G3, 2025, jkaf064
https://doi.org/10.1093/g3journal/jkaf064
Advance Access Publication Date: 19 March 2025
Genome Report

The genome assembly of the westslope cutthroat trout, *Oncorhynchus lewisi*, reveals interspecific chromosomal rearrangements with the rainbow trout, *Oncorhynchus mykiss*

Anne-Marie Flores,[1,†] Kris A. Christensen ,[1,*,†] Theresa Godin,[2] Yniv Palti ,[3] Matthew R. Campbell,[4] Geoffrey C. Waldbieser,[5] Sheron A. Simpson,[6] Brian E. Scheffler,[6] Seth R. Smith,[7,8] Andrew R. Whiteley,[8] Ryan P. Kovach,[9] Gordon Luikart,[8,10] Matthew C. Boyer,[11] Marty Kardos,[8,12] Scott Relyea,[13] Craig Wells ,[14] Ben F. Koop [1,*]

[1]Department of Biology, University of Victoria, Victoria, Canada, V8W 2Y2 BC
[2]Science Division, Freshwater Fisheries Society of BC, 80 Regatta Landing, Suite 101, Victoria, BC, Canada, V9A 7S2
[3]USDA-ARS National Center for Cool and Cold Water Aquaculture, Kearneysville, WV 25430, USA
[4]Eagle Fish Genetics Lab, Idaho Department of Fish and Game, 1800 Trout Road, Eagle, ID 83616, USA
[5]USDA-ARS Warmwater Aquaculture Research Unit, 141 Experimental Station Road, Stoneville, MS 38776, USA
[6]USDA-ARS Genomics and Bioinformatics Research Unit, 141 Experimental Station Road, Stoneville, MS 38776, USA
[7]Washington Department of Fish and Wildlife, Molecular Genetics Laboratory, 1111 Washington St. SE, Olympia, WA 98501, USA
[8]Wildlife Biology Program, W.A. Franke College of Forestry and Conservation, University of Montana, Missoula, MT 59812, USA
[9]Montana Fish, Wildlife and Parks, University of Montana Conservation Genetics Laboratory, University of Montana, Missoula, MT 59812, USA
[10]Flathead Lake Biological Station, Division of Biological Sciences, University of Montana, Polson, MT 59860, USA
[11]Montana Fish, Wildlife and Parks, Kalispell, MT 59901, USA
[12]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA 98112, USA
[13]Sekokini Springs Hatchery, Montana Fish, Wildlife and Parks, Kalispell, MT 59901, USA
[14]Bioinformatics and Computational Biology, University of Idaho, Moscow, ID 83844, USA

*Corresponding author: Kris A. Christensen, Department of Biology, University of Victoria, 3800 Finnerty Rd., Victoria, Canada, V8W 2Y2. Email: kris.christensen@wsu.edu ;
*Corresponding author: Ben F. Koop, Department of Biology, University of Victoria, 3800 Finnerty Rd., Victoria, Canada, V8W 2Y2. Email: bkoop@uvic.ca
[†]These authors contributed equally to this work.

Cutthroat trout (*Oncorhynchus clarkii*) are popular among anglers throughout their native range along the West Coast and interior of North America. As they colonized the interior of North America, cutthroat trout diverged into several genetically distinct groups. Many of these groups are now threatened by habitat destruction, hybridization with rainbow trout (*Oncorhynchus mykiss*), and competition from introduced species. These groups were previously classified as subspecies, but recent research suggests that they may represent distinct species. In this study, we produced a chromosomal-level genome assembly and a genetic map for one of the species in the cutthroat trout species complex, the westslope cutthroat trout (*Oncorhynchus lewisi*—formerly *Oncorhynchus clarkii lewisi*). We also constructed haplotype-resolved assemblies from a westslope cutthroat-rainbow trout F1 hybrid. We used the new genome assemblies to identify major interspecific chromosomal rearrangements between the 2 sister species, including fusions, fissions, and inversions. These genome assemblies and chromosome data provide valuable insights regarding genetic variation within cutthroat trout and in hybrids between rainbow and cutthroat trout.

Keywords: subspecies; species complex; cutthroat trout; rainbow trout; stocking; karyotype; chromosomal rearrangements

Introduction

Cutthroat trout (*Oncorhynchus clarkii*) are an iconic species of western North America. They hold cultural significance for Indigenous communities and are highly valued for their recreational and economic importance (DFO 2009; Mallet and Thurow 2022). Cutthroat trout are particularly valued by anglers, due to their surface feeding behavior and high catchability (Mallet and Thurow 2022). A 2015 survey by the Freshwater Fisheries Society of BC (published in the Economic Impact Report—2019) ranked cutthroat trout as the 4th favorite sport fish among anglers in British Columbia (BC), Canada (gofishbc.com).

Cutthroat trout form a species complex (i.e. a group that is closely related without clear species boundaries) that is native to the coastal and interior waters of western North America (Allendorf and Leary 1988; Behnke 1992; Whiteley *et al.* 2019). These trout exhibit remarkable phenotypic and genetic variability, diverse life history strategies, and significant evolutionary diversity (Allendorf and Leary 1988; Behnke 2002; Campbell *et al.* 2011). They occupy various aquatic habitats, including small headwater streams, large rivers, lakes, and estuaries. These habitats share the characteristics of being cold, clean, and oxygen-rich (Behnke 1992; Trotter 2008).

The extensive diversity within cutthroat trout has led to disagreements among researchers regarding its description and classification, particularly concerning the number of species and

Received on 21 October 2024; accepted on 03 March 2025
Published by Oxford University Press on behalf of The Genetics Society of America 2025.

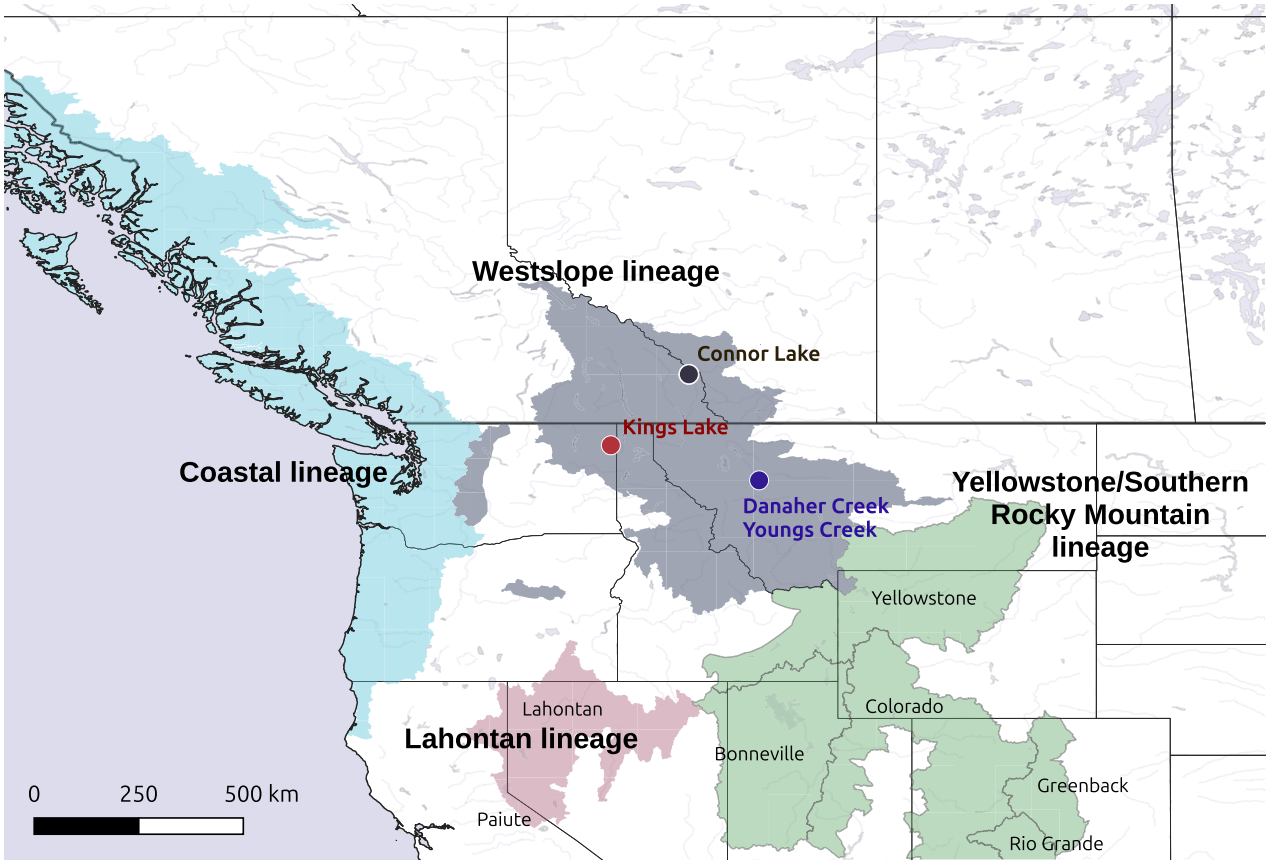

Fig. 1. Historical and current distributions of cutthroat trout in western North America. The 4 major evolutionary lineages of cutthroat trout are labeled on the map. Westslope cutthroat trout (WCT) were sampled from Connor Lake, British Columbia (Canada), Kings Lake, Idaho (USA), and Youngs Creek and Danaher Creeks in the headwaters of the South Fork of the Flathead River, Montana (USA). Map adapted from Penaluna *et al.* (2016).

subspecies (Behnke 1992; Metcalf *et al.* 2007; Penaluna *et al.* 2016; Trotter *et al.* 2018). Previously, 14 subspecies of cutthroat trout were recognized, 2 of which are now extinct (Behnke 1992; Behnke 2002). Recent studies (see Wilson and Turner 2009; Loxterman and Keeley 2012; Metcalf *et al.* 2012; Trotter *et al.* 2018; Bestgen *et al.* 2019) have re-evaluated the earlier classification. These studies continue to support the finding that modern cutthroat trout diverged from a common ancestor into 4 major evolutionary lineages—Coastal, Lahontan Basin, Upper Columbia/Missouri River (Westslope), and the Yellowstone/Southern Rocky Mountain Lineage/Upper Snake River (Fig. 1) (Allendorf and Leary 1988; Behnke 1992; Trotter *et al.* 2018). Furthermore, there may be at least 25 unique groups within these lineages (Trotter *et al.* 2018). The nomenclature and classification of this complex are still subject to debate, and further research is needed to determine the appropriate classification (e.g. species or subspecies).

The westslope cutthroat trout (WCT), *Oncorhynchus lewisi*—formerly *Oncorhynchus clarkii lewisi* (Page *et al.* 2023)—is one of the 4 major evolutionary lineages of cutthroat trout (Fig. 1), and is renowned for its ecological, economic, and cultural significance (Quinn 2005; Cosewic 2006). While the historical distribution of WCT is somewhat uncertain, their range included portions of the Fraser, South Saskatchewan, Missouri, and Columbia River basins (Behnke 1992; Shepard *et al.* 2005; McPhail 2007). Although these 4 major basins are currently isolated, some degree of historical connectivity or migration among them has been proposed (Young *et al.* 2018). The WCT lineage can be distinguished from other lineages of cutthroat trout through differences in morphology (Behnke 1992), karyotype (Gold *et al.* 1977; Loudenslager and Thorgaard 1979), and genomic divergence (Leary *et al.* 1987; Allendorf and Leary 1988; Utter and Allendorf 1994).

Molecular studies have revealed divergence within the WCT lineage as well (Drinan *et al.* 2011; Loxterman and Keeley 2012). Young *et al.* (2018) proposed that the WCT lineage has differentiated into at least 9 unique groups (Table 1). The genetic structure of WCT across its entire range aligns with major watershed boundaries and cycles of the Pleistocene glaciation (Drinan *et al.* 2011; Young *et al.* 2018). Further divergence within the WCT lineage may exist at smaller spatial scales. For example, populations from the Upper Kootenay, Elk, Columbia, and Fraser Rivers could potentially be classified as separate groups, as they are genetically distinct and associated with major drainage basins in BC (Taylor *et al.* 2003; B.C. Ministry of Environment 2014).

Modern populations and the distribution of WCT have significantly declined due to several factors, including habitat loss and fragmentation, barriers to migration, overexploitation, competition from introduced species, climate change, and hybridization (Shepard *et al.* 2005; Cosewic 2006; Trotter 2008; Muhlfeld *et al.* 2014; Kovach *et al.* 2022). Among these, hybridization poses the primary threat to the genetic integrity of native WCT populations (Allendorf and Leary 1988; Behnke 1992; Shepard *et al.* 2005). Hybridization between cutthroat trout, including the WCT lineage, and introduced rainbow trout (RT, *Oncorhynchus mykiss*), is

Table 1. List of the proposed uniquely identifiable evolutionary units (UIEUs) that have differentiated from the westslope (Upper Columbia–Missouri River) evolutionary lineage of cutthroat trout.

Name	Location
John Day cutthroat trout	Upper portion of the John Day River basin
Coeur d'Alene cutthroat trout	Upper portion of the Coeur d'Alene River basin
St. Joe cutthroat trout	Upper portion of the St. Joe River basin
North Fork Clearwater cutthroat trout	Upper portion of the North Fork Clearwater River
Salmon cutthroat trout	Salmon River basin
Clearwater Headwaters cutthroat trout	Headwaters of the Selway and South Fork Clearwater Rivers
Clearwater-Eastern Cascades cutthroat trout	Lochsa River, lower Selway River and lower portion of tributaries to the Middle Fork Clearwater River, and the Wenatchee River, Lake Chelan, and Methow River basins
Neoboreal cutthroat trout	Columbia River basin from the Sanpoil River upstream (excluding the Spokane River), Fraser River basin and the South Saskatchewan River basin
Missouri River cutthroat trout	Missouri River basin

The names primarily correspond to the major river basins. Adapted from Young *et al.* (2018).

widespread in both Canada and the United States (Young 1995; Rubidge *et al.* 2001; Boyer *et al.* 2008; Bennett and Kershner 2009; Rasmussen *et al.* 2010). In Canada, populations of WCT have been reduced by approximately 80% (Cosewic 2006).

Rainbow and cutthroat trout are sister species (Crespi and Fulton 2004; Wilson and Turner 2009; Crête-Lafrenière *et al.* 2012), sharing a common ancestor approximately 10.2 million years ago (Stearley and Smith 2016). Despite differences in their karyotypes, Robertsonian chromosomal rearrangements (involving the entire arm of a chromosome) have maintained the same number of diploid chromosome arms (n = 104) in both species (Loudenslager and Thorgaard 1979; Thorgaard 1983). The consistency in chromosome arm number may contribute to their ability to produce viable, fertile hybrid offspring. Diploid chromosome numbers in cutthroat trout vary from 64 to 68, while in RT, they can vary from 58 to 64 (Loudenslager and Thorgaard 1979; Thorgaard 1983).

Hybridization occurs due to the absence of reproductive barriers and temporal and spatial overlap in spawning (Allendorf and Leary 1988; Behnke 1992). Hybridization between introduced RT and native WCT can lead to the loss of locally adapted traits (Muhlfeld *et al.* 2009a), reduced fitness (Muhlfeld *et al.* 2009b; Kovach *et al.* 2016a), and decreased resilience to environmental changes (Yau and Taylor 2013; Muhlfeld *et al.* 2014; Kovach *et al.* 2016b), all of which threaten the survival of WCT populations (Allendorf and Leary 1988; Allendorf *et al.* 2001; Boyer *et al.* 2008). Additionally, the introduction of hatchery strains of WCT may affect the integrity of wild WCT populations (Young *et al.* 2018).

The RT genome assembly was the first published salmonid assembly (Berthelot *et al.* 2014). Since then, several high-quality assemblies have been produced for various RT strains (e.g. Pearse *et al.* 2019; Gao *et al.* 2021). In the absence of a genome assembly, cutthroat trout genomic research has relied on reference-free methods or alignment of sequencing data to divergent reference genomes. To facilitate modern genetic and genomics techniques, we produced a genome assembly of a WCT from a BC lake commonly used as a source for broodstock (i.e. a group of individuals used for breeding). We also produced haplotype-resolved assemblies of a WCTxRT F1 hybrid. Hi-C data from the hybrid and a high-density WCT genetic map were used to confirm chromosomal fusions, fissions, and inversions among RT and cutthroat trout assemblies. This study provides groundwork for research evaluating the genetic basis for reproductive isolation between RT and cutthroat trout, and local adaptation within cutthroat trout lineages.

Methods

Sample collection of non-hybridized WCT

Several bodies of water in BC serve as sources of WCT broodstock, with most stocked WCT in BC originating from Connor Lake in the upper Elk System since the 1970s (B.C. Ministry of Environment 2014). Connor Lake, originally barren, was stocked in 1950 with WCT from Kiakho Lake, which itself had been stocked earlier with WCT from various BC sources, including Munroe Lake and Peavine Creek. Although the exact origin of Connor Lake's WCT is unclear, previous genetic testing has confirmed the trout in Connor Lake are non-hybridized WCT (B.C. Ministry of Environment. 2014). While these trout were once thought to belong to the Yellowstone cutthroat trout lineage (Stenton 1960), genetic and chromosomal analyses have shown that only coastal and westslope lineages exist in Canada (McPhail 2007).

In June 2023, milt samples were collected from 3 male wild WCT at Connor Lake (Fig. 1) by Freshwater Fisheries Society of British Columbia (FFSBC). Provincial authorization to transfer samples was given to FFSBC by Fisheries and Oceans Canada (license number: 134373). Liver tissue was dissected from female cutthroat trout that did not survive egg retrieval. Liver samples were stored in ethanol on ice until they could be transferred to a freezer where they were stored at −80 °C before processing.

DNA extraction of WCT and Oxford Nanopore Technologies (ONT) sequencing

High molecular weight genomic DNA was extracted from the milt using the Nanobind CBB kit (PacBio) according to the manufacturer's protocol. The Short Read Eliminator Kit (PacBio) was used to reduce the number of small DNA fragments according to the manufacturer's instructions. Sequencing libraries were then prepared using the Ligation Sequencing Kit V14 (SQK-LSK114, ONT) and sequenced on a R10.4.1 flow cell (FLO-PRO114M) of a PromethION 2 Solo system (ONT) on site (University of Victoria). Sequences were saved as FASTQ format using the MinKNOW software (ONT).

Hi-C library and whole genome shotgun sequencing of WCT

A Hi-C library was prepared from female WCT liver tissue at Canada's Michael Smith Genome Sciences Centre in Vancouver, BC, Canada. The library was prepared with the Arima High Coverage Hi-C kit (Arima Genomics) according to the manufacturer's instructions and outlined in the User Guide for Animal Tissues Doc A160162 v01. The resulting proximally-ligated DNA served as the basis for library construction, which also used the NEBNext Ultra II DNA Library Prep Kit (New England Biolabs), Q5 PCR Master Mix, and custom IDT indexed primer pairs. Subsequent library products were amplified with 10 reaction cycles using NEBNext Q5 Master Mix (New England Biolabs) supplemented with 2 mM $MgSO_4$. The amplified products were bead cleaned, quantified, and then sequenced on the Illumina

NovaSeq platform (Illumina) using paired-end 150 bp sequencing (S4 flow cell).

Genome shotgun sequencing was also performed at Canada's Michael Smith Genome Sciences Centre using the TruSeq DNA PCR-free library prep kit (Illumina) automated via a Microlab NIMBUS liquid handling robot (Hamilton Robotics). This method was chosen to reduce bias and coverage gaps from PCR amplification in high GC or AT-rich regions. Briefly, 500 ng of genomic DNA (extracted from milt of a different male from ONT sequencing—due to limited DNA) was placed into a 96-well microtiter plate and fragmented using sonication (Covaris LE220). The fragmented DNA was end-repaired and size-selected using paramagnetic PCRClean DX beads (C-1003-450, Aline Biosciences), which targeted a 300–400 bp range. After 3′ A-tailing, full-length TruSeq adapters were added. The libraries were purified with paramagnetic beads from Aline Biosciences and quantified using a qPCR Library Quantification kit (KAPA, KK4824). The libraries were then sequenced with paired-end 150 bp reads on an S4 (v1.0) flow cell of the Illumina NovaSeq 6000 sequencer following the manufacturer's protocol.

Genome assembly—WCT

Flye (version 2.9.2) (Kolmogorov *et al.* 2019) was used to produce the initial WCT contig genome assembly from the ONT reads. The --nano-hq, --read-error 0.03, -g 2.5g, and --asm-coverage 40 parameters were used for this initial assembly (metrics—estimated coverage: 69×, read N50: 29541, genome assembly size: 2.4 Gb, fragments: 4,641, and contig N50: 5 Mb). This assembly was then polished with racon (version 1.5.0) (Vaser *et al.* 2017) once and Pilon twice (version 1.24) (Walker *et al.* 2014). Details of the polishing steps are in the following paragraph.

Minimap2 (version 2.17) (Li 2018; Li 2021) was used to align the ONT reads to the initial assembly (parameters: -ax map-ont) before the alignment was polished using racon (parameters: -u). Short reads were used for polishing using Pilon after they were trimmed using Trimmomatic (version 0.39) (Bolger *et al.* 2014) with the following parameters: TruSeq3-PE.fa:2:30:10:2: keepbothReads, LEADING:3, TRAILING:3, and MINLEN:36. The trimmed reads were aligned to the racon polished assembly with bwa mem (version 0.7.10) (Li and Durbin 2009; Li and Durbin 2010; Li 2013) using default settings. After the alignments were sorted with SAMtools (version 1.9) (Li *et al.* 2009; Danecek *et al.* 2021), default Pilon parameters were used to polish the assembly.

After polishing, the Arima Genomics mapping_pipeline (version 3, see github.com) was used to map the Hi-C data to the assembly. The alignments were sorted using SAMtools, and Matlock and Juicebox utilities (Phase Genomics, see github.com) were used to convert the alignment file to a Hi-C contact map. Juicebox (version 1.11, Durand *et al.* 2016) was used to visualize the Hi-C contact map and to make manual changes to the order and orientation. Alignments of the assembly to the Swanson line rainbow trout (*O. mykiss*) genome assembly (Omyk_2.0, GCA_025558465.1) were produced using Dgenies (Cabanettes and Klopp 2018) and were used to verify order and orientation of scaffolds. Juicebox utilities were used to output the modified assembly.

Sample collection and production of F1 hybrid WCTxRT

A cross was generated between a female WCT (Kings Lake strain) from the Cabinet Gorge Fish Hatchery, Idaho and a coastal male RT (*Oncorhynchus mykiss irideus*, Hayspur strain) at the Hayspur Hatchery, Idaho, United States of America in May 2022 (Fig. 1—Kings Lake). Fin tissue samples were collected from the sire and dame of the cross. Blood was sampled from a single F1 hybrid offspring of unknown sex when it was approximately 1 year in age. The Kings Lake strain was tested for introgression with RT, and RT alleles were identified at a frequency of less than 1% (Idaho Fish and Game, unpublished data).

Haplotype-resolved genome assembly—F1 hybrid WCTxRT

DNA was extracted from a blood sample of a single F1 hybrid offspring of unknown sex (NCBI BioSample accession SAMN43529089 and BioProject PRJNA1157974) using a TE-NaCl-SDS Proteinase K overnight digestion, protein precipitation by ammonium acetate, isopropanol precipitation of nucleic acids, ethanol wash, gentle treatment of DNA with wide pore tips, and mostly decanting instead of pipetting. HiFi reads were produced using the CCS mode on the PacBio Sequel II system (GBRU Stoneville, MS). A total of 117.5 Gb sequence data (49×) was generated from 7.6 million HiFi reads (NCBI SRA accessions SRX26069220 and SRX26069221). The Hi-C libraries were prepared from a frozen blood sample and sequenced by a commercial vendor (Phase Genomics, Seattle, WA) to produce 868 million Illumina paired-end reads (2 × 150 bp) (accessions SRX26061379, SRX26061380, SRX26061381). Illumina whole genome sequencing data were generated by a commercial vendor (Admera Health, South Plainfield, NJ) from the WCT female parent (SAMN43529090) and RT male parent (SAMN43529091) using a KAPA Hyper Prep—PCR Free Kit (Roche). In total, 89.1 Gb (37×) and 94.4 Gb (39×) were generated from the WCT (SRX26028960) and RT (SRX26028961) parents, respectively. The genomic DNA from the 2 parents was extracted from tail fin clips using the salting-out procedure as previously described (Palti *et al.* 2002).

The genome assembler Hifiasm (Cheng *et al.* 2021) (version 0.19.8-r603, parameters for trio-binning assembly: -t 72 -z 20 -1 <paternal kmers produced using yak> -2 <maternal kmers>) was used to generate 2 genome-wide haplotypes (WCT and RT) from the F1 hybrid, using input data from PacBio HiFi long read reads and whole genome short reads sequence data from both parents. The total length of the RT contigs assembly was 2.33 Gb in 3,489 contig sequences (contig N50: ~2.0 Mb), and the WCT contigs assembly was composed of 3,808 sequences for a total combined length of 2.35 Gb (contig N50: ~2.0 Mb). The order and orientation of contigs were inferred manually from Hi-C data (using the same protocol that was used for the WCT genome assembly).

Restriction site associated DNA sequencing (RADSeq) genetic map

The genetic map was constructed using genotypes of parents and offspring from 2 haploid families and 1 diploid family bred at the Sekokini Springs Fish Hatchery operated by Montana Fish, Wildlife, and Parks (MTFWP). Linkage mapping families were spawned using parents collected from Youngs Creek and Danaher Creeks in the headwaters of the South Fork of the Flathead River, Montana, United States of America (Fig. 1). The linkage map was constructed following the methods of Blumstein *et al.* (2020) and Waples *et al.* (2016). The linkage map and a detailed description of the linkage mapping methods are available in the Supplementary Data (Supplementary Files 1 and 2). The WCT genetic map was compared to version 1 of the Swanson RT genome assembly (GCA_002163495.1—version 2 was not

Table 2. Genome sequencing and assembly results for a non-hybridized westslope cutthroat trout (WCT) and haplotype-resolved genome assemblies from a WCT × rainbow (RT) F1 hybrid.

	WCT	Hybrid WCT	Hybrid RT
Total length (Gb)	2.4	2.4	2.3
Contig N50 (Mb)	5.0	2.0	2.0
Scaffold N50 (Mb)	73.5	62.2	75.0
Contigs	4,647	3,808	3,489
Sequence in Chr.	90.1%	88.1%	88.6%
Complete BUSCOs	98.2% (S: 55.1, D: 43.1)	98.1% (S: 55.6, D: 42.5)	98.1% (S: 55.5, D: 45.6)
QV[a]	43.42	43.62	43.63
Error rate	$4.6e^{-05}$	$4.3e^{-05}$	$4.3e^{-05}$
Completeness[b]	97.1%	Cutthroat: 98.27% Rainbow: 0.43%	Cutthroat: 1.98% Rainbow: 98.75%

Complete BUSCOs are further divided into single and duplicated (in parentheses).
[a] Assembly consensus quality value—log-scaled probability of error (QV of 40 has 99.99% accurate consensus base calls).
[b] kmer based estimation of the percent of short-read kmers compared to the genome assembly kmers. The hybrid assemblies were haplotype-resolved genome assemblies, and the 2 values represent the percent of kmers from each parental genotype within each assembly (to better understand how well the haplotypes were separated from each other and the completeness of both).

available at the time it was created). Version 1 and 2 are similar, with the exception of an inversion on Omy05.

Comparison of genome assemblies and genomic metrics

Dgenies (Cabanettes and Klopp 2018) was used to align the 3 genome assemblies produced in this study to the second version of the Swanson RT genome assembly (Pearse *et al.* 2019, GCA_025558465.1). The Arlee (Gao *et al.* 2021, GCF_013265735.2), Whale Rock (GCA_029834435.1), and Keithly Creek RT (GCA_034753235.1) genome assemblies were also aligned given the substantial genetic variation between RT strains (Palti *et al.* 2014). We also verified the structural changes of the WCT genome assemblies with the genetic map.

The WCT genome assembly was also compared with a genetic map generated from a Yellowstone cutthroat trout (YCT, *Oncorhynchus clarkii bouvieri*) hybrid (Ostberg *et al.* 2013). We aligned markers from the YCT genetic map (Ostberg *et al.* 2013), originally taken from Rexroad *et al.* (2008), to the WCT genome assembly using BLASTN (Altschul *et al.* 1990, using the outfmt 6 parameter). The output was filtered for the best alignments (based on bit score), and we were able to assign linkage groups from the genetic map to chromosomes in the genome assembly.

Length and contig N50 values were calculated using the index files generated by SAMtools from the genome assemblies. BUSCO (version 5.5.0, Manni *et al.* 2021) scores were used to assess genome completeness. These analyses were conducted on the public Galaxy server (usegalaxy.org; Afgan *et al.* 2016) using the actinopterygii_odb10 gene set. We also used Mequry (version 1.4, Rhie *et al.* 2020) to determine completeness (kmer based, k = 21) and to determine the estimated error rate. These analyses used trimmed short reads for the comparison (see above). For the haplotype-resolved genome assemblies of the WCTxRT F1 hybrid, we used short reads from the parents and the progeny to generate hap-mers and identify the completeness of each haplotype-resolved assembly.

Sex chromosomes

The salmonid sex-determining locus, sdY, is located approximately 5–6 Mb from the start of Omy29 (a.k.a. OmyY) in the Swanson and Arlee RT genome assemblies (Pearse *et al.* 2019; Gao *et al.* 2021). We used BLASTN (Altschul *et al.* 1990) and the sdY gene sequence from RT (Genbank AB626896.1) to try to locate the scaffold containing sdY in the WCT and the F1 WCTxRT hybrid genome assemblies.

Results

We generated a chromosome-level reference genome assembly for WCT and haplotype-resolved genome assemblies from a WCTxRT F1 hybrid (Table 2). The WCT genome assembly was 2.4 Gb in length, and it had a contig N50 of 5.0 Mb with 98.2% complete BUSCOs (Table 2). The hybrid WCT assembly was also 2.4 Gb long and had 98.1% complete BUSCOs. The contig N50 was 2.0 Mb. Similarly, the hybrid RT assembly was 2.3 Gb long, with a contig N50 of 2.0 Mb and 98.1% complete BUSCOs. The high number of duplicate BUSCOs (Table 2) is consistent with the ancestral autopolyploid whole genome duplication common to salmonids (Allendorf and Thorgaard 1984) and previous rainbow trout genome assemblies (e.g. Gao *et al.* 2021).

We identified interspecific chromosomal rearrangements between WCT and RT with alignments and confirmed putative rearrangements using the hybrid Hi-C data (Figs. 2 and 3). In contrast to the RT genome assemblies, WCT displayed no variation in chromosomal arrangements (Fig. 2 and Supplementary Fig. 1). The YCT, from Ostberg *et al.* (2013), had 1 less chromosome than WCT as they lack a fission that WCT have (Supplementary Fig. 1).

WCT, in this study, have 33 chromosomes, compared to the 29 chromosomes observed in the Swanson RT strain, and they lack the double inversion on chromosome 5 (Omy05) found in some RT (Pearse *et al.* 2019). Five fissions and 1 fusion were identified in comparisons of WCT with RT. WCT also have interspecific pericentric inversions on Ocl20, Ocl22, and a potential double paracentric inversion on Ocl29. The inversion on Ocl20 occurs in the same region as the Omy20 chromosomal inversion previously characterized in Hale *et al.* (2024) (Figs. 4 and 5). Smaller interspecific inversions were also present in the WCT genome, though these were not well supported by the Hi-C data.

The hybrid RT genome assembly is similar to the Swanson strain assembly; however, it lacks the Omy05 inversion and the fusion event between chromosomes Omy25 and Omy31 (Gao *et al.* 2021). The hybrid RT also has 30 chromosomes, similar to the Keithly Creek RT genome assembly (Fig. 2). The key difference between the hybrid genome assembly and the Keithly Creek genome assembly is the fusion of different chromosome arms, with the hybrid having Omy4 fused with Omy30 instead of Omy25 fused with Omy31.

We located the sdY gene in the WCT genome assembly. Our results suggest that the sex chromosome is Ocl30 (which is different from RT). This evidence is weak due to limited Hi-C contact points observed in Juicebox (data not shown). We were unable to locate the sdY gene in either of the haplotype-resolved genome

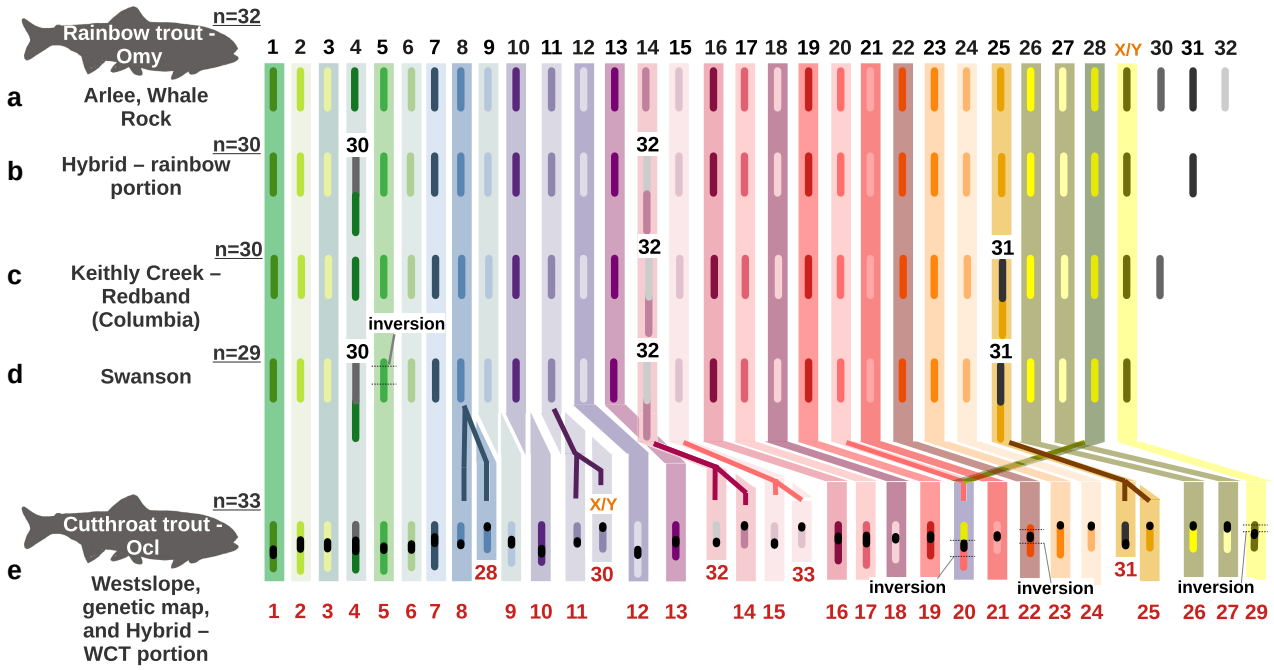

Fig. 2. Comparison of syntenic chromosomes among multiple strains of rainbow trout (RT) and westslope cutthroat trout (WCT). This figure highlights karyotype variation among Arlee and Whale Rock RT a), the RT portion from the WCTxRT hybrid b), Keithly Creek RT c), and Swanson RT d) strains. Only 1 RT intraspecific inversion was identified on Omy5 d). The WCT genome assembly (Connor Lake), the WCT genetic map, and the WCT portion of the WCTxRT hybrid all share the same karyotype e). The potential sex chromosome for WCT is indicated with X/Y. Chromosome numbering is shown in black for RT (top) and red for WCT (bottom). Centromeres illustrated on the WCT (black ovals) are depicted relative to their size (based on the genetic map centromere positions). Interspecific inversions, relative to RT, are shown with dashed lines e). They include pericentric inversions on Ocl20 and Ocl22, and a paracentric inversion on Ocl29.

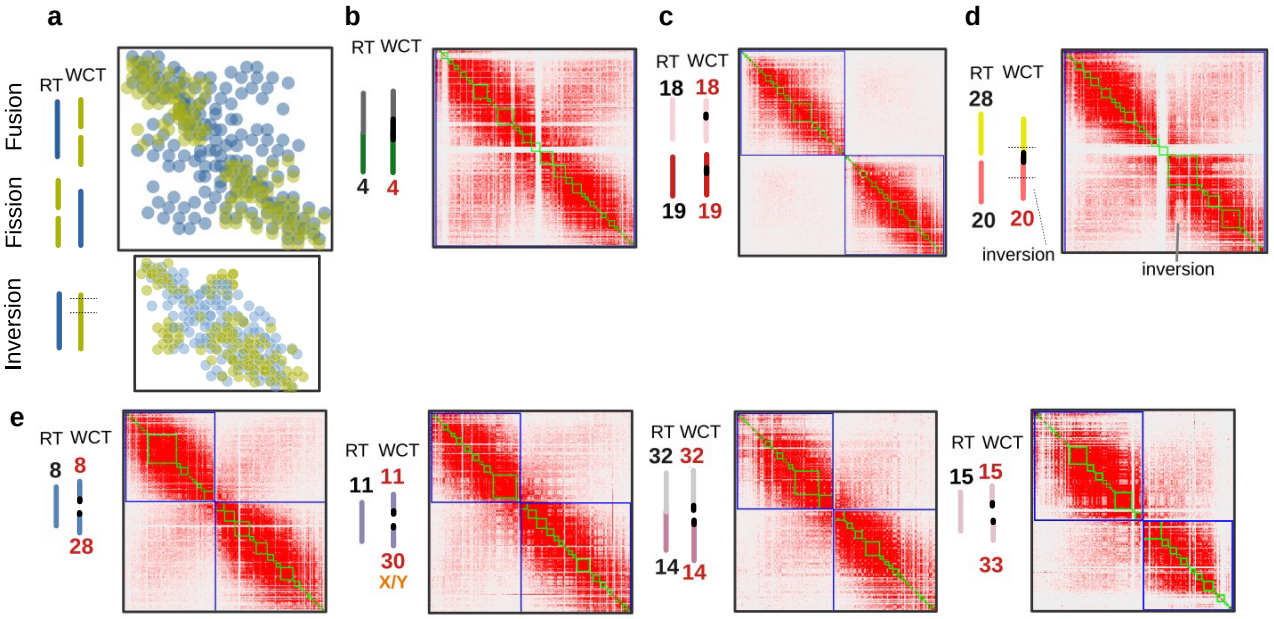

Fig. 3. Verification of fusions, fissions and inversions between rainbow trout (RT) and westslope cutthroat trout (WCT) using WCTxRT hybrid Hi-C data. a) An illustration to demonstrate how Hi-C interaction patterns (right) from 2 different parental chromosomes (left) are visualized in Juicebox as the hybrid chromosome when the parental chromosomes hypothetically have different colors. In this example, RT is represented by a fused chromosome (blue—bottom layer on contact map), and WCT by 2 unfused chromosomes (green—overlapped layer). The density of the points reflects the degree of interaction between sections of a chromosome (a proxy for physical distance between those sections). Where the RT chromosome is fused, there are fewer interactions in the hybrid overall because of the lack of interactions from the WCT unfused chromosomes. A RT fission would have a similar appearance except the colors of the chromosomes would be opposite. An inversion would have high-density areas of contact that are not on the typical diagonal. In practice, Juicebox uses a single color scale, so identifying these features depends entirely on variations of the intensity of the interaction matrix. b) An example in the hybrid where both parental chromosomes are not polymorphic. Chromosome boundaries (exterior/blue boxes) and interior boundaries of contigs (green boxes) are relative to the WCT part of the hybrid assembly. The points (red) represent data from the hybrid (i.e. contributions from both RT and WCT parental chromosomes). c) An example of 2 unfused chromosomes in both parental genomes. d) An example of a fusion in the WCT parental genome and an interspecific inversion. Notice the decreased intensity of the interactions near the center relative to b). e) Examples of fissions in the WCT parental genome.

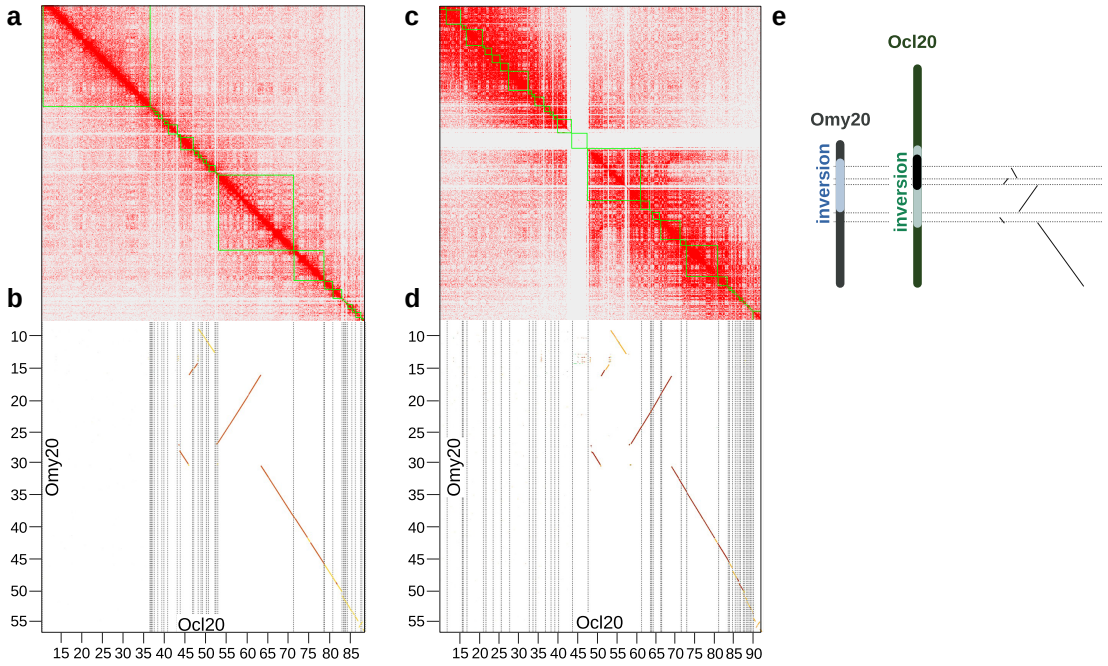

Fig. 4. Ocl20 interspecific chromosomal inversion relative to Omy20. a) Hi-C contact map for part of the WCT Ocl20 (Connor Lake, WCT assembly), with each point representing a contact along the chromosome between locations on the vertical and horizontal axes (~10–90 Mb). Darker points represent more contacts (a proxy for the distance between points; more contacts are expected for locations closer together). Boxes serve as contig boundaries that comprise the Ocl20 chromosome in this region. b) Dot plot of alignments from contigs on the Connor Lake, WCT assembly to the rainbow trout Omy20 (axis units—Mb). Each vertical line symbolizes a contig boundary and the diagonal lines represent the alignment to Omy20. c) Hi-C contact map of the WCTxRT hybrid. Since the WCTxRT hybrid had parental haploid genomes from both species, the Hi-C contact map from the hybrid will have conflicting data where there are differences between species. Contacts from the WCTxRT hybrid in this region are dense in conflicting locations, suggesting that the WCT and RT chromosomes in the hybrid are different from each other (specifically, the contig within the 47–60 Mb interval). This supports the rearrangement identified from alignments to the rainbow trout genome assembly. d) Dot plot of the WCT haplotype-resolved genome assembly (WCT portion of the WCTxRT genome) to Omy20. e) Cartoon depiction of the double interspecific pericentric inversions on Omy20 (RT characterized in Hale *et al.* 2024) and Ocl20 (WCT) with a representation of a simplified alignment dot plot.

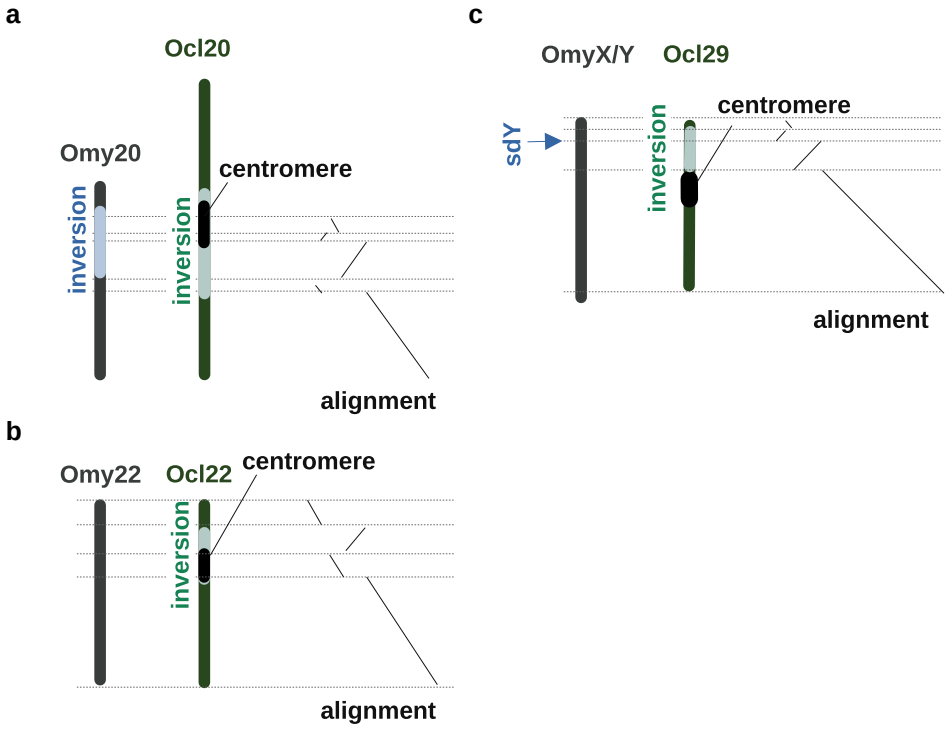

Fig. 5. Cartoon depictions of major interspecific chromosomal inversions. a) Interspecific pericentric inversions on Omy20 (RT) and Ocl20 (WCT) with a representation of a simplified alignment dot plot. b) Interspecific pericentric inversion(s) on Ocl22 (WCT). c) Interspecific pericentric inversion(s) on Ocl29 (WCT). The inferred location of sdY, from the Arlee genome assembly (GCF_013265735.2), is also shown.

assemblies from the F1 hybrid, which was from an individual with unknown sex.

Discussion

The goal of this study was to facilitate modern genetic and genomics techniques by producing genomic resources for WCT. The 3 genome assemblies generated in this study demonstrated high completeness, with BUSCO scores above 98%. The high number of duplicated BUSCOs was consistent with the ancestral whole genome duplication common to salmonids (Allendorf and Thorgaard 1984). The 2 hybrid assemblies had lower contig N50 values compared with the WCT assembly. This could be due to differences in sequencing technologies, different assembly strategies, or differences in sequencing coverage. The chromosome-level assembly of the WCT and the assemblies from the WCTxRT F1 hybrid provide valuable insights into the genomic structure and evolutionary dynamics of cutthroat and rainbow trout.

Comparative analysis between the WCT portion of the hybrid genome, the WCT assembly, and the Swanson RT assembly revealed several distinctive interspecific chromosomal rearrangements. The WCT assembly has 33 haploid chromosomes, consistent with earlier karyotypic studies by Loudenslager and Thorgaard (1979) and the WCT genetic map produced in this study. Despite differences in chromosome number, the number of chromosome arms remains constant, indicating that chromosomal variations are primarily due to fusions or fissions (Thorgaard 1983; Phillips and Rab 2001). The higher chromosome number in WCT compared to the Swanson RT results from the fission of 5 chromosomes. The only fusion in WCT relative to RT was the fusion of Omy20 and Omy28 into Ocl20.

The chromosomal rearrangements in WCT closely resemble those reported by Ostberg *et al.* (2013) in YCT, which belong to the Yellowstone/Southern Rocky Mountain/Upper Snake River evolutionary lineage (Fig. 1). In that study, a genetic map, produced using YCTxRT hybrids, was used to identify several chromosomal rearrangements that differed between the YCT and RT genomes. Here, we compared the genomes of YCT and WCT by mapping the flanking sequences of the microsatellites that were used by Ostberg *et al.* (2013) to generate the YCTxRT genetic map (Rexroad *et al.* 2008; Ostberg *et al.* 2013) onto the WCT genome assembly. The main difference between WCT and YCT is their chromosome number. The YCT genome has 32 haploid chromosomes compared to 33 in the WCT genome (Loudenslager and Thorgaard 1979). This difference is attributed to the absence of a fission of chromosome Ocl08 in YCT.

Chromosomal inversions play a significant role in behavior, local adaptation, and diversification by suppressing recombination, which can drive evolutionary change (Ostberg *et al.* 2013; Wellenreuther and Bernatchez 2018; Pearse *et al.* 2019). The WCT genome has 3 inversions relative to RT (Figs. 2 and 5): a double pericentric inversion (i.e. involving the centromere) on chromosome Ocl20 (these are separate inversions in each species that overlap), a pericentric inversion on Ocl22, and a paracentric inversion on Ocl29. The ~10 Mb inversion on Ocl20 in WCT relative to RT overlaps with the ~14 Mb inversion on Omy20 (Fig. 5) that was previously documented in RT populations (Pearse *et al.* 2019; Campbell *et al.* 2021; Gao *et al.* 2021; Hale *et al.* 2024). Although the 2 inversions overlap, they appear to have originated in separate events as they do not share common start and end positions (Fig. 5). Interestingly, a pericentric inversion of the entire q-arm of Omy20 in YCT was hypothesized as one of the rearrangements that occurred before the Omy20–Omy28 fusion in YCT and WCT (Ostberg *et al.* 2013). Ostberg *et al.* (2013) suggested that this inversion was necessary for the fusion to occur. In rainbow trout, the Omy20 inversion predates the Omy05 inversion and protein-coding genes found within the inversion are associated with growth, reproduction, immune function, and early development (Cádiz *et al.* 2021; Hale *et al.* 2024).

The WCT genome and the hybrid RT genome lack the derived form of the Omy05 inversion previously described in Swanson RT (Pearse *et al.* 2019; Weinstein *et al.* 2019). The Omy05 double inversion spans approximately 55 Mb (Fig. 2—inversion on Swanson) and is associated with adaptive traits such as life-history development (e.g. residency vs anadromy), sexual maturation, and behavior (Sundin *et al.* 2005; Miller *et al.* 2012; Pearse *et al.* 2019; Rundio *et al.* 2021). The absence of the Omy05 inversion in the 2 WCT genomes and the genetic map examined in the current study is likely a consequence of the age of the Omy05 inversion, which occurred after the divergence of rainbow and cutthroat trout (Hale *et al.* 2024).

For the remaining inversions observed in the WCT genome relative to the RT, it is unclear whether they are unique to WCT or shared with the other cutthroat trout lineages. Smaller inversions were identified in the WCT genome relative to the RT, though these were not well supported by Hi-C data. Further research is needed to establish whether these smaller inversions represent true genomic variants or are artifacts of contig misorientation during genome scaffolding.

Chromosome rearrangements are known to suppress recombination events in rainbow and cutthroat trout hybrids (Ostberg *et al.* 2013). In this study, up to 9 WCT chromosomes would be expected to have recombination suppression in an F1 hybrid between WCT and RT. While evidence suggests reduced recombination near polymorphic fusion/fission events, we lack data from post-F1 generations to support this. In a North American Atlantic salmon strain, non-Robertsonian translocations showed recombination suppression near fusions, but polymorphic Robertsonian translocations only exhibited this suppression in 1 out of 10 female genetic maps (MacLeod-Bigley and Boulding 2023). If these rearrangements become common following hybridization, Robertsonian translocations may exhibit infrequent recombination suppression, as seen in Atlantic salmon.

Conclusion

Our study provides new insights into the genomic architecture of cutthroat trout and its divergence from rainbow trout. The unique chromosomal features of WCT, combined with comparisons to previous studies on hybrid trout genomes, enhance our understanding of the evolutionary and adaptive processes shaping these trout species. The 3 genome assemblies and the genetic map offer a valuable resource for future research into the genetics, ecology, and conservation of cutthroat trout and their hybrids with rainbow trout.

Data availability

All WCT sequence data and the genome assembly are associated with the NCBI BioProject: PRJNA1151023. The F1 hybrid and parental DNA sequences are associated with BioProject PRJNA1157974. The hybrid assemblies were deposited as BioProjects PRJNA1174131 and PRJNA1174132. The genetic map is available as supplemental material (Supplementary Files 1 and 2). Pipelines used in this work and which are not published can be

found at https://github.com/ArimaGenomics/mapping_pipeline and https://github.com/phasegenomics/juicebox_scripts.

Supplemental material available at G3 online.

Acknowledgments

We thank Dr. Guangtu Gao with our utmost gratitude. Sadly, Dr. Gao passed away last year. Before his passing, he produced the haplotype-resolved genome assemblies of the WCTxRT hybrid. We would like to take this opportunity to acknowledge his incredible talents and his important contribution to this work and to salmonids genomics research. We thank the Michael Smith Genome Science Centre and their technicians for producing the Hi-C and WGS data. Thanks also to the Digital Research Alliance of Canada (alliancecan.ca) and its regional partner at the University of Victoria (BC DRI Group) for computational resources. We appreciate the extensive help in sample collection that the Freshwater Fisheries Society of BC provided. We thank Vibha Tripathi for her assistance with sequence data submission to NCBI-SRA. We thank Jim Seeb and the Sekokini Hatchery for their help making crosses. We also thank Brooke Penaluna for sharing GIS files to produce the map. Finally, we would like to thank the 2 anonymous reviewers, editors, and staff at G3: Genes | Genomes | Genetics. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US government. USDA is an equal opportunity provider and employer.

Funding

Funding was provided by the Natural Sciences and Engineering Research Council of Canada to BFK and the USDA Agricultural Research Service in house project number 8082-31000-013.

Conflicts of interest

The author(s) declare no conflicts of interest.

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Editor: K. Verhoeven