The pink salmon genome: uncovering the genomic consequences of a strict two-year life-cycle

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Abstract

Pink salmon (*Oncorhynchus gorbuscha*) adults are the smallest of the five Pacific salmon native to the western Pacific Ocean. Pink salmon are also the most abundant of these species and account for a large proportion of the commercial value of the salmon fishery worldwide. A strict two-year life-history of most pink salmon generates temporally isolated populations that spawn either in even-years or odd-years. To uncover the influence of this genetic isolation, reference genome assemblies were generated for each year-class and whole genome re-sequencing data was collected from salmon of both year-classes. The salmon were sampled from six Canadian rivers and one Japanese river. At multiple centromeres we identified peaks of Fst between year-classes that were millions of base-pairs long. The largest Fst peak was also associated with a million base-pair chromosomal polymorphism found in the odd-year genome near a centromere. These Fst peaks may be the result of centromere drive or a combination of reduced recombination and genetic drift, and they could influence speciation. Other regions of the genome influenced by odd-year and even-year temporal isolation and tentatively under selection were mostly associated with genes related to immune function, organ development/maintenance, and behaviour.

Introduction

Pink salmon are an economically important species under heavy exploitation and have been the subject of intense mitigation efforts. Commercial catches of pink salmon comprise roughly half of all Pacific salmon catches by weight and a much greater percentage by count as they are the smallest of the commercially important Pacific salmon (1,2). Since the late 1980s, more than a billion pink salmon are released annually from hatcheries (1) to maintain the abundance of this fishery.

The native range of pink salmon encompasses parts of the southern Arctic Ocean between North America and Asia as well as much of the northern Pacific Ocean (3). Recently, Arctic climate warming has opened previously inaccessible Arctic territory to pink salmon as well (4–6). Pink salmon have been introduced to the Great Lakes in North America (7) and drainage basins of the White Sea (reviewed in (8)), near the border of Russia and Finland.

Pink salmon spend a year and a half at sea before returning to rivers to spawn at two-years of age. This strict two-year life history, unique to this species among salmon, has wide-ranging implications for their evolution, conservation, and possibly for their future as a species. Populations that spawn in an even-numbered year (e.g., 2020) return in an even-year (e.g., 2022) to spawn as adults. Similarly, odd-year spawned pink salmon return in odd-numbered years. Gene flow between year-classes/lineages is consequently limited (9) (this phenomenon is known as allochronic or temporal isolation). Rare exceptions to a strict two-year life-cycle of pink salmon in their native range have been reported (10–12). Outside their native range, three-year-old pink salmon have been observed in the Great Lakes following introduction (7,13). One hypothesis based on experimental rearing in heated sea water is that temperature may play a role in precocious development (i.e., one-year life-cycle) (14).

Within a year-class, population genetic differentiation among rivers tends to be lower than that of other salmon species, which is a possible consequence of increased straying of pink salmon from natal streams during spawning (15,16). Increased straying itself may be a repercussion of the reduced time that pink salmon spend in their natal streams compared to most other salmon species (chum salmon – *Oncorhynchus keta* being an exception) (3). Pink salmon are ready for sea migration as soon as they emerge from gravel and after yolk-sac absorption (17).

In contrast to the regional reduced heterogeneity observed within year-class populations, there is a high level of divergence between year-classes as a result of limited gene flow (9,18–22). Genetic differentiation between odd and even lineages from the same river is greater than within year-class

66 differentiation, a phenomenon observed across the species natural range (23). There are also
67 phenotypic differences that have been reported between lineages such as gill raker counts (19),
68 length/size (with even-year fish tending to be smaller in Canada) (24–26), and survival/alevin growth
69 in low temperature environments (27).

70 The divergence of pink salmon from other Pacific salmon species has been estimated to have
71 occurred several million years ago (28–32); this provides a maximal time of odd and even lineage
72 divergence. Based on mitochondrial nucleotide diversity, divergence times between odd and even-year
73 lineages have previously been estimated as 23,600 years (33), 150 – 608 thousand years ago (34), and
74 0.9 – 1.1 million years ago (22). The relatively recent estimates of divergence are inconsistent with
75 complete temporal isolation between odd and even lineages (potentially for several million years). It
76 has been suggested that low-level gene flow or recolonization of extirpated year-classes by alternate
77 year-classes could account for recent estimates of divergence, with recolonization being a favoured
78 explanation (33). Both low-level gene flow and recolonization have been observed in introduced pink
79 salmon in the North American Great Lakes (7,35,36), revealing that it is possible that environment and
80 temperature (suggested in (36)) can alter the strict allochronic isolation observed in modern times.
81 Maturation in pink salmon has been verified to be sensitive to temperature and photoperiod under
82 experimental conditions (37).

83 While odd-year and even-year pink salmon populations may occupy the same environment
84 (during different years), these lineages can still have different selective pressures (38). For example,
85 the density of pink salmon is known to vary between years (38,39), and density may influence the
86 composition of pink salmon predators, prey, and the number of fish on the spawning grounds (40–42).
87 In years with a high abundance of pink salmon, some studies have reported a decrease in body size of
88 pink salmon at sea (other species of salmon and seabirds have also been adversely influenced during
89 these high abundance years) (41–45). These studies reveal that the intraspecific competition among

other pink salmon and interspecific competition among other species can vary significantly between odd and even-years.

In this study, we present genome assemblies for both odd-year and even-year lineages, develop a transcriptome to annotate these assemblies, and analyze polymorphisms found between groups. We identified regions of the genome that have diverged between odd and even-year lineages with some possibly as a response to selection. We were also able to identify large F_{st} peaks adjacent to many centromeres and to verify one major fusion or deletion on LG15_El12.1-15.1 by combining polymorphism data with long-read sequencing of both year-classes. These regions of the genome are important aspects of pink salmon biology and provide greater insight into the evolutionary divergence of the lineages.

Materials and Methods

Animal care

Fisheries and Oceans Canada Pacific Region Animal Care Committee (Ex. 7.1) was the authorizing body for animal care carried out in this study. All salmon were reared, collected, or euthanized in compliance with the Canadian Council on Animal Care Guidelines.

Genome assemblies

A mature male pink salmon was sampled from the Big Qualicum River Hatchery (NCBI BioSample: SAMN16688056) on September 19, 2019 (odd-year) by hatchery personnel and euthanized by concussion as specified in section 5.5 of the Canadian Council on Animal Care guidelines. A mature male pink salmon was also sampled from the Quinsam River Hatchery (NCBI BioSample:

112 SAMN18987060) by hatchery personnel in the same manner on July 28, 2020 (even-year). We
 113 dissected liver, spleen, kidney, and heart tissues from the carcasses and flash-froze them on dry ice
 114 immediately and stored them at -80°C. We used a Nanobind Tissue Big DNA Kit (Circulomics) to
 115 isolate high-molecular DNA following the manufacturer's protocol from multiple tissues. In addition,
 116 Short Read Eliminator Kits (Circulomics) were used to reduce the fraction of small DNA fragments in
 117 the DNA extractions following the kit protocol for DNA samples to be sequenced on Oxford Nanopore
 118 Technologies (ONT) platforms.

119 We generated sequencing libraries with the prepared DNA using a Ligation Sequencing Kit
 120 (SQK-LSK109 ONT) following the manufacturer's protocol. The libraries were sequenced on a Spot
 121 On Flow Cell MK1 R9 with a MinION (ONT) or a PromethION (R9.4.1 flow cell). Libraries
 122 sequenced on the PromethION were size selected using magnetic beads (0.4:1 ratio). DNase flushes
 123 were performed to increase yield according to manufacturer's instructions. We also tried to add 1%
 124 DMSO immediately before sequencing to reduce secondary structures that might block pores and
 125 reduce sequencing efficiency for one flow-cell (with a minor increase in pore occupancy, more titration
 126 will be needed to identify if there are benefits of adding DMSO). FASTQ sequence files were
 127 generated either using the Guppy Basecalling Software (version 3.4.3+f4fc735 for sequences from the
 128 MinION) with default settings or MinKNOW v3.4.6 (for sequences from the PromethION).

129 Short-read sequence data were generated for genome polishing for the even-year genome
 130 assembly (NCBI SRA accession: SRX10913279 – SRX10913282) and the odd-year genome assembly
 131 (NCBI SRA accession: SRX6595859 – SRX6595860). We generated the short-read data for the even-
 132 year genome by shearing 1ug of DNA (pink even-year male described above) with a COVARIS LE220
 133 (Covaris) using the following configuration in a 96 microTUBE plate (Covaris): duty 20, pip450,
 134 cycles/burst 200, total time 90s, pulse spin in between 45s treatment. The library was then constructed

135 using the MGIEasy PCR-Free DNA Library Set (MGI) following the manufacturer's protocol. The
136 library was then sequenced on an MGISEQ-200RS Sequencer (150 + 175 PE).

137 We generated the short-read sequence data for polishing the odd-year genome assembly for a
138 previous assembly that was not published because the contiguity of the assembly was low. The
139 sequences were from an odd-year haploid female produced at Fisheries and Oceans Canada using
140 source material from the Quinsam River Hatchery (NCBI BioSample: SAMN12367892). To produce
141 the haploid salmon, we applied UV irradiation (560 uW/cm² for 176 s) to sperm from a Quinsam River
142 male pink salmon (to destroy parental DNA) immediately before fertilizing eggs from a Quinsam River
143 female pink salmon. Prior to sequencing, the individual was confirmed to be haploid using a panel of
144 11 microsatellites. The details of the library preparations and sequencing technology can be found on
145 the NCBI website (NCBI SRA accession: SRX6595859 – SRX6595860).

146 We created a Hi-C library for the even-year genome assembly using the Arima-HiC 2.0 kit
147 (Arima Genomics – manufacturer's protocol) with liver tissue from the even-year male (NCBI SRA
148 accession: SRR14496776). The library was then sequenced on an Illumina HiSeq X (PE150). A Hi-C
149 library was only successfully generated for the even-year genome assembly.

150 After sequencing, we produced initial genome assemblies with the Flye genome assembler
151 (version 2.7-b1587 – odd, 2.8.2-b1695 – even) (46) using ONT sequences (parameters: -g 2.4g, --asm-
152 coverage 30). Racon (version 1.4.16) (47) was then used to find consensus sequences of the Flye
153 assemblies (parameters: -u) after aligning the respective ONT reads to the assemblies using minimap2
154 (48) (version 2.13, parameters: -x map-ont). We polished the assemblies with Pilon (version 1.22) (49)
155 using the following methods. Paired-end reads were filtered and trimmed using Trimmomatic (50)
156 (version 0.38) (parameters for the odd-year reads: ILLUMINACLIP: TruSeq3-PE-2.fa:2:30:10
157 LEADING:28 TRAILING:28 SLIDINGWINDOW:4:15 MINLEN:200; parameters for the even-year
158 reads: ILLUMINACLIP:TruSeq3-PE.fa:2:30:10:2:keepBothReads LEADING:3 TRAILING:3

MINLEN:36). The respective reads were aligned to each of the Racon-corrected assemblies using bwa (51,52) (version 0.7.17) with the -M parameter and sorted and indexed using Samtools (53) (version 1.9) prior to polishing with Pilon (default parameters).

After the genome assemblies were polished, we identified the order and orientation of contigs/scaffolds on pseudomolecules/chromosomes for the odd-year genome using a genetic map (54) and synteny to the coho salmon genome (NCBI: GCF_002021735.2). Chromonomer (55) (version 1.10) was used to order the contigs/scaffolds using the genetic map (parameters: --disable_splitting). Ragtag (56) (version 1.0.1) was used to order the contigs/scaffolds using synteny to the coho salmon genome (default parameters). We used a custom script (57) to compare the contig order files output by Chromonomer and Ragtag (.agp files) and manually reviewed the output for discrepancies. The manually curated order and .fasta files were submitted to the NCBI.

To order and orient contigs and scaffolds on pseudomolecules for the even-year genome, we mapped Hi-C reads to the polished assembly using scripts from Arima Genomics (58). The output alignment file was then converted to a .bed file using BEDtool bamtobed (version 2.27.1) (59) with default parameters and sorted using the Unix command 'sort -k 4.' After the Hi-C reads were mapped to the genome assembly, Salsa2 (60,61) was used to further scaffold the contigs and initial scaffolds (parameters: -e GATCGATC,GANTGATC,GANTANTC,GATCANTC). After scaffolding, we mapped the remaining contigs and scaffolds onto pseudomolecules/chromosomes using the same strategy as for the odd-year genome assembly (see above) except a newer genetic map was used (62) (an odd-year genetic map was the only available) and the rainbow trout genome assembly (NCBI: GCF_013265735.2, (63)) was chosen for synteny. The proposed order and orientation was then reviewed manually using Juicebox (version 1.11.08) (64) before submission to the NCBI. The .hic and .assembly files used by Juicebox were produced using the pipeline from Phase Genomics (65). The

182 nomenclature for the chromosomes was based on the linkage group from the genetic maps and from the
183 Northern pike orthologous chromosomes in an attempt to standardize nomenclature across salmonids
184 (66).

185 A BUSCO (Benchmarking Universal Single-Copy Orthologs) version 3.0.2 analysis (67) was
186 used to assess assembly quality. We performed these analyses after polishing assemblies, but before
187 mapping contigs/scaffolds onto chromosomes. The lineage dataset used in this analysis was
188 actinopterygii_odb9 (4584 BUSCOs). The parameters used were: -m genome and -sp zebrafish.

189 A Circos plot was generated from the odd-year genome assembly using Circos software version
190 0.69-8 (68). We identified homeologous regions of the genome with SyMap version 5.0.6 (69) using a
191 repeat-masked version of the assembly without unplaced scaffolds or contigs (default settings).
192 Repeats had previously been identified by NCBI and were masked by us using Unix commands. The
193 output from SyMap was formatted and summarized using scripts from Christensen et al. (2018) (70).
194 A histogram of repetitive sequence was generated using a python script (71). The Marey map (genetic
195 map markers aligned to a genome) was generated using the methods from Christensen et al. (2018)
196 (70). Centromere positions were taken from the genetic map after it was converted into a Marey map.
197

198 **Whole-genome re-sequencing**

199 Samples were previously collected by Fisheries and Oceans Canada personnel from the
200 following bodies of water (British Columbia unless otherwise noted): Quinsam River Hatchery,
201 Atnarko River, Kitimat River Salmon Hatchery, Deena River, Yakoun River Hatchery, Snootli Creek
202 Hatchery, Kushiro River (Japan) (S1 File). Samples were chosen to encompass odd-year and even-year
203 samples from the same body of water or from nearby streams (even-year n=30, odd-year n=31).

some analyses, which are sensitive to linkage disequilibrium. Variants were filtered if heterozygous allele counts were not evenly represented — also known as allele balance (minor allele count < 20% of the major allele count, see (77) for python script). Variants in linkage disequilibrium were thinned using BCFtools (79) (parameters: +prune, -w 20kb, -l 0.4, and -n 2). Custom scripts, bwa mem, and Samtools index were used to map the variants to different genome assemblies (80).

Transcriptome

To better annotate the genome assemblies, we collected a dataset of 19 tissues from a juvenile female pink salmon (NCBI Accessions: SRX6595821-SRX6595839). Euthanasia of this salmon was performed by placing the salmon in a bath of 100 mg/L tricaine methanesulfonate buffered with 200 mg/L sodium bicarbonate. Team dissection was used to quickly remove tissues and each tissue was stored in RNAlater Stabilization Solution (ThermoFisher) as recommended by the manufacturer.

We extracted RNA from the tissue stored in RNAlater Stabilization Solution using the Qiagen RNeasy kit (QIAGEN). Stranded mRNASeq libraries were generated at McGill University and Génome Québec Innovation Centre, with NEBNext dual index adapters. Libraries were then sequenced as a 1/39 fraction of a NovaSeq 6000 S4 PE150 lane at McGill University and Génome Québec Innovation Centre. These datasets were deposited to NCBI for use in gene annotation (BioProject: PRJNA556728).

Population structure

As clustering techniques are sensitive to linkage disequilibrium, we used variants that were hard-filtered (including for allele balance) and filtered for linkage disequilibrium for all population

249 structure analyses. A DAPC analysis (81) was used to cluster individuals in R (82) using the following
250 packages: adegenet (83), vcfR (84), and ggplot2 (85). The number of DAPC clusters was determined
251 using the find.clusters function and choosing the cluster count with the lowest Bayesian information
252 criterion. Thirty principal components were retained with the dapc function. The variants used for the
253 DAPC analysis were not yet mapped to chromosomes.

254 To complement the DAPC analysis, we also performed an Admixture (version 1.3.0) analysis
255 (86) to identify clusters of individuals and quantify the admixture between the identified groups. To
256 format the linkage disequilibrium thinned .vcf file, we used a custom Python script to rename the
257 chromosomes to numbers (77) and PLINK (version 1.90b6.15) (87,88) was used to generate .bed files
258 (parameters: --chr-set 26 no-xy, --double-id). PLINK was also used to generate a principal components
259 analysis. The Admixture software was then used to identify the optimal cluster number based on the
260 lowest cross-validation error value. The admixture values from this analysis were plotted in R.

261 To examine population structure based on the mitochondrion sequence, we generated a
262 phylogenetic tree based on full mitochondria sequences. The genome assembly included a
263 mitochondrion sequence, and this region of the genome was subset from the variant file using vcftools.
264 The resulting file and the SNPRelate (89) package in R was used to generate the phylogenetic tree.
265 The snpgdsVCF2GDS and snpgdsOpen functions were used to import the data, the snpgdsDiss
266 function was used to calculate the individual dissimilarities for pairwise comparisons between samples,
267 the snpgdsHCluster function was used to generate a hierarchical cluster of the dissimilarity matrix, the
268 snpgdsCutTree function was used to determine subgroups, and the snpgdsDrawTree function was used
269 to plot the dendrogram.

270 From the variants with minimal filtering and the variants after all filters had been applied, the
271 heterozygosity ratio was separately calculated based on the number of heterozygous genotypes divided

272 by the number of alternative homozygous genotypes (90,91). The number of heterozygous and
 273 homozygous genotypes were counted using a python script from Christensen et al. (2020) (77).
 274 Heterozygous genotypes per kilobase pair (kbp) was calculated by dividing the heterozygous genotype
 275 counts by the genome size (2,528,518,120 bp) and then multiplied by 1000. This calculation was used
 276 on the variants with minimal filtering not yet mapped to chromosomes.

277 The number of shared alleles was calculated as a metric for relatedness using custom scripts for
 278 the variants with minimal filtering and which were mapped to chromosomes (92). This value is
 279 calculated by counting the number of alleles an individual has in common with another individual and
 280 is similar to previous work (93–95). The percent shared alleles was calculated in R (number of shared
 281 alleles divided by the total allele count multiplied by 100) and plotted using the reshape2 (96) and
 282 pheatmap (97) R packages.

283 Fst, nucleotide diversity (within populations— π and between— d_{xy}), and Tajima’s D were
 284 calculated and plotted using the R packages PopGenome (98), dplyr (99), tidyr (100), stringr, and
 285 qqman. In PopGenome, all metrics were calculated using a sliding window of 10 kbp and the data
 286 were visualized as a Manhattan plot using qqman. We used the populations module from Stacks
 287 version 2.54 (101) to calculate the number of private alleles, percent polymorphic variants, Fis
 288 (inbreeding coefficient), and Pi (nucleotide diversity within a population) for odd and even year class
 289 samples grouped as populations. These metrics were compared with the sample from Japan as an odd-
 290 year sample or as its own population. A comparison was also performed to see how filtering
 291 influenced these metrics.

292

293 **Genomic regions associated with population structure under selection**

294 To identify regions of the genome associated with population structure identified in the DAPC
 295 analysis, we performed an eigenGWAS analysis (102). The format of the hard-filtered variants was
 296 converted to the appropriate format in PLINK, and the GEAR (103) software was used to run the
 297 eigenGWAS analysis (this was performed on a slightly different version of the genome assembly
 298 available on the NCBI, but only positions on chromosome 9 were minimally affected). Significance
 299 was corrected for using the genomic inflation factor to better identify markers potentially under
 300 selection rather than a result of genetic drift between populations. The genomic inflation factor
 301 corrected p-values were then plotted in R using the qqman (104) and stringr (105) packages. A
 302 Bonferroni correction was applied as a multiple test correction ($\alpha = 0.05$). Only peaks with at least
 303 5 SNPs within 100 kbp of each other were retained to reduce false-positives (nucleotide variants under
 304 selection are expected to be in linkage disequilibrium with surrounding variants and significant single
 305 variants not in linkage may be a consequence of spurious alignments).

306

307 **Sex determination and sdY**

308 We utilized a genome-wide association (GWA) of phenotypic sex to identify the region of the
 309 genome associated with sex for all pink salmon (individual year-classes were checked as well). This
 310 analysis was also used to identify where the contig from the genome assembly with the sdY gene
 311 should be placed. This was confirmed with synteny from the rainbow trout Y-chromosome
 312 (NC_048593.1) and manual inspection of the Hi-C data (it was placed in the even-year genome
 313 assembly). The GWA analyses were performed using PLINK (parameters: --logistic --perm). Synteny
 314 was identified from alignments to the rainbow trout genome assembly (GCF_013265735.2, (63)) using
 315 CHROMEISTER (106) (default settings).

When manually genotyping the presence/absence of the sdY gene by visualizing alignments in IGV (107), we noticed some males had increased coverage of the sdY gene, and two haplotypes were identified (4 variants in non-coding DNA). The haplotypes were manually genotyped. To estimate the copy number of the sdY gene, we first used a python script to determine the average coverage of all hard-filtered variants (108). The average coverage of the four variants in the sdY gene was then divided by the average coverage of all variants.

Results

Genome assemblies

The odd-year assembly (GCA_017355495.1) had a combined length of ~2.5 Gbp, with 20,664 contigs and a contig N50 of ~1.8 Mbp. The even-year assembly had similar metrics, with a contig N50 of ~1.5 Mbp, 24,235 contigs, and a length of ~2.7 Gbp. We used a BUSCO analysis of known conserved genes to determine the completeness and quality of the genome assembly. Of the 4584 BUSCOs, 95.3% were found to be complete in the odd-year genome assembly (54.9% single-copy and 40.4% duplicated), 1.4% were fragmented, and 3.3% were missing. The even-year assembly also had 95.3% complete BUSCOs (51.5% single-copy and 43.8% duplicated), but more fragmented (1.6%) and fewer missing BUSCOs (3.1%).

The odd-year assembly had 26 linkage groups and extensive homeologous regions between chromosomes (Fig 1). The odd-year genome assembly contained similar levels of repetitive DNA and duplicated regions compared to other salmonids (Fig 1, (70,77,109)). Like other salmon species, increased sequence similarity was also observed at telomeres between duplicated chromosomal arms

(Fig 1). Peaks of increased F_{st} between odd and even-year lineages were commonly found at putative centromere locations (Fig 1, Table 1).

Fig 1. Circos plot of pink salmon genome assembly. Positions are all based on the odd-year genome assembly. Chromosomes/linkage groups are noted with blue boxes representing the centromere identified in Tarpey et al. (2017) (62). Links between chromosomes are homeologous regions identified using SyMap. A) F_{st} values between all odd-year and even-year salmon greater than 0.25. Values greater than 0.5 are highlighted red. B) The fraction of repetitive DNA as identified by NCBI (odd-year). Values greater than 0.65 are highlighted red. C) The percent identity between homeologous regions identified by SyMap (scale 75-100%). Values greater than 90% are highlighted red. D) A Marey map with markers from the genetic map (y-axis, 0 – 1, with 1 being the marker with the greatest cM value) placed onto the genome (x-axis, odd-year).

Table 1. Largest F_{st} peaks between odd and even-year lineages.

Linkage group/ chromosome	Region (Mbp)	Size of peak (Mbp)	Frequency Odd (p*)	Frequency Even (p*)	HWE	Potential cause
LG04_El13.1-02.1	50-53	~3	0.98	0.43	Both	Centromere
LG10_El12.1-15.1	46.5-50	~3.5	0.69	0.22	Both	Centromere
LG14_El18.2-23.2	49-55	~6	0.77	0.12	Both	Centromere
LG15_El08.2-20.1	50-54	~4 Centromere ~1.26 Deletion	0.5**	0**	Both	Centromere/ Deletion-Fusion
LG18_El09.2-17.1	45.5-46.5	~1	1	0	Both	Selection
LG21_El24.2-22.1	31-34.5	~3.5	0.63	0	Both	Centromere
LG25_El23.1-24.1	17.5-19	~1.5	0.65	0.15	Both	Centromere
LG26_El09.1-11.1	11.3-18	~3.5 Centromere ~7.3 Misassembly	0.89	0.67	Both	Centromere/ Misassembly

The odd and even allele frequencies (p) were based on the most clearly defined sub-region rather than the entire region. It is unclear which individuals have the deletion or fusion on LG15_El08.2-20.1.

*reference genome allele frequency

**alternative (to reference) allele frequency

355 **Population structure**

356 A shared allele analysis (Fig 2) and both Admixture and DAPC analyses (Fig 3) revealed a clear
357 delineation between odd and even-year lineages. Parent-progeny and sibling relationships
358 (relationships known during sampling) are highlighted by increased levels of shared alleles, but the
359 majority of clustering appears to be related to geographical distance (Fig 2, S1 File). No apparent
360 admixture was observed in the even-year class (Fig 3B). In the odd-year lineage, estimated ancestry
361 from the even-year group varied from zero to over forty percent (Fig 3B).

362

363 **Fig 2. Percent of shared alleles among pink salmon.** A heatmap of shared alleles between salmon is
364 shown with clustering and a dendrogram. Each square represents the percent shared alleles after minor
365 filtering of variants (bi-allelic SNPs). In addition to the legend displaying the colour representation of
366 percent shared alleles, the sex, year-class, and river system sample information is colour-coded and
367 shown on both rows and columns.

368 **Fig 3. Population structure of pink salmon.** A) Sampling locations for odd and even-year pink
369 salmon. B) An admixture analysis based on an optimal group number of two. Sampling site is
370 specified on the left (y-axis) by colour and fraction of alleles inherited from a lineage is shown on the
371 x-axis (orange – even-year, blue – odd-year). On the right, DAPC groups are shown (see S1 File for
372 group and coordinate positions). The DAPC groups matched year-class/lineage designations.

373

374 A separate analysis of mitochondrial DNA was performed to further investigate the
375 relationships between the odd and even-year lineages. Odd-year pink salmon had longer branch
376 lengths in mitochondria dendrograms and haplotype networks with more uniform distributions of
377 haplotypes (Figs 4A and B). The even-year salmon had two major haplotypes (Fig 4B). Mitochondrial

sequence analyses revealed 21 unique haplotypes from the 61 mitochondria sequences with 1-19 steps between haplotypes (Fig 4). Based on the length of the sequence analyzed (16,822 bp) this represents a mutation frequency between 0.006% to 0.1%. One haplotype was shared between lineages and the closest haplotype that was not shared had 5 steps between year-classes (Fig 4). The mitochondrial analyses illustrate divergence between the odd and even-year lineages, but also raises questions regarding possible recent admixture based on a shared haplotype and an odd-year haplotype most closely related to an even-year haplotype.

385

Fig 4. Whole mitochondrial genome comparisons between lineages. A) A dendrogram based on full mitochondrial sequences. The y-axes show dissimilarity scores on the left and coancestry values on the right, which were used to cluster individuals. Year-class/lineage is specified below the dendrogram. B) A full mitochondrial genome haplotype network is shown for the 21 unique haplotypes identified. River names are shown for the haplotype shared between lineages.

391

Several metrics were calculated to quantify genetic divergence between and within year-classes: heterozygosity ratios, heterozygous genotype per kbp, polymorphic sites, private alleles, and nucleotide diversity. Heterozygosity ratios in odd-year fish ranged from 1.5-4.56, with an average of 2.54 (excluding haploid individuals generated for a previous project) (S1 File). Even-year class individuals ranged from 1.09-1.78, with an average of 1.44 (S1 File). The average heterozygous genotype per kbp (excluding haploids) was 0.71 for odd-year salmon (range: 0.55 – 0.85) and 0.58 for even-year (range: 0.45 – 0.69) pink salmon. The Pearson correlation between heterozygosity ratio and heterozygosity per kb was 0.91 (excluding haploids). Salmon from odd-years had on average higher levels of polymorphic sites, increased private alleles, and increased nucleotide diversity (Table 2). These values varied based on sample inclusion and filtering parameters used for filtering nucleotide variants (Table

2). The average percent of shared alleles among odd-year fish was 76.13%, 74.42% among even-year individuals, and 71.04% between year-classes (S1 File). Most analyses revealed increased genetic diversity among odd-year pink salmon than among even-year pink salmon and fewer shared alleles between odd and even-year populations than within year-class.

Table 2. Population metrics of the two lineages.

	Odd with sample from Japan	Odd without sample from Japan	Even
Number of variants		3,817,721 (101,594)	
% polymorphic	93.23 (97.91)	92.61 (97.64)	90.66 (87.86)
Private alleles	356,634 (12,333)	302,507 (10,486)	258,335 (2,124*)
Pi	0.276 (0.337)	0.276 (0.337)	0.269 (0.283)
Fis	0.075 (0.143)	0.068 (0.135)	0.122 (0.195)

Variants with minimal filtering are shown first and variants after all filters are shown in parentheses. *2,126 without sample from Japan

Genomic regions associated with odd and even-year lineages

We identified regions of the genome with divergence between odd and even-year lineages using an eigenGWAS and Fst analysis. Seventeen significant regions of the genome were discovered to contribute to the divergence between odd and even-year lineages (Fig 5, Table 3). These regions are putatively under selection as genetic drift is partially accounted for through the genomic inflation factor. Multiple candidate genes under selection were identified in these regions (Table 3).

Fig 5. Genome-wide divergence between odd and even-year pink salmon lineages. A Manhattan plot of eigenGWAS results, with chromosome positions on the x-axis and p-values (corrected for genetic drift using the genomic inflation factor) on the y-axis identifies region of the genome potentially under selection. The red horizontal line represents a Bonferroni correction at $\alpha=0.01$ and the blue line at $\alpha=0.05$. All positions are from the odd-year genome assembly.

422 **Table 3. Top eigenGWAS peaks identified between lineages.**

Chromosome	BP range	SNP position with lowest P- value	Candidate Gene	Gene Symbol
LG01_El19.1-16.1	51653225-51738345	51716026	protein tyrosine phosphatase receptor type J	<i>PTPRJ</i>
LG02_El19.2-07.2	18075760-18095551	18075760	AT-rich interactive domain- containing protein 3A	<i>arid3a</i>
LG02_El19.2-07.2	46961740-47008290	46969254	protein-methionine sulfoxide oxidase mical2b	<i>mical2b</i>
LG02_El19.2-07.2	110392052-110493632	110449484	multidrug and toxin extrusion protein 2-like	<i>SLC47A2</i>
LG08_El03.2-06.2	60715584-60782108	60767399	polh polymerase (DNA directed), eta	<i>POLH</i>
LG14_El18.2-23.2	29365137-29414547	29411435	uncharacterized gene	
LG14_El18.2-23.2	50053735-50217619	50217619	Unknown	
LG15_El08.2-20.1	42753852-42762230	42758791	cystathionine gamma-lyase	<i>CTH</i>
LG15_El08.2-20.1	51106314-52224901	51106314	multiple candidates*	
LG18_El09.2-17.1	45516859-45534019	45524530	cell division control protein 42 homolog*	<i>CDC42</i>
LG18_El09.2-17.1	46342891-46450909	46347678	H-2 class II histocompatibility antigen, A-U alpha chain*	<i>H2-Aa</i>
LG19_El20.2-01.2	22831059-22843129	22836628	B-cell receptor CD22	<i>CD22</i>
LG21_El24.2-22.1	9281799-9398462	9391795	histidine N-acetyltransferase	<i>hisat</i>
LG22_El03.1	10576168-10595358	10595038	purine nucleoside phosphorylase	<i>PNP</i>
LG22_El03.1	15334053-15411821	15405819	multiple candidates	
LG24_El10.1-25.1	47355926-47430898	47398112	microtubule-associated protein 9	<i>map9</i>
LG25_El23.1-24.1	37126251-37202999	37137812	uncharacterized gene (ncRNA)	

423 All positions are relative to the odd-year genome.

424 *associated with an Fst peak

425

426 Seven of the eight largest Fst peaks between year-classes were located in the vicinity of a
427 centromere (Fig 1, Table 1). More detail is presented on one of the largest Fst peaks. This peak is also
428 associated with a large deletion or fusion. The Fst peak on LG15_El12.1-15.1 (Fig 6A) is in Hardy-
429 Weinberg equilibrium in the odd-year lineage ($p=0.984$ with a chi-square test), but fixed in the even-
430 year lineage (Figs 6B and 6C). When Oxford Nanopore reads from the two year-classes were aligned

431 to the genome assembly, a heterozygous deletion or fusion from 51,670,144 – 52,926,328 was found in
432 this region of the odd-year male used for genome assembly (S1 Fig.). The ~1.2 Mbp deletion/fusion
433 may explain why the LG15_El12.1-15.1 Fst peak was one of the largest and widest (Fig 1, S1 Fig.).

434

435 **Fig 6. Chromosome LG15_El12.1-15.1 Fst peak.** A) A Manhattan plot of 10 kbp sliding-window Fst
436 values between odd and even-year pink salmon lineages on chromosome LG15_El12.1-15.1. B)
437 Genotypes visualized in IGV. Each row represents an individual pink salmon and each column
438 represents a nucleotide variant (dark blue – homozygous reference, light blue – heterozygous reference,
439 green – homozygous alternative, and white – missing genotype). Individuals were sorted by year-class
440 (shown on the right) and then by assigned genotype (shown on the left). C) Counts of genotypes of the
441 chromosomal polymorphism based on manual genotyping.

442

443 It is difficult to distinguish between a deletion and a chromosomal fusion in these analyses and
444 this may represent a fusion instead of a deletion. Previous research support chromosomal variants in
445 pink salmon (110) and a species specific fusion of this chromosome (66), but further research will be
446 needed to confirm this hypothesis. From these analyses, many highly divergent regions of the genome
447 were identified, either from selection or from genetic isolation/population dynamics. The largest
448 reservoirs of divergence between odd and even-year classes appears to be associated with centromeres,
449 but not exclusively and uncommonly for regions putatively under selection (Table 3).

450

451 **Sex determination and sdY**

452 The sex-determination gene in salmonids, *sdY* (111), was located on a ~110 kbp contig in the
 453 pink salmon odd-year genome assembly (NCBI accession: JADWMN010014055.1) and on a contig
 454 ~367 kbp that was placed onto a chromosome in the even-year genome assembly. The *sdY* gene can
 455 be placed at one of the ends of LG20_El14.2 by using genome-wide association with sex as the trait of
 456 interest, Hi-C contact data (even-year genome), and synteny with the rainbow trout Y-chromosome and
 457 chromosome 29 (an autosome) of the coho salmon (Fig 7A, S1 and S3 Figs.). LG20_El14.2, has the
 458 reverse orientation in the odd-year assembly compared to the genetic map (Fig 1), but was corrected to
 459 have the same orientation in the even-year assembly.

460

461 **Fig 7. The location and counts of the sex-determining gene, *sdY*, in pink salmon.** A) A genome-
 462 wide association analysis with sex as the phenotype under investigation shown as a Manhattan plot.
 463 The putative sex-determining region is indicated with an arrow. B) A scatterplot with the average
 464 coverage of the variants across the genome on the x-axis for all the pink salmon, and the estimated *sdY*
 465 count on the y-axis (*sdY* has previously been identified as the sex-determining gene in most salmonids).
 466 The different colour points represent different year-classes and *sdY* haplotypes.

467

468 In both genome assemblies there is only one copy of the *sdY* gene, confirmed with a BLAST
 469 alignment of a *sdY* gene available in the NCBI database (KU556848.1) to the respective assemblies.
 470 From a self-alignment of the *sdY*-containing contig, the majority of this contig is highly repetitive, > 90
 471 kbp out of ~110 kbp. From the alignment of the *sdY*-containing region in pink salmon to the coho
 472 salmon chromosome 29, only a small portion of the Y-chromosome appears to be unique to the Y-
 473 chromosome (S3 Fig.). Genotypes were called for the majority of this region for males and females,

474 and the main difference related to sex was that all females had large runs of homozygosity while many
475 males had large runs of heterozygosity (S4 Fig, S1 File).

476 From previous research (112,113), a pseudo growth hormone 2 gene was shown to be tightly
477 linked to sex-determination in pink salmon. Four tandem duplicates of this gene (NCBI: DQ460711.1)
478 were identified on the same contig in the even-year genome assembly, but only two copies were found
479 in the odd-year genome assembly on separate contigs (S1 File). As these contigs were not mapped to a
480 chromosomal position, it is likely that parts of the Y-chromosome specific region remain incomplete in
481 these two assemblies.

482 There were two *sdY* haplotypes (variants found in non-coding DNA) observed in both odd and
483 even male pink salmon (Fig 7B, S1 File). Additionally, some males possessed multiple copies of the
484 *sdY* gene (Fig 7B). The CGGA *sdY* haplotype was only identified in a single odd-year male pink
485 salmon, while the TTAC haplotype was evenly distributed between year-classes (S1 File).

486 Based on manual inspection of the genotypes, long stretches of heterozygosity were observed
487 near the *sdY* gene in some males, but not in others. In males with the TTAC *sdY* haplotype, there were
488 extended or short runs of heterozygosity evenly distributed between year-classes (S1 File). Even-year
489 males with the TTAC *sdY* haplotype and a short run of heterozygosity were more likely to have
490 multiple copies of the *sdY* gene ($n=4$, average=2.7) than the same group with long runs of
491 heterozygosity ($n=4$, average=0.9, $p=0.017$ with one-tailed, unpaired t-test). Any individuals with the
492 CGGA *sdY* haplotype did not have stretches of heterozygosity near the putative location of *sdY*. One
493 hypothesis to explain these results is that individuals with the CGGA *sdY* haplotype have an alternative
494 sex chromosome.

495

496 Discussion

497 Population structure

498 Similar to previous studies (23,54), pink salmon population structure divergence was found at
 499 the whole-genome level to be greater between year-classes rather than based on geography. Shared
 500 allele, DAPC, and Admixture analyses point to a clear delineation of odd and even lineages, with the
 501 exception of the only sample from Japan. Further sampling will need to be performed to provide an
 502 improved picture of the diversity of this species at the whole-genome level within year-class and across
 503 their Pacific Rim distribution. In British Columbia, however, the even-year lineage appeared to be
 504 more homogeneous than the odd-year lineage based on the admixture analysis and several population
 505 metrics such as nucleotide diversity. A similar result was previously observed with microsatellite (15)
 506 and SNP markers (23).

507 Divergence between lineages was also revealed by whole mitochondrial sequences. There were
 508 21 unique mitochondria genotypes among the 61 individuals sampled, and only one of these haplotypes
 509 was shared between lineages. While the number of unique haplotypes was the same between lineages,
 510 most of the even-year class haplotypes (8 out of 10) were similar in sequence. The two major
 511 haplotypes seen in the even-year class were consistent with the Alaskan A and AA haplotypes seen in
 512 Churikov and Gharrett (2002) (33), as were the numerous and more distantly related odd-year
 513 haplotypes.

514 The low nucleotide diversity of mitochondrial haplotype networks and the increase of rare
 515 haplotypes have led previous studies to conclude that pink salmon (with some local exceptions) have
 516 undergone a bottleneck during the Pleistocene interglacial period and rapid expansion since the last
 517 glacial maximum or earlier (33,34,114). The interconnected mitochondrial networks in these studies

518 have inner shared haplotypes between year-classes. Churikov and Gharrett (2002) suggested that these
 519 observations supported a model where a year-class might go extinct and an alternate year-class would
 520 then replace that population rather than continued gene flow between year-classes that would be
 521 necessary to otherwise explain the shared haplotypes (incomplete lineage sorting was tested) (33). The
 522 mitochondrial network seen in this study is consistent with that hypothesis. An alternative hypothesis
 523 is that environmental factors influence maturation timing and the strict two year life-cycle of pink
 524 salmon, and gene flow between year-classes only occurs when environmental conditions favour
 525 changes to the two year life-cycle, as that seen in the introduction of pink salmon to the Great Lakes
 526 (7,35,36).

527 Estimates of divergence based on mitochondrial sequences suggest that odd and even-year
 528 lineages (from East Asia and Alaska) are relatively recent for pink salmon as a species (generally less
 529 than 1 million years ago) and divergence likely began during the Pleistocene interglacial period or later
 530 (22,33,34). If the two-year life-cycle is environmentally influenced, these estimates could be distorted
 531 by phases of gene-flow and would suggest that the interglaciation period was the last major period of
 532 gene-flow between odd and even-year classes (but does not necessarily mean that was when the strict
 533 two year life-cycle evolved).

534 It has previously been reported that the odd-year lineage of pink salmon has higher levels of
 535 heterozygosity, private alleles, and allelic richness (23,54). A similar trend was observed in this study
 536 with the heterozygosity ratio, heterozygous genotypes per kbp, private alleles, and other metrics
 537 assessing nucleotide diversity. Several factors could help explain the reduced levels of nucleotide
 538 diversity seen in the sampled even-year populations. Tarpey et al. (2018) suggested three possibilities,
 539 1) the odd-year lineage was older and the even lineage was derived from the odd-year lineage, 2) there
 540 was a past reduction in even-year lineage(s), and 3) genetic variation was lost during adaptation (23).

541 Further sampling will be required to understand if this phenomenon is seen in all even-year populations
 542 (especially as lower heterozygosity in the even-year lineage is not universally supported, e.g. (20)).
 543 This information is important to interpret which hypothesis is better supported or if another model is
 544 better suited (e.g., extirpated lineage replaced by alternate year or that recent population demographics
 545 are more important).

546

547 **Fst peaks between odd and even-year lineages**

548 A single major chromosomal polymorphism (either a fusion or deletion) was identified
 549 proximal to a centromere on LG15_El12.1-15.1. This region was characterized by ~4 Mbp runs of
 550 homozygosity/heterozygosity. This region was identified from an Fst analysis because nearly the entire
 551 region was fixed in the even-year lineage, but appeared to segregate as a single locus in Hardy-
 552 Weinberg equilibrium in the odd-year lineage.

553 Interestingly, pink salmon runs of homozygosity/heterozygosity were common at centromeres
 554 rather than an effect of chromosomal polymorphisms. Six other major runs of
 555 homozygosity/heterozygosity were also located near centromeres and they differed between lineages.
 556 All of these Fst peaks extend for at least 1 Mbp. It is expected that regions with reduced
 557 recombination, such as centromeres, will have increased runs of homozygosity and reduced genetic
 558 diversity (reviewed in (115)). This may help explain why there are long runs of homozygosity at
 559 centromeres, but not why there are differences between lineages at these loci. Genetic drift or selection
 560 such as centromere drive would also need to be considered.

561 The centromere drive hypothesis posits that a centromere can be retained in a female gamete
 562 more often than an alternative centromere during meiosis due to an advantageous DNA sequence

563 mutation at the centromere or from mutations in centromere associated proteins (reviewed in (116–
564 118)). In populations that become isolated, the competition between centromere sequences can quickly
565 drive differentiation at these regions between the populations and result in hybrid defects should they
566 come into contact again (117). These observations reveal that the pink salmon lineages may be at a
567 point where speciation is a likely outcome as these large centromere differences could cause hybrid
568 defects. In medaka, genomic diversity at non-acrocentric repeats in centromeres were associated with
569 speciation (119).

570 The centromere drive hypothesis may further shed light on the fixation of the Fst peak on
571 LG15_El12.1-15.1. Robertsonian fusions (assuming that the Fst peak on LG15_El12.1-15.1 is indeed
572 associated with a fusion rather than a deletion) can generate centromeres that are preferentially able to
573 segregate to the egg during female meiosis (118). This could help drive the fusion to fixation in a
574 population. Alternatively, if the telocentric chromosomes instead of the fused metacentric chromosome
575 had more effective centromeres, the telocentric chromosomes would become fixed. Further studies
576 will be needed to confirm if there is indeed a fusion instead of a deletion and that the fusion leads to
577 fixation by centromere drive.

578

579 **Genomic regions putatively under selection**

580 A large component of the genetic and phenotypic diversity between pink salmon year-classes
581 likely originates from genetic drift as there is little evidence for gene flow between lineages. However,
582 in addition to genetic drift, these lineages may experience different selective pressures even if they
583 occupy the same streams. As mentioned in the Introduction, population density between lineages is
584 often different and this can generate different ecological environments. EigenGWAS and Fst analyses
585 were used to identify regions of the genome potentially responsive to these environmental differences

586 between pink salmon year-classes. Candidate genes under selection were organized into three broad
587 categories (immune system, organ development/maintenance, and behavior), and each is discussed
588 below.

589 **Immune system**

590 Variation in immune related genes is a common phenomenon between salmonid populations
591 (e.g., (77,120,121)). Between odd and even-year pink salmon, five eigenGWAS peaks were identified
592 near or in genes with immune related functions. These include the H-2 class II histocompatibility
593 antigen, A-U alpha chain (*H2-Aa*) (122–126), B-cell receptor CD22 (*CD22*) (127,128), polh
594 polymerase (DNA directed), eta (*POLH*) (129–133), AT-rich interactive domain-containing protein 3A
595 (*arid3a*) (134,135), and purine nucleoside phosphorylase (*PNP*) (136–140).

596 Several factors could influence why these immune related genes might be under selection
597 between odd-year and even-year populations of pink salmon. For example, altered migration patterns
598 (reviewed in (141,142)), increased pathogen loads between year classes due to increased density
599 (reviewed in (142,143)), and increased physiological stress from competition and increased number of
600 predators during years with larger returns (e.g., (142)) could all influence the differences observed in
601 immune related genes. Further investigations into the nature of these genes in pink salmon may
602 uncover the environmental factors and selective pressures relevant to the evolutionary history of these
603 pink salmon lineages.

604 **Organ development/maintenance**

605 Salmon go through nutritional and behavioural changes that require organ-level alterations and
606 maintenance throughout their life-cycle. This can be observed in developing salmon that transition
607 from plankton to other fish as food sources. In the eye, this transition requires the development of new

608 functionality such as night vision to chase prey. One example of such a transition is the change of UV
609 opsins observed in hatched Pacific salmon to blue later in life by opsin changeover (144,145).

610 Variation in vision related genes have previously been observed between sockeye salmon
611 populations (77). In Atlantic salmon, *six6*, a gene related to eye development, daylight vision
612 (146,147), and fertility (148) was also found to be associated with age at maturity (149,150) and later
613 with stomach fullness during migration (151). These studies suggest that genetic variation influencing
614 organ development, transition, or maintenance are important components influencing salmonid
615 evolution.

616 Similar to *six6* in Atlantic salmon, Protein tyrosine phosphatase receptor type J (*PTPRJ*) (152),
617 histidine N-acetyltransferase (*hisat*) (153–160), and microtubule-associated protein 9 (*MAP9* or *ASAP*)
618 (161) all appear to play roles in proper vision. The variation in these genes may represent differences
619 in selective pressure between odd and even-years and could be driven by the different population
620 dynamics observed between odd and even-year populations.

621 Cystathionine gamma-lyase (*CTH*) may have, among other roles, a function in hearing (162–
622 164), and could have been influenced by similar population dynamics as those suggested for vision-
623 related genes. Multidrug and toxin extrusion protein 2 gene (*SLC47A2*) is not related to a specific
624 organ, though it may have a special role in the blood-brain barrier (165,166). Instead, it may help in
625 removing toxins, which might accumulate in more dense and stressed populations.

626 **Behaviour**

627 Fish display consistent behavioural differences from each other, analogous to human
628 personalities (167). Personality variation in a population may represent adaptive solutions to different
629 environmental pressures (167). In high density populations, such as the odd-year populations, more
630 aggressive behaviours during high-density spawning conditions (40) could result in more offspring, but

might waste energy in lower-density conditions. Associations to genes related to behaviour have previously been identified among sockeye salmon populations (77), and under selection between wild and farmed Atlantic salmon (168). In the present study, protein-methionine sulfoxide oxidase *mical2b* (*mical2b*) (169,170) and cell division control protein 42 homolog (*CDC42*) (171), both putative genes found in the eigenGWAS analysis between even and odd-year pink salmon, have previously been found to be associated with anxiety/reactiveness and schizophrenia, respectively.

Another gene, we were not able to unambiguously identify as a candidate gene from other nearby genes, on LG15_El08.2-20.1 is worth noting because of its association with behaviour. The gene, 5-hydroxytryptamine receptor 2B (*5-HT2B* and also known as serotonin receptor 2B), is associated with impulsivity and impulsive aggression in humans and mice (172,173). This region of the genome was fixed in the even-year class pink salmon where population density is often lower (in many North American populations, but not necessarily in all of the rivers in this study) and aggression might be maladaptive. These associations suggest that population dynamics might influence the frequency of certain personality traits in pink salmon populations, with density as a possible driving force.

Sex determination and sdY

With the discovery of a novel sex-determining gene in salmonids (111), and previously with closely linked genetic markers (174,175) researchers have been able to identify instances of sex-determination switching between chromosomes in salmonids (176–180). As suggested in Yano et al. (2013), Y-chromosome switching may act in response to (expected) degeneration of the Y-

652 chromosome due to mutation accumulation from reduced recombination (181). In pink salmon, *sdY*
653 was located on LG20_El14.2, but we suggest there may be an alternative location as well.

654 Several pieces of information indicate that LG20_El14.2 may not be the only location of the
655 sex-determining gene, *sdY*, in the pink salmon genome. For instance, there were two *sdY* haplotypes
656 and several males had multiple copies of this gene. Also, all males with the CGGA *sdY* haplotype had
657 a run of homozygosity similar to most females on the LG20_El14.2 chromosome near the putative
658 location of *sdY*. It is expected that near the *sdY* gene, recombination is reduced and mutations would
659 accumulate between the X and Y-chromosomes as a result of reduced recombination. Females tend to
660 have long runs of homozygous genotypes where recombination is reduced and males tend to have long
661 stretches of heterozygous genotypes when reads from the X and Y-chromosome align at the same
662 location (77). Since the males with the CGGA *sdY* haplotype have long runs of homozygous genotypes
663 at the LG20_El14.2 region, as most of the females do, we suggest that the CGGA *sdY* is at another
664 location in the genome in these individuals. We were unable to identify a precise putative alternative
665 location because there were too few individuals with the CGGA *sdY* to obtain a signal from a genome
666 wide association analysis, however, the potential discovery of another salmon species with alternative
667 *sdY* locations, further supports the hypothesis of Y-chromosome switching put forth by Yano et al.
668 (2013) for salmonids (181).

669

670 **Conclusions**

671 We generated reference genome assemblies for both pink salmon lineages, RNA-seq data for
672 genome annotation, and whole genome re-sequencing data to expand the available resources for this
673 commercially important and evolutionarily interesting species. The coupled whole genome re-

674 sequencing study of 61 individuals from several streams in British Columbia (and one from Japan)
 675 helped us to characterize regions of the genome that have diverged between the temporally isolated
 676 groups. The amount and degree of lineage-specific genomic variation suggests that there is little gene-
 677 flow between the year-classes, but the shared variants such as whole mitochondrial and *sdY* haplotypes
 678 suggests that there has been enough recent gene-flow or alternative year-class replacement to maintain
 679 these similarities. Divergence at centromeres between the two lineages may be a consequence of
 680 centromere drive (or genetic drift and reduced recombination) and represent early stages of speciation.
 681 Genes related to the immune system, organ development/maintenance, and behaviour were divergent
 682 between odd and even-year classes as well. These example lineage defining differences offer us a
 683 glimpse into the evolutionary landscape and the selective pressures or demographic histories that may
 684 have driven the divergence in these genes and the differences between odd and even lineages of pink
 685 salmon.

686

687 **Acknowledgements**

688 Extensive sample preparation and sequencing was performed at McGill University and Génome
 689 Québec Innovation Centre (now the Centre d'expertise et de services Génome Québec) and we would
 690 like to thank the staff and scientists there for their efforts. We would also like to thank the generous
 691 computing resources provided by Compute Canada (www.computecanada.ca). Fisheries and Oceans
 692 Canada and the University of Victoria facilities and personnel made this work possible. Finally, the
 693 authors would like to thank the many Fisheries and Oceans Canada staff who collected samples for
 694 analysis in this study.

695

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697 **Supporting information**

698 **S1 File. Sample information.** The sample tab has metadata about each sample, including information
 699 on sex, river, and year-class (latitude and longitude locations are approximate). The StatsAllFilters

shows metrics from the .vcf file after filtering for LD (see methods). Stats1stFilter has the same information, but from the .vcf file after only preliminary filtering (see methods). The eigenGWAS tab contains the DAPC values used in the eigenGWAS analysis (see methods). The Mitochondrion tab shows metadata used to generate the mitochondria figures. The GPS tab shows the coordinates used in the sample map. The Admixture tab has the values output from the admixture analysis. For each tab with LG, these sheets have manually genotyped areas and calculations of HWE. The PrivateAlleles tab has metrics output from Stacks. The SharedAlleles tab has a matrix of shared alleles between individuals in long format and statistics on the right. The Y-Chrom tab has information about the *sdY* haplotypes. The GWAS tab has metadata used in the GWAS analysis. The GHp tab displays the alignments of the growth hormone pseudogene and *sdY* gene to the odd and even genome. The even genome placements will change after processing by NCBI.

S1 Fig. Chromosomal polymorphism at centromere on LG15_El08.2-20.1. Depiction of LG15_El08.2-20.1 and a chromosomal polymorphism, either a deletion or evidence of a chromosomal fusion. At the top, LG15_El08.2-20.1 is depicted with the distance and location of the purported polymorphism. Scaffolds/contigs that comprise the region surrounding the polymorphism are shown below the chromosomal depiction, with a blue arrow showing where multiple small contigs were placed. Below the scaffolds, synteny with rainbow trout and Northern pike is shown based on CHROMEISTER (106) alignments. Finally, ONT/Nanopore reads that were used to generate the genome assemblies were aligned back to the odd-year genome and visualized with IGV. Reads in the odd-year individual are shown flanking the deletion (the display was split because the region was too large to adequately visualize otherwise).

S2 Fig. Sex determining region of the even-year pink salmon compared to the rainbow trout Y-chromosome. A) A CHROMEISTER (106) dotplot between the Y-specific portion (top) and shared

723 portion (bottom) of LG20_El14.2 of the even-year pink salmon genome assembly and the rainbow
724 trout Y-chromosome (63). The location of the *sdY* gene is shown based on the position in the rainbow
725 trout chromosome and the genes annotated by NCBI are shown on the x-axis at the bottom. B) A plot
726 of the Hi-C contact map of the even-year pink salmon genome assembly produced by Juicebox (64).
727 There are multiple inversions between the pink salmon and rainbow trout genome, but the contact map
728 supports the order and orientation for the pink salmon genome assembly and these could represent
729 actual inversions between species instead of assembly errors.

730 **S3 Fig. Sex determining region of the even-year pink salmon compared to the coho salmon**
731 **chromosome 29 autosome.** A) A CHROMEISTER (106) dotplot between the Y-specific portion (top)
732 and shared portion (bottom) of LG20_El14.2 of the even-year pink salmon genome assembly and coho
733 salmon chromosome 29. B) A plot of the Hi-C contact map of the even-year pink salmon genome
734 assembly produced by Juicebox (64). There are multiple inversions between the pink salmon and coho
735 salmon genome, but the contact map supports the order and orientation for the pink salmon genome
736 assembly and these could represent actual inversions between species instead of assembly errors.

737 **S4 Fig. Sex determining region of the even-year pink salmon with genotype information.**
738 Genotypes are shown from an IGV (107) screenshot for the 61 samples of pink salmon for the region
739 with the *sdY* sex-determining gene. The top portion shows the distance of the Y-specific genome
740 region (~3.2 Mbp) and the contig/scaffold boundaries that make up this region are shown as vertical
741 lines. Below the distances, allele frequencies for each locus are shown, and below that individual
742 genotypes. The x-axis of the genotypes represent loci and each line on the Y-axis represents an
743 individual pink salmon. The dark-blue colour is a homozygous reference genotype, the light-blue
744 colour a heterozygous genotype, and the green genotype is for a homozygous alternative locus. There
745 are large stretches (1-2 Mbp) of heterozygosity and homozygosity based on sex. Please note that there

746 is a possible inversion (from a mis-assembly) in this region as the runs of homozygosity and
747 heterozygosity are broken by a section from ~600 kbp and ~1,300 kbp.

Fig1

Fig2

Fig3

Fig4

Fig5

Fig6

A

B

Fig7