials and Methods

Coho salmon samples for genome assembly

All animals were reared in compliance with the Canadian Council on Animal Care Guidelines, under permit from the Fisheries and Oceans Canada Pacific Region Animal Care Committee (under Ex.7.1). Using Inch Creek coho salmon, we generated fully homozygous diploid gynogenetic individuals (doubled haploids) to help improve genome assembly quality (as noted in [9]). For details on doubled haploid generation and DNA extraction methods, please see the Supplemental Methods section. Tissues were also collected for RNA-seq and included kidney, heart, head kidney, spleen, gill, nares, ovary, white muscle, brain, eye, gut, liver, skin, stomach, and pyloric caecum. See the Supplemental Methods for further details on RNA extraction.

Genome sequence and assembly – Version 1

A common sequencing and assembly pipeline for salmonids was used for this version of the genome assembly (e.g., [10–12]). Full details of sequencing and genome assembly can be found in the Supplemental Methods section. A brief description of the assembly involved generating Illumina libraries (mate-pair and paired end), generating PacBio data, and assembling the Illumina sequence data using Allpaths-LG [13] followed by scaffolding with PacBio data using PBJelly [14]. All sequencing data related to this genome assembly and annotation were submitted to the NCBI under BioProject Accession: PRJNA352719. Genome completeness was assessed using BUSCO (v3.0) [15], with default settings aside from "-sp zebrafish" using the ODB9 Actinopterygian database.

A circos plot (v0.69-4) was generated to show the relationship of homeologous chromosome resulting from the salmonid specific genome duplication [16, 17] for both versions of the genome assembly. For further details of the circos plot see Supplemental Methods.

Genome sequence and assembly – Version 2

Version 2 of the coho salmon genome assembly incorporated 10X chromium data, Hi-C data, and new PacBio data with the previous Illumina sequencing and PacBio data generated for the first version. Some of this data came from a different doubled-haploid coho salmon individual compared to the first version. For full details of sequencing and assembly methodology see Supplemental Methods.

Transciptome and annotation

RNA-seq data was generated from 15 tissues taken from the same doubled-haploid coho salmon used to produce the first genome assembly. RNA-seq data was also generated from two other coho salmon for this project, including: spleen, head kidney, kidney, gill, and gut (gut was only from one of the two salmon). In total, RNA from 24 tissues were sent for library construction and sequencing at the Michael Smith Genome

Sciences Centre (Vancouver, BC, Canada). Eukaryotic single-strand RNAseq libraries were prepared and sequenced across 7 lanes of PE125 sequencing on an Illumina HiSeq2500. Four tissues were pooled per lane except brain, ovary, liver and gut from the genome individual (DH3), which were pooled two per lane. Sequences were submitted to NCBI under SRR5333359-SRR5333382 for eventual inclusion in the standard NCBI Eukaryotic Genome Annotation pipeline, which has been used on many genome assemblies. This data was generated for use in the NCBI annotation (Version 2), but was not used in any other way in this study.

Repeat library

A species-specific repeat library was generated for coho salmon using the methodology developed for salmonids in [18], and fully described in [10]. In brief, the Atlantic salmon repeat library [18], was combined with repetitive sequences from the RepBase database [19]. The RepBase sequences were derived from the Salmoniformes family. They excluded simple repeats. RepeatModeler v1.0.8 [20] was also used together with the genome assembly in a *de novo* approach. The repetitive sequences were then aligned to the coho genome with BLASTN [21]. Sequences were classified into either high-confidence or low-confidence categories based on frequency and length. Low-confidence repeats were removed, and after filtering all of the sequences were compared to each other using an all-by-all BLASTN search. A redundancy filter was applied, prioritizing longest and highest-confidence repeats where two sequences were considered to overlap.

Whole-genome resequencing and nucleotide variant calling

Whole-genome resequencing was used to characterize broad genomic characteristics across the coho salmon's North American range. Table 1 contains a list of sampled locations (see File S1 for more information). We included one commercial strain from Chile as well (Table 1).

DNA was extracted from fin-clips using the DNeasy Blood and Tissue extraction kit (Qiagen) or a MagMAX DNA Multi-Sample Ultra Kit with a KingFisher (ThermoFisher Scientific). Following DNA extraction, samples were quantified by Qubit BR DNA assay (ThermoFisher) and integrity validated by agarose gel electrophoresis. At McGill University and Genome Québec Innovation Centre (Montreal, QC, Canada), individual Illumina libraries were constructed with Illumina TruSeq LT sample preparation kits, and each individual was sequenced separately on a lane of Illumina HiSeq2500 PE125 or in batches of four on a HiSeqXTen (PE150) lane, targeting approximately 15-30X coverage. Resequenced genomes were submitted to the NCBI under BioProject:PRJNA401427 and PRJNA808051 (File S1).

Nucleotide variant calling on the dataset followed GATK3 best practices where possible. BWA-MEM v0.7.17 [22] was used to align Illumina data to the reference genome (version 2), with -M option for Picard compatibility. The Picard v2.18.9 [23] AddOrReplaceReadGroups program was used to add read group IDs, and the MarkDuplicates program was used to mark duplicates (default settings). GATK v3.8 [24, 25] was then used to call genotypes. Base and variant recalibration were each performed once (for two rounds through genotyper). The variants used for recalibration were from 1) a reduced set of very high-confidence calls following default “hard-filtering” guidelines from GATK documentation from the first round of genotyping with a particular focus on coding regions, and 2) validated SNPs on a 200K Affymetrix SNParray [26].

Following genotyping, VCFtools v0.1.15 [27] was used to additionally thin data to only include biallelic SNPs with a minor allele frequency of 0.05 or greater, variants with fewer than 10% missing genotypes, and variants with a mean coverage between 5 and 200. Minor allele frequency was not used for filtering in the SMC++ analysis (below). Some individuals were removed at this point from the VCF file because they were not intended for this study (see NCBI BioProject: PRJNA808051 and File S1 for removed samples). They were included as it was more computationally efficient to call all

224 individuals at the same time. Finally, the SNPs were filtered for linkage disequilibrium to
225 reduce the influence of large haploblocks in the principal components analysis (PCA)
226 (bcftools [28] version 1.9-102-g958180e +prune -w 20kb -l 0.4 -n 2).
227
228 *Whole-genome analyses*
229 A PCA was performed with variants that had been filtered for linkage
230 disequilibrium (see previous paragraph) using PLINK [29, 30] v1.90b6.15 with default
231 parameters. PLINK was also used to identify and quantify runs of homozygosity using
232 default settings (Figure S1). For comparison, we also performed the analysis with the
233 following parameters (Figure S2): min SNP count – 100, min length – 100 kb, max
234 inverse density – 50 kb/SNP, max internal gap 100 kb, max heterozygous genotypes 1,
235 SNP scanning window size – 100, min scanning window hit rate – 0.05, max missing calls
236 – 20.
237 Private allele counts per river were tallied using the populations module in
238 Stacks [31, 32] version 2.54 with default parameters. Populations with more than five
239 individuals were randomly subsampled to five to reduce the influence of uneven
240 sampling on the number of private alleles identified. Stacks was also used to calculate
241 other population level metrics such as observed heterozygosity, nucleotide diversity (Pi),
242 and Fis with default settings.
243 A genome-wide association (GWA) analysis was performed to characterize the
244 extent of isolation-by-distance previously reported by several authors (e.g., [7, 33]). The
245 trait of interest under investigation was latitude (the Chile strain of coho salmon was
246 excluded from this analysis). We used PLINK with default settings to perform this
247 analysis. Population structure was not included in this analysis as a covariate because
248 we were trying to characterize the fraction of the genome with a north – south gradient
249 and adding this covariate would remove much of that variation. R [34] and the qqman
250 package in R [35] were used to visualize the GWA analysis.
251 We tested for gene ontology enrichment based on the annotated variants that

were associated with the north – south gradient (for variants that were 'moderately' likely to influence gene function and for those having 'low' or 'moderate' likelihood). SnpEff [36] version 5.0e and the gene annotation from the NCBI were used to annotate nucleotide variants for potential function alterations using default settings. Blast2GO [37] and OmicsBox [38] version 2.0.36 were used to test for enriched GO categories using default parameters.

To infer demographic histories of the salmon from the various rivers, we used SMC++ [39] version 1.15.4.dev18+gca077da. In this analysis, we set the mutation rate to 8e-9 bp/generation and the generation time to 3 years. These parameters were previously used in another coho salmon study examining demographic histories [7]. We used nucleotide variants that were not filtered for rare variants (e.g., MAF < 0.05). We also used the --missing-cutoff option (50 kbp) in SMC++ to reduce the influence of missing genotypes (e.g., in centromeres).

Results

Genome assemblies

The size of both versions of the coho salmon genome assembly was 2.3 Gb, which is also the same size of the closely related Chinook salmon (*O. tshawytscha*) genome assembly [40]. However, version 2 of the coho salmon genome assembly was much more contiguous than version 1 and had a more complete gene set (inferred from BUSCO completeness). There was an almost 20x fold increase in contig N50 between version 1 (58 kb) and version 2 (1,159 kb) of the genome assembly (Table 2). Likely as a consequence of the increase in contiguity, the number of complete BUSCOs rose from 91% to 99%, which is comparable to the human genome assembly at 99% [41]. The proportion of repeats also rose from 44.82% to 53.12% (compared to 52.94% in Chinook salmon), and the number of annotated genes increased from 41,179 to 60,330 (47,105 in Chinook salmon). The NCBI reported that from version 1 to version 2, 37% of the

genome annotations were new and that 16% of the annotations on version 2 required major changes from the previous version [42]. We note that the genome assembly was produced from sequence data from two coho salmon and therefore not haplotype resolved but chimeric in nature.

The coho salmon genome has extensive signatures of chromosomal duplication (Figure 1, Table 2), which have been retained from the whole genome duplication common to all salmonids [17]. The majority of duplicated regions from the salmonid-specific genome duplication have diverged to a point where it is relatively easy to differentiate between the copies (Figure 1, ≤90% identity), but certain sections of the genome have retained high-sequence similarity where it is difficult to distinguish between copies (Figure 1). Regions with very high-sequence similarity remain as unplaced scaffolds as it was not possible to resolve which sequence belonged to which duplicated region (see assembly methods; available on the NCBI website [43]). The number of duplicate BUSCOs increased from 37% to 42.2% between versions (Table 2), which suggests that the second assembly was able to distinguish between similar paralogs/homeologs better whereas the first assembly likely collapsed them into a single gene/BUSCO.

The coho salmon genome also has a high retention of repetitive elements (Figure 1, Table 2), which is another commonality of studied salmonids (e.g., [12, 18]). This is especially true in regions near the centromere where the fraction of repetitive elements is roughly 75% (Figure 1). That value is high compared to the genome average of 53% (Table 2). For comparison, the most recent version of the Chinook salmon genome also has a repeat content of 53% [44].

Population genomics

A PCA of 83 resequenced coho salmon genomes sampled from across North America (and aquaculture samples), revealed that coho salmon clustered by region with the exceptions of the Salmon River and Inch Creek (Figure 2). On the first principal

component of the PCA, the Salmon River clustered away from all the other samples. This river belongs to the Thompson River watershed, and coho salmon from this region have previously been observed to cluster in a similar manner [7]. Inch Creek salmon might cluster separately as an artifact since the genome assembly was derived from an Inch Creek salmon. This might increase read-alignment scores and influence SNP-calling in some regions of the genome.

Excluding the Salmon River and Inch Creek samples, all other samples clustered by region and by latitude in a manner consistent with isolation-by-distance suggested by [7]. The Salmon River group have the lowest private allele counts (1,876 vs. a median of 4,188) and observed heterozygosity (0.22966 vs. a median of 0.285565). They also have the highest total runs of homozygosity (Figures S1 and S2). The region with the highest private allele count appears to be around the Puget Sound (e.g., Wallace River, private allele count = 5,546) and Strait of Georgia regions (e.g., Capilano River, private allele count = 6,415). Most of the northern rivers have low private allele counts with the exception of the Kitimat River (private allele count = 6,341), which has the second highest count (Figure 2).

To investigate how much of the genome has been influenced by isolation-by-distance, we quantified the number of SNPs associated with the latitude gradient observed from the PCA above (Figure 3, File S2). Roughly 13.9-33.8% of the 5,631,459 variants were associated with latitude at a significance level of 0.01-0.1 without multiple test corrections (Figure 3). The proportion of variants associated with latitude dropped to 0.07% after the alpha threshold was set to 0.05 with a Bonferroni correction (these variants were widely distributed throughout the genome).

In Table 3, the most common nucleotide variant annotations from SNPeff are shown, with intronic and intergenic variants being the most common type of variant annotation. The variants that were significantly associated with latitude (see previous paragraph, 0.07%) have a similar broad distribution of annotations relative to the entire genome rather than enriched for variants that are likely to influence gene function

(Table 3, File S2). For instance, the percent of intergenic nucleotide variants remained at 31.2% of the total number of variants for the whole genome and for variants that were significantly associated with latitude (Table 3). We would expect that if variants were influencing traits under selection (e.g., based on latitude), the distribution would change between all variants and those significantly associated with latitude if those SNPs influenced gene function (e.g., 3' UTR and missense annotations).

Significant latitude-associated nucleotide variants (0.07%) identified in the GWA analysis that were annotated as having a 'Moderate' likelihood to influence gene function by SnpEff were found in 45 genes (File S2). Of these 45 genes, 4 genes had two or more variants that were annotated as 'Moderate' in their impact on gene function (Figure 4). No enriched gene ontologies were identified from genes with 'Moderate' or even 'Moderate' + 'Low' (87 genes) nucleotide variant annotations (File S2). Only when all genes with associated variants were tested, regardless of influencing function, did we observe enriched GO terms (data not shown).

To put the nucleotide variation generated by isolation-by-distance into a broader context, we identified possible times when northern populations could have recolonized after the last glaciation period. By modeling demographic histories from genome sequences using the SMC++ program, we were able to identify major decreases in effective population size (Ne) that correspond with the Cordilleran Ice Sheet maximum and the presumed penultimate global glacial maximum (Figure 5). We also observed that for some populations, mostly northern, there was an additional drop in effective population size between 3,750 and 8,000 years ago (Figure 5).

Discussion

As with previous analyses of salmonid genomes [10, 12, 17, 18, 45, 46], the retention of duplicated chromosomes (i.e., homeologs) from the salmonid-specific whole genome duplication [17] is a defining feature of the coho salmon genome. Some

of the duplicated regions have likely retained very high sequence similarity for roughly 90 million years (time estimate from [17, 45, 47]). A possible mechanism for high sequence similarity retention is through tetrasomic inheritance [48].

The second version of the coho salmon genome assembly resolved a greater number of duplicated regions of the genome compared to the first version. The better resolution of duplicated regions can be observed with the increase in gene count and the number of duplicated BUSCOs identified. Finer detail in these regions may help us in future studies to better understand the residual impacts of whole genome duplication on the biology of salmon.

From resequenced coho salmon genomes, we were able to better understand population structure of coho salmon and its relationship with isolation-by-distance. One of the striking features of the PCA of coho salmon populations was how divergent Salmon River salmon were to all other populations. The Salmon River is part of the Thompson River watershed and coho salmon from this system were thought to be isolated from all other populations for potentially 150,000 years before secondary contact roughly 13,500 years ago (essentially during the previous glacial period) [7]. This would be consistent with findings in kokanee (*O. nerka*, a landlocked sockeye salmon ecotype) in the upper Columbia River that similarly appear divergent from all other populations of sockeye salmon and kokanee [12]. Taken together, these pieces of evidence might be interpreted as support for a glacial refugium near the intersection of the Cordilleran Ice Sheet and the Laurentide Ice Sheet.

A more likely alternative is that another unknown factor was influencing past analyses and the PCA from the current study. The Salmon River coho salmon have increased runs of homozygosity, reduced heterozygosity, and reduced private alleles, which are indicators of a recent and extensive bottleneck. We were also able to infer the demographic history from whole genome sequences of the Salmon River coho salmon and found evidence of a bottleneck (from ~Ne 16,227 to ~Ne 1,749) around 4,000 years ago. These results could help explain why the Salmon River coho salmon

appear so divergent in a PCA, as low genetic diversity might be expected to increase the amount of variation in the analysis since most of the other individuals do not have low genetic diversity. We only collected samples from one tributary of the Thompson River (a part of a much larger basin) and can only suggest that a plausible hypothesis from this data is that recolonization of the Salmon River from a small founding population took place after glaciers receded. We did not account for the influence of hatcheries, which could also influence many of the metrics discussed above. Also, we did not incorporate linked selection in demographic modeling as the type of analysis that we used was not amenable. Without linked selection accounted for, there could be biases in times and effective population sizes from our estimates [49]. Based on all the genetic diversity metrics (above), demographic modeling, and what has previously been published on the time of the most recent glacial maximum [50], however, recent recolonization of the Salmon River remains a likely alternative hypothesis to a glacial refugium between ice sheets.

Most other streams, except Inch Creek, clustered in the PCA based largely on latitude for both PC1 and PC2 of the PCA. With a much more extensive sampling strategy, Rougemont et al. (2020) found a similar trend and tested various demographic histories [7]. The authors of that study found that the best supported model was a glacial refugia to the south with recolonization of the northern streams after glacial retreat – generating genomic signatures of isolation-by-distance. The private allele analyses from the current study also supports this interpretation. The private allele analysis identified that most of the northern streams have low private allele counts compared to southern streams.

To better understand the pattern of genomic isolation-by-distance along the latitude gradient, we performed a GWA analysis based on stream latitude. We found that 13.9% of the variants were associated with latitude based on a *p*-value of 0.01 without multiple test correction. To put this into perspective, the north – south gradient likely formed after the last Cordilleran Ice Sheet maximum 19-20,000 years ago [50] but

could have formed later between 3,750-8,000 years ago based on our demographic history modeling. This would suggest that a large fraction of the nucleotide variants responded within less than 8,000 years to the influence of isolation-by-distance.

We analyzed the influence of significantly associated nucleotide variants on gene function and also the distribution of annotated variants to better understand if selection played a large role in establishing the north – south gradient. We tested if genes with significantly associated variants ($\alpha = 0.05$, Bonferroni-correction), with 'Low' to 'Moderate' likelihoods of influencing gene function, belonged to any enriched GO categories. If a trait was under selection based on latitude, we might expect enriched GO terms associated with that trait. We did not find any enriched GO categories from the GWA analysis. This may indicate that selection may not have contributed much to establishing the north – south gradient.

When comparing the distribution of the most common variant annotations of the full dataset with the variants that were significantly associated with latitude, the largest fold difference was between the missense mutations (Table 3). We observed a ~2x difference from 0.8% missense mutation rate in the entire genome to 1.7% in the variants associated with latitude. While this increase does suggest selection may have contributed to the development of the latitude gradient, we interpret that, because the majority of the other annotations have similar frequencies, the majority of the variants that make up the gradient are not under direct selection. Further, the increase in missense mutations may represent slightly deleterious variants that escaped selective pressure during postglacial recolonization and expansion. Linked selection could still play a larger role but was not investigated here.

With or without selection, the north – south gradient of nucleotide variants likely influences some phenotypic differences in a similar gradient. As an example, we identified genes that had multiple nucleotide variants that are predicted to moderately influence gene function, and which also have an association with latitude. These included the rhotekin-like (*RTKN*, unknown function [51]), plectin-like (*PLEC*, giant

448 cytoskeleton scaffold [52]), PH and SEC7 domain-containing protein 4-like (*PSD4*, tight
449 junctions maintenance [53]), and GTPase IMAP family member 9-like (*Gimap9*, possibly
450 involved in T-cell development [54, 55]) genes. The nucleotide diversity of these genes
451 largely arises from the frequency of the alternative allele in the four most northern
452 streams – regions that would have likely been recolonized most recently assuming a
453 main southern glacial refugium.

454 Interestingly, *PLEC* is a candidate gene associated with migration distance in
455 brown trout (*Salmo trutta*), perhaps through its role in osmoregulation [56]. Cells
456 without *PLEC* were found to be more sensitive to changes in osmolarity (shrinking more
457 after exposure to urea) [57], and hatch-stage whitefish (*Coregonus lavaretus*) exposed
458 to high salinity have significantly higher *PLEC* protein expression [58]. Two of the other
459 genes with multiple variants moderately-likely to modify gene function and which were
460 associated with the north – south latitudinal gradient, *RTKN* and *PSD4*, may also have
461 roles in salinity tolerance. An Atlantic cod (*Gadus morhua* L.) nucleotide variant in the
462 intron of *PSD4* was found to be associated with a salinity gradient between the North
463 Sea and the Baltic Sea [59]. Likewise, researchers discovered that Rho (RTKN is an
464 effector protein of RhoA [51, 60]), is activated by hyperosmotic stress [61].

465 *PLEC*, *PSD4*, and *RTKN* all appear to be involved in cell junction functionality. Cell
466 junctions observed in a *PLEC* knockout cell line appeared to be compromised [62], *PSD4*
467 (also known as EFA6B) is required for efficient tight junction formation [63], and *RTKN*
468 influences cell junctions through PIST [51] and Septin proteins [51, 64]. It is thought that
469 cellular tight junctions play an important role in water and salt balance in teleost fishes
470 [65]. Considering that three of the four genes with multiple latitude associated
471 nucleotide variants and which are moderately likely to alter gene function could impact
472 cell junctions and osmoregulation, we hypothesize that ocean salinity may have
473 influenced coho salmon recolonization of northern streams.

474 While, the Pacific Ocean salinity was thought to be ~4% higher during the last
475 glacial maximum as freshwater was stored in glaciers [66], it is difficult to predict how

476 the salinity gradient observed in modern times [67] might have been influenced as
477 glaciers began to retreat (when northern recolonization would have been possible). If
478 there was a difference in salinity between northern and southern regions, nucleotide
479 variation in these genes may have facilitated northern colonization in some way.
480 From inferred demographic histories, we were able to estimate a recolonization
481 date of some northern streams (based on the founder effect that would be expected to
482 accompany recolonization) to between 3,750-8,000 years ago. This places an upper limit
483 on the age of the latitude gradient and how swiftly such a gradient can form. These
484 values are based on assumptions of a mutation rate of 8e-9 bp/generation and a
485 generation time of three years. Linked selection may also bias our time and effective
486 population estimates [49] as we did not account for them in modeling.
487 While it is important to remember that time and population estimates are
488 influenced by many factors when inferring demographic histories from sequence data,
489 multiple lines of evidence can be used to strengthen these inferences or put them in a
490 more realistic context. Radiometric evidence supports that the Cordilleran Ice Sheet
491 maximum occurred between 19,000 and 20,000 years ago [50]. Likewise, chemical
492 properties of gases in Antarctic ice cores support this termination of the last glaciation
493 period (Termination I) to roughly the same time period, as well as a previous
494 termination of the penultimate glaciation period around 138,000 and 148,000 years ago
495 (Termination II) [68]. In the demographic histories of the coho salmon, we noted
496 dramatic declines of nearly all salmon populations for both these time periods. This
497 observation supports the parameters used for modeling the demographic histories as
498 we expect that populations might decline in response to increased glaciation or rapid
499 climate change.
500 The overall trend we observed from modeling demographic histories was major
501 drops in effective population size at each transition from glaciation to inter-glaciation
502 period with increases for nearly all populations after the penultimate glaciation period
503 and uncommon increases for specific rivers after the most recent glaciation period. At a

504 species level, these transitional drops likely influence multiple aspects of coho salmon
505 biology since genetic variability can contribute to many characteristics of a species.
506
507 **Conclusions**
508 In this study, we generated two reference genome assemblies as tools for
509 conservation and management of coho salmon. Additionally, we resequenced the
510 genomes of a wide distribution of coho salmon from rivers along North America. We
511 were able to identify a north – south gradient in the nucleotide variation of the
512 genomes, which had been observed in previous studies. To add to previous
513 observations, we quantified that approximately 13.9% of the variation in the
514 resequenced genomes followed the north – south gradient. We also were able to
515 estimate that the age of the north – south gradient is likely under 8,000 years of age.
516 This gradient likely contributes to phenotypic diversity between northern and southern
517 rivers since we identified gene modifying variants that were associated with the latitude
518 gradient. Finally, we modeled demographic histories of the coho salmon from different
519 rivers and discovered that major drops in effective population size were related to
520 changes between glacial and inter-glacial periods. We believe the coho salmon genome
521 assemblies will facilitate research to better understand coho salmon biology and may
522 enhance management of this culturally and economically important species.
523
524 **Data availability**
525 Raw data for the genome assembly was submitted to the NCBI under the BioProject
526 PRJNA352719. Whole genome resequencing data was submitted under PRJNA401427
527 and PRJNA808051 to the NCBI BioProjects (see File S1 for specific samples used in this
528 study). The VCF file used for analyses in this study was submitted to the GSA Figshare
529 portal.
530
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561

562 **Author Contributions**

563 EBR, KAC, JSL, and BFK performed genome assembly, chromosome assembly,
564 genome submission, and generated genome metrics. DRM performed repeat library
565 construction. EBR, CAD, MTC, AM, and DS performed wet-lab work including DNA and
566 RNA extractions and mitochondrial sequencing. EBR, QR, EN, DRM, and JSL performed
567 SNP calling and population genomic analyses. RHD, MTC, DS, and CB generated, raised,
568 and dissected doubled-haploid samples for the genome assembly and transcriptome.
569 REB, TDB, KAN, and JMY provided samples used in resequencing work. KAN provided
570 early access to linkage map and additional guidance on its use. RHD, REW, TDB, KAN,
571 JMY, RN, LB, WSD, SJMJ, and BFK initiated, planned and supervised the project. EBR,
572 KAC, DRM and BFK wrote first draft of the manuscript.

573

574

575 **References**

1. Irvine JR, Fukuwaka M. Pacific salmon abundance trends and climate change. ICES J Mar Sci. 2011;68:1122–30.

2. Beamish RJ, Sweeting RM, Lange KL, Noakes DJ, Preikshot D, Neville CM. Early Marine Survival of Coho Salmon in the Strait of Georgia Declines to Very Low Levels. Mar Coast Fish. 2010;2:424–39.

3. Beamish RJ, McFarlane GA, Thomson RE. Recent declines in the recreational catch of coho salmon (Oncorhynchus kisutch) in the Strait of Georgia are related to climate. Can J Fish Aquat Sci. 2011. https://doi.org/10.1139/f98-195.

4. Kristianson G, Strongitharm D. The Evolution of Recreational Salmon Fisheries in British Columbia. Vancouver, BC: Pacific Fisheries Resource Conservation Council; 2006.

5. Noakes DJ, Beamish RJ, Sweeting R, King J. Changing the balance: Interactions between hatchery and wild Pacific coho salmon in the presence of regime shifts. North Pac Anadromous Fish Commision Bull. 2000;:155–63.

6. Beacham TD, Wetklo M, Deng L, MacConnachie C. Coho Salmon Population Structure in North America Determined from Microsatellites. Trans Am Fish Soc. 2011;140:253–70.

7. Rougemont Q, Moore J-S, Leroy T, Normandeau E, Rondeau EB, Withler RE, et al. Demographic history shaped geographical patterns of deleterious mutation load in a broadly distributed Pacific Salmon. PLOS Genet. 2020;16:e1008348.

8. Rougemont Q, Xuereb A, Dallaire X, Moore J-S, Normandeau E, Rondeau EB, et al. Long-distance migration is a major factor driving local adaptation at continental scale in Coho salmon. Mol Ecol. 2022;n/a n/a.

9. Zhang H, Tan E, Suzuki Y, Hirose Y, Kinoshita S, Okano H, et al. Dramatic improvement in genome assembly achieved using doubled-haploid genomes. Sci Rep. 2014;4:6780.

10. Christensen KA, Leong JS, Sakhrani D, Biagi CA, Minkley DR, Withler RE, et al. Chinook salmon (Oncorhynchus tshawytscha) genome and transcriptome. PLOS ONE. 2018;13:e0195461.

11. Rondeau EB, Minkley DR, Leong JS, Messmer AM, Jantzen JR, Schalburg KR von, et al. The Genome and Linkage Map of the Northern Pike (Esox lucius): Conserved Synteny Revealed between the Salmonid Sister Group and the Neoteleostei. PLOS ONE. 2014;9:e102089.

12. Christensen KA, Rondeau EB, Minkley DR, Sakhrani D, Biagi CA, Flores A-M, et al. The sockeye salmon genome, transcriptome, and analyses identifying population defining regions of the genome. PLOS ONE. 2020;15:e0240935.

13. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, et al. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci. 2011;108:1513–8.

14. English AC, Richards S, Han Y, Wang M, Vee V, Qu J, et al. Mind the Gap: Upgrading Genomes with Pacific Biosciences RS Long-Read Sequencing Technology. PLOS ONE. 2012;7:e47768.

15. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31:3210–2.

16. Krzywinski MI, Schein JE, Birol I, Connors J, Gascoyne R, Horsman D, et al. Circos: An information aesthetic for comparative genomics. Genome Res. 2009. https://doi.org/10.1101/gr.092759.109.

17. Allendorf FW, Thorgaard GH. Tetraploidy and the Evolution of Salmonid Fishes. In: Turner BJ, editor. Evolutionary Genetics of Fishes. Springer US; 1984. p. 1–53.

18. Lien S, Koop BF, Sandve SR, Miller JR, Kent MP, Nome T, et al. The Atlantic salmon genome provides insights into rediploidization. Nature. 2016;533:200–5.

19. Jurka J, Kapitonov VV, Pavlicek A, Klonowski P, Kohany O, Walichiewicz J. Repbase Update, a database of eukaryotic repetitive elements. Cytogenet Genome Res. 2005;110:462–7.

20. Smit A, Hubley R. RepeatModeler Open-1.0. 2013. http://www.repeatmasker.org/. Accessed 18 Dec 2017.

21. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, et al. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10:1–9.

22. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. ArXiv13033997 Q-Bio. 2013.

23. github.com/broadinstitute/picard. Java. Broad Institute; 2020.

24. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Curr Protoc Bioinforma. 2013;43:11.10.1-11.10.33.

25. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303.

26. Barría A, Christensen KA, Yoshida G, Jedlicki A, Leong JS, Rondeau EB, et al. Whole Genome Linkage Disequilibrium and Effective Population Size in a Coho Salmon (Oncorhynchus kisutch) Breeding Population Using a High-Density SNP Array. Front Genet. 2019;10:498.

27. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011;27:2156–8.

28. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10:giab008.

29. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. 2015;4.

30. PLINK 1.9. http://www.cog-genomics.org/plink/1.9/. Accessed 1 Jun 2018.

31. Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. Stacks: an analysis tool set for population genomics. Mol Ecol. 2013;22:3124–40.

32. Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlethwait JH. Stacks: Building and Genotyping Loci De Novo From Short-Read Sequences. G3 GenesGenomesGenetics. 2011;1:171–82.

33. Smith CT, Nelson RJ, Wood CC, Koop BF. Glacial biogeography of North American coho salmon (Oncorhynchus kisutch). Mol Ecol. 2001;10:2775–85.

34. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2020.

35. Turner SD. qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots. bioRxiv. 2014;:005165.

36. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 2012;6:80–92.

37. Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, et al. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Res. 2008;36:3420–35.

38. OmicsBox - BioBam | Bioinformatics Made Easy. BioBam. https://www.biobam.com/omicsbox/. Accessed 1 Mar 2022.

39. Terhorst J, Kamm JA, Song YS. Robust and scalable inference of population history from hundreds of unphased whole genomes. Nat Genet. 2017;49:303–9.

40. Oncorhynchus tshawytscha genome assembly Otsh_v2.0. NCBI. https://ncbi.nlm.nih.gov/data-hub/assembly/GCF_018296145.1/. Accessed 6 Apr 2022.

41. Homo sapiens genome assembly GRCh38.p13. NCBI. https://ncbi.nlm.nih.gov/data-hub/assembly/GCF_000001405.39/. Accessed 6 Apr 2022.

42. Oncorhynchus kisutch Annotation Report. https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Oncorhynchus_kisutch/101/. Accessed 30 Mar 2022.

43. Oncorhynchus kisutch genome assembly Okis_V2. NCBI. https://ncbi.nlm.nih.gov/data-hub/assembly/GCF_002021735.2/. Accessed 6 Apr 2022.

44. Oncorhynchus tshawytscha Annotation Report. https://www.ncbi.nlm.nih.gov/genome/annotation_euk/Oncorhynchus_tshawytscha/101/. Accessed 6 Apr 2022.

45. Berthelot C, Brunet F, Chalopin D, Juanchich A, Bernard M, Noël B, et al. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat Commun. 2014;5.

46. De-Kayne R, Zoller S, Feulner PGD. A de novo chromosome-level genome assembly of Coregonus sp. "Balchen": One representative of the Swiss Alpine whitefish radiation. Mol Ecol Resour. 2020;20:1093–109.

47. Macqueen DJ, Johnston IA. A well-constrained estimate for the timing of the salmonid whole genome duplication reveals major decoupling from species diversification. Proc R Soc B Biol Sci. 2014;281.

48. Allendorf FW, Bassham S, Cresko WA, Limborg MT, Seeb LW, Seeb JE. Effects of Crossovers Between Homeologs on Inheritance and Population Genomics in Polyploid-Derived Salmonid Fishes. J Hered. 2015;106:217–27.

49. Pouyet F, Aeschbacher S, Thiéry A, Excoffier L. Background selection and biased gene conversion affect more than 95% of the human genome and bias demographic inferences. eLife. 2018;7:e36317.

50. Clark PU, Dyke AS, Shakun JD, Carlson AE, Clark J, Wohlfarth B, et al. The Last Glacial Maximum. Science. 2009;325:710–4.

51. Ito H, Morishita R, Nagata K. Functions of Rhotekin, an Effector of Rho GTPase, and Its Binding Partners in Mammals. Int J Mol Sci. 2018;19:2121.

52. Winter L, Wiche G. The many faces of plectin and plectinopathies: pathology and mechanisms. Acta Neuropathol (Berl). 2013;125:77–93.

53. Zangari J, Partisani M, Bertucci F, Milanini J, Bidaut G, Berruyer-Pouyet C, et al. EFA6B Antagonizes Breast Cancer. Cancer Res. 2014;74:5493–506.

54. Wang Z, Li X. IAN/GIMAPs are conserved and novel regulators in vertebrates and angiosperm plants. Plant Signal Behav. 2009;4:165–7.

55. Limoges M-A, Cloutier M, Nandi M, Ilangumaran S, Ramanathan S. The GIMAP Family Proteins: An Incomplete Puzzle. Front Immunol. 2021;12.

56. Lemopoulos A, Uusi-Heikkilä S, Hyvärinen P, Alioravainen N, Prokkola JM, Elvidge CK, et al. Association Mapping Based on a Common-Garden Migration Experiment Reveals

Candidate Genes for Migration Tendency in Brown Trout. G3 GenesGenomesGenetics. 2019;9:2887–96.

57. Osmanagic-Myers S, Gregor M, Walko G, Burgstaller G, Reipert S, Wiche G. Plectin-controlled keratin cytoarchitecture affects MAP kinases involved in cellular stress response and migration. J Cell Biol. 2006;174:557–68.

58. Papakostas S, Vasemägi A, Vähä J-P, Himberg M, Peil L, Primmer CR. A proteomics approach reveals divergent molecular responses to salinity in populations of European whitefish (Coregonus lavaretus). Mol Ecol. 2012;21:3516–30.

59. Berg PR, Jentoft S, Star B, Ring KH, Knutsen H, Lien S, et al. Adaptation to Low Salinity Promotes Genomic Divergence in Atlantic Cod (Gadus morhua L.). Genome Biol Evol. 2015;7:1644–63.

60. Thumkeo D, Watanabe S, Narumiya S. Physiological roles of Rho and Rho effectors in mammals. Eur J Cell Biol. 2013;92:303–15.

61. Ciano-Oliveira CD, Sirokmány G, Szászi K, Arthur WT, Masszi A, Peterson M, et al. Hyperosmotic stress activates Rho: differential involvement in Rho kinase-dependent MLC phosphorylation and NKCC activation. Am J Physiol-Cell Physiol. 2003;285:C555–66.

62. Jirouskova M, Nepomucka K, Oyman-Eyrilmez G, Kalendova A, Havelkova H, Sarnova L, et al. Plectin controls biliary tree architecture and stability in cholestasis. J Hepatol. 2018;68:1006–17.

63. Théard D, Labarrade F, Partisani M, Milanini J, Sakagami H, Fon EA, et al. USP9x-mediated deubiquitination of EFA6 regulates de novo tight junction assembly. EMBO J. 2010;29:1499–509.

64. Kim J, Cooper JA. Junctional Localization of Septin 2 Is Required for Organization of Junctional Proteins in Static Endothelial Monolayers. Arterioscler Thromb Vasc Biol. 2021;41:346–59.

65. Chasiotis H, Kolosov D, Bui P, Kelly SP. Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: A review. Respir Physiol Neurobiol. 2012;184:269–81.

66. Insua TL, Spivack AJ, Graham D, D'Hondt S, Moran K. Reconstruction of Pacific Ocean bottom water salinity during the Last Glacial Maximum. Geophys Res Lett. 2014;41:2914–20.

67. Durack PJ, Wijffels SE, Matear RJ. Ocean Salinities Reveal Strong Global Water Cycle

Intensification During 1950 to 2000. Science. 2012;336:455–8.

68. Kawamura K, Parrenin F, Lisiecki L, Uemura R, Vimeux F, Severinghaus JP, et al. Northern Hemisphere forcing of climatic cycles in Antarctica over the past 360,000 years. Nature. 2007;448:912–6.

69. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York; 2016.

70. Becker RA, Wilks AR, Brownrigg R, Minka TP, Deckmyn A. maps: Draw Geographical Maps. 2018.

71. Chappellaz J, Barnola JM, Raynaud D, Korotkevich YS, Lorius C. Ice-core record of atmospheric methane over the past 160,000 years. Nature. 1990;345:127–31.

72. Alexander DH, Novembre J, Lange K. Fast model-based estimation of ancestry in unrelated individuals. Genome Res. 2009;19:1655–64.

576

577 **Figures**

578 **Figure 1. Circos plot of the first version of the coho salmon genome assembly.** In the
579 interior of the Circos plot are the links between duplicated regions of the
580 chromosomes/linkage groups (i.e., homeologous regions). A) Representations of the
581 chromosomes with the approximate position of the centromere marked by a filled
582 circle. The tick marks represent 10 Mbp intervals. B) The percent identity between
583 duplicated regions of the chromosome. The orange-red color represents very high
584 similarity (> 94%), the orange color high similarity (91-94%), the yellow moderate (88-
585 91%), and the green low (< 88%). C) The fraction of repetitive elements, with red
586 representing high (> 60%), yellow as moderate (35-60%), and green as low (< 35%).

587

588 **Figure 2. Influence of isolation-by-distance on coho salmon population structure.** A) A
589 PCA of coho salmon based on variants that were filtered for linkage disequilibrium
590 (plotted using ggplot [69]). Circles were drawn around Inch Creek and Salmon River
591 individuals to highlight that much of the variation of this PCA was due to differences of

592 salmon from these rivers. B) The same figure as A, with Salmon and Inch Creek salmon
593 removed. When Inch Creek and Salmon River salmon are removed from the graph, the
594 influence of latitude/isolation-by-distance can be observed on PC1 and PC2. Individuals
595 from the same river (same colour) also tended to cluster near each other. C) A map of
596 the various rivers sampled for this study and the corresponding private allele counts
597 (plotted with the maps [70] package in R). The private allele counts are also displayed to
598 the side as a bar graph.
599
600 **Figure 3. Fraction of the genome responsive to isolation-by-distance.** A) A Manhattan
601 plot of the latitude genome-wide association analysis of all coho salmon except for the
602 Chile strain. The red line represents a significance level of 0.01 after a Bonferroni
603 correction and the blue line a 0.05 level with the same correction. B) A histogram of
604 variant counts for different significance levels. C) The percent of the variants at different
605 significance levels. The percentage represents all the variants at or above the line. There
606 are 33.8% of the variants that have a significant association with latitude at $p \leq 0.1$
607 without multiple test correction.
608
609 **Figure 4. Genes with multiple 'moderate' nucleotide variants that may influence**
610 **function.** Four genes were found to be associated with latitude and also contained
611 multiple SNPs that likely modify gene function. The four genes are: rhotekin-like (RTKN
612 LOC109895613), plectin-like (PLEC LOC109904478), PH and SEC7 domain-containing
613 protein 4-like (PSD4 LOC109868337), and GTPase IMAP family member 9-like (Gimap9
614 LOC109880231). A) Pie diagrams showing the distribution of reference (blue) and
615 alternative alleles (red) for each gene and location. B) Map produced with the maps
616 package in R showing the sampling sites.
617
618 **Figure 5. Demographic histories of coho salmon populations based on genome**
619 **sequencing.** A) Each labeled line represents multiple individuals from the same river or

620 strain. The X-axis represents calendar years based on a generation time of 3 years for
621 coho salmon. The Y-axis is the effective population size (Ne) estimate. The estimated
622 age of the Cordilleran ice sheet maximum was taken from [50]. The approximate age of
623 the last interglacial period was based on [71]. B) For each location, a number nearby
624 indicates a drop of at least 5,000 in the Ne for one of the time points noted in A. The
625 colour of the river label indicates if the river had a drop in Ne during the 1st time interval
626 (orange – yes, blue – no, the Klamath River was the only southern river with a drop
627 during the 1st time period).
628
629 **Tables**
630 **Table 1.** Whole-genome resequencing sources

Source	Country	State/Province	Count Female, Male
Klamath River (Hatchery)	US	CA/OR	1F, 4M
Deschutes River (Hatchery)	US	CA/OR	2F, 3M
Big Quilcene River (Hatchery)	US	WA	2F, 3M
Wallace River (Hatchery)	US	WA	6M, 4?
Tsoo-Yess River (Hatchery)	US	WA	1F, 4M
Inch Creek (Hatchery)	Canada	BC	3F, 5M
Capilano River (Hatchery)	Canada	BC	5F
Robertson Creek (Hatchery)	Canada	BC	5M
Salmon River (Hatchery)	Canada	BC	5F
Pallant Creek	Canada	BC	5M
Kitimat River (Hatchery)	Canada	BC	5F, 5M
Berners River	US	AK	2F, 3M
Kwethluk River	US	AK	1F, 4M
AquaChile (Strain)	Chile	NA	5F

631 State/Province Abbreviations: CA - California, OR - Oregon, WA - Washington, BC -

632 British Columbia, AK – Alaska

633

634 **Table 2.** Genome statistics

	Contig N50	Contig #	BUSCO	% Repeats	Genes
Ver 1	58,118	97,074	91%-55:37*	44.82†	41,179†
Ver 2	1,159,298	8,770	99.2%-57.1:42.2*†	53.12†	60,330†

635 Ver 1, NCBI: GCF_002021735.1; Ver 2, NCBI: GCF_002021735.2

636 *Percent complete-single:duplicate

†Reported by NCBI (NCBI used actinopterygii_odb10 for BUSCO)

637

638 **Table 3.** Distribution of common nucleotide variant annotations

	Entire genome*	Associated with latitude*
Intron	44.0%	42.1%
Intergenic	31.2%	31.2%
Upstream	10.4%	11.2%
Downstream	7.3%	7.6%
3' UTR	2.8%	3.2%
Missense	0.8%	1.7%
Synonymous	1.1%	1.1%

639 *5,631,459 genomic SNPs, 3,940 significant SNPs from GWA analysis

640

641 **Supplemental Material**

642 **Figure S1. Runs of homozygosity and admixture among coho salmon from different**

643 **streams.** A) The top figure shows the total runs of homozygosity (ROH) for each

644 individual (default settings in PLINK). The bottom figures show the admixture of each

645 individual based on cluster counts of k=2 and k=3 (with Admixture software [72] using

default settings with LD filtered SNPs). Streams are shown at the bottom and delineated by the alternating blue bar. B) A map (generated using the maps package in R) showing the locations in A.

Figure S2. The relationship between runs of homozygosity and latitude. A) Counts of runs of homozygosity (ROH) and the total length of the ROH when combined (see Methods for parameters used). There is a distinct cluster of Salmon River individuals with higher counts and lengths of ROH. B) The relationship between the average length of ROH per individual and latitude. The line was plotted using the geom_smooth function in ggplot2 with the linear model method. Latitude significantly (p = 0.029) explained variation in the average length of ROH (~6% of the variation, Adjusted R^2 = 0.04886).

File S1. Sample information and SRA accession numbers.

File S2. Nucleotide variants significantly associated with latitude. The SignificantVariants tab in this spreadsheet file has information on all of the significantly associated SNPs with latitude. The ModerateGenes tab has information on SNPs that were both associated with latitude and also have annotations from SNPeff that were moderately likely to influence gene function. The Moderate+LowGenes tab has information on SNPs that were both associated with latitude and also have an annotation from SNPeff that were likely to have moderate or low influences on gene function. The GO tab has two lists of genes that were used in the GO enrichment analyses. The Frequency of MultiVariantGenes tab has information on the genes with multiple SNPs thought to moderately influence gene function and which were also associated with latitude. This information was used to generate pie charts. The GenotypeGenes tab has the genotypes for each individual for genes in the Frequency of MultiVariantGenes tab. The Regions tab has information on the latitudes used for each

674 stream. The DistributionOfVariantAnnotations tab has information on SNPeff
675 annotations from the SNPs that were significantly associated with latitude as well as the
676 SNPs from the entire genome.