The potential role of chromosomal inversions in the persistence of small, isolated populations of Brook Trout (*Salvelinus fontinalis*)

Cait M. Nemeczek[1*], M. Lisette Delgado[1], Meg E. Smith[1], John MacMillan[2], Mallory Van Wyngaarden[1+], Daniel E. Ruzzante[1*]

[1] Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada

[2] Inland Fisheries Division, Nova Scotia Department of Fisheries and Aquaculture, Halifax, Nova Scotia, Canada

+ Current address: Fisheries and Oceans Canada, Bedford Institute of Oceanography, Dartmouth, Nova Scotia, Canada

*: Correspondence: cnemeczek@gmail.com and daniel.ruzzante@dal.ca

ORCID numbers
Cait M. Nemeczek https://orcid.org/0009-0009-3245-7295
M. Lisette Delgado https://orcid.org/0000-0001-5710-9961
Daniel E. Ruzzante https://orcid.org/0000-0002-8536-8335
Mallory Van Wyngaarden https://orcid.org/0000-0002-3789-2351

Funding statement: This work was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant award to DER (Award Number: RGPIN-2019-04679) and by grants from the Nova Scotia Department of Fisheries and Aquaculture.

Abstract

Understanding the role of structural variants such as chromosomal inversions in local adaptation among small, isolated populations is an important addition to robust conservation strategies, as most studies investigating inversions to date have been conducted on high gene flow systems. Brook Trout (*Salvelinus fontinalis*), an economically important top sportfish, is extremely vulnerable to thermal stress. Local adaptation with respect to this trait warrants investigation as climate change accelerates the loss of cold-stream ecosystems. We performed low-coverage whole genome sequencing (lcWGS) on N=192 Brook Trout from nine small, isolated streams in Nova Scotia, Canada. Using the indirect structural variant detection framework, we detected four potential chromosomal inversions in the three westernmost populations which differ from all other streams in water temperature, streamflow, and surficial geology. These genomic regions exhibited high linkage disequilibrium (LD) and principal component analyses (PCA) revealed the presence of three karyotypes (inverted and non-inverted homokaryotype, and heterokaryotype). Heterozygosity was lowest among inverted homokaryotypes, providing further support of the presence of inversions. Mitogenome analyses suggest that a single glacial lineage recolonized the region. The mtDNA haplotypes of individuals carrying potential inversions were shared among individuals with two copies of non-inverted chromosomal regions, suggesting these inversions were derived post-recolonization. These novel inversions comprised genes involved in different biological processes including thermal adaptations.

Keywords: structural variants (SVs), chromosomal inversions, adaptation, intraspecific variation.

Introduction

Genetic diversity is important and chromosomal structural variants are an important component of diversity

Genetic diversity is an essential component of a species or population's ability to adapt to changing environmental conditions. Accordingly, it is included in the Convention on Biological Diversity (Hoban et al., 2022) but is not yet at the forefront of goals set by the Convention (Hoban et al., 2020; Laikre et al., 2020). Conservation management often focuses on the species level, excluding the fine-grained intraspecific variation found within and among populations. Local adaptation or phenotypic plasticity among populations, for example, can allow populations to persist in rapidly changing environments (Des Roches et al., 2018, 2021). Many recent studies highlight the importance of intraspecific variation (see Lujan et al., 2022; Shaney et al., 2020; von Takach et al., 2024) while few have begun to incorporate it into conservation management through conservation units (CUs) and designatable units (DUs) but see Lehnert et al. (2023) and Waples et al. (2022). Genomic structural variants (SVs) including inversions, copy number variants, and transposable elements are known to contribute significantly to the genetic variation observed within species (Dorant et al., 2020; Mérot et al., 2023; Wellenreuther et al., 2019; Wellenreuther & Bernatchez, 2018) and are thus a critical component of intraspecific variation.

Chromosomal inversions (CIs) are a type of SV that occur when part of the chromosome breaks, and the orientation of that segment becomes reversed (Kirkpatrick, 2010). Research on CIs, has uncovered frequent and sometimes large chromosomal inversions with an average size of 8.4 megabases across species (An et al., 2024; Harringmeyer & Hoekstra, 2022; Wellenreuther & Bernatchez, 2018). When an inversion develops, it evolves by natural selection and over time, genetic drift, mutations, and recombination will have varying effects that can cause changes in the inversion arrangement frequencies leading to the maintenance, fixation, or removal of inversions (Faria, Johannesson, et al., 2019). Inversions can play a large role in local adaptation and speciation which comes primarily from the suppressed recombination that takes place in heterokaryotypes carrying one copy of the inverted and one of the non-inverted sequence arrangements. In some cases, inversions can reach fixation by selection or drift leading to speciation for example if heterokaryotypes are selected against because of epistatic interactions. They can also be maintained by balancing selection leading to polymorphic inversions among populations (Faria, Johannesson, et al., 2019; Hoffmann & Rieseberg, 2008; Kirkpatrick, 2010). Recent research on seahorses (Meyer et al., 2024) and stick insects (Nosil et al., 2023) highlight situations in which inversions evolve and are maintained by factors including balancing selection. The role of inversions in diversification and local adaptation has also been well documented in monkey flowers where they are responsible for annual and perennial ecotypes (Twyford & Friedman, 2015), common quails where a large inversion controls phenotypic differences in size, throat colouration, wings, migration, and flight efficiency (Sanchez-Donoso et al., 2022) and in differences in migratory behavior in Atlantic Cod (Matschiner et al., 2022) as

well as more broadly in adaptation to environmental differences in willow warbler (Lundberg et al., 2023), and Atlantic Herring (Fuentes-Pardo et al., 2024).

Inversions can be detected using a direct SV detection approach (Mérot et al., 2020) or an indirect detection approach (Faria, Chaube, et al., 2019; Mérot et al., 2021). The indirect approach involves the examination of patterns of linkage disequilibrium (LD) by chromosome, principal components analysis (PCA), estimation of genetic differentiation with F_{ST}, and an examination of patterns of heterozygosity within the groups identified in the PCA. As limited recombination is indicative of a potential inversion, LD patterns are the first step of this method. A PC plot across individuals is expected to show three distinct groups; the middle comprised of heterokaryotypes for the inversion, while left and right groups will either be homokaryotypes with or without inversions as seen in many studies (Huang et al., 2020; Nosil et al., 2023; Reeve et al., 2023). Heterozygosity is also used as supporting evidence for potential inversions as heterozygous individuals will have one copy of the inverted arrangement and thus have higher heterozygosity than homokaryotypes.

Inversions have been discovered in several salmonid species including Lake Trout (Smith et al., 2020), Arctic Charr (Hale et al., 2021), Rainbow Trout (Pearse et al., 2019), and Chum Salmon (McKinney et al., 2020). However, studies of local adaptation and chromosomal inversions in Brook Trout are relatively scarce (Brookes et al., 2022; Sutherland et al., 2016). Brook Trout, an economically important sportfish, are vulnerable to habitat loss, overexploitation, and invasive species as well as to climate change leading to decreased streamflow and concomitant increases in water temperature, a particularly challenging combination given the species' narrow thermal tolerance (Cherry et al., 1977; MacMillan et al., 2008; Nova Scotia Department of Agriculture and Fisheries Inland Fisheries Division, 2005). Here we have examined Brook Trout populations inhabiting small streams along the North Mountain region of Nova Scotia, all of which drain into the Bay of Fundy. These populations are largely isolated (Ruzzante et al., 2016), they thus provide a unique scenario to examine the role of inversions in pristine populations exhibiting limited to no gene flow (Ruzzante et al., 2016). Unlike other inversion studies on species with high gene flow and low levels of genetic differentiation such as Atlantic Cod (Matschiner et al., 2022) and Atlantic Silversides (Akopyan et al., 2022) these Brook Trout populations have small N_e and thus are more impacted by genetic drift (Ruzzante et al., 2016, 2019) than the former adding to the uniqueness of this study system.

Knowledge of potential inversions and their role in local adaptation could improve our understanding of Brook Trout population decline and help build a basis for new conservation efforts, building on the current use stocking in Nova Scotia to maintain angling opportunities (Lehnert et al., 2020). As such, here we used lcWGS to examine genetic diversity among nine isolated small Brook Trout populations. We aim to improve our understanding of how SVs promote and maintain intraspecific diversity. Using whole mitogenome data, we aim to determine whether the pattern of inversion discovery can be explained by post-glacial dispersion history. We also explore genes within potential inversion regions and suggest possible roles in local adaptation.

Materials and Methods

Sampling and Environmental Data

Brook Trout fin clip samples were collected from nine streams along the North Mountain (Nova Scotia) in June 2021 (N=40 individuals per stream, total N=360) and stored in 95% ethanol (Fig 1). These streams differ in environmental and physical characteristics such as surficial geology, drainage area, stream gradient, and pH (Table S1, S2). HOBO pendent temperature loggers were installed and kept (April-November 2021) on streambeds of five of the nine streams recording temperature every two hours and maximum daily water temperatures calculated (Figure S6).

DNA Extraction, Low-Coverage Whole-Genome (lcWGS) Sequencing and Quality Control

DNA was extracted from N=21-22 individuals per stream using the Omega E.Z.N.A tissue DNA kit (Omega Bio-Tek) following the manufacturer's protocol. DNA was quantified using the Quanti-iT Picogreen dsDNA assay (Invitrogen). Six libraries were prepared for lcWGS with a target depth of 3X following the Illumina DNA Prep protocol with modifications of the Illumina Nextera-Flex protocol, Hackflex (Gaio et al., 2022), multiplexed library prep from Baym et al. (2015), and the protocol from Therkildsen and Palumbi (2017). The six pooled library of 192 individuals was sequenced on four lanes of paired-end 2x 150bp reads on a NovaSeq S4 flowcell (Illumina) at The Centre for Applied Genomics (The Hospital for Sick Children, Toronto Ontario). Raw fastq files were assessed using FastQC v.0.11.9 (Andrews, 2010) and MultiQC v.1.11 (Ewels et al., 2016) and adapters removed with Trimmomatic v.0.39 (Bolger et al., 2014) in paired end mode. Fastp v.0.23.1 (S. Chen et al., 2018) was used to remove poly-G tails.

Genome Mapping and Quality Filtering

Filtered reads were mapped to the Brook Trout (*Salvelinus fontinalis*) genome ASM2944872v1 (GenBank assembly accession: GCA_029448725.1) and reference mitogenome (NCBI Reference Sequence: NC_000860.1) with Bowtie2 v.2.4.4 (Langmead & Salzberg, 2012). The indices of the reference genome and dictionary were generated using SAMtools v.1.13 (Li et al., 2009) and Picard tools v.2.26.3 ("Picard Toolkit," 2019). Aligned BAM files were filtered with SAMtools v.1.13 (Li et al., 2009) to remove discordantly mapped pairs and those with mapping quality scores below 20 before being merged. Picard tools v.2.26.3 ("Picard Toolkit," 2019) was used to identify and remove PCR and optical duplicate reads and overlapping paired reads were removed using Bamutils v.1.0.14 (Jun et al., 2015). Filtered reads were aligned around indels with GATKs v.3.7 (McKenna et al., 2010). Pilon v.1.24 (Walker et al., 2014) was used to detect and correct residual errors in the aligned mitogenomic sequences. Average read depth was calculated by stream (population) using SAMtools v.1.17 (Li et al., 2009). Average depth per stream for lcWGS data across 21-22 individuals was 2.2x-3x**.** Average depth per stream for mitogenomic samples was 391.4x-2484x.

SNP Calling and Genotype Likelihoods

Scripts used are available on GitHub (https://github.com/cnemeczek). ANGSD v.0.940 (Korneliussen et al., 2014) -doDepth was used to get depth counts at every position for all populations together and populations individually and plotted as histograms in R v.4.3.0 (R

Studio Team, 2021) to select appropriate maximum and minimum depths. These parameters were used to estimate genotype likelihoods and allele frequencies. Global genotype likelihoods were calculated using the following parameters -uniqueOnly 1, -remove_bads 1, -only_proper_pairs 1, -trim 0, -C 50, -baq 1, -minInd 96, -minMapQ 20, -minQ 20, -setMinDepth 474, -setMaxDepth 676, -doCounts 1, -GL 1, -doGLF 2, -doMajorMinor 1, -doIBS 1, -doCov 1, -makeMatrix 1, -doMaf 1, -SNP_pval 1e-6, -minMaf 0.05. SAMtools identified 2,620,282 SNPs from individuals aligned to the Brook Trout genome. The covariance matrices produced by ANGSD were used to perform a PCA in R v.4.1.2 (R Studio Team, 2021) with ggplot2 v.3.3.5 (Wickham, 2016), dplyr v.1.0.7 (Wickham et al. 2021), and tidyverse v.1.3.1 (Wickham et al. 2019). Eigenvectors and values were calculated from the covariance matrices and used to plot principal components explaining the highest percentage of variation in the SNP data. The allele frequencies and genotype likelihood files produced from running ANGSD with a supplied SNP list were used for the estimation of LD.

Linkage Disequilibrium

LD was calculated with ngsLD v.1.1.1 (Fox et al., 2019) using a -max_kb_dist of 100 and the decay rate determined as 15kb. LD was calculated again using -max_kb_dist of 15kb and SNPs in LD were pruned using ngsLD's prune_graph.pl resulting in 391,154 SNPs. For pruning, the minimum weight for assuming SNPs were connected based on r^2 values (--min_weight) was set to 0.4 based on the LD decay plot and was done by chromosome.

The existence of potential inversions was explored on a chromosome by chromosome basis. ngsLD v.1.1.1 (Fox et al., 2019) was used to calculate LD for each chromosome using –max_kb_dist 0 and –max_snp_dist of 0 while randomly sampling 50% of SNPs. The r^2 values from the LD data were divided into percentiles and plotted as heatmaps in R v.4.0.2 (RStudio Team, 2021) using ggplot2 v.3.3.6 (Wickham, 2016) following scripts from Mérot et al. (2021). Heatmaps of each chromosome were used to find areas of high LD (r^2 above ~0.6) and genotype likelihoods were calculated using ANGSD on SNPs only in those regions. PCAs for chromosomes with high LD were produced using ggplot2 v.3.3.6 (Wickham, 2016) in R v.4.0.2 (RStudio Team, 2021).

A subset of each of chromosomes 12, 19, 27, 31 (CM055694.1, CM055701.1, CM055709.1, CM055713.1) from the Brook Trout genome was chosen for further analyses based on the size of blocks of LD. LD was calculated for all nine populations pooled and for the six easternmost populations pooled (ie. excluding Poole, Healeys, Sheep Shearer) using ngsLD v.1.1.1 (Fox et al. 2019) and heatmaps were produced as described above.

Heterozygosity

ANGSD v.0.940 was used to calculate allele frequencies and Hardy-Weinberg equilibrium with -doHWE on individuals representing the three potential inversion groups for each of Brook Trout chromosomes 12, 19, 27, and 31 using SNP lists for entire chromosomes. Observed heterozygosity (H_{obs}) was calculated followed by average H_{obs} in 10kb windows with a 10kb slide using the R package WindowScanR (Tavares, 2022) across entire chromosomes following scripts from (Mérot et al., 2021). H_{obs} was also calculated within potential inverted regions and average H_{obs} calculated in windows the size of potential inversion regions. Boxplots were generated using R v.4.0.2 (RStudio Team, 2021) and ggplot2 v.3.3.6 (Wickham, 2016).

mtDNA Haplotypes and Phylogeny

Mitogenomic sequences from Pilon (Walker et al., 2014) were loaded into Geneious Prime 2023.2.1 (https://www.geneious.com) and aligned using the MUSCLE alignment tool. Thirty haplotypes were identified and extracted using the "Find Duplicates" function and named by frequency. A neighbour-joining tree was constructed using Geneious Prime 2023.2.1 with default parameters and bootstraps were calculated with 100,000 replicates. The Lake Trout (*Salvelinus namaycush*) reference mitogenome (NCBI Reference Sequence: NC_ 036392.1) was used as the outgroup. A median-joining haplotype network was constructed using PopArt v.1.7 (J. W. Leigh & Bryant, 2015) run with an epsilon value of 0.

Gene Annotation

The UCSC genome browser (Kent et al., 2002) was used to identify genes within the potential inverted regions on chromosomes 12, 19, 27, and 31 of the Brook Trout genome. The resulting gene table was sorted in R v.4.3.3 (RStudio Team, 2021) to search for unique gene symbols. The Brook Trout genome is not fully annotated and as such resulting loci not corresponding to any genes were removed from further analyses. Lists of gene symbols were searched in Metascape v.3.5 (Zhou et al., 2019) with input species as "any" and analysis as "human" to find GO terms of biological processes, pathways, and protein interactions.

Results

Genome Mapping ANGSD SNP Calling/Genotype Likelihoods

Target sequencing depth per individual was 3x and the average read depth per stream (population) after filtering out low quality reads was 2.2x-3x (Fig S1). Principal component analysis representing genotype likelihoods from more than 2.6 million SNPs show individuals for 8 of the 9 populations, grouped by population and surficial geology type. Brook Trout from "groups 1 and 2" are distinguishable from Brook Trout in group 3 along PC1 (8.21% of total variance). The 9th population, Poole Brook is distinguishable from the two other populations in its substrate group along PC2 (5.93 % of variation) and from the two other groups along both axes (Fig 2).

Potential Inversion Discovery and Support

Linkage Disequilibrium

Heatmaps of LD for all populations pooled show multiple blocks of LD ($r^2 > 0.60$) with distinct blocks seen on each of four chromosome 12, 19, 27, and 31 (CM055694.1, CM055701.1, CM055709.1, CM055713.1) (Fig S2). All four LD blocks are larger than 10 Mb (Table 1). The patterns of large LD blocks disappear when Brook Trout from the three westernmost populations (Healeys, Sheep Shearer, Poole brooks) are excluded from the analysis (Fig 3).

Genotype Likelihoods for Potential Inversion Regions

PCA of chromosomes 12, 19, 27, and 31 show 3 groups with individuals clustering as homokaryotypes with and without potential inversions on the left and right side and heterokaryotypes in the middle (Fig 4). For all four chromosomes, the number of individuals

with the potential inversion is ≤ 8 and most individuals are homokaryotypes without potential inversions. On chromosome 12 Brook Trout are found in each of the 3 groups in Sheep Shearer and Healeys but not in Poole (Fig 4a). This indicates that both Sheep Shearer and Healeys have Brook Trout that are homozygous for the potential inverted and non-inverted arrangement as well as heterozygous Brook Trout containing a copy of each. A similar pattern is seen on chromosome 19 (Fig 4b). Brook Trout are found in each of the 3 groups in Poole and Healeys but not in Sheep Shearer (Fig 4b). On chromosomes 27 and 31 only Brook Trout from Healeys brook are found in each of the 3 groups suggesting Brook Trout from Healeys are homozygous for the potential inversion and non-inverted arrangements and heterozygous for potential inversion arrangements. (Fig 4c,d).

Heterozygosity

The potential inversions breakpoints were more precisely estimated from plotting H_{obs} across entire chromosomes (Fig S3) and used to determine H_{obs} within potential inversion regions. Average H_{obs} within potential inversion regions on each of the four chromosomes is highest among heterozygous individuals for the inversion as these individuals have one copy of the non-inverted arrangement and one copy of the potentially inverted arrangement, intermediate among individuals without the potential inversions with two copies of the non-inverted arrangement and lowest among individuals with two copies of the potential inversion arrangement (Fig 5).

Haplotype Diversity

Thirty mtDNA haplotypes were identified in this system and the median-joining haplotype network showed 29 of these 30 radiating from a single node by 1-7 mutational steps (Fig 6), suggesting they share recent common ancestry or lineage. Individuals with potential inversions were identified in haplotypes 2, 3, 6, 10, and 14 (Fig 6). These haplotypes contain homokaryotypes with and without potential inversions suggesting the potential inversions were derived after colonization (Fig 6).

Gene Annotation

Two hundred and thirteen genes and biological processes/pathways were identified among the four potential inversion regions (Table S3, S4). The inversion in chromosome 31 is the largest containing 110 different genes and 13 GO terms (Table S4, S5). In comparison, the smallest inversion in chromosome 19 contained only 12 genes and no GO terms were identified (Table S3, S4). Chromosomes 12 and 27 are of similar size and four GO terms were identified in each while chromosome 12 contains 66 genes and chromosome 27 contains 25 genes (Table S3, S4).

Water Temperature

Amongst five of nine streams that temperature was recorded, Healeys and Poole brook (westernmost streams) consistently had higher maximum daily water temperatures with Healeys reaching the highest while Ross Creek (easternmost) consistently had the lowest temperatures (Figure S6).

Discussion

Four large potential chromosomal inversions representing 2.72% of the Brook Trout genome were discovered among western populations Brook Trout as assessed by the indirect detection method of SVs. All Brook Trout originate from one lineage and some genes within potential inversion regions likely have an adaptive function. LD patterns are similar among chromosomes 12, 19, 27, and 31 showing individuals from western streams exhibiting large LD blocks while those in the east do not (Fig 3). PCAs of potential inversion regions show the three groups suggestive of heterokaryotypes, and homokaryotypes with and without potential inversions where H_{obs} is lower among individuals with potential inversions (Fig 4,5). The low frequency of individuals with potential inversions from only the western populations suggests that the potential inversions are rare. Below, we first consider some caveats with our approach and then discuss the implications of our study in detail.

While the use of whole genome sequencing has facilitated the detection of SVs including chromosomal inversions (Liu et al., 2021; Waldbieser et al., 2023), the use of indirect detection methods including the use of high LD regions can be biased towards the preferential consideration of large genomic regions as large SVs will hold more SNPs that could be adaptive and have more impact on frequency, establishment, and persistence than small regions (Hale et al., 2021; Wellenreuther & Bernatchez, 2018). While the size of potential inversions can affect their evolution, N_e may also play a role and should be considered when using this technique (Connallon & Olito, 2022) where drift can lead to false positive detection of adaptive loci (Leigh et al., 2021). High levels of LD can also be evidence of other types of SVs such as copy number variants and fusions, and in addition could be the result of other factors such as selective sweeps (Mérot et al., 2020). In the present study, however, further support for inversions arises from the relatively low H_{obs} exhibited by both homokaryotypes. Long read sequencing would need to be performed, however, to confirm these are indeed chromosomal inversions (Mérot et al., 2020; Warburton & Sebra, 2023). In addition, gene expression and common garden experiments (Bernatchez et al., 2024; Hämälä et al., 2021; Ma et al., 2024; Thomas et al., 2022) can help identify the role of genes within potential inversions or if there is evidence of phenotypic plasticity affecting local adaptation which is possible given past research on Brook Trout in Newfoundland (Wood et al., 2014, 2016; Wood & Fraser, 2015).

Chromosomal inversions and species identification

Our research adds to the growing understanding of inversions among salmonid species and provides new findings specific to Brook Trout. Inversions were discovered among Brook Trout linkage groups (LG) using RADseq (Sutherland et al., 2016) and research on the Lake Trout linkage map and genome (Smith et al., 2020, 2022) highlights synteny between Lake Trout inversions with other salmonids, however to our knowledge, this study provides the first evidence of potential inversions with a full genome assembly of Brook Trout. Sutherland et al. (2016) identified a potential inversion on LG03- chromosome 3 in a genetic map produced from a Laval River, Québec female, a domestic Québec aquaculture strain male, and their offspring, however in our populations in North Mountain, Nova Scotia chromosome 3 did not show evidence of any large LD block (Fig S2). Recent work suggests that inversions differentiating Brook Trout from Lake Trout are found on Brook Trout's LG 09, 25, and 35 corresponding to Lake Trout chromosomes 12, 23, and 28 (Smith et al., 2020; Sutherland et al., 2016). Heatmaps

of LD show small blocks on Brook Trout chromosomes 9 and 25 (Fig S2) which supports the finding of Smith et al. (2020), however our heterozygosity estimates do not support the hypothesis that these are potential inversions (data not shown). Lake Trout chromosome 24 has inversions that are known to be diagnostic between Arctic Charr, Rainbow Trout, and Atlantic Salmon. The potential inversion we found on Brook Trout chromosome 31 is, however, polymorphic and not diagnostic for species differentiation with Lake Trout chromosome 24 (Smith et al., 2020). Lake Trout chromosome 11 is known to be polymorphic with Brook Trout and there is evidence of higher LD in an area on the corresponding chromosome 22 of the Brook Trout genome (Fig S2).

Did the inversions originate post-colonization?

The presence of the inversions only in the western populations suggests that deglaciation of the North Mountain, Nova Scotia and colonization history might have influenced this pattern. Different patterns of ice advance and retreat took place during the late Wisconsinan phase approximately 14,000-10,000 years before present (Stea et al., 1998). During this period, different marine incursion and recession events generated a post-glacial history that led the western streams (Healeys, Sheep Shearer, Poole) to deglaciate first (Shaw et al., 2006; Stea, 2004, John Gosse, personal communications, November 2022). Glaciation and postglacial colonization history play a role in population distribution and structure among fishes (Bernatchez & Wilson, 1998; Ruzzante et al., 2020) as has also been seen in Brook Trout (Ferchaud et al., 2020; Pilgrim et al., 2012). In addition, eastern Canada exhibits evidence of having been colonized by Brook Trout originating from Atlantic and Acadian refugia (Danzmann et al., 1998) but mtDNA evidence suggests that Brook Trout from New Brunswick and Cape Breton, Nova Scotia originate from a single lineage (Jones et al., 1996). To our knowledge, this is the first time Brook Trout from the North Mountain are included in mtDNA analyses and the evidence we report in the present study suggests they originate from a single lineage as well (Fig 6). The group of individuals carrying the potential inversions exhibits five different mtDNA haplotypes, but these haplotypes are shared with individuals that do not exhibit the potential inversions, suggesting the inversions took place post-colonization. Lower H_{obs} among homokaryotypes with potential inversions than homokaryotypes without them (Fig 5) also provides support for the hypothesis that the potential inversions are likely the derived state as we expect a younger arrangement to have relatively low diversity due to shorter time for the accumulation of mutations (Faria, Johannesson, et al., 2019).

Chromosomal inversions and local adaptation

The potential inversions among western populations likely contribute to local adaptation due to advantageous genes within the inverted regions. Two hundred and thirteen genes were identified within the four potential inversions and gene annotation suggests some key pathways of potential adaptive value such as response to hypoxia and response to oxidative stress (Table S5,6). Of particular interest was the discovery of the cerkl gene (ceramide kinase like) on chromosome 19. The cerkl protein is related to cerk (ceramide kinase) both of which have a role in the metabolism of signaling lipids, however cerkl does not phosphorylate ceramide (Bornancin et al., 2005; Chalfant & Spiegel, 2005). The cerk gene has been linked to thermal adaptation in Rainbow Trout due to its role in metabolic and wound-healing rates (Willoughby et al., 2018). In addition, cerk's role in stress related signaling pathways suggests it might be involved in cardiovascular function from reduced oxygen at higher water temperatures and heat

shock response in Redband Trout (Z. Chen & Narum, 2021). Maximum daily water temperatures in Healeys and Poole brook where potential inversions were found in our study were highest, sometimes exceeding 20°C (Fig S6) which approaches Brook Trout's upper thermal limit (MacMillan et al., 2008). While cerk has been studied among salmonids, cerkl's role is less clear, however it is known to play a role in stress-induced apoptosis and is regulated during oxidative stress such as hypoxia among cells involved in retinal degeneration (Tuson et al., 2009). We do not know cerkl's specific role among fishes suggesting an important area of research. Another gene of interest is the pdhx gene pyruvate dehydrogenase complete component x on chromosome 12, which plays a role in carbohydrate metabolism and has also been found within an inversion in Walleye (Thorstensen et al., 2022). Although its function was not explored in the Walleye study (Thorstensen et al., 2022), pdhx was found to be one of six genes with a potential effect on hyperthermia resistance in reared rainbow trout (Lagarde et al., 2023) suggesting some adaptive advantage. The potential inversion on chromosome 27 contains sesn1 (sestrin 1), a gene linked to potential stress response in Atlantic Salmon and Rainbow Trout (Kousoulaki et al., 2015; Syahputra et al., 2020). While we did not directly assess the potential of adaptation, some genes found within potential inversion regions might be important and should be further assessed.

The finding of four potential inversions in only westerns streams is critical to our understanding of intraspecific population variation and conservation of such populations. Recent research on Brook Trout in Nova Scotia showed that there is limited introgression between stocked and wild populations and that there is strong population structuring (Lehnert et al., 2020). Highly structured populations are very common among Brook Trout in other areas of its native range (Castric et al., 2001; Erdman et al., 2022; Morgan II et al., 2021). Brook Trout have historically been stocked in Nova Scotia to sustain fisheries and restore populations (Nova Scotia Department of Agriculture and Fisheries Inland Fisheries Division, 2005) and while Lehnert et al. (2020) found limited introgression, there was evidence for some in the Margaree River system in which a locally derived strain is used, and they suggested that these individuals likely have an adaptive advantage. Other research also finds limited hatchery introgression with wild populations (Erdman et al., 2022; White et al., 2018) but recent research shows introgression can be affected by habitat (Bruce et al., 2020). If populations of Brook Trout in Nova Scotia are locally adapted as evidence in the present study suggests is likely, then introgression from stocking could become problematic for intraspecific population variation especially when they have small N_e such as in the current study system (Ruzzante et al., 2016) as locally adapted genetic diversity could be reduced by admixture (Ryman & Laikre, 1991). This study also has implications at a broader scale in terms of the designation of CUs and DUs representing intraspecific population variation. While Brook Trout are not listed under the Species at Risk Act (SARA), our study provides additional reasoning for the incorporation of intraspecific genomic-based adaptation such as SVs in delineating DUs which could promote maintenance of genetically diverse populations. Other research on salmonids have already begun to assess how for example, large effect loci could or could not be used to designate CUs among Chinook Salmon and Steelhead (Waples et al., 2022). In addition, recent research has used evidence of genomic-based adaptation such as SVs to designate DUs in eastern Canada for anadromous Atlantic Salmon (Lehnert et al., 2023).

In conclusion, this study provides evidence of four potential inversions that are unique to western populations of Brook Trout in the North Mountain region of Nova Scotia. Understanding the role of these inversions and intraspecific variation can provide an important avenue of research for

conservation of Brook Trout. More effort could be placed on understanding how inversions could be advantageous to Brook Trout, a thermally sensitive species that faces challenges due to ongoing temperature increases in their habitats here in Nova Scotia (MacMillan et al., 2008).

Acknowledgements

We thank Sam Andrews for his help conducting backpack electrofishing and sampling of Brook Trout. We also thank Claire Mérot for her guidance and advice on the use of her scripts for summarizing LD and calculating H_{obs}.

References

Akopyan, M., Tigano, A., Jacobs, A., Wilder, A. P., Baumann, H., & Therkildsen, N. O. (2022). Comparative linkage mapping uncovers recombination suppression across massive chromosomal inversions associated with local adaptation in Atlantic silversides. *Molecular Ecology*, *31*(12), 3323–3341. https://doi.org/10.1111/mec.16472

An, X., Mao, L., Wang, Y., Xu, Q., Liu, X., Zhang, S., Qiao, Z., Li, B., Li, F., Kuang, Z., Wan, N., Liang, X., Duan, Q., Feng, Z., Yang, X., Liu, S., Nevo, E., Liu, J., Storz, J. F., & Li, K. (2024). Genomic structural variation is associated with hypoxia adaptation in high-altitude zokors. *Nature Ecology & Evolution*, 1–13. https://doi.org/10.1038/s41559-023-02275-7

Andrews, S. (2010). *FastQC: A quality control tool for high throughput sequence data* [Computer software]. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Baym, M., Kryazhimskiy, S., Lieberman, T. D., Chung, H., Desai, M. M., & Kishony, R. (2015). Inexpensive Multiplexed Library Preparation for Megabase-Sized Genomes. *PLOS ONE*, *10*(5), e0128036. https://doi.org/10.1371/journal.pone.0128036

Bernatchez, L., Ferchaud, A.-L., Berger, C. S., Venney, C. J., & Xuereb, A. (2024). Genomics for monitoring and understanding species responses to global climate change. *Nature Reviews Genetics*, *25*(3), 165–183. https://doi.org/10.1038/s41576-023-00657-y

Bernatchez, L., & Wilson, C. C. (1998). Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, *7*(4), 431–452. https://doi.org/10.1046/j.1365-294x.1998.00319.x

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bornancin, F., Mechtcheriakova, D., Stora, S., Graf, C., Wlachos, A., Dévay, P., Urtz, N., Baumruker, T., & Billich, A. (2005). Characterization of a ceramide kinase-like protein. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1687*(1), 31–43. https://doi.org/10.1016/j.bbalip.2004.11.012

Brookes, B., Jeon, H.-B., Derry, A. M., Post, J. R., Rogers, S. M., Humphries, S., & Fraser, D. J. (2022). Neutral and adaptive drivers of genomic change in introduced brook trout (Salvelinus fontinalis) populations revealed by pooled sequencing. *Ecology and Evolution*, *12*(2), e8584. https://doi.org/10.1002/ece3.8584

Brown, C. (2018). *na.tools: Comprehensive Library for Working with Missing (NA) Values in Vectors*. https://CRAN.R-project.org/package=na.tools

Bruce, S. A., Kutsumi, Y., Van Maaren, C., & Hare, M. P. (2020). Stocked-Fish Introgression into Wild Brook Trout Populations Depends on Habitat. *Transactions of the American Fisheries Society*, *149*(4), 427–442. https://doi.org/10.1002/tafs.10239

Castric, V., Bonney, F., & Bernatchez, L. (2001). LANDSCAPE STRUCTURE AND HIERARCHICAL GENETIC DIVERSITY IN THE BROOK CHARR, SALVELINUS FONTINALIS. *Evolution*, *55*(5), 1016–1028. https://doi.org/10.1111/j.0014-3820.2001.tb00618.x

Chalfant, C. E., & Spiegel, S. (2005). Sphingosine 1-phosphate and ceramide 1-phosphate: Expanding roles in cell signaling. *Journal of Cell Science*, *118*(20), 4605–4612. https://doi.org/10.1242/jcs.02637

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, *34*(17), i884–i890. https://doi.org/10.1093/bioinformatics/bty560

Chen, Z., & Narum, S. R. (2021). Whole genome resequencing reveals genomic regions associated with thermal adaptation in redband trout. *Molecular Ecology*, *30*(1), 162–174. https://doi.org/10.1111/mec.15717

Cherry, D. S., Dickson, K. L., Cairns Jr., J., & Stauffer, J. R. (1977). Preferred, Avoided, and Lethal Temperatures of Fish During Rising Temperature Conditions. *Journal of the Fisheries Research Board of Canada*, *34*(2), 239–246. https://doi.org/10.1139/f77-035

Connallon, T., & Olito, C. (2022). Natural selection and the distribution of chromosomal inversion lengths. *Molecular Ecology*, *31*(13), 3627–3641. https://doi.org/10.1111/mec.16091

Danzmann, R. G., Morgan II, R. P., Jones, M. W., Bernatchez, L., & Ihssen, P. E. (1998). A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook trout (Salvelinus fontinalis): Distribution and postglacial dispersal patterns. *Canadian Journal of Zoology*, *76*(7), 1300–1318. https://doi.org/10.1139/z98-056

Des Roches, S., Pendleton, L. H., Shapiro, B., & Palkovacs, E. P. (2021). Conserving intraspecific variation for nature's contributions to people. *Nature Ecology & Evolution*, *5*(5), 574–582. https://doi.org/10.1038/s41559-021-01403-5

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology & Evolution*, *2*(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5

Dorant, Y., Cayuela, H., Wellband, K., Laporte, M., Rougemont, Q., Mérot, C., Normandeau, E., Rochette, R., & Bernatchez, L. (2020). Copy number variants outperform SNPs to reveal genotype–temperature association in a marine species. *Molecular Ecology*, *29*(24), 4765–4782. https://doi.org/10.1111/mec.15565

Dowle, M., & Srinivasan, A. (2021). *data.table: Extension of `data.frame`*. https://CRAN.R-project.org/package=data.table

Erdman, B., Mitro, M. G., Griffin, J. D. T., Rowe, D., Kazyak, D. C., Turnquist, K., Siepker, M., Miller, L., Stott, W., Hughes, M., Sloss, B., Kinnison, M. T., Wilson, C. C., & Larson, W. (2022). Broadscale Population Structure and Hatchery Introgression of Midwestern Brook Trout. *Transactions of the American Fisheries Society*, *151*(1), 81–99. https://doi.org/10.1002/tafs.10333

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Faria, R., Chaube, P., Morales, H. E., Larsson, T., Lemmon, A. R., Lemmon, E. M., Rafajlović, M., Panova, M., Ravinet, M., Johannesson, K., Westram, A. M., & Butlin, R. K. (2019). Multiple chromosomal rearrangements in a hybrid zone between Littorina saxatilis ecotypes. *Molecular Ecology*, *28*(6), 1375–1393. https://doi.org/10.1111/mec.14972

Faria, R., Johannesson, K., Butlin, R. K., & Westram, A. M. (2019). Evolving Inversions. *Trends in Ecology & Evolution*, *34*(3), 239–248. https://doi.org/10.1016/j.tree.2018.12.005

Ferchaud, A.-L., Leitwein, M., Laporte, M., Boivin-Delisle, D., Bougas, B., Hernandez, C., Normandeau, É., Thibault, I., & Bernatchez, L. (2020). Adaptive and maladaptive genetic diversity in small populations: Insights from the Brook Charr (Salvelinus fontinalis) case study. *Molecular Ecology*, *29*(18), 3429–3445. https://doi.org/10.1111/mec.15566

Fox, E. A., Wright, A. E., Fumagalli, M., & Vieira, F. G. (2019). ngsLD: Evaluating linkage disequilibrium using genotype likelihoods. *Bioinformatics*, *35*(19), 3855–3856. https://doi.org/10.1093/bioinformatics/btz200

Fuentes-Pardo, A. P., Stanley, R., Bourne, C., Singh, R., Emond, K., Pinkham, L., McDermid, J. L., Andersson, L., & Ruzzante, D. E. (2024). Adaptation to seasonal reproduction and environment-associated factors drive temporal and spatial differentiation in northwest Atlantic herring despite gene flow. *Evolutionary Applications*, *17*(3), e13675. https://doi.org/10.1111/eva.13675

Gaio, D., Anantanawat, K., To, J., Liu, M., Monahan, L., & Darling, A. E. (2022). Hackflex: Low-cost, high-throughput, Illumina Nextera Flex library construction. *Microbial Genomics*, *8*(1), 000744. https://doi.org/10.1099/mgen.0.000744

Hale, M. C., Campbell, M. A., & McKinney, G. J. (2021). A candidate chromosome inversion in Arctic charr (Salvelinus alpinus) identified by population genetic analysis techniques. *G3: Genes, Genomes, Genetics*, *11*(10). https://doi.org/10.1093/g3journal/jkab267

Hämälä, T., Wafula, E. K., Guiltinan, M. J., Ralph, P. E., dePamphilis, C. W., & Tiffin, P. (2021). Genomic structural variants constrain and facilitate adaptation in natural populations of Theobroma cacao, the chocolate tree. *Proceedings of the National Academy of Sciences*, *118*(35), e2102914118. https://doi.org/10.1073/pnas.2102914118

Harringmeyer, O. S., & Hoekstra, H. E. (2022). Chromosomal inversion polymorphisms shape the genomic landscape of deer mice. *Nature Ecology & Evolution*, 1–15. https://doi.org/10.1038/s41559-022-01890-0

Hoban, S., Archer, F. I., Bertola, L. D., Bragg, J. G., Breed, M. F., Bruford, M. W., Coleman, M. A., Ekblom, R., Funk, W. C., Grueber, C. E., Hand, B. K., Jaffé, R., Jensen, E., Johnson, J. S., Kershaw, F., Liggins, L., MacDonald, A. J., Mergeay, J., Miller, J. M., … Hunter, M. E. (2022). Global genetic diversity status and trends: Towards a suite of Essential Biodiversity Variables (EBVs) for genetic composition. *Biological Reviews*, *97*(4), 1511–1538. https://doi.org/10.1111/brv.12852

Hoban, S., Bruford, M., D'Urban Jackson, J., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P. A., Paz-Vinas, I., Sjögren-Gulve, P., Segelbacher, G., Vernesi, C., Aitken, S., Bertola, L. D., Bloomer, P., Breed, M., Rodríguez-Correa, H., Funk, W. C., Grueber, C. E., Hunter, M. E., Jaffe, R., … Laikre, L. (2020). Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation*, *248*, 108654. https://doi.org/10.1016/j.biocon.2020.108654

Hoffmann, A. A., & Rieseberg, L. H. (2008). Revisiting the Impact of Inversions in Evolution: From Population Genetic Markers to Drivers of Adaptive Shifts and Speciation? *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 21–42. https://doi.org/10.1146/annurev.ecolsys.39.110707.173532

Huang, K., Andrew, R. L., Owens, G. L., Ostevik, K. L., & Rieseberg, L. H. (2020). Multiple chromosomal inversions contribute to adaptive divergence of a dune sunflower ecotype. *Molecular Ecology*, *29*(14), 2535–2549. https://doi.org/10.1111/mec.15428

Jones, M. W., Clay, D., & Danzmann, R. G. (1996). Conservation genetics of brook trout (Salvelinus fontinalis): Population structuring in Fundy National Park, New Brunswick, and eastern Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(12), 2776–2791. https://doi.org/10.1139/f96-237

Jun, G., Wing, M. K., Abecasis, G. R., & Kang, H. M. (2015). An efficient and scalable analysis framework for variant extraction and refinement from population scale DNA sequence data. *Genome Research*, gr.176552.114. https://doi.org/10.1101/gr.176552.114

Kent, W. J., Sugnet, C. W., Furey, T. S., Roskin, K. M., Pringle, T. H., Zahler, A. M., & Haussler, and D. (2002). The Human Genome Browser at UCSC. *Genome Research*, *12*(6), 996–1006. https://doi.org/10.1101/gr.229102

Kirkpatrick, M. (2010). How and Why Chromosome Inversions Evolve. *PLOS Biology*, *8*(9), e1000501. https://doi.org/10.1371/journal.pbio.1000501

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*, *15*(1), 356. https://doi.org/10.1186/s12859-014-0356-4

Kousoulaki, K., Østbye, T.-K. K., Krasnov, A., Torgersen, J. S., Mørkøre, T., & Sweetman, J. (2015). Metabolism, health and fillet nutritional quality in Atlantic salmon (Salmo salar) fed diets containing n-3-rich microalgae. *Journal of Nutritional Science*, *4*. https://doi.org/10.1017/jns.2015.14

Lagarde, H., Lallias, D., Patrice, P., Dehaullon, A., Prchal, M., François, Y., D'Ambrosio, J., Segret, E., Acin-Perez, A., Cachelou, F., Haffray, P., Dupont-Nivet, M., & Phocas, F. (2023). Genetic architecture of acute hyperthermia resistance in juvenile rainbow trout (Oncorhynchus mykiss) and genetic correlations with production traits. *Genetics Selection Evolution*, *55*(1), 39. https://doi.org/10.1186/s12711-023-00811-4

Laikre, L., Hoban, S., Bruford, M. W., Segelbacher, G., Allendorf, F. W., Gajardo, G., Rodríguez, A. G., Hedrick, P. W., Heuertz, M., Hohenlohe, P. A., Jaffé, R., Johannesson, K., Liggins, L., MacDonald, A. J., OrozcoterWengel, P., Reusch, T. B. H., Rodríguez-Correa, H., Russo, I.-R. M., Ryman, N., & Vernesi, C. (2020). Post-2020 goals overlook genetic diversity. *Science*, *367*(6482), 1083–1085. https://doi.org/10.1126/science.abb2748

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Lehnert, S. J., Baillie, S. M., MacMillan, J., Paterson, I. G., Buhariwalla, C. F., Bradbury, I. R., & Bentzen, P. (2020). Multiple decades of stocking has resulted in limited hatchery introgression in wild brook trout (Salvelinus fontinalis) populations of Nova Scotia. *Evolutionary Applications*, *13*(5), 1069–1089. https://doi.org/10.1111/eva.12923

Lehnert, S. J., Bradbury, I. R., Wringe, B. F., Van Wyngaarden, M., & Bentzen, P. (2023). Multifaceted framework for defining conservation units: An example from Atlantic salmon (Salmo salar) in Canada. *Evolutionary Applications*, *16*(9), 1568–1585. https://doi.org/10.1111/eva.13587

Leigh, D. M., Lischer, H. E. L., Guillaume, F., Grossen, C., & Günther, T. (2021). Disentangling adaptation from drift in bottlenecked and reintroduced populations of Alpine ibex. *Molecular Ecology Resources*, *21*(7), 2350–2363. https://doi.org/10.1111/1755-0998.13442

Leigh, J. W., & Bryant, D. (2015). popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116. https://doi.org/10.1111/2041-210X.12410

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Liu, S., Gao, G., Layer, R. M., Thorgaard, G. H., Wiens, G. D., Leeds, T. D., Martin, K. E., & Palti, Y. (2021). Identification of High-Confidence Structural Variants in Domesticated Rainbow Trout Using Whole-Genome Sequencing. *Frontiers in Genetics*, *12*. https://doi.org/10.3389/fgene.2021.639355

Lujan, N. K., Colm, J. E., Weir, J. T., Montgomery, F. A., Noonan, B. P., Lovejoy, N. R., & Mandrak, N. E. (2022). Genomic population structure of Grass Pickerel (Esox americanus vermiculatus) in Canada: Management guidance for an at-risk fish at its northern range limit. *Conservation Genetics*, *23*(4), 713–725. https://doi.org/10.1007/s10592-022-01450-w

Lundberg, M., Mackintosh, A., Petri, A., & Bensch, S. (2023). Inversions maintain differences between migratory phenotypes of a songbird. *Nature Communications*, *14*(1), Article 1. https://doi.org/10.1038/s41467-023-36167-y

Ma, L.-J., Cao, L.-J., Chen, J.-C., Tang, M.-Q., Song, W., Yang, F.-Y., Shen, X.-J., Ren, Y.-J., Yang, Q., Li, H., Hoffmann, A. A., & Wei, S.-J. (2024). Rapid and Repeated Climate Adaptation Involving Chromosome Inversions following Invasion of an Insect. *Molecular Biology and Evolution*, *41*(3), msae044. https://doi.org/10.1093/molbev/msae044

MacMillan, J. L., Caissie, D., Marshall, T.J, & Hinks, L. (2008). *Population indices of brook trout (Salvelinus fontinalis), Atlantic salmon (Salmo salar), and salmonid competitors in relation to summer water temperature and habitat parameters in 100 streams in Nova Scotia*. Department of Fisheries and Oceans Gulf Region.

Matschiner, M., Barth, J. M. I., Tørresen, O. K., Star, B., Baalsrud, H. T., Brieuc, M. S. O., Pampoulie, C., Bradbury, I., Jakobsen, K. S., & Jentoft, S. (2022). Supergene origin and maintenance in Atlantic cod. *Nature Ecology and Evolution*, *6*(4), 469–481. https://doi.org/10.1038/s41559-022-01661-x

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

McKinney, G., McPhee, M. V., Pascal, C., Seeb, J. E., & Seeb, L. W. (2020). Network Analysis of Linkage Disequilibrium Reveals Genome Architecture in Chum Salmon. *G3 Genes|Genomes|Genetics*, *10*(5), 1553–1561. https://doi.org/10.1534/g3.119.400972

Mérot, C., Berdan, E. L., Cayuela, H., Djambazian, H., Ferchaud, A.-L., Laporte, M., Normandeau, E., Ragoussis, J., Wellenreuther, M., & Bernatchez, L. (2021). Locally Adaptive Inversions Modulate Genetic Variation at Different Geographic Scales in a Seaweed Fly. *Molecular Biology and Evolution*, *38*(9), 3953–3971. https://doi.org/10.1093/molbev/msab143

Mérot, C., Oomen, R. A., Tigano, A., & Wellenreuther, M. (2020). A Roadmap for Understanding the Evolutionary Significance of Structural Genomic Variation. *Trends in Ecology & Evolution*, *35*(7), 561–572. https://doi.org/10.1016/j.tree.2020.03.002

Mérot, C., Stenløkk, K. S. R., Venney, C., Laporte, M., Moser, M., Normandeau, E., Árnyasi, M., Kent, M., Rougeux, C., Flynn, J. M., Lien, S., & Bernatchez, L. (2023). Genome assembly, structural variants, and genetic differentiation between lake whitefish young species pairs (Coregonus sp.) with long and short reads. *Molecular Ecology*, *32*(6), 1458–1477. https://doi.org/10.1111/mec.16468

Meyer, L., Barry, P., Riquet, F., Foote, A., Der Sarkissian, C., Cunha, R. L., Arbiol, C., Cerqueira, F., Desmarais, E., Bordes, A., Bierne, N., Guinand, B., & Gagnaire, P.-A. (2024). Divergence and gene flow history at two large chromosomal inversions underlying ecotype differentiation in the long-snouted seahorse. *Molecular Ecology*, *n/a*(n/a), e17277. https://doi.org/10.1111/mec.17277

Morgan II, R. P., Kazyak, D. C., King, T. L., Lubinski, B. A., Sell, M. T., Heft, A. A., & Jones, J. W. (2021). Genetic Structure of Maryland Brook Trout Populations: Management Implications for a Threatened Species. *North American Journal of Fisheries Management*, *41*(4), 1097–1119. https://doi.org/10.1002/nafm.10618

Neuwirth, E. (2022). *RColorBrewer: ColorBrewer Palettes*. https://CRAN.R-project.org/package=RColorBrewer

Nosil, P., Soria-Carrasco, V., Villoutreix, R., De-la-Mora, M., de Carvalho, C. F., Parchman, T., Feder, J. L., & Gompert, Z. (2023). Complex evolutionary processes maintain an ancient chromosomal inversion. *Proceedings of the National Academy of Sciences*, *120*(25), e2300673120. https://doi.org/10.1073/pnas.2300673120

Nova Scotia Department of Agriculture and Fisheries Inland Fisheries Division. (2005). *Nova Scotia Trout Management Plan*. Nova Scotia Department of Agriculture and Fisheries.

Pearse, D. E., Barson, N. J., Nome, T., Gao, G., Campbell, M. A., Abadía-Cardoso, A., Anderson, E. C., Rundio, D. E., Williams, T. H., Naish, K. A., Moen, T., Liu, S., Kent, M., Moser, M., Minkley, D. R., Rondeau, E. B., Brieuc, M. S. O., Sandve, S. R., Miller, M. R., … Lien, S. (2019). Sex-dependent dominance maintains migration supergene in rainbow trout. *Nature Ecology & Evolution*, *3*(12), Article 12. https://doi.org/10.1038/s41559-019-1044-6

Picard toolkit. (2019). In *Broad Institute, GitHub repository*. Broad Institute. https://broadinstitute.github.io/picard/

Pilgrim, B. L., Perry, R. C., Keefe, D. G., Perry, E. A., & Marshall, H. D. (2012). Microsatellite variation and genetic structure of brook trout (Salvelinus fontinalis) populations in Labrador and neighboring Atlantic Canada: Evidence for ongoing gene flow and dual routes of post-Wisconsinan colonization. *Ecology and Evolution*, *2*(5). https://www.proquest.com/docview/2300642616/abstract/366ED1C467B4470APQ/1

R Studio Team. (2021). *R Studio: Integrated Development Environment for R* [Computer software]. http://www.rstudio.com/

Reeve, J., Butlin, R. K., Koch, E. L., Stankowski, S., & Faria, R. (2023). Chromosomal inversion polymorphisms are widespread across the species ranges of rough periwinkles (Littorina saxatilis and L. arcana). *Molecular Ecology*, *n/a*(n/a). https://doi.org/10.1111/mec.17160

Ruzzante, D. E., McCracken, G. R., Førland, B., MacMillan, J., Notte, D., Buhariwalla, C., Mills Flemming, J., & Skaug, H. (2019). Validation of close-kin mark–recapture (CKMR) methods for estimating population abundance. *Methods in Ecology and Evolution*, *10*(9), 1445–1453. https://doi.org/10.1111/2041-210X.13243

Ruzzante, D. E., McCracken, G. R., Parmelee, S., Hill, K., Corrigan, A., MacMillan, J., & Walde, S. J. (2016). Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, Salvelinus fontinalis. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1823), 20152601. https://doi.org/10.1098/rspb.2015.2601

Ruzzante, D. E., Simons, A. P., McCracken, G. R., Habit, E., & Walde, S. J. (2020). Multiple drainage reversal episodes and glacial refugia in a Patagonian fish revealed by sequenced microsatellites. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1928), 20200468. https://doi.org/10.1098/rspb.2020.0468

Ryman, N., & Laikre, L. (1991). Effects of Supportive Breeding on the Genetically Effective Population Size. *Conservation Biology*, *5*(3), 325–329.

Sanchez-Donoso, I., Ravagni, S., Rodríguez-Teijeiro, J. D., Christmas, M. J., Huang, Y., Maldonado-Linares, A., Puigcerver, M., Jiménez-Blasco, I., Andrade, P., Gonçalves, D., Friis, G., Roig, I., Webster, M. T., Leonard, J. A., & Vilà, C. (2022). Massive genome inversion drives coexistence of divergent morphs in common quails. *Current Biology*, *32*(2), 462-469.e6. https://doi.org/10.1016/j.cub.2021.11.019

Shaney, K. J., Diaz-Ramirez, L. G., Espindola, S., Castañeda-Rico, S., Berovides-Álvarez, V., & Vázquez-Domínguez, E. (2020). Defining intraspecific conservation units in the endemic Cuban Rock Iguanas (Cyclura nubila nubila). *Scientific Reports*, *10*(1), 21607. https://doi.org/10.1038/s41598-020-78664-w

Shaw, J., Piper, D. J. W., Fader, G. B. J., King, E. L., Todd, B. J., Bell, T., Batterson, M. J., & Liverman, D. G. E. (2006). A conceptual model of the deglaciation of Atlantic Canada. *Quaternary Science Reviews*, *25*(17), 2059–2081. https://doi.org/10.1016/j.quascirev.2006.03.002

Smith, S. R., Amish, S. J., Bernatchez, L., Le Luyer, J., C. Wilson, C., Boeberitz, O., Luikart, G., & Scribner, K. T. (2020). Mapping of Adaptive Traits Enabled by a High-Density Linkage Map for Lake Trout. *G3 Genes|Genomes|Genetics*, *10*(6), 1929–1947. https://doi.org/10.1534/g3.120.401184

Smith, S. R., Normandeau, E., Djambazian, H., Nawarathna, P. M., Berube, P., Muir, A. M., Ragoussis, J., Penney, C. M., Scribner, K. T., Luikart, G., Wilson, C. C., & Bernatchez, L. (2022). A chromosome-anchored genome assembly for Lake Trout (Salvelinus namaycush). *Molecular Ecology Resources*, *22*(2), 679–694. https://doi.org/10.1111/1755-0998.13483

Stea, R. R. (2004). The appalachian glacier complex in maritime canada. In J. Ehlers & P. L. Gibbard (Eds.), *Developments in Quaternary Sciences* (Vol. 2, pp. 213–232). Elsevier. https://doi.org/10.1016/S1571-0866(04)80199-4

Stea, R. R., Piper, D. J. W., Fader, G. B. J., & Boyd, R. (1998). Wisconsinan glacial and sea-level history of Maritime Canada and the adjacent continental shelf: A correlation of land and sea events. *GSA Bulletin*, *110*(7), 821–845. https://doi.org/10.1130/0016-7606(1998)110<0821:WGASLH>2.3.CO;2

Sutherland, B. J. G., Gosselin, T., Normandeau, E., Lamothe, M., Isabel, N., Audet, C., & Bernatchez, L. (2016). Salmonid Chromosome Evolution as Revealed by a Novel Method for Comparing RADseq Linkage Maps. *Genome Biology and Evolution*, *8*(12), 3600–3617. https://doi.org/10.1093/gbe/evw262

Syahputra, K., Kania, P. W., Al-Jubury, A., Marnis, H., Mathiessen, H., Dirks, R. P., & Buchmann, K. (2020). Association between stress, metabolism, and growth in *Ichthyophthirius multifiliis* infected rainbow trout gills: Transcriptomic evidence. *Aquaculture*, *526*, 735384. https://doi.org/10.1016/j.aquaculture.2020.735384

Tavares, H. (2022). *windowscanr: Apply functions using sliding windows*.

Thomas, L., Underwood, J. N., Rose, N. H., Fuller, Z. L., Richards, Z. T., Dugal, L., Grimaldi, C. M., Cooke, I. R., Palumbi, S. R., & Gilmour, J. P. (2022). Spatially varying selection between habitats drives physiological shifts and local adaptation in a broadcast spawning coral on a remote atoll in Western Australia. *Science Advances*, *8*(17), eabl9185. https://doi.org/10.1126/sciadv.abl9185

Thorstensen, M. J., Euclide, P. T., Jeffrey, J. D., Shi, Y., Treberg, J. R., Watkinson, D. A., Enders, E. C., Larson, W. A., Kobayashi, Y., & Jeffries, K. M. (2022). A chromosomal inversion may facilitate adaptation despite periodic gene flow in a freshwater fish. *Ecology and Evolution*, *12*(5), e8898. https://doi.org/10.1002/ece3.8898

Tuson, M., Garanto, A., Gonzàlez-Duarte, R., & Marfany, G. (2009). Overexpression of CERKL, a gene responsible for retinitis pigmentosa in humans, protects cells from apoptosis induced by oxidative stress. *Molecular Vision*, *15*, 168–180.

Twyford, A. D., & Friedman, J. (2015). Adaptive divergence in the monkey flower Mimulus guttatus is maintained by a chromosomal inversion. *Evolution*, *69*(6), 1476–1486. https://doi.org/10.1111/evo.12663

von Takach, B., Cameron, S. F., Cremona, T., Eldridge, M. D. B., Fisher, D. O., Hohnen, R., Jolly, C. J., Kelly, E., Phillips, B. L., Radford, I. J., Rick, K., Spencer, P. B. S., Trewella, G. J., Umbrello, L. S., & Banks, S. C. (2024). Conservation prioritisation of genomic diversity to inform management of a declining mammal species. *Biological Conservation*, *291*, 110467. https://doi.org/10.1016/j.biocon.2024.110467

Waldbieser, G. C., Liu, S., Yuan, Z., Older, C. E., Gao, D., Shi, C., Bosworth, B. G., Li, N., Bao, L., Kirby, M. A., Jin, Y., Wood, M. L., Scheffler, B., Simpson, S., Youngblood, R. C., Duke, M. V., Ballard, L., Phillippy, A., Koren, S., & Liu, Z. (2023). Reference genomes of channel catfish and blue catfish reveal multiple pericentric chromosome inversions. *BMC Biology*, *21*(1), 67. https://doi.org/10.1186/s12915-023-01556-8

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., & Earl, A. M. (2014). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PloS One*, *9*(11), e112963. https://doi.org/10.1371/journal.pone.0112963

Waples, R. S., Ford, M. J., Nichols, K., Kardos, M., Myers, J., Thompson, T. Q., Anderson, E. C., Koch, I. J., McKinney, G., Miller, M. R., Naish, K., Narum, S. R., O'Malley, K. G., Pearse, D. E., Pess, G. R., Quinn, T. P., Seamons, T. R., Spidle, A., Warheit, K. I., & Willis, S. C. (2022). Implications of Large-Effect Loci for Conservation: A Review and Case Study with Pacific Salmon. *Journal of Heredity*, *113*(2), 121–144. https://doi.org/10.1093/jhered/esab069

Warburton, P. E., & Sebra, R. P. (2023). Long-Read DNA Sequencing: Recent Advances and Remaining Challenges. *Annual Review of Genomics and Human Genetics*, *24*(Volume 24, 2023), 109–132. https://doi.org/10.1146/annurev-genom-101722-103045

Wellenreuther, M., & Bernatchez, L. (2018). Eco-Evolutionary Genomics of Chromosomal Inversions. *Trends in Ecology & Evolution*, *33*(6), 427–440. https://doi.org/10.1016/j.tree.2018.04.002

Wellenreuther, M., Mérot, C., Berdan, E., & Bernatchez, L. (2019). Going beyond SNPs: The role of structural genomic variants in adaptive evolution and species diversification. *Molecular Ecology*, *28*(6), 1203–1209. https://doi.org/10.1111/mec.15066

White, S. L., Miller, W. L., Dowell, S. A., Bartron, M. L., & Wagner, T. (2018). Limited hatchery introgression into wild brook trout (Salvelinus fontinalis) populations despite reoccurring stocking. *Evolutionary Applications*, *11*(9), 1567–1581. https://doi.org/10.1111/eva.12646

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org
Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686
Wickham, H., François, R., Henry, L., & Müller, K. (2022). *dplyr: A Grammar of Data Manipulation*. https://CRAN.R-project.org/package=dplyr
Wickham, H., & Girlich, M. (2022). *tidyr: Tidy Messy Data*. https://CRAN.R-project.org/package=tidyr
Willoughby, J. R., Harder, A. M., Tennessen, J. A., Scribner, K. T., & Christie, M. R. (2018). Rapid genetic adaptation to a novel environment despite a genome-wide reduction in genetic diversity. *Molecular Ecology*, *27*(20), 4041–4051. https://doi.org/10.1111/mec.14726
Wood, J. L. A., Belmar-Lucero, S., Hutchings, J. A., & Fraser, D. J. (2014). Relationship of habitat variability to population size in a stream fish. *Ecological Applications*, *24*(5), 1085–1100. https://doi.org/10.1890/13-1647.1
Wood, J. L. A., & Fraser, D. J. (2015). Similar plastic responses to elevated temperature among different-sized brook trout populations. *Ecology*, *96*(4), 1010–1019. https://doi.org/10.1890/14-1378.1
Wood, J. L. A., Yates, M. C., & Fraser, D. J. (2016). Are heritability and selection related to population size in nature? Meta-analysis and conservation implications. *Evolutionary Applications*, *9*(5), 640–657. https://doi.org/10.1111/eva.12375
Xie, Y. (2015). *Dynamic documents with R and Knitr* (Second edition). CRC Press/Taylor & Francis.
Zhou, Y., Zhou, B., Pache, L., Chang, M., Khodabakhshi, A. H., Tanaseichuk, O., Benner, C., & Chanda, S. K. (2019). Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. *Nature Communications*, *10*(1), 1523. https://doi.org/10.1038/s41467-019-09234-6

Data Accessibility Statement

Genetic Data:

Raw sequence reads
Individual filtered bam files
?

Author Contributions

Cait Nemeczek and Daniel Ruzzante designed the study with input from John MacMillan. Field work was undertaken by John MacMillan, Cait Nemeczek and Daniel Ruzzante. Cait Nemeczek produced the libraries for sequencing with input and guidance from Mallory Van Wyngaarden. Meg Smith analyzed the mitochondrial DNA and completed the corresponding sections of the paper. Cait Nemeczek led the writing of the manuscript and Lisette Delgado and Daniel Ruzzante contributed critically to the drafts. Lisette Delgado provided substantial guidance for analyses of low-coverage whole genome data.

Tables and Figures

Table 1 Size of LD blocks on chromosomes from the Brook Trout genome. The size was estimated from the r^2 of LD shown in heatmaps for these chromosomes using all 192 Brook Trout.

Chromosome	Size of LD block (mb)
12 (CM055694.1)	14-35
19 (CM055701.1)	22-39
27 (CM055709.1)	13-35
31 (CM055713.1)	15-40

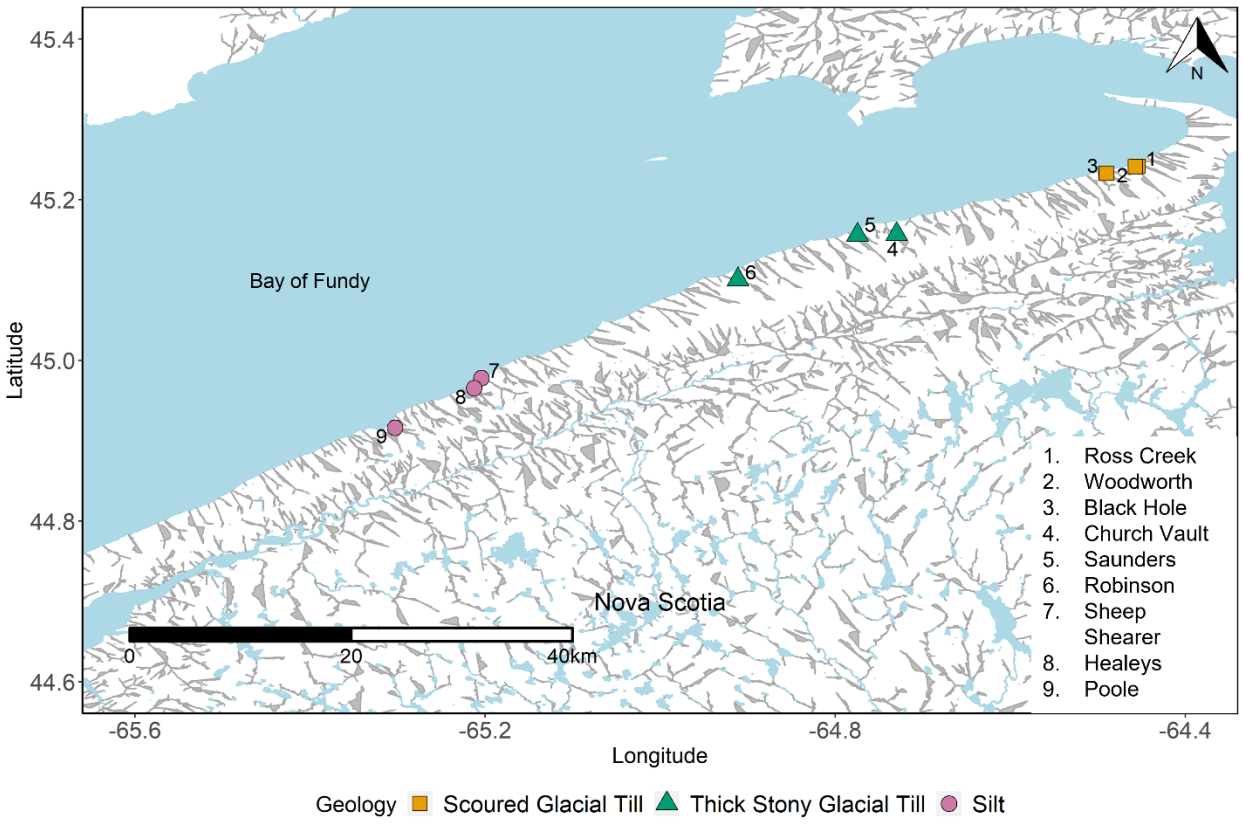

Figure 1 Nine streams in the North Mountain, Nova Scotia sampled for Brook Trout. Shapes indicate the type of surficial geology that make up the streams.

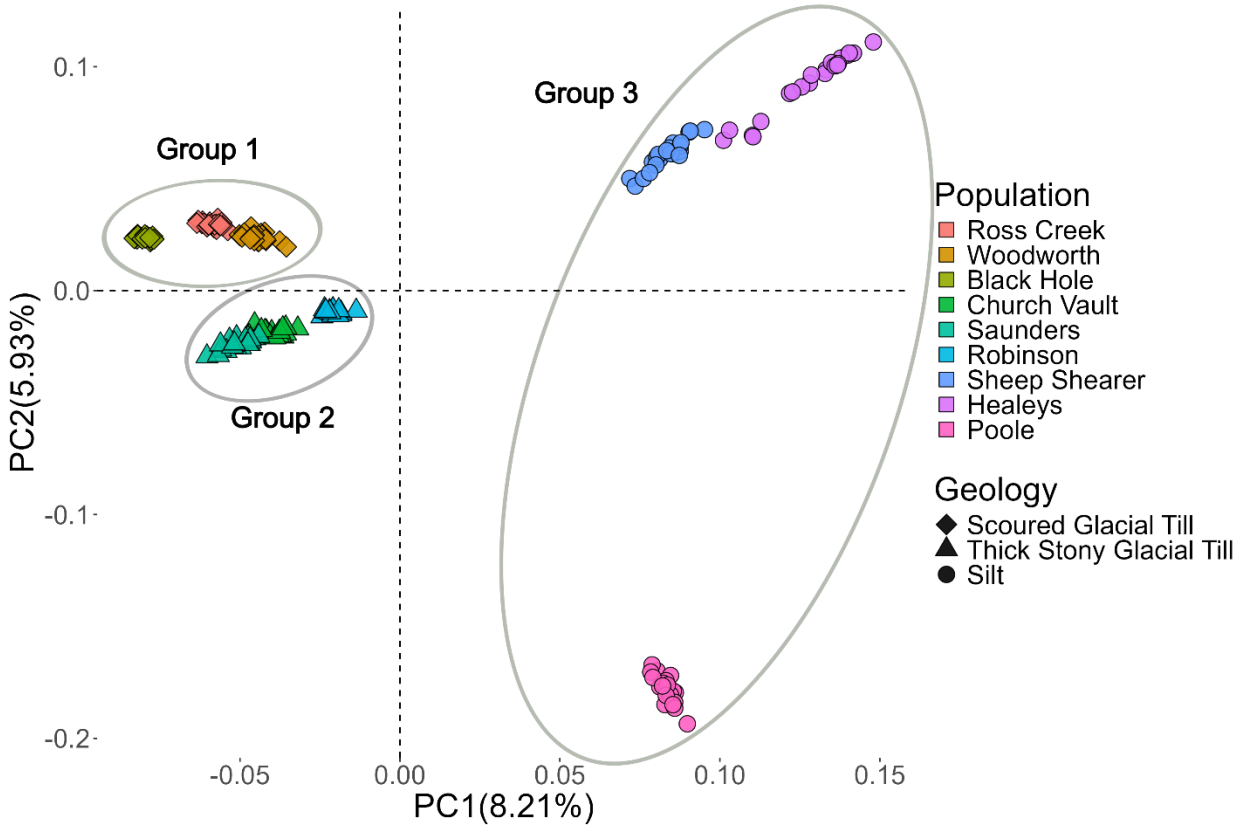

Figure 2 Principal Component plot using genotype likelihoods for N=192 Brook Trout. Covariance matrix based on 2,620,282 SNPs found with reads aligned to the Brook Trout genome.

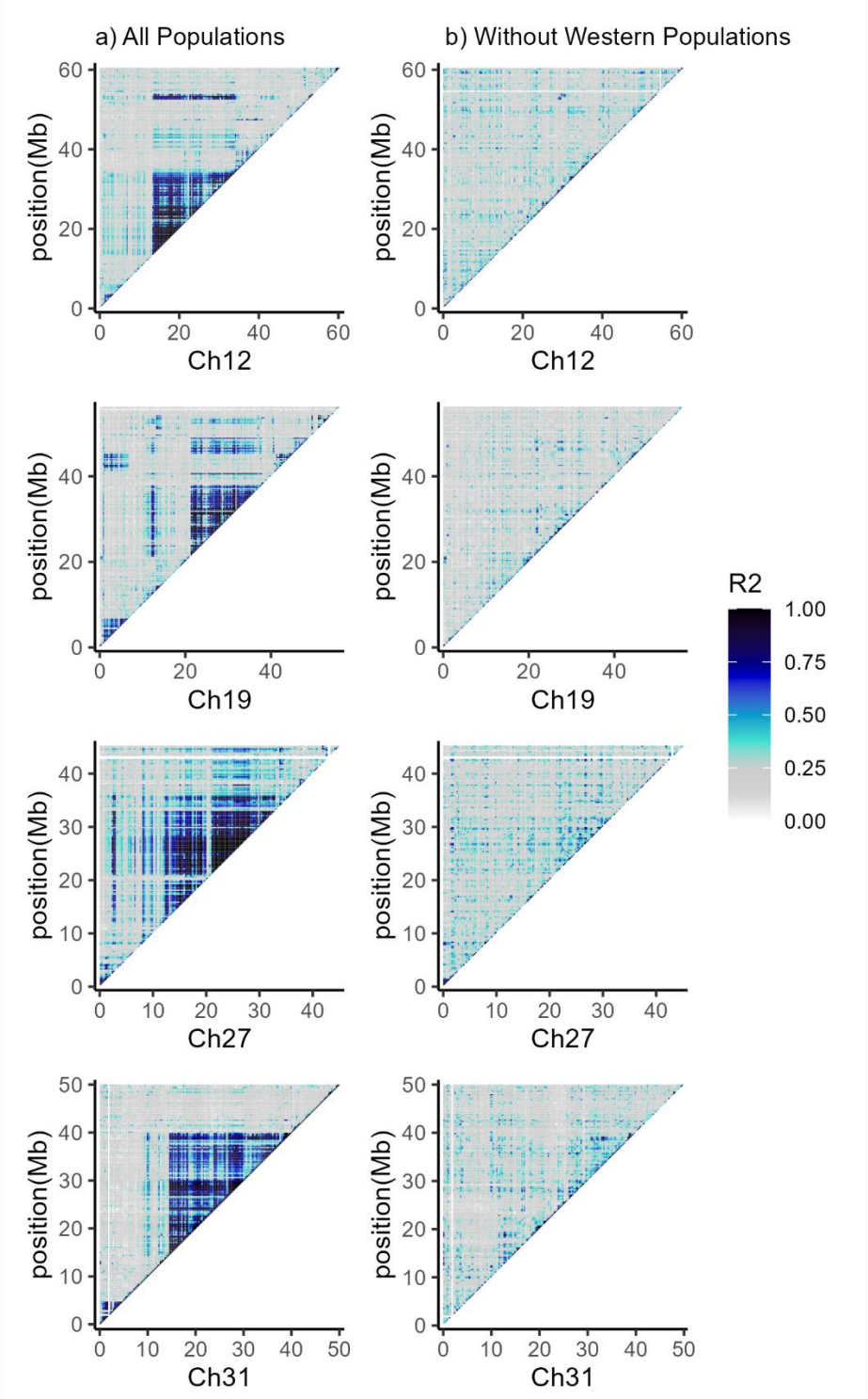

Figure 3 Heatmaps of chromosomes 12, 19, 27, 31 (CM055694.1, CM055701.1, CM055709.1, CM055713.1) from the Brook Trout genome. r^2 values in 250kb windows of the second percentile from LD calculations are shown. a) Heatmaps of all 9 populations pooled b) 6 populations excluding individuals from western streams (Healeys, Sheep Shearer, Poole). High r^2 values are those with dark colours and represent areas of high LD.

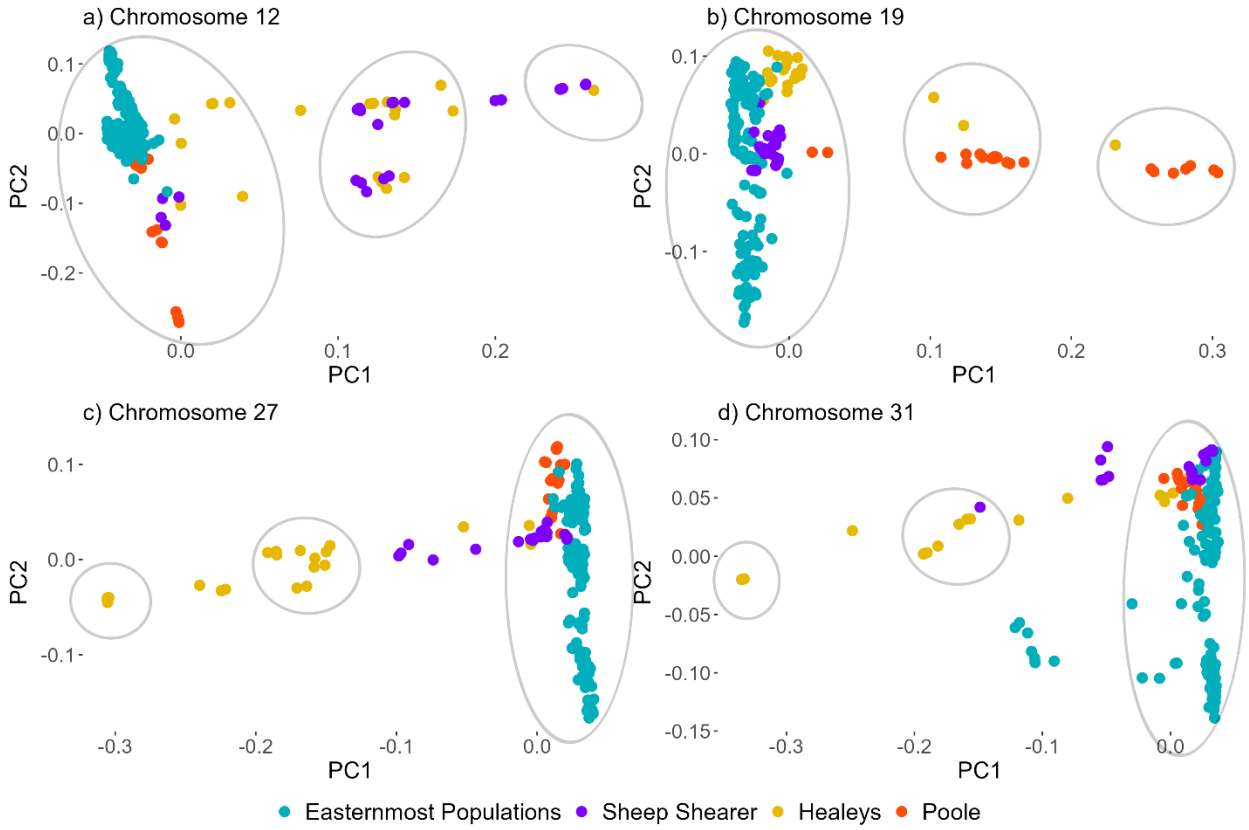

Figure 4. a-d PCAs for LD blocks on Chromosomes 12, 19, 27, and 31 of the Brook Trout genome. The 3 clusters in a-d represent the different karyotypes expected when there is an inversion. The middle group on each PCA represents the heterokaryotypes which have one copy of the potential inversion and one of the non-inverted chromosome, the left and right groups represent either individuals with two copies of the potential inversion arrangement or no potential inversion arrangements. Percent variation explained for chromosome 12 PC1 19.6%, PC2 6.0%, chromosome 19 PC1 18.5%, PC2 6.5%, chromosome 27 PC1 20.9%, PC2 6.2%, chromosome 31 PC1 17.3%, PC2 6.4%.

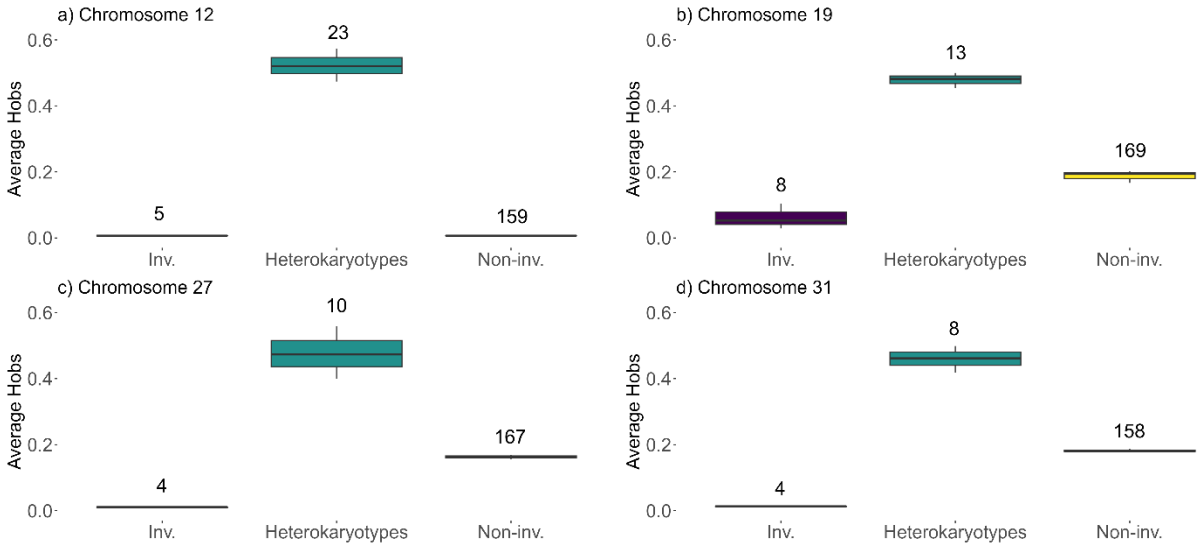

Figure 5 Average observed proportion of heterozygotes (H_{obs}) in windows the size of potential inversion regions. Individuals included in each of homokaryotypes with potential inversions (Inv.), heterokaryotypes, and homokaryotpyes without potential inversions (Non-inv.) are those represented by the 3 different karyotype groups in PCAs of LD blocks (Figure 5). Number of individuals in each group are indicated above each bar. H_{obs} is calculated as a population representing homokaryotypes with potential inversions and without, and heterokaryotypes where the proportion of homozygotes and heterozygotes are calculated at each SNP and then averages taken in windows the size of inversion regions.

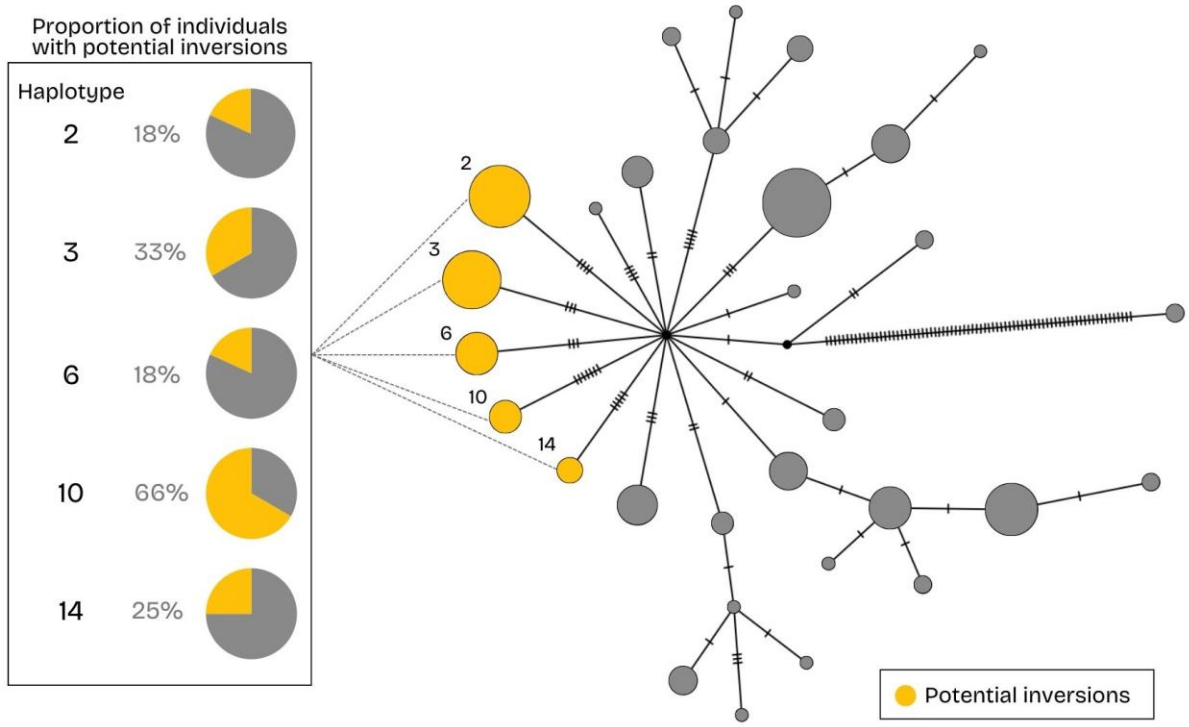

Figure 6 Median-joining haplotype network associating the 30 mtDNA haplotypes identified among North Mountain Brook Trout. Mutational steps are represented by hashmarks on the edges and nodes scaled to represent frequency. Haplotypes containing individuals with potential inversions are highlighted in yellow and the frequency of these individuals within each haplotype is shown.

Supplemental

Table S1 Physical characteristics of the nine streams sampled for Brook Trout in the North Mountain, Annapolis Valley, Nova Scotia and average and median fork lengths of 40 Brook Trout sampled from each stream.

Stream	Surficial Geology	Area of drainage basin (km^2)	Stream gradient % (slope)	Average fork length (cm)	Median fork length (cm)
Ross Creek	Scoured layer of glacial till	6.9	3.42	10.33	9.5
Woodworth	Scoured layer of glacial till	10.78	2.93	11.01	10.8
Black Hole	Scoured layer of glacial till	6.42	3.66	11.77	10.6
Church Vault	Thick stony-granite derived till	10.7	2.70	11.32	11
Saunders	Thick stony-granite derived till	8.50	3.33	11.63	11cm
Robinson	Thick stony-granite derived till	12.3	3.08	10.80	10.45
Sheep Shearer	Silt	7.90	3.77	15.11	14.4
Healeys	Silt	4.9	4.5	11.48	10.95
Poole	Silt	6.89	6.03	12.87	12.4

Table S2 Average pH calculated from pH measured in the middle and at both banks of each stream where streamflow was measured using a pHep^{+} H198108 (Hanna instruments) once a month.

Stream	Average pH July 14 2021	Average pH August 9 2021	Average pH September 13 2021	Average pH October 6 2021	Average pH November 17 2021
Ross Creek	7.60	7.79	7.73	7.67	7.62
Woodworth Creek	7.78	7.87	7.84	7.65	7.56
Black Hole Brook	7.74	7.83	7.92	7.80	7.64
Church Vault Brook	8.00	8.10	7.98	8.01	7.70
Saunders Brook	8.09	8.16	8.00	8.08	7.82
Robinson Brook	7.88	7.81	7.73	7.86	7.86
Sheep Shearer Brook	7.84	7.51	7.50	7.44	7.70
Healeys Brook	7.42	7.55	7.47	7.36	7.77
Poole	7.59	7.50	7.43	7.17	7.77

Table S3 Biological processes and pathways involved in statistically significant gene symbols from chromosomes 12, 19, 27, and 31. Processes with a -log10(P) greater than two were included in the table and greater than three for chromosome 31.

Chromosome	GO biological process, KEGG Pathways, Reactome Gene Sets
12	Cytokinetic process, cilium assembly, carbohydrate metabolic processes
19	none
27	Mitochondrion organization, negative regulation of intracellular signal transduction
31	Negative regulation of cellular catabolic process, regulation of TP53 activity, positive regulation of autophagy of mitochondrion in response to mitochondrion depolarization, PID ERBB2 ERBB3 pathway, 1p36 copy number variation syndrome, ERAD pathway, response to hypoxia, glutathione metabolism, positive regulation of protein localization, response to oxidative stress, positive regulation of cellular component biogenesis, negative regulation of DNA metabolic process, response to UV

Table S4 Genes identified within potential inversion regions on each chromosome using the UCSC genome browser.

Chromosome 12	Chromosome 19	Chromosome 27	Chromosome 31

agbl2	arsh	acat2	acot7
arl2bp	cerkl	afg1lb	adnpb
armc10	dnajc10	ak9	agmat
bend7	hspbap1	cep57l1	alas2
bhlhe41	itprid2	epm2a	ano11
btbd11b	nifk	fam166c	apex2
c12h15orf40	pdia5	fig4a	arhgef16
calub	pou1f1	foxo3b	brpf3b
cax2	slc49a4	glrx5	casz1
ccdc113	tfcp2l1	opn8a	ccdc30
cdk10	tsn	opn8b	chchd6a
cfdp1	ttn.1	otofa	chrna4b
ch25hl1.1		ppil6	clstn1
chd2		sash1a	cntn3a.1
chmp1a		sesn1	cntn3a.2
chst6		slc25a47a	ctnnbip1
cmip		snx3	ddx19a
coq9		sod2	dedd
exoc3l1		tcp1	dffa
fam185a		tdrd6	dnajc16
gabarapl2		ubxn2a	dpm1
gdpgp1		utrn	edem2
hydin		wasf1	efhd2
kiaa0513		wtap	ela2
lrrn2		zgc:112001	ela2l
mapk12a			espn
mlc1			exosc10
neto2b			fam217ba
pdhx			fam43b
pdp2			fance
pgpep1l			fhad1
phrf1			gata5
plcg2			gdap1l1
plin1			gss
pllp			her3
polr2c			hm13
prmt7			hspg2
prr5a			igf3
rabl2			ints11
rad52			kif1b
rcn2			lamb2
rgma			lamb2l
rxylt1			lemd1
sephs1			lzic
si:ch1073-390k14.1			mapk13
si:ch211-59o9.10			masp2

si:dkey-30c15.13	mesd
si:dkey-30c15.2	mfn2
slco3a1a	mtor
sntb2	mul1b
spata2l	nfascb
spire2	nkain5
st8sia2	nmnat1
tat	noc2l
terb1	nppal
tmem231	nppb
tmem263	otud3
tmem60	pdyn
trabd	pex14
tsnaxip1	pgd
vps4a	pik3cd
zfpm1	pink1
zgc:112052	plod1a
zgc:56622	ppih
zgc:77752	ppp1r3da
znf276	prdm16
	prdm2a
	prex1
	ptpn22
	rer1
	rtel1
	samd11
	si:ch211-167b20.8
	si:ch211-218o21.4
	si:ch73-206d17.1
	si:dkey-178k16.1
	si:dkey-32e6.3
	si:dkeyp-100a1.6
	si:dkeyp-110g5.4
	si:rp71-17i16.5
	si:rp71-17i16.6
	sike1
	slc35c2
	smim1
	srm
	taf10
	taf11
	taf13
	tafa5l
	tardbpa
	tlnrd1
	tmco4

		tmem201 tmem51a tnnc2.2 tp73 tpk2 tprg11 troap ttll9 ube4b usp19 vwa1 vwa5b1 wdr13 wrap73 ybx1 zgc:154075 zgc:56699 zmynd12

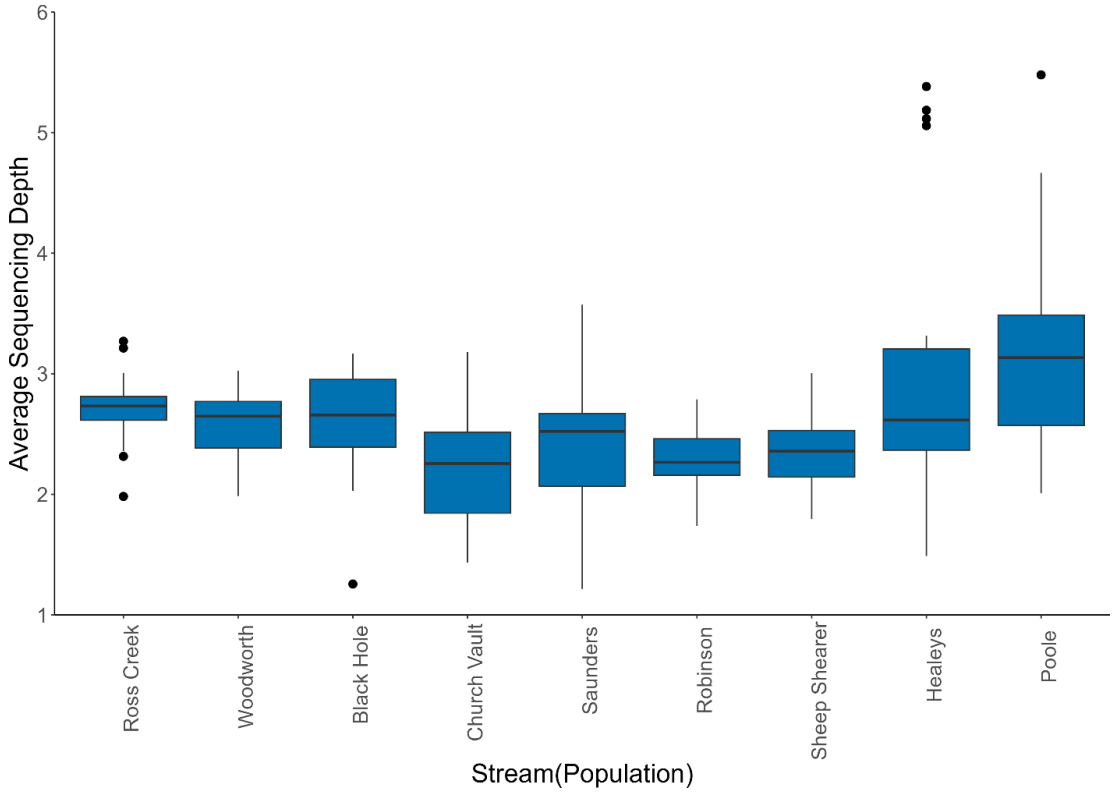

Figure S1 Average sequencing depth per stream (population) calculated at all positions covered by reads using the Brook Trout (*Salvelinus fontinalis*) reference genome. Error bars are 95% confidence intervals.

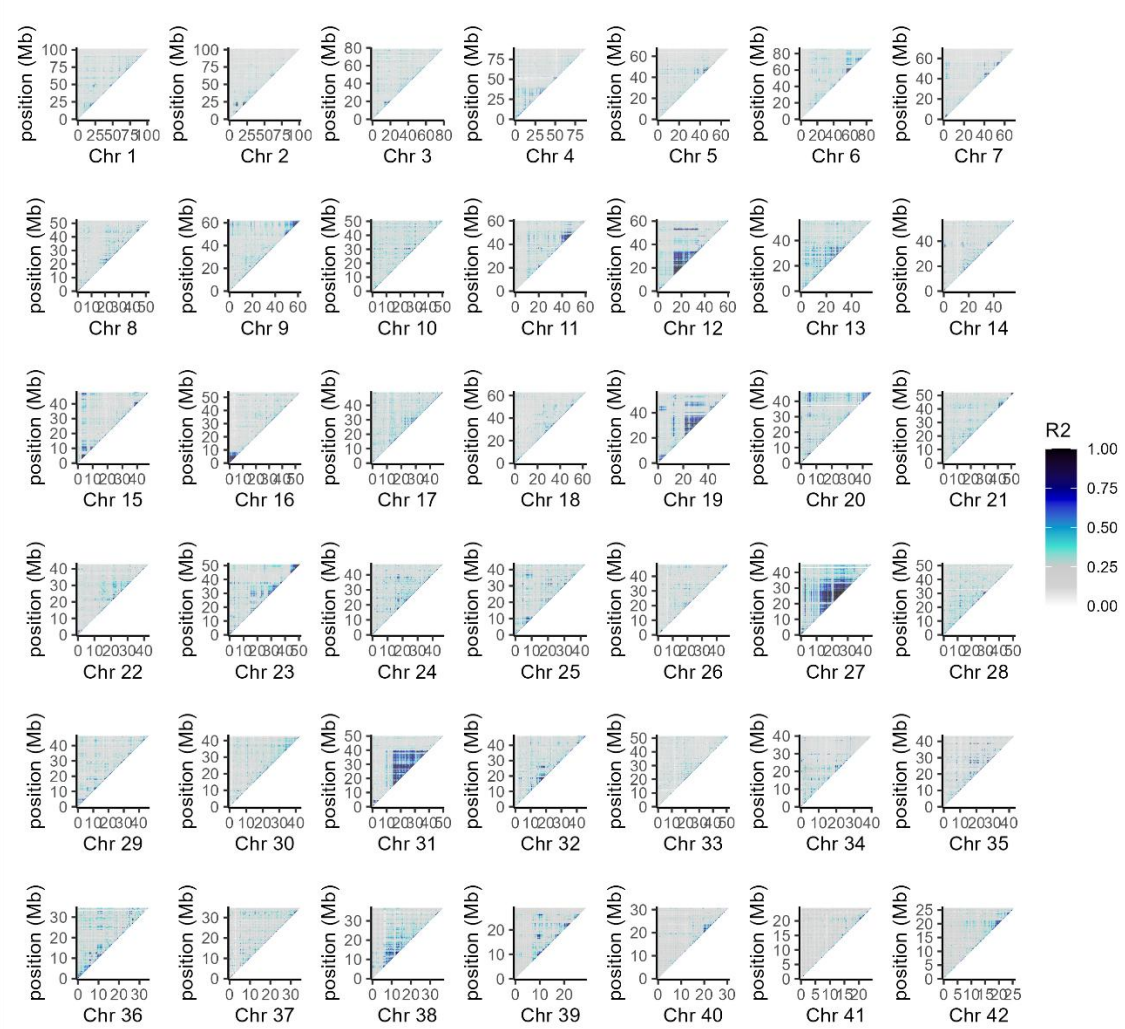

Figure S2 Heatmaps of r^2 from LD calculations for 192 Brook Trout on all 42 chromosomes in the Brook Trout genome. The second percentile of r^2 values in 250kb windows were used for plotting. High r^2 values and dark colours represent areas on a chromosome with high LD.

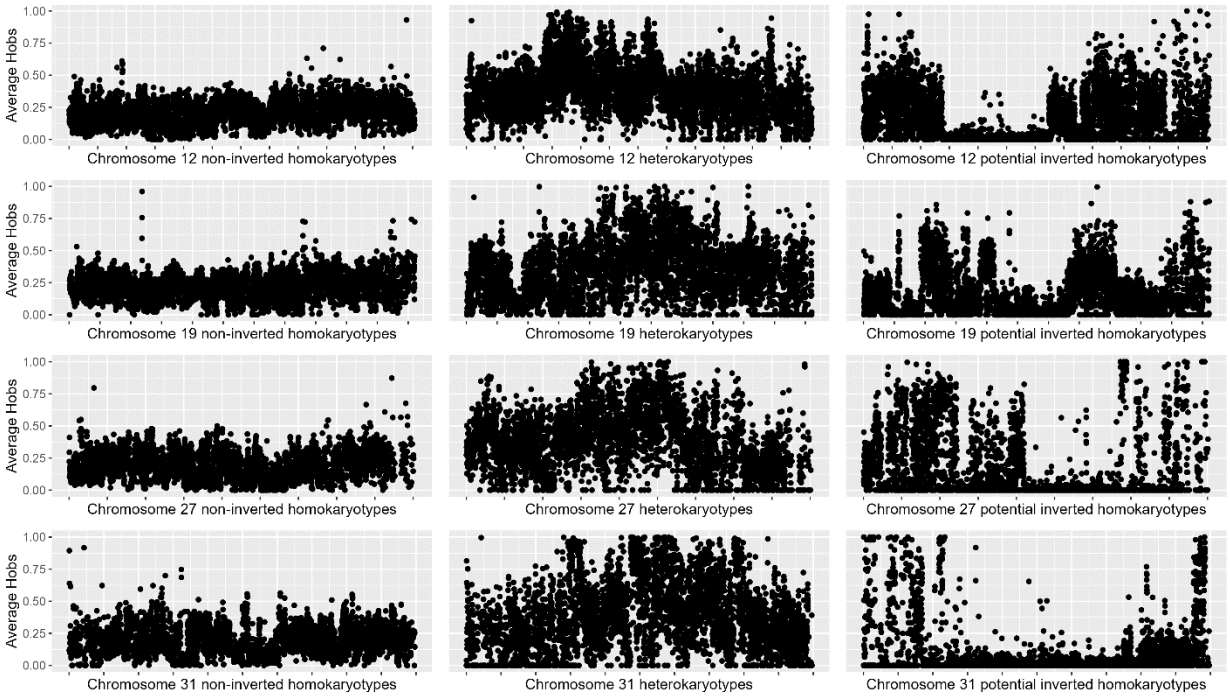

Figure S3 Average observed proportion of heterozygotes (H_{obs}) across entire chromosomes of individuals from the 3 different potential inversion groups from PCAs of LD blocks. All individuals from each of the inverted and non-inverted homokaryotypes and heterokaryotypes were used to calculate H_{obs}. Average H_{obs} was calculated in 10kb windows using a 10kb slide and points on the plot represent H_{obs} among groups for SNPs in these windows.

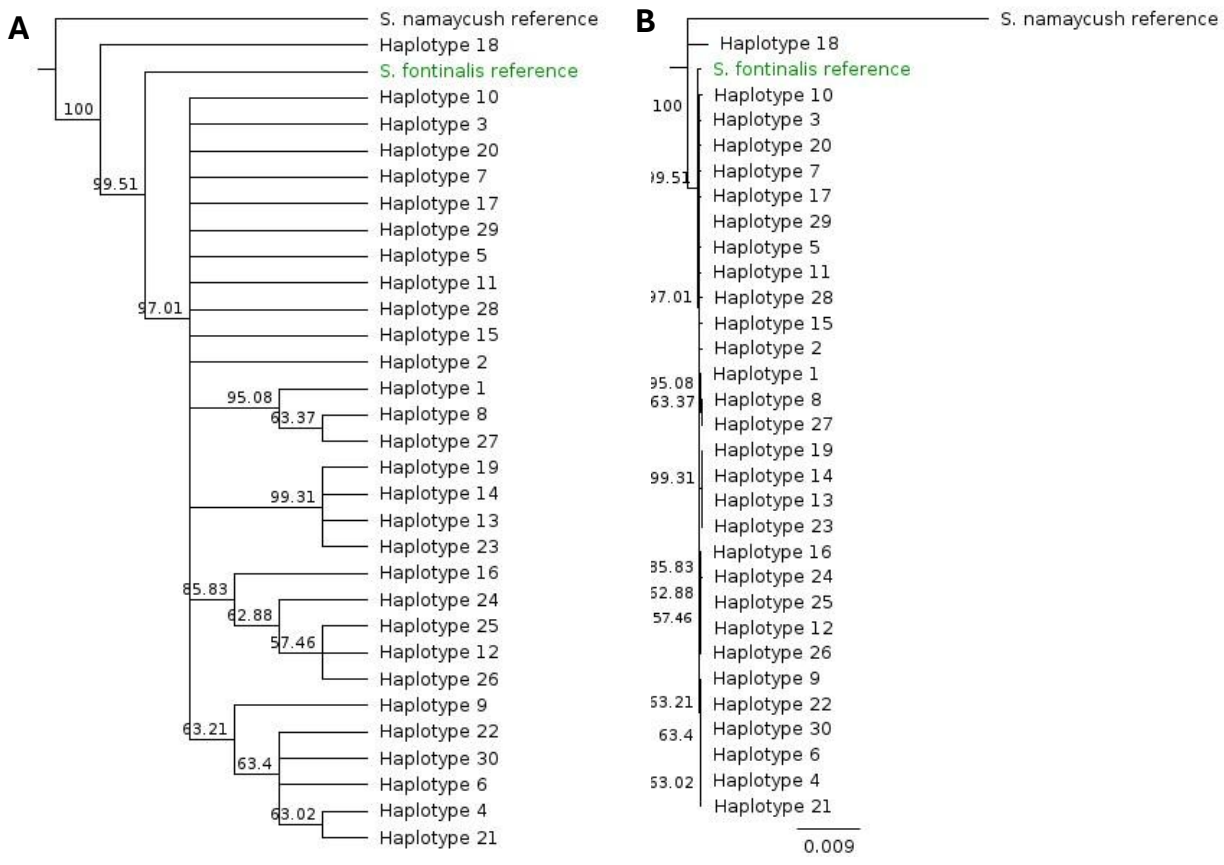

Figure S4 (A) Neighbour-joining tree containing 30 haplotype sequences identified among North Mountain Brook Trout (*Salvelinus fontinalis*) populations, the Brook Trout reference mitogenome and the Lake Trout (*Salvelinus namaycush*) reference mitogenome as the outgroup. Bootstraps indicated at branch nodes. (B) Branch lengths scaled to represent sequence divergence.

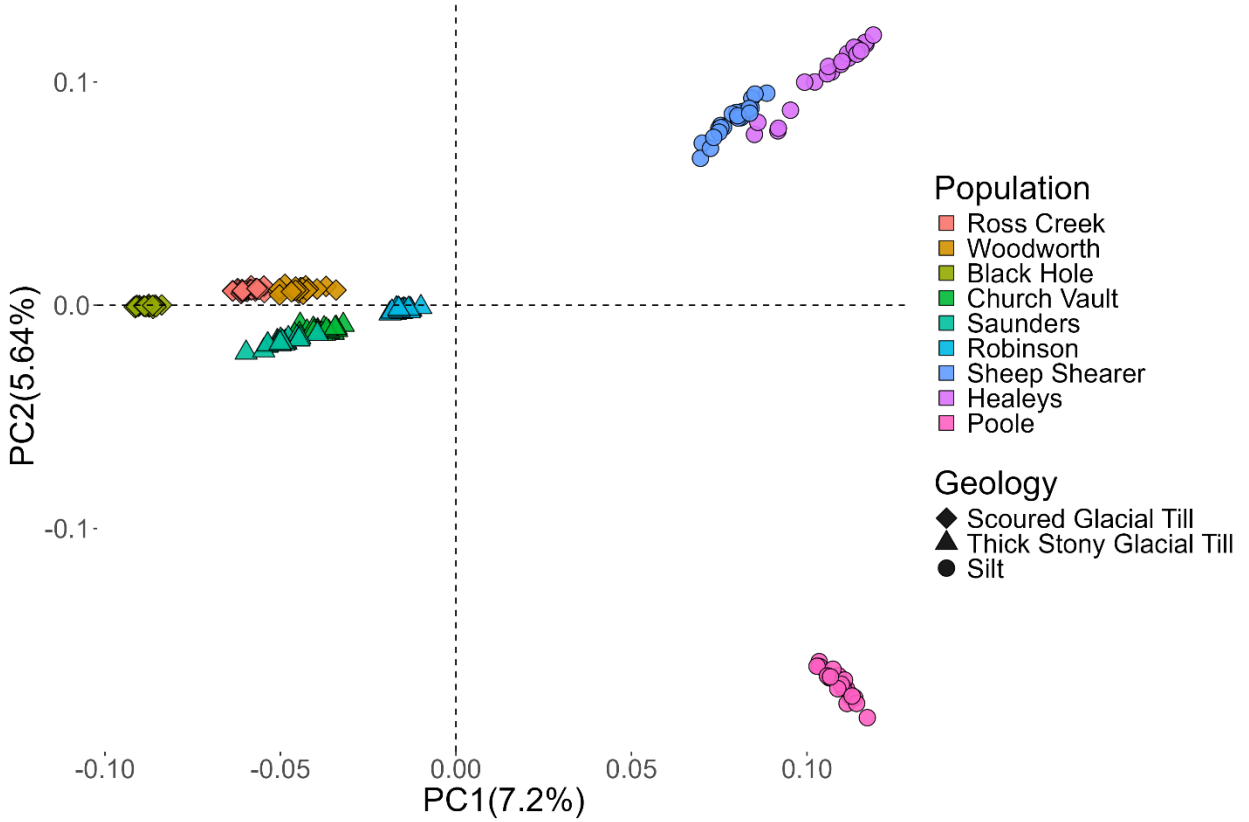

Figure S5 PCA with genotype likelihoods for N=192 Brook Trout produced from the covariance matrix of 391,155 SNPs representing those after LD pruning.

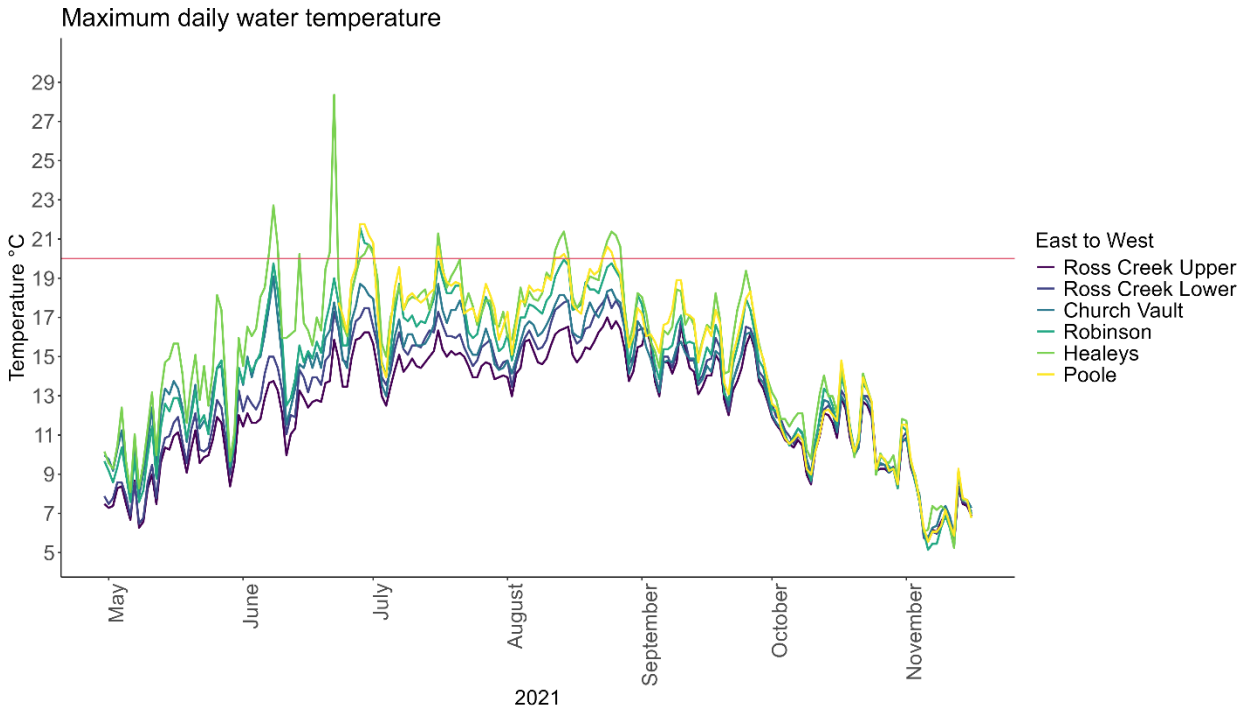

Figure S6 Maximum daily water temperatures calculated from water temperature recorded every two hours from May-November 2021 with HOBO pendant loggers. Temperature was recorded in a subset of the nine streams. The red line represents temperature that Brook Trout avoid.

Data Filtering and Reference Genome Mapping

FastQC v.0.11.9 (Andrews, 2010) and MultiQC v.1.11 (Ewels et al., 2016) were used on demultiplexed, raw, and barcode removed fastq files to examine quality of reads and test for the presence of adaptor sequences. Trimmomatic v.0.39 (Bolger et al., 2014) was used to remove i5 and i7 IDT Illumina and Illumina Nextera adapters. Fastp v.0.23.1 (Chen et al., 2018) was used on the paired, adaptor trimmed fastq.gz files to remove poly-G tails. Unplaced scaffolds were not use for alignment to reduce the effects of reads mapping in duplicate locations and reads were mapped using Bowtie2. For indel realignment, GATKs RealignerTargetCreator function was used to make an intervals file with locations of indels and potential indels which the IndelRealigner function uses as input. Reads with minimum base quality < 20 and minimum mapping quality < 20 were removed for depth calculations using SAMtools v.1.13 (Li et al., 2009) The resulting depth files were analyzed in R v.4.1.2 (RStudio Team, 2021) using tidyverse v.1.3.1 (Wickham et al., 2019), ggplot2 v.3.3.5 (Wickham, 2016), dplyr v.1.0.7 (Wickham et al., 2022), knitr v.1.36 (Xie, 2015), and RColorBrewer v.1.1.2 (Neuwirth, 2022).

Depth and Genotype Likelihood Parameters for ANGSD

ANGSD v.0.940 (Korneliussen et al., 2014) was used for depth calculation and -maxDepth was used to set an initial maximum depth of 10x per individual. Briefly, the distribution of depth counts from the .depthGlobal files were plotted as histograms using R v.4.1.2 (RStudio Team, 2021) with tidyr v.1.2.0 (Wickham & Girlich, 2022), data.table v.1.14.2 (Dowle & Srinivasan,

2021), ggplot2 v.3.3.6 (Wickham, 2016), na.tools v.0.3.1 (Brown, 2018), and dyplr v.1.0.9 (Wickham et al., 2022). Data was filtered based on the normal distribution and the mean and standard deviation was calculated. The maximum and minimum depth were then calculated as the mean + and – the standard deviation. This was done for each stream and for all streams combined. ANGSD v.0.940 was then used to calculate genotype likelihoods. After determining the number of SNPs, genotype likelihoods were calculated again using the SNP list generated from the previous step in discovering SNPs.

Linkage Disequilibrium Calculation and Percentile Plotting

Individual chromosome minor allele frequency and genotype likelihood files discussed in the methods were used to calculate LD by chromosome with ngsLD v.1.1.1 on all 192 Brook Trout. R^2 values were summarized into percentiles using scripts from Mérot et al. (2021) and plotted in R v.4.0.2 (RStudio Team, 2021) using ggplot2 v.3.3.6 (Wickham, 2016). The second percentile of r^2 values was plotted in windows of 250kb.

Brook Trout chromosomes 12, 19, 27, and 31 (CM055694.1, CM055701.1, CM055709.1, CM055713.1) with blocks of LD (r^2 above ~0.6) were further analyzed using PCA to look for patterns of inversions in all individuals (3 groups representing homozygotes for the inversion, homozygotes without the inversion, and heterozygotes with one copy of the inversion). SNPs within LD blocks were used to calculate genotype likelihoods using ANGSD v.0.940. PCA was performed in R Studio v.4.0.2 with the eigen function and plotted with ggplot2 v.3.3.6. Heatmaps were made for chromosomes 12, 19, 27, and 31 using all populations, and all populations excluding the westernmost streams (Poole, Healeys, Sheep Shearer). LD was calculated as described above using ngsLD v.1.1.1.

Heterozygosity Among Potential Inversion Polymorphisms

Allele frequencies of major and minor alleles across chromosomes 12, 19, 27, and 31 for individuals representing the 3 different groups of a potential inversion were calculated with ANGSD v.0.940. Observed and expected proportions of heterozygotes and homozygotes were calculated using the following script https://github.com/clairemerot/angsd_pipeline/blob/master/01_scripts/Rscripts/Hobs_sliding.r (Mérot, 2021) and the R package WindowScanr v.0.1 (Tavares, 2022) was used to calculate average observed proportion of heterozygotes (H_{obs}) in 10kb windows with a 10kb step. Average H_{obs} across each chromosome was plotted in R v.4.0.2 (RStudio Team, 2021) using ggplot2 v.3.3.6 (Wickham, 2016). The size of the potential inversion could be more accurately estimated from plots of raw H_{obs} data compared to the LD heatmaps and the SNP lists were adjusted accordingly. H_{obs} was calculated within potential inversion regions using a window size the size of potential inversions and plotted as boxplots in R v.4.0.2 (RStudio Team, 2021) using ggplot2 v.3.3.6 (Wickham, 2016).