Comparative mapping between coho salmon (*Oncorhynchus kisutch*) and three other salmonids suggests a role for chromosomal rearrangements in the retention of duplicated regions following a whole genome duplication event

Miyako Kodama\(^1\)§, Marine S O Brieuc\(^1\), Robert H Devlin\(^2\), Jeffrey J Hard\(^3\) and Kerry A Naish\(^1\) §

\(^1\) School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105, USA
\(^2\) Fisheries and Oceans Canada, West Vancouver, British Columbia, V7K 1N6, Canada
\(^3\) National Marine Fisheries Service, Northwest Fisheries Science Center, Seattle, WA 98112, USA

§ Corresponding authors

Email addresses:

MK: mkodama@uw.edu
MSOB: mbrieuc@uw.edu
RHD: robert.devlin@dfo-mpo.gc.ca
JJH: Jeff.Hard@noaa.gov
KAN: knaish@uw.edu
ABSTRACT

Whole genome duplication has been implicated in evolutionary innovation and rapid diversification. In salmonid fishes, however, whole genome duplication significantly pre-dates major transitions across the family, and re-diploidization has been a gradual process between genomes that have remained essentially collinear. Nevertheless, pairs of duplicated chromosome arms have diverged at different rates from each other, suggesting that the retention of duplicated regions through occasional pairing between homeologous chromosomes may have played an evolutionary role across species pairs. Extensive chromosomal arm rearrangements have been a key mechanism involved in re-diploidization of the salmonid genome and so we investigated their influence on degree of differentiation between homeologs across salmon species. We derived a linkage map for coho salmon and performed comparative mapping across syntenic arms within the genus Oncorhynchus, and with the genus Salmo, to determine the phylogenetic relationship between chromosome arrangements and the retention of undifferentiated duplicated regions. A 6596.7cM female coho salmon map, comprising 34 linkage groups with 7415 and 1266 non-duplicated and duplicated loci respectively, revealed uneven distribution of duplicated loci along and between chromosome arms. These duplicated regions were conserved across syntenic arms across Oncorhynchus species, and were identified in metacentric chromosomes likely formed ancestrally to the divergence of Oncorhynchus from Salmo. These findings support earlier studies, where observed pairings involved at least one metacentric chromosomes. Rediploidization in salmon may have been prevented or retarded by the formation of metacentric chromosomes following the whole genome duplication event, and may explain lineage-specific innovations in salmon species if functional genes are found in these regions.
INTRODUCTION

Whole genome duplication (WGD) is a mutational mechanism that can serve as a primary driver of evolutionary novelty (Ohno 1970; Zhang 2003; Crow and Wagner 2006; Lynch 2007; Edger and Pires 2009). Changes in ploidy levels following WGD can lead to dramatic alterations at the cellular and phenotypic level (Mayfield-Jones et al. 2013), and provide additional genetic variation for mutation, drift and selection to act upon. These evolutionary processes can result in new adaptations and species diversification (Van de Peer et al. 2009; Storz et al. 2013). Genome sequencing projects are increasingly revealing that WGD is widespread in many key lineages, such as flowering plants and vertebrates, and represents an ongoing phenomenon in many species (Otto and Whitton 2000; Van de Peer et al. 2009). Understanding the processes governing the return to a diploid mode - diploidization - by comparing the genomes of species descended from a WGD event can provide insights into the event’s role in evolutionary innovation and persistence of duplicated regions (Jaillon et al. 2009; Mayfield-Jones et al. 2013).

The stabilization of the duplicated genome through diploidization can be achieved by rearrangements (such as translocations, fissions, fusions and transpositions), gene loss, and sequence deletion and divergence (Hufton and Panopoulou 2009; Schubert and Lysak 2011). These processes tend to reduce the similarity of the duplicated ohnologs (Wolfe 2001), and the homeologous chromosomes resulting from WGD, but the exact mechanisms vary across lineages (Hufton and Panopoulou 2009). Whole genome duplication has been frequently implicated in evolutionary innovation in eukaryotic genomes of paleopolyploids (ancient polyploids; Ohno 1999; Lynch and Conery 2000; Jaillon et al. 2004; Cañestro et al. 2013), but evidence in plants suggests that the rate of diversification and extinction of neopolyploids can be lower than that of related diploid lineages (Mayrose et al. 2011). Increasing the number of studies on mesopolyploids - organisms in the intermediate process of diploidization (Mayfield-Jones et al. 2013) - will provide a clearer understanding of contribution of WGD events to evolutionary innovation.

Salmonid fishes are descended from a whole genome duplication event in an autotetraploid ancestor (Allendorf and Thorgaard 1984), distinct from the second round of duplication (2R) that occurred basal to the vertebrate tree and the third round (3R) early in the evolution of the teleosts, 225 to 333 Mya (Hurley et al. 2007; Postlethwait 2007; Santini et al. 2009; Near et al. 2012). This fourth round (4R) of duplication was recently estimated as occurring 88-103 million years ago (Macqueen and Johnston 2014; see also Crête-Lafrenière et al. 2012; Alexandrou et al. 2013; Berthelot et al. 2014). Although the genomes of these species are returning to a stable diploid state through chromosomal rearrangements and divergence of homeologous chromosomes, evidence of tetrasomic inheritance in males and extensive rearrangements among chromosomes has shown that restoration of diploidy is not yet complete (Wright et al. 1983; Allendorf and Thorgaard 1984; Allendorf and Danzmann 1997). Comparative genome sequencing between ohnologs in rainbow trout has revealed extensive
collinearity between the duplicated chromosomes, loss of about half the protein-coding regions through pseudogenization, but retention of most of the duplicated miRNA genes (Berthelot et al. 2014).

The role of the WGD event in salmonid trait innovation and diversification is unclear. Recent evidence based on molecular clock estimates suggest that duplication is unlinked to a major transition in life history, anadromy (Alexandrou et al. 2013; Macqueen and Johnston 2014), and preceded rapid species diversification by several million years (Berthelot et al. 2014; Macqueen and Johnston 2014). Rather, both transitions appear to correspond with climate cooling (Macqueen and Johnston 2014). Re-diploidization has been subsequently characterized as a gradual process unlinked to significant genome rearrangements (Berthelot et al. 2014). However, it has also been argued that the duplication event might have provided the raw material for evolution to act upon, and that differential divergence of duplicated regions might have promoted speciation at varying time points (Macqueen and Johnston 2014). Large-scale genome characterization in the salmonids is increasingly revealing the location of genes or regions that may have played a role in adaptation and diversification (Davidson et al. 2010; Bourret et al. 2013; Larson et al. 2013). Therefore, it is important to combine these studies with an understanding of the mechanism and timing of divergence between homeologous chromosome arms across salmon lineages so that it will be possible to understand how duplication played a role in evolution of salmon, and whether key genomic regions might explain innovation across a subset of species.

It has been known for some time that one of the key mechanisms for diploidization within the subfamilies Coregoninae and Salmoninae (which includes Salmo, Salvelinus, and Oncorhynchus) has occurred through Robertsonian rearrangements of whole chromosome arms (Ohno 1999; Phillips and Ráb 2001). Most chromosome arms are syntenic between Salmoninae species, and the combined efforts of genome mapping, and karyotyping have permitted alignment of chromosome arms among several species within this subfamily (Danzmann et al. 2005; Phillips et al. 2009; Lubieniecki et al. 2010; Lien et al. 2011; Timusk et al. 2011; Guyomard et al. 2012; Naish et al. 2013; Phillips et al. 2013). Chromosome arm number is largely conserved (NF = approx. 100) but the numbers of chromosomes vary substantially across species as a result of the Robertsonian rearrangements (Phillips and Ráb 2001). The exception is Atlantic salmon, with reduced chromosome arm number compared to the other species (NF = 72-74). However, large syntentic blocks within the arms of this species correspond to whole arms in other members of the Salmoninae, making comparative studies feasible across this subfamily as a whole.

Comparative mapping between Chinook salmon (O.tshawytscha) and rainbow trout (O.mykiss) has revealed evidence for the retention of at least eight metacentric chromosomes and four acrocentric chromosomes that are ancestral to species divergence within the genus Oncorhynchus (Naish et al. 2013; Phillips et al. 2013). One of the eight metacentric chromosomes and one of the acrocentric chromosomes are also ancestral to the divergence
between *Salmo* and *Oncorhynchus*. There is also further evidence for another ancestral metacentric and an ancestral acrocentric chromosome, but these have undergone subsequent rearrangements within at least one descendant species (Naish *et al.* 2013; Ostberg *et al.* 2013; Brieuc *et al.* 2014). High-density linkage maps have revealed that recently diverged or undifferentiated duplicated loci are not uniformly distributed among chromosomes (Atlantic salmon, Lien *et al.* 2011; Chinook salmon, Brieuc *et al.* 2014), and the biased distribution of duplicated loci along chromosome arms provides evidence that pairs of homeologous arms have diverged at different rates from each other (Brieuc *et al.* 2014). This finding confirms observations from previous studies conducted with lower marker densities (e.g. Danzmann *et al.* 2005; Guyomard *et al.* 2012). Intriguingly, homeologous pairings have been observed to include at least one metacentric chromosome (Wright *et al.* 1983), and duplicated markers map to such chromosomes (Brieuc *et al.* 2014), supporting the view that metacentric chromosomes play an important role in homeologous pairing (Phillips *et al.* 2009; Brieuc *et al.* 2014). These observations raise the interesting possibility that the evolutionary timing of metacentric chromosome formation during re-diploidization following the WGD event might influence the retention, and hence the evolutionary role, of duplicated regions across species. Therefore, by comparing chromosomal arrangements and distribution of duplicated regions across salmon species, we aim to provide a contextual framework for the further investigation of loci involved in species diversification.

The development of a high density linkage map for a less-described salmon species will contribute further information on chromosome rearrangements that have already been defined in several salmon species, and enhance our understanding of the timing of these arrangements in a phylogenetic context. Examining the distribution of duplicated regions across individual chromosome arms in a second species - beyond Chinook salmon (Brieuc *et al.* 2014) - will also facilitate an understanding of the relationship between timing of metacentric chromosome formation and diversification between homeologs. Coho salmon (*Oncorhynchus kisutch*) is a species whose genome has not been extensively described to date. A low density linkage map of coho salmon has been constructed using microsatellites (McClelland and Naish 2008), but this map is not sufficiently resolved to study the consequences of WGD because there is a low number of duplicated loci mapped. A high density map in this species is feasible, given recently emerged sequencing technologies (e.g. Baird *et al.* 2008). Coho and Chinook salmon are sister species (Crête-Lafrenière *et al.* 2012); therefore, comparative mapping across coho and Chinook salmon, as well as more divergent species in the genus *Oncorhynchus* and *Salmo* will help validate the hypotheses and provide more robust evidence on the process of chromosomal evolution following WGD.

The aim of our research is to determine the relationship between chromosome arrangements and the retention of recently diverged or undifferentiated duplicated regions by deriving a linkage map for coho salmon and comparing this map with those of Chinook salmon, rainbow trout, and Atlantic salmon. We therefore constructed high-density linkage maps for coho
salmon using restriction site associated DNA (RAD) sequencing (Baird et al. 2008). By achieving this objective we also produced a reference database of RAD markers that can be used for alignment of sequences generated in future work, and described in detail the properties of the coho linkage map. Coho salmon chromosome arms were identified by comparative mapping with Chinook salmon using markers in common between the species, and whole chromosome arm homologies were described across species to improve our current understanding of chromosome arm rearrangement within the genera *Oncorhynchus* and *Salmo*. Linkage groups representing homeologous chromosome arms in coho salmon were discovered using duplicated markers, and regions of duplicated markers were compared across species to determine the extent to which these regions were conserved across lineages. By identifying genomic regions that are in the process of diploidization, and linking these regions to chromosomal rearrangements, we aim to provide the basis for determining the role of duplication in maintaining ongoing polymorphisms and explaining processes of diversification across Salmoninae species.

**METHODS**

*Justification and description of sample collection and experimental crosses*

A two-step approach was used to develop genomic resources and construct linkage maps. First, RAD sequences from individuals sampled from multiple populations were used to construct a reference database for aligning loci across mapping families. This reference database was screened for errors, duplicated loci, and repeat regions following approaches described by (Brieuc et al. 2014), and loci were subsequently named to ensure consistency across mapping families. Second, specific cross types were used to perform the mapping: gynogenetic haploid crosses were used to map both duplicated and non-duplicated loci, and diploid crosses were used to construct sex-specific maps.

The reference database of RAD loci was constructed using sequences from 583 individuals representing four populations in the Pacific Northwest of the United States and Canada: 1) the Washington Department of Fish and Wildlife’s (WDFW) Wallace River Hatchery (47°87′N, 121°71′W), 2) the Domsea broodstock population, which originated in 1973 and 1974 from Wallace River Hatchery, 3) Bingham Creek (47°15′N, 123°40′W), a tributary to the Satsop River in the Southwest Washington Coast/Lower Columbia ESU, and 4) Chehalis River located in British Columbia, Canada (49°29′N, 121°94′W).

An initial framework map was constructed using two haploid crosses (Haploid Family 1 and 2) comprising 64 and 62 individuals respectively. These types of crosses have the advantage of identifying duplicated loci, because these loci will appear as heterozygotes in the offspring if they are polymorphic, while non-duplicated loci will be homozygous. Haploid families were created at the University of Washington hatchery facility (47°65′N, 122°31′W), following the
Sex-specific maps were created using two F3 outbred diploid crosses and one outbred diploid cross. Specifically, F3 diploid crosses were created from a cultured line originally derived from an outbred cross between two populations in Washington State, USA (McClelland and Naish 2010). F0 Males were collected from Bingham Creek in Southwest Washington (47°15′N, 123°40′W). F0 Females were obtained from the Domsea broodstock farm. Two F3 crosses were established in December 2010 by mating two F2 full-sibs to create one family, and two F2 half-sibs to create the other. The two families comprised 55 and 67 offspring respectively (Diploid Family 1 and 2). An additional diploid outbred cross was created from an aquaculture population using coho salmon derived from the Chehalis River located in British Columbia, Canada (49°29′N, 121°94′W). Specifically, cultured individuals were repeatedly backcrossed with wild individuals from the Chehalis River for six generations, and diploid crosses were created in January 2011. One diploid family from these crosses, comprising 99 individuals was used for further analyses (Diploid Family 3).

DNA extraction, sequencing and amplification of sex-linked markers

Genomic DNA from the sampled individuals was extracted using the DNeasy extraction kit (QIAGEN, Valencia, CA, USA) following the manufacturer’s procedures. The DNA was digested with SbfI, and a 6-nucleotide barcode was added to each sample for individual identification following protocols described in Baird et al. (2008). Between 24 to 36 individuals were pooled in a single library and sequenced with 100 bp single-read lengths using the Illumina HiSeq 2000 sequencer. The sequences were separated by individual using PROCESS_RADTAGS implemented in STACKS (Catchen et al. 2011; Catchen et al. 2013). Because the quality score of sequences decreased beyond 74 nucleotides, sequences were trimmed to 74 nucleotides in order to remove low quality sequences. A locus was defined as a 74-nucleotide RAD sequence for the purpose of this study.

Genetic sex was determined in the two diploid families (Diploid Family 1 and 2) using a Y-linked growth hormone pseudogene, (GH5 and GH6; Devlin et al. 2001) and sex-determining gene, sdY (sdY E2S1 and sdY E2AS4; Yano et al. 2012). Polymerase chain reactions were performed for each set of primers using a QIAGEN Multiplex PCR kit. Specifically, reaction mixtures consisted of 10-200ng genomic DNA, 1x QIAGEN Multiplex PCR Master Mix, 0.25 μM of GH5 and GH6, or 0.4μM of sdY E2S1 and sdY E2AS4, making up a total volume of 10μl. Cycling conditions consisted of a 15-min, initial activation step at 95°C, 30 cycles of 30-s denaturing step at 94°C, 90s annealing step at 60 °C and 60-s extension step at 72 °C, and a 10-min, final extension step at 72°C.
Reference database of RAD loci

RAD loci that are found within repeat regions, and loci containing repeat units, can confound the identification of unique loci. Therefore, a reference sequence database comprising a set of pre-screened RAD loci was first created from the survey of four populations following bioinformatic procedures fully described in Brieuc et al. (2014). This database served as a resource for aligning loci across studies. In brief, sequences from all 583 individuals sampled across the four populations described earlier were extracted using STACKS 0.9995 (Catchen et al. 2011). Both monomorphic and polymorphic loci that were sequenced with a depth greater than 5X in more than 496 individuals (85%) were retained in a temporary database and used for further screening.

Loci in the temporary database that corresponded to repeat regions and loci containing repeat units were removed using two alignment-based strategies, following the protocol of Brieuc et al. (2014). First, loci in the temporary database were aligned against themselves using BOWTIE (Langmead et al. 2009) by allowing a maximum of three nucleotide mismatches per locus. A locus that aligned to several loci, or a locus that did not align to itself was removed from the temporary database. Then, a BLAST search (Altschul et al. 1990) of the temporary database was conducted against itself. Loci that did not return a match, or loci where the best match were not themselves, were removed from the temporary database.

Using the updated temporary database of RAD loci, polymorphic duplicated loci were identified based on two haploid families. First, sequences from these haploid families were aligned to the temporary database using BOWTIE, allowing a maximum of three nucleotide mismatches per locus. Sequences from the haploid individuals that aligned to more than one locus in the database could not be confidently relied upon in further analyses; they were thus identified as “blacklisted” loci and removed from the temporary database. Subsequently, polymorphic loci sequenced with a depth greater than 10X per haploid individual were identified using STACKS and retained for further screening. Among these polymorphic loci, a locus was identified as being putatively duplicated when more than one haploid offspring in a family was heterozygous at this particular locus (Brieuc et al. 2014). A final reference database comprising named duplicated and non-duplicated loci, as well as loci removed from the alignment-based screening steps and “blacklisted” loci, was created.

Genotyping of individuals in map crosses

Haploid individuals were genotyped at both non-duplicated and duplicated loci. Sequences from all haploid individuals were aligned to the non-duplicated and duplicated loci from the final reference database using BOWTIE by allowing a maximum of three nucleotide mismatches per locus. In haploids, we have shown reliable identification of single loci that have up to three SNPS - we have confirmed this result with genome mapping. To remain consistent, we used up to three mismatches so that we could differentiate between non-duplicated loci and
duplicated loci. Both this study and a previous one (Brieuc et al. 2014) have shown that very reliable linkage results can be obtained in haploids using these criteria. Polymorphic loci sequenced with a depth greater than 10X per individual were identified using STACKS. Both non-duplicated and duplicated markers in the haploid families were used for mapping, described below and following the protocol of (Brieuc et al. 2014). Polymorphic duplicated loci were mapped when one of the paralogs was polymorphic (OPP - one paralog polymorphic, parental genotypes aa and ab, or aa and bc) or when both paralogs were polymorphic (BPP - parental genotypes ab and ac, or ab and cd; see table 1 in Brieuc et al. 2014).

Diploid individuals were only genotyped at non-duplicated loci. Sequences from all diploid individuals were aligned to the non-duplicated loci identified in the final reference database using BOWTIE by allowing a maximum of three nucleotide mismatches per locus. Subsequently, polymorphic loci were identified in each diploid family using STACKS, and genotypes at these loci were determined when alleles were sequenced with a depth greater than 10X per individual.

STACKS employs a maximum likelihood statistical model to identify sequence polymorphisms and determine individual genotypes (Catchen et al. 2011; Catchen et al. 2013). This approach can be biased towards heterozygous genotypes when sequence depths differ between the two alleles. To correct this bias against heterozygous genotypes, genotypes were corrected after running STACKS with the Python script developed by (Brieuc et al. 2014). Specifically, individuals were determined as heterozygotes at a locus if both alleles had a depth of more than two and the total read depth was 10X or greater.

**Linkage mapping**

Linkage maps in all haploid and diploid families were constructed using software for genetic mapping, ONEMAP 2.0-3 (Margarido et al. 2007), implemented in R version 3.0.2 (R Development Core Team 2013). Because coho salmon has 30 chromosome pairs (Phillips and Ráb 2001), each mapping family was expected to have at least 30 linkage groups. Linkage groups were named “Co”, following the convention used in mapping studies in salmonids: this practice uses abbreviated common names for groups that are not yet anchored to chromosomes (e.g. Danzmann et al. 2005; Naish et al. 2013). RAD loci with 20% or less missing values among individuals within a family were employed for linkage analyses, and these loci were assigned to linkage groups in each family separately using a minimum log of odd ratio (LOD) score of 4.0 and a maximum recombination fraction of 0.25. The LOD score was subsequently increased by 1.0 until the number of linkage groups reached 30 or higher. An integrated haploid map was first constructed from the two haploid families using MERGEMAP (Wu et al. 2011) because genotypes at duplicated loci were only determined in these families. This integrated haploid map was later used to examine the distribution of duplicated loci across all linkage groups and identify linkage groups involved in recent or ongoing homeologous pairing.
Recombination rates in male salmonids tend to be smaller than those observed for females (Sakamoto et al. 2000; Ostberg et al. 2013), but these differences tend to decrease with high marker density and genome coverage (Rexroad et al. 2008; Lien et al. 2011). We used the female meiosis from the three diploid families to estimate marker order in these crosses. The data from all haploid and diploid female parents were then combined to calculate an integrated female haploid/diploid map using MERGEMAP.

Ordering markers in the diploid male map was computationally difficult, potentially due to reduced recombination and occasional tetrasomic inheritance in males (Allendorf and Danzmann 1997). Therefore, information from the integrated female map constructed with haploid and diploid mothers was used to infer the order and map the male meiosis in the three diploid families (Diploid Family 1, 2, 3). Polymorphic loci in common between the male parents and the integrated female map, as well as the Y-linked growth hormone pseudogene and sex-determining gene, sdY were grouped using a log of odd ratio (LOD) score of 4.0 and a maximum recombination fraction of 0.25 using ONEMAP. The LOD score was subsequently increased by 1.0 until the number of linkage groups reached 30 or higher. Grouped loci were then ordered based on the known order on the integrated female map using the make.seq and map functions implemented in ONEMAP. The position of a Y-linked growth hormone pseudogene and sex-determining gene, sdY on the male map was estimated in the two diploid families (Diploid Family 1 and 2) using the try.seq and map functions implemented in ONEMAP. The data from all diploid male parents were then combined to calculate an integrated male map using MERGEMAP.

Comparative mapping with Chinook salmon and comparison with other salmonid species

The reference database for coho salmon containing duplicated and non-duplicated RAD loci was aligned to the 54,937 filtered RAD loci identified in Chinook salmon (Brieuc et al. 2014) using BOWTIE, allowing no more than 3 nucleotide mismatches per locus. Homologies between Chinook and coho salmon were determined by examining the chromosomal arm locations of shared loci between the two species. Putative centromere positions on coho linkage groups were estimated based on markers mapped in the gynogenetic diploid families in Chinook salmon (Brieuc et al. 2014). The order of mapped loci between the Chinook and coho salmon map was compared to determine if marker orders for chromosomes or chromosomal arms between the species were conserved. Finally, homologies identified between Chinook and coho salmon were used to infer homologies across coho salmon, rainbow trout and Atlantic salmon using molecular markers in common between published maps (Phillips et al. 2009; Lien et al. 2011; Miller et al. 2012; Brieuc et al. 2014).

Homeologous relationships and the distribution of duplicated loci across genomes

As we point out earlier, two categories of duplicated loci were identified in this study: where one of the paralogs was polymorphic (OPP) or both paralogs were polymorphic (BPP).
Duplicated loci with both paralogs polymorphic (BPP) were used to infer homeologous linkage groups, since both paralogs could be mapped. The positions of duplicated loci were subsequently examined on the integrated haploid map to determine whether there was a bias in the distribution of these loci across linkage groups. A kernel smoothing approach using a sliding window of 2cM was used to determine whether there was a regional bias in distribution of these loci for each linkage group, following methods described in (Brieuc et al. 2014). Homeologous relationships detected in coho salmon were also compared to those identified in Chinook and Atlantic salmon (Lien et al. 2011; Brieuc et al. 2014).

RESULTS

Reference database of RAD loci

A reference database comprising a unique set of RAD loci was created for the purpose of sequence alignment and identification of polymorphisms across individuals. A total of 70,037 loci were sequenced with a depth greater than 5X per individual in at least 496 individuals. These loci formed the temporary reference database of RAD loci, and they were retained for further screening. Sequence alignment using BOWTIE showed that 4075 loci did not align uniquely to themselves and likely corresponded with repeat regions; therefore these loci were removed from the temporary database. After performing the BLAST search of the temporary reference database against itself, 2085 loci did not return a match or the best match score was not the locus itself. It was possible that these loci contained repeat sequences; therefore, these loci were also removed from the temporary reference database. Sequences from the haploid individuals were aligned to the reference database using BOWTIE; 3706 loci from the haploid individuals aligned to several other loci, and these loci were thus blacklisted and removed from the reference database. Additionally, 7235 loci were identified as polymorphic duplicated loci in the haploid families. The final reference database comprising 52,936 non-duplicated loci and 7235 duplicated loci, as well as the 9866 loci that were removed by screening, are given in Supplementary material S1.

Linkage mapping

An initial framework map was constructed with two haploid families. Haploid Family 1 and 2 had 3976 and 4048 biallelic polymorphic RAD loci respectively, comprising a total of 6652 unique RAD loci. Among these loci, a mixture of duplicated and non-duplicated loci (3897 loci in Haploid Family 1; 3996 loci in Haploid Family 2) were successfully assigned to 30 linkage groups with a LOD score of 5.0 to 7.0. The total map length for the Haploid Family 1 and 2 was 3040.1 cM and 3185.5 cM, respectively. The integrated haploid map had 5377 non-duplicated markers and 1266 duplicated markers with a total map length of 3602.6 cM (Supplementary material S2.1).

Linkage analyses were conducted in the diploid families, following the construction of the integrated haploid map. Diploid Family 1, 2 and 3 had 1360, 1176 and 1931 biallelic non-
duplicated loci that were polymorphic in each female parent, respectively. Among these loci, a set of loci (1214 loci in Diploid Family 1; 1138 loci in Diploid Family 2; 1765 loci in Diploid Family 3) were successfully assigned to 30 linkage groups with a LOD score of 4.0 to 8.0. The total map length for the Diploid Family 1, 2 and 3 was 3714.1cM, 3068.9cM and 5047.2cM, respectively. Although the Diploid Family 3 had the largest total map length, it also had the highest number of markers mapped. Since more recombination events are captured with more markers (Liu 1998), it is not surprising that the Diploid Family 3 had the largest total map length. Finally, data from the haploid parents and diploid female parents were combined; an integrated haploid/diploid female map measured 6596.7 cM, and it comprised 7415 non-duplicated markers and 1266 duplicated markers (Table 1; Figure 1; Supplementary material S2.2).

The male meiosis was mapped using linkage analyses in the diploid families. Among the 8681 loci placed on the integrated haploid/diploid female map, Diploid Family 1, 2 and 3 had 846, 814 and 879 polymorphic loci in common for each male parent. Among these loci, a set of loci (792 loci in Diploid Family 1; 790 loci in Diploid Family 2; 851 loci in Diploid Family 3), as well as the Y-linked growth hormone pseudogene and sex-determining gene, sdY were successfully assigned to 30 linkage groups with a LOD score of 4.0 to 7.0. Both the growth hormone pseudogene and sdY mapped to the beginning of the linkage group, Co30. All linkage groups were successfully merged, except for Co22 which was split into two linkage groups (Co22_1 and Co22_2; Supplementary material S2.3). The number of markers in common between the integrated male and female maps varied for each linkage group, ranging from 25 to 106 markers per linkage group (Supplementary material S3). The male map had a total map length of 4141.76 cM (Supplementary material S3).

The comparison between the male and female linkage groups reflected different recombination patterns between the sexes (Figure 2; Supplementary material S4). Although telomeres were not mapped in males due to a lack of duplicated markers, many male linkage groups were expanded in size toward the terminal regions relative to the female, as seen by the increased distance in these regions reflecting more recombination events. Such patterns were particularly prominent for several linkage groups (Co02, Co04, Co05, Co08 - Co10, Co13 - Co15, Co17 - Co19, Co21 - Co29). Although qualitative, there was also evidence of suppressed recombination around the region containing the centromere in male compared to female integrated map for all linkage groups, as the male map had reduced distance in these regions compared to the female map.

Comparative mapping with Chinook salmon and comparisons with other salmonid species

We mapped 664 RAD loci in coho salmon that had been previously placed on the Chinook salmon map, which permitted the identification of homologous chromosomal arms between the two species (Table 1). On the basis of this comparison, we also identified the putative locations of centromeres within coho salmon linkage groups (Figure 1). Two homologous relationships between the species were inferred. An arm of the linkage group Co07a
had 3 markers that mapped to Ots34 in Chinook salmon. On the other hand Co12b had 6 markers that mapped to Ots34 and 3 markers that mapped to Ots11p. Ots11p and Ots34 are likely involved in recent or ongoing homeologous pairing in Chinook salmon (Brieuc et al., 2014), therefore it was not surprising that loci on Ots34 mapped to both homologous arms in coho salmon. In this case, we assumed that Co07a was homologous to Ots11p, and Co12b was homologous to Ots34 for reasons given in the discussion.

Comparative mapping with Chinook salmon permitted inference of the structure of coho linkage groups. Twenty linkage groups in coho salmon corresponded to putative bi-armed metacentric chromosomes, and 10 linkage groups corresponded to putative uni-armed acrocentric chromosomes (Figure 1). These inferred structures are in agreement with the known chromosome structures in coho salmon (Phillips and Ráb 2001). The short (p) arm of an acrocentric chromosome is usually uncharacterized in mapping studies because there are often insufficient markers describing this region (Brieuc et al. 2014). In this study, we identified the small arm for two putative acrocentric chromosomes (Co22, Co29) through comparative mapping with Chinook salmon.

Comparative mapping between the Chinook and coho salmon maps also provided information on chromosomal arrangements that are shared between the two species. Eighteen chromosomes are conserved between the species (Table 1); specifically, 9 metacentric chromosomes and 9 acrocentric chromosomes were conserved between the species. The remaining chromosome structures likely support independent Robertsonian rearrangements that occurred after descent from a common ancestor.

Five metacentric linkage groups in coho salmon (Co10 - Co14) consist of one acrocentric chromosome and one arm from a metacentric chromosome in Chinook salmon. Four metacentric linkage groups in coho salmon (Co15 - Co18) comprise arms that are found in two separate metacentric chromosome pairs in Chinook salmon. Two metacentric linkage groups (Co19, Co20) comprise two acrocentric chromosome pairs in Chinook salmon. Finally, one acrocentric linkage group (Co30) corresponds to an arm that is a part of a metacentric chromosome pair in Chinook salmon.

The orders of the RAD loci on the Chinook and coho salmon maps were compared across each linkage group or for each chromosome arm to determine whether any chromosomal inversions occurred following divergence between the species. There was a strong linear relationship among mapped loci for all the linkage groups or arms (Supplementary material S5), suggesting that marker orders were conserved for all chromosomes or chromosomal arms. Such analyses provide additional evidence for the occurrence of centrometric inversion in Omy20 in rainbow trout following divergence between rainbow trout and Chinook/coho salmon, and this chromosomal inversion may be exclusive to rainbow trout (Naish et al. 2013; Ostberg et al. 2013; Brieuc et al. 2014).
The homologies we observed between Chinook and coho salmon permitted alignment of coho linkage groups to those of rainbow trout and the Atlantic salmon, and the results are summarized in Table 1. Three acrocentric and 8 metacentric chromosomes were conserved among coho salmon, Chinook salmon and rainbow trout. Comparison between the Oncorhynchus species and the Atlantic salmon revealed that one metacentric and one acrocentric chromosomes were conserved across all compared species.

**Homeologous relationships and the distribution of duplicated loci across linkage groups**

The identification of linkage groups involved in homeologous pairing, as well as the localization of duplicated loci across individual linkage groups, was examined using the integrated haploid female map. A total of 1169 duplicated loci (1066 OPP and 103 BPP) were placed on this map. These loci were not distributed evenly among the linkage groups ($\chi^2$ test for uniform distribution across linkage groups, after correction for the number of markers per linkage group: $p$-value $\sim 0$, df = 29); 87.0% of the duplicated loci were located on 16 linkage groups (Figure 3). There was a bias in distribution of these loci along the 16 linkage groups; duplicated loci were mostly found in the distal regions of all 16 linkage groups (Figure 4). Homeologies were identified between putative chromosome arms using marker pairs in which both paralogs were polymorphic (Table 2). All 8 homeologous arm pairs with a high retention of duplicated loci detected in coho salmon were also observed in Chinook salmon, and 4 homeologous arm pairs were conserved in Atlantic salmon (Table 2). All of these chromosome arms, likely involved in recent or ongoing homeologous pairing, involved at least one metacentric chromosome ancestral to the divergence between Pacific salmon species.

**DISCUSSION**

Here, we aimed to examine the relationship between chromosomal evolution and retention of duplicated regions within the genus Oncorhynchus, and between this genus and Salmo, by deriving a linkage map for coho salmon, and comparing this map to that of Chinook salmon, rainbow trout and Atlantic salmon. Thirty linkage groups including 20 putative metacentric and 10 putative acrocentric chromosomes were described across two haploid and three diploid families. Chromosomal rearrangements were identified by comparing homologous arms between coho salmon, Chinook salmon, rainbow trout and Atlantic salmon. Results confirmed the conservation of at least one metacentric chromosome between Oncorhynchus and Salmo (Co09), seven metacentric chromosomes across the genus Oncorhynchus (Co01 - Co07; Naish et al. 2013; Phillips et al. 2013; Brieuc et al. 2014), and detected a polymorphism in another across coho and Chinook salmon and rainbow trout (Co14 and Co15). Another metacentric chromosome was detected as ancestral to coho and Chinook salmon only (Co08). The placement of 1166 duplicated loci on the consensus haploid map of 7415 markers revealed that these loci were not evenly distributed across all linkage groups, supporting an earlier finding in Chinook salmon (Brieuc et al. 2014); namely, that homeologous pairs diverged from each other at different rates following the whole genome duplication event. Regions of the genome
with polymorphic duplicated markers were found on the same eight pairs of homologous chromosome arms (16 arms in total) across coho and Chinook salmon. Each of the eight pairs of chromosomes likely involved in ongoing or recent homeologous pairing included at least one of the ancestral metacentric chromosomes that are conserved between the two species. The other chromosome arm involved in the pairing may be part of either an acrocentric chromosome or metacentric chromosome. The data suggest that Robertsonian rearrangements that result in metacentric chromosome formation prior to the diversification of homeologous pairs might partly explain the uneven retention of duplicated regions across the genome, at least within Pacific salmon.

The consensus male map, constructed with three diploid families, was significantly smaller (4141.7 cM) than the consensus female map (6596.7 cM) constructed with two haploid families and three diploid families. There are three main reasons that might explain the reduced map size in male compared to the female map. First, the difference could simply be a function of more markers being placed on the female map (8581 for the female and 2041 for the male), as map size tends to increase when more markers are added (Liu 1998). Second, some male linkage groups only represented a portion of those in female; for example, in three metacentric chromosomes (Co07, Co09, Co13) only one arm and a region containing the centromere were mapped, the duplicated markers were not mapped in male. Third, recombination in males is suppressed relative to females, and male maps in salmonids often tend to be smaller. Comparisons between the consensus male map and the consensus (haploid and diploid) female map indicated that recombination in the male was suppressed around the region containing the centromere in some linkage groups while recombination in female seemed suppressed toward telomeric regions.

Accurate identification of chromosome structure in coho salmon relied on aligning homologous chromosome arms with Chinook salmon. In addition, regions containing the centromere were inferred through comparative mapping between coho and Chinook salmon as no gynogenetic diploid families were employed for this study to identify the exact location of the centromere. Homology of Co07a and Co12b with Chinook salmon chromosome arms Ots11p and Ots34 was not completely resolved. These arms are homeologous to each other within both species, and markers on Ots34 mapped to both Co07a and Co12b in coho salmon. We inferred that Co07a was homologous to Ots11p, as the arm Co07a is part of a metacentric chromosome that is conserved in Chinook salmon (Ots11) and rainbow trout (Omy19, Table 1). In fact, (Naish et al. 2013) speculate that these are conserved across the genus because Chinook salmon and rainbow trout are distantly related. In contrast, the metacentric chromosome Co12 is not conserved in Chinook salmon and rainbow trout. We assumed these structures in the subsequent discussion.
Coho salmon map coverage, size and differences in sex-specific recombination

The coho salmon linkage map constructed in this study has 8681 markers, spanning all predicted 30 linkage groups. This coverage is comparable to recently published maps across a number of salmon species (Lien et al. 2011; Everett et al. 2012; Miller et al. 2012; Brieuc et al. 2014). We observed different map sizes in the consensus female maps; the map constructed with combined haploid and diploid families had a size of 6596.7 cM, which is significantly larger than the coho map created with haploids alone (3602.6 cM). There are several reasons that might explain the differences such as nonrandom missing values (Jorgenson et al. 2005), genotyping errors (Hackett and Broadfoot 2003) and numbers of markers mapped. Potential bias against heterozygotes in RAD sequencing (Brieuc et al. 2014) may also partly explain the inflated map distances in the haploid/diploid map, especially since the size of the map created with only haploid families in this study was much smaller.

Male salmonids are the heterogametic sex (Allendorf and Thorgaard 1984). In this study, both the Y-linked growth hormone pseudogene and sex-determining gene, sdY mapped to the telomeric region on the acrocentric chromosome, Co30. This finding is in agreement with earlier findings (Phillips et al. 2005); that the sex chromosome in this species is acrocentric and the SEX locus was located on the telomeric region of this chromosome. Mapping has shown that the sex chromosome is not conserved across the species, and that a small male-specific region including the sex determining gene has been repeatedly transposed to different chromosomes in different salmon species (Phillips et al. 2001; Woram et al. 2003; Yano et al. 2012).

Our results showed that suppressed recombination around the region containing the centromere in male was widely apparent, while higher recombination was observed in telomeric regions for some male linkage groups relative to female. The results are in agreement with a number of studies performed on other salmonid species (Sakamoto et al. 2000; Moen et al. 2008; Rexroad et al. 2008; Lien et al. 2011). Male recombination rate in telomeric regions of certain rainbow trout linkage groups has been shown to be higher than that of female, but lower in centromeric regions (Sakamoto et al. 2000). Such different recombination patterns might be in part explained by occasional multivalent formation during male meiosis, in which crossovers between homeologous chromosomes are increased in the telomeric regions while crossovers between homologous chromosomes are hindered in the centromeric regions through structural constraints (Sakamoto et al. 2000). In our study, however, suppressed recombination around the centromere and increased recombination in telomeric regions were apparent for many male linkage groups including the ones not likely involved in homeologous pairing. Some studies have also found notable clustering of markers in centromeric regions for many male linkage groups (Nichols et al. 2003; Lien et al. 2011; Miller et al. 2012). In Atlantic salmon, high marker densities were also involved in regions close to the centromere for male linkage groups with a lower frequency of duplicated markers (Lien et al. 2011). Recombination rates are known to differ between the sexes in a wide range of species (Lenormand and Dutheil 2005); while
homeologous chromosome pairing during male meiosis could certainly account for some of the differences, the origin of the sex differences observed in this study still remains unclear.

**Comparative genome mapping**

Comparative mapping provided insights into the process of chromosomal evolution occurring after the whole genome duplication event, and this is the first study that characterizes chromosomal evolution between coho and Chinook salmon. Nine metacentric and nine acrocentric chromosomes appear to be conserved between these two species. Among these conserved chromosomes, one metacentric (Co08 in coho and Ots12 in Chinook) and five acrocentric chromosomes are unique to coho and Chinook salmon, suggesting that the structure of these linkage groups is ancestral to the divergence of these species relative to rainbow trout and Atlantic salmon. The remaining linkage groups are not conserved, reflecting the chromosomal rearrangements since coho and Chinook salmon diverged.

Syntenic relationships between the Chinook salmon and rainbow trout maps permitted comparisons across the genus *Oncorhynchus* (Naish et al. 2013; Phillips et al. 2013). There are four acrocentric chromosomes that are conserved across all three *Oncorhynchus* species (Co26 - Co29). Similarly, eight metacentric chromosomes are conserved among all three species (Co01 - Co07, Co09). The results support the hypothesis of (Naish et al. 2013) that the arm rearrangements that resulted in these metacentric chromosomes are ancestral to the divergence of the species and could be conserved across the genus *Oncorhynchus*. There is one interesting extension to these earlier observations. One metacentric chromosome in Chinook salmon, Ots08, sometimes occurs as a metacentric chromosome (Omy25p and q) or as two acrocentric arms (Omy25 and Omy 29) in rainbow trout (Figure 5). Here, we found that the homologous arms in coho salmon occur in two separate unrelated metacentric linkage groups (Co14a and Co15a; Figure 5). In Atlantic salmon, these two arms are fused together along with a third arm to form a large acrocentric chromosome (Figure 5). Robertsonian fusions are common, and can also form acrocentric chromosomes: this outcome is likely more frequent in Atlantic salmon than in Pacific salmon (Phillips and Råb 2001). Taken together, the configurations of these particular chromosomes suggest they may have undergone recurrent fusions and fissions across species.

One metacentric and one acrocentric chromosome, Co09 and Co26, respectively, appear to be conserved across coho salmon, Chinook salmon, rainbow trout and Atlantic salmon (Phillips et al. 2009; Brieuc et al. 2014). Our study supports the earlier findings that this metacentric chromosome is likely ancestral to the divergence of the two genera, *Salmo* and *Oncorhynchus*. It also appears that this chromosome is conserved in Arctic charr and Brook charr within the genus *Salvelinus* (Timusk et al. 2011), both of which share a more recent common ancestor with *Oncorhynchus*. In addition, earlier results provided evidence for the occurrence of centrometric inversion in Omy20 in rainbow trout following divergence between rainbow trout and Chinook/coho salmon, and this chromosomal inversion may be exclusive to rainbow trout (Naish et al. 2013; Ostberg et al. 2013; Brieuc et al. 2014). In the current study,
marker orders are fully conserved between coho and Chinook salmon chromosome arms, Co25 and Ots25, respectively, further supporting this earlier observation (Supplementary material S5).

Conservation of reduced divergence between homeologous chromosomes across species

While our results confirm that the divergence rates of homeologs following the whole genome duplication event have not been uniform (Brieuc et al. 2014), the key finding of this study is that the ancestral metacentric chromosomes retain recently diverged duplicates and are the ones likely involved in recent or ongoing homeologous pairing (Co01 - Co07, conserved among all three *Oncorhynchus* species; Co09, conserved among all four species). Such findings suggest that homeologies may be preferentially retained between larger metacentric chromosomes (Phillips et al. 2009), and the involvement of at least one metacentric chromosome provides the stability required for the formation of multivalents (Wright et al. 1980; Wright et al. 1983; Brieuc et al. 2014). These results support the hypothesis of Phillips et al. (2009), which suggested that diploidization of chromosomes not involved in homeologous pairings may have occurred in the ancestral salmonid before the divergence between *Salmo* and *Oncorhynchus*. We speculate that this process also differed to some extent following the divergence of the two genera. Although the exact distribution of duplicated markers along chromosome arms in Atlantic salmon has not been described (Lien et al. 2011), only four out of eight homeologous pairings appear to share polymorphic duplicated loci between the Atlantic salmon and Chinook and coho salmon grouping (Table 2).

The implications of our findings for species divergence within the subfamily Salmoninae will become clearer once we gain a greater understanding of the role of duplicated regions in evolution. If the duplicated regions we detected have genes that permit greater flexibility for adaptation by providing the opportunity to acquire additional or novel functions (Soltis and Soltis 2000; Koop and Davidson 2008; Van de Peer et al. 2009; Feldman et al. 2012; Alexandrou et al. 2013; Macqueen and Johnston 2014), then retention of particular duplicated regions within certain lineages may explain their subsequent innovation and diversification. On the other hand, the physical formation of metacentric chromosomes may inhibit diploidization because of ongoing recombination; such chromosomes may continue to exhibit tetrasomic inheritance, thus becoming “evolutionary dead ends”. Whole genome sequencing of duplicated chromosome arms in rainbow points towards the fact that duplicated protein coding loci have simply become lost through gradual change (Berthelot et al. 2014); the conserved metacentric chromosomes may continue exhibiting tetrasomic inheritance and prevent functional divergence of protein coding regions. In this study, we provide preliminary evidence that the evolutionary timing of metacentric chromosome formation varied, which might have impacted the rate of diploidization across different lineages. As comparative genome sequencing of salmon species continues, comparing the rates of differentiation along certain chromosome arms between species and identifying the location of genes involved in diversification will provide insights into the role of WGD in salmon evolution. Here, we have identified chromosome arms of interest for further efforts addressing such questions.
CONCLUSION

Here, we developed an extensive set of genomic resources for coho salmon: a reference database of unique RAD loci, two types of consensus female linkage maps and a consensus male linkage map. A dense female map constructed in this study permitted alignment of linkage groups in this species with that of Chinook salmon, enabling interspecies comparisons with related salmon species. Syntenic relationships across multiple salmonid species identified in this study provided strong evidence for chromosomal rearrangements and conservation of metacentric and acrocentric chromosomes following the divergence between Salmo and Oncorhynchus. We have also identified linkage groups that have been recently or may be involved in ongoing homeologous pairing in coho salmon. Such pairings were conserved with related Pacific salmon species. Ancestral metacentric chromosomes appear to retain recently diverged duplicated regions and be potentially involved in homeologous pairings; such results indicate that diploidization may have been prevented or retarded in these ancestral metacentric chromosomes following the whole genome duplication event. The resources developed here will facilitate genome-wide studies in coho salmon, such as genome scans, QTL mapping and genome-wide association studies (Naish and Hard 2008), as well as provide resources for studies concerning ecology and evolution in related salmon species.

COMPETING INTERESTS

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

We thank Isadora Jimenez-Hidalgo for assistance with genomic data processing. Dan Drinan provided thoughtful discussions and helpful suggestions. Initial experimental lines were obtained from SweetSpring Salmon / Aquaseed Corporation, suppliers of the Domsea broodstock, and we particularly thank Per Heggelund, Greg Hudson and Patty Munsell for their help. We also thank Linda Park and Jim Myers for help in setting up the initial crosses and David Rose for maintaining the lines. Finally, we are grateful to two anonymous referees and the Associate Editor for their very helpful comments on the first draft of the manuscript. Funding for this study was provided by NOAA Fisheries/Federal Columbia River Power System (FCRPS) Biological Opinion Remand Funds to KAN and JJH, School of Aquatic and Fishery Sciences and Graduate Opportunities and Minority Achievement Program (GO-MAP, University of Washington) award to MK.
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Table 1 - Description of the coho salmon consensus linkage map constructed with haploid and diploid female parents, and comparison with chromosome arms of Chinook salmon, rainbow trout and Atlantic salmon. Linkage groups (Co) were randomly assigned numbers, and arm names are given as “a” and “b”. Homologous arms in Chinook salmon, rainbow trout and Atlantic salmon are based on chromosome names for each species (Ots, Omy and Ssa respectively), with known orientations (p is the short arm, q the long arm). * and ** denote inferred relationship; there were no markers in common between Co07a and Ots11p(*), and there are markers in common between Co12b and Ots34/Ots11p (**). *** denotes a chromosomal arm that is composed entirely of ribosomal DNA. The final column designates chromosomal rearrangements conserved across species; letter corresponds to phylogenetic placement in Figure 5.

1 indicates incompletely resolved relationships between Atlantic salmon and rainbow trout according to published studies.

| Coho linkage group | Size (cM) | Number of markers | Coho linkage arms | Chinook chromosome (Philips et al., 2013; Brieuc et al., 2014) | Rainbow trout chromosome (Philips et al., 2009; Brieuc et al., 2014) | Atlantic salmon chromosome (Danzmann et al., 2008; Philips et al., 2009) | Chromosomal rearrangement conserved across species |
|-------------------|-----------|-------------------|-------------------|-------------------------------------------------------------|------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------|
| Co01              | 267.51    | 393               | Co01a             | Ots02p                                                      | Omy17p                                                           | Ssa02q                                                                 | B, metacentric                              |
|                   |           |                   | Co01b             | Ots02q                                                      | Omy17q                                                           | Ssa12qb 1                                                                  |                                |
| Co02              | 248.6     | 344               | Co02a             | Ots03p                                                      | Omy03p                                                           | Ssa02p                                                                 | B, metacentric                              |
|                   |           |                   | Co02b             | Ots03q                                                      | Omy03q                                                           | Ssa25                                                                  |                                |
| Co03              | 274.51    | 360               | Co03a             | Ots04p                                                      | Omy06p                                                           | Ssa24                                                                  | B, metacentric                              |
|                   |           |                   | Co03b             | Ots04q                                                      | Omy06q                                                           | Ssa26                                                                  |                                |
| Co04              | 250.26    | 395               | Co04a             | Ots06p                                                      | Omy01p                                                           | Ssa16qa                                                                 | B, metacentric                              |
|                   |           |                   | Co04b             | Ots06q                                                      | Omy01q                                                           | Ssa18qa                                                                 |                                |
| Co05              | 268.59    | 376               | Co05a             | Ots07p                                                      | Omy07p                                                           | Ssa17qb                                                                 | B, metacentric                              |
|                   |           |                   | Co05b             | Ots07q                                                      | Omy07q                                                           | Ssa22                                                                  |                                |
| Co06              | 267.8     | 415               | Co06a             | Ots09p                                                      | Omy12p                                                           | Ssa13qb                                                                 | B, metacentric                              |
|                   |           |                   | Co06b             | Ots09q                                                      | Omy12q                                                           | Ssa03q                                                                  |                                |
| Co07              | 211.09    | 241               | Co07a             | Ots11p*                                                     | Omy19p                                                           | Ssa04p                                                                 | B, metacentric                              |
|                   |           |                   | Co07b             | Ots11q                                                      | Omy19q                                                           | Ssa01p                                                                  |                                |
| Co08              | 295.21    | 382               | Co08a_1           | Ots12p                                                      | Omy11p                                                           | Ssa20qa                                                                 | C, metacentric                              |
|                   |           |                   | Co08a_2           |                                                             | Omy11q                                                           |                                                                        |                                |
|                   |           |                   | Co08b             | Ots12q                                                      | Omy26                                                            | Ssa11qb                                                                  |                                |
| Co  |  Value  |  |  | Co09  |  Value  |  |  | Co09a  |  Value  |  | Co09b  |  Value  |  |  |
|-----|---------|---|---|------|---------|---|---|------|---------|---|---|------|---------|---|---|
| Co09| 196.03  | 207|  | Co09a |  Ots15p |  |  | Omy21p |  |  | Ssa07p |  |  | A, metacentric |
| Co09b| Ots15q |  |  | Omy21q |  |  | Ssa07q |  |  |  |  |  |  |
| Co10| 239.27  | 346|  | Co10a |  Ots01p |  |  | Omy04p |  |  | Ssa23 |  |  |  |
| Co10b| Ots27 |  |  | Omy13q |  |  | Ssa06q |  |  |  |  |  |  |
| Co11| 280.26  | 419|  | Co11a |  Ots01q |  |  | Omy23 |  |  | Ssa01q |  |  |  |
| Co11b| Ots29 |  |  | Omy15p |  |  | Ssa29 |  |  |  |  |  |  |
| Co12| 215.12  | 286|  | Co12a |  Ots05p |  |  | Omy08p |  |  | Ssa15q |  |  |  |
| Co12b| Ots34** |  |  | Omy10q |  |  | Ssa08q |  |  |  |  |  |  |
| Co13| 288.32  | 350|  | Co13a |  Ots05q |  |  | Omy05q |  |  | Ssa10q |  |  |  |
| Co13b| Ots23 |  |  | Omy02p |  |  | Ssa05q |  |  |  |  |  |  |
| Co14| 270.54  | 356|  | Co14a |  Ots08p |  |  | Omy25p |  |  | Ssa09q |  |  |  |
| Co14b| Ots31 |  |  | Omy14p |  |  | Ssa14qb |  |  |  |  |  |  |
| Co15| 282.89  | 355|  | Co15a |  Ots08q |  |  | Omy25q | (Omy29) |  | Ssa09qb |  |  |  |
| Co15b| Ots13q |  |  | Omy27 |  |  | Ssa20qb |  |  |  |  |  |  |
| Co16| 211.31  | 220|  | Co16a |  Ots10p |  |  | Omy09p |  |  | Ssa18qb |  |  |  |
| Co16b| Ots14p |  |  | Omy18p |  |  | Ssa16qb |  |  |  |  |  |  |
| Co17| 181.92  | 292|  | Co17a |  Ots13p |  |  | Omy18q |  |  | Ssa27 |  |  |  |
| Co17b| Ots16q |  |  | Omy09q |  |  | Ssa15qb |  |  |  |  |  |  |
| Co18| 209.02  | 323|  | Co18a |  Ots14q |  |  | Omy24 |  |  | Ssa09qc |  |  |  |
| Co18b| Ots16p |  |  | Omy11p |  |  | Ssa19qa |  |  |  |  |  |  |
| Co19| 217.65  | 315|  | Co19a |  Ots17 |  |  | Omy15q |  |  | Ssa17qa |  |  |  |
| Co19b| Ots21 |  |  | Omy14q |  |  | Ssa05p |  |  |  |  |  |  |
| Co20| 203.33  | 247|  | Co20a |  Ots24 |  |  | Omy16p |  |  | Ssa19qb |  |  |  |
| Co20b| Ots32 |  |  | Omy13p |  |  | Ssa12qa |  |  |  |  |  |  |
| Co21| 130.25  | 184|  | Co21 |  Ots18 |  |  | Omy04q |  |  | Ssa06p |  |  | C, acrocentric |
| Co22| 194.97  | 243|  | Co22 |  Ots19 |  |  | Omy02q |  |  | Ssa10qb |  |  | C, acrocentric |
|   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|
|Co23| 212.27 | 213 | Co23| Ots20 | Omy05p | Ssa01qb | C, acrocentric|
|Co24| 127.18 | 187 | Co24| Ots22 | Omy16q | Ssa13qa | C, acrocentric|
|Co25| 171.35 | 196 | Co25_1| Ots25 | Omy20p | Ssa08p*** | A, acrocentric|
|      |   |   | Co25_2| Ots25 | Omy20q | Ssa28 |   |
|Co26| 184.21 | 225 | Co26| Ots26 | Omy22 | Ssa21 | A, acrocentric|
|Co27| 164.56 | 198 | Co27| Ots28 | Omy28 | Ssa03p | B, acrocentric|
|Co28| 169.82 | 214 | Co28| Ots30 | Omy10p | Ssa04q | C, acrocentric|
|Co29| 173.74 | 181 | Co29| Ots33 | OmySex | Ssa11qa | B, acrocentric|
|Co30| 189.15 | 218 | Co30| Ots10q| Omy08q | Ssa14qa |   |
|Total| 6596.7 | 8681 |   |   |   |   |   |   |
Table 2 - Homeologous chromosome arm pairs identified in coho salmon and the number of marker pairs supporting the homeologous relationship. Corresponding known homeologous relationships in Chinook salmon, rainbow trout and Atlantic salmon are shown. The final column designates known conservation of metacentric chromosome with high frequency of duplicated markers; letter corresponds to phylogenetic placement in Figure 5. Star (*) denotes possible earlier chromosomal arrangement (A) and subsequent rearrangement.

^: homeologous relationships with little or no support in Atlantic salmon (Lien et al. 2011)
+: we have corrected the homeologous relationship between Omy17q and Omy13p; evidence suggests that this relationship was incorrectly reported as being between Omy17p and Omy13p in previous studies

| Homeology in coho salmon | Number of marker pairs supporting homeolog pairings | Homeology in chinook salmon (Brieuc et al., 2014) | Homeology in rainbow trout (Phillips et al., 2006) | Homeology in Atlantic salmon (Lien et al., 2011) | Known phylogenetic placement of Metacentric arrangement |
|-------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|------------------------------------------------|
| Co01b - Co20b           | 15                                            | Ots02q - Ots32                                | Omy17q^+ - Omy13p                             | Ssa02q - Ssa12qa^+                            | B                                            |
| Co02a - Co13b           | 11                                            | Ots03p - Ots23                                | Omy03p - Omy02p                               | Ssa02p - Ssa05q                               | B*                                           |
| Co03b - Co08b           | 15                                            | Ots04q - Ots12q                               | Omy06q - Omy26                                | Ssa26 - Ssa11qa^+                             | B, C                                         |
| Co04b - Co11a           | 17                                            | Ots06q - Ots01q                               | Omy01q - Omy23                                | Ssa18qa - Ssa01qa^+                           | B                                            |
| Co05a - Co16b           | 10                                            | Ots07p - Ots14p                               | Omy07p - Omy18p                               | Ssa17qa – Ssa16qb^+                           | B                                            |
| Co06b - Co10b           | 7                                             | Ots09q - Ots27                                | Omy12q - Omy13q                               | Ssa03q - Ssa06p                               | B*                                           |
| Co07a - Co12b           | 9                                             | Ots11p - Ots34                                | Omy19p - Omy10q                               | Ssa04p - Ssa08q                               | B*                                           |
| Co09a - Co19a           | 16                                            | Ots15p - Ots17                                | Omy21p - Omy15q                               | Ssa07p - Ssa17qa                              | A                                            |
Figure 1 - Graphical representation of 30 consensus linkage groups in haploid and diploid female coho salmon. Co01 to Co20 are metacentric, and Co21 to Co30 are acrocentric, inferred from comparative mapping. The size of linkage groups ranges from 127.18 to 295.21 centiMorgan (Kosambi), and each line corresponds to the location of one or more markers. The putative location of the centromere, estimated by comparative mapping with Chinook salmon, is represented in red.
Figure 2 - Comparison of map distances between common markers mapped in male and female coho salmon. Linkage groups, Co04 (a), Co17 (b), Co18 (c) and Co26 (d) are given as examples. The putative region containing the centromere is represented by the cross-hatched area. All linkage groups are presented in Supplementary Material S4.
Figure 3 - Number of markers and distribution of duplicated markers across each coho salmon linkage groups. Non-duplicated loci are represented in light grey. Duplicated loci are represented in dark grey (loci with only one paralog polymorphic) or black (both paralogs polymorphic).
Figure 4 - Distribution of duplicated and non-duplicated loci along the 16 linkage groups with a high proportion of duplicated loci. Non-duplicated loci are represented in light grey. Duplicated loci are represented in dark grey (loci with one paralog polymorphic) or in charcoal (loci with both paralogs polymorphic). The putative region containing the centromere is represented by the cross-hatched area.
Figure 5 - Phylogenetic tree showing the orientation of homologous arms, Ssa09qab, Omy25/29, Co14a, Co15a and Ots08 in Atlantic salmon, rainbow trout, coho salmon and Chinook salmon, respectively. Chromosomal rearrangements and homeologous relationships conserved across species at phylogenetic nodes A, B, C are summarized in Table 1 and 2.