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INVESTIGATION

A Dense Brown Trout (Salmo trutta) Linkage Map Reveals Recent Chromosomal Rearrangements in the Salmo Genus and the Impact of Selection on Linked Neutral Diversity

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ABSTRACT High-density linkage maps are valuable tools for conservation and eco-evolutionary issues. In salmonids, a complex rediploidization process consecutive to an ancient whole genome duplication event makes linkage maps of prime importance for investigating the evolutionary history of chromosome rearrangements. Here, we developed a high-density consensus linkage map for the brown trout (Salmo trutta), a socioeconomically important species heavily impacted by human activities. A total of 3977 ddRAD markers were mapped and ordered in 40 linkage groups using sex- and lineage-averaged recombination distances obtained from two family crosses. Performing map comparison between S. trutta and its sister species, S. salar, revealed extensive chromosomal rearrangements. Strikingly, all of the fusion and fission events that occurred after the S. salar/S. trutta speciation happened in the Atlantic salmon branch, whereas the brown trout remained closer to the ancestral chromosome structure. Using the strongly conserved synteny within chromosome arms, we aligned the brown trout linkage map to the Atlantic salmon genome sequence to estimate the local recombination rate in S. trutta at 3721 loci. A significant positive correlation between recombination rate and within-population nucleotide diversity (π) was found, indicating that selection constrains variation at linked neutral sites in brown trout. This new high-density linkage map provides a useful genomic resource for future aquaculture, conservation, and eco-evolutionary studies in brown trout.

KEYWORDS linkage mapping Salmo trutta salmonids RAD markers chromosomal rearrangements recombination rate

A renewed interest for linkage mapping studies has recently occurred thanks to the simplified procedures for generating large genotype datasets in nonmodel organisms (Slate et al. 2008; Stapley et al. 2010; Ekblom and Galindo 2011). High-density linkage maps have been particularly developed in teleost fishes because of their high number of species, economic importance for aquaculture (e.g., Sauvage et al. 2012; Zhao et al. 2013; Wang et al. 2015; Liu et al. 2016; Kumar and Kocour 2017), phylogenetic position within vertebrates (Amores et al. 2011; Rondeau et al. 2014; Wang et al. 2015; Kuang et al. 2016), and broad interests for eco-evolutionary and conservation/management issues (Everett et al. 2012; Gagnaire et al. 2013a; Kai et al. 2014).

The salmonid family, which has 11 genera and >60 species (Crête-Lafrenière et al. 2012), is a group of fish in which numerous initiatives have been developed to construct high-density linkage maps (Brieuc et al. 2014; Gonen et al. 2014; Limborg et al. 2015b; Larson et al. 2016; Tsai et al. 2016). Besides their applications for mapping traits of importance for aquaculture (Houston et al. 2012; Sauvage et al. 2012; Tsai et al. 2013), salmonid linkage maps have catalyzed molecular ecology...
and genome evolution studies over recent years. Several studies have used linkage map information for addressing eco-evolutionary and related conservation biology issues at the species level or among hybridizing taxa (Lamaze et al. 2012; Bourret et al. 2013; Perrier et al. 2013; Gagnaire et al. 2013b; Limborg et al. 2014; McKinney et al. 2015). A second category of studies have focused on understanding the evolutionary consequences of the whole genome duplication event (salmonid-specific fourth vertebrate whole genome duplication; WGD-Ss4R) that occurred within the salmonid family ~60 MYA (Crête-Lafrenière et al. 2012), especially with regards to the partial rediploidization process, which is still underway and not totally understood yet (Berthelot et al. 2014; Brieuc et al. 2014; Kodama et al. 2014; Sutherland et al. 2016; Allendorf et al. 2015; Lien et al. 2016; Nugent et al. 2017). A significantly improved understanding of this process has been achieved through increased efforts to map and compare chromosomal rearrangements among species (inversions, translocations, fissions, and fusions), which have helped to reconstruct the evolutionary history of salmonid genome architecture (Kodama et al. 2014; Sutherland et al. 2016). Furthermore, linkage mapping approaches have also improved the characterization of the genomic regions that still exhibit tetrasomic inheritance patterns (Allendorf et al. 2015; Kodama et al. 2014; Lien et al. 2016; May and Delany 2015; Waples et al. 2016). The partial rediploidization of salmonid genomes is also known to challenge the development of genome-wide marker data sets. Indeed, duplicated loci that mostly reside in tetrasomic regions (Allendorf et al. 2015) are usually underrepresented or even excluded in salmonid genomic studies (Hohenlohe et al. 2011; Hecht et al. 2012; Gagnaire et al. 2013b; Larson et al. 2014; Perrier et al. 2013; Leitwein et al. 2016).

The Eurasian brown trout (Salmo trutta L. 1758), is one of the most widespread freshwater species in the Northern Hemisphere. It presents high levels of phenotypic diversity linked to its complex evolutionary history (Elliott 1994; Bernatchez and Osinov 1995; Bernatchez 2001). Natural brown trout populations have been intensely studied using molecular markers (e.g., Hansen et al. 2000; Sanz et al. 2006; Thauлов et al. 2014; Berrebi 2015; Leitwein et al. 2016), but previous population genetics and phylogeography studies that usually relied on a limited number of markers did not integrate linkage map information (but see Hansen and Mensberg 2009; Hansen et al. 2010). The microsatellite linkage map available until now in brown trout (Gharbi et al. 2006) contains ~300 markers (288 microsatellites and 13 allozymes), distributed over 37 of the 40 linkage groups (LGs) expected in this species (2n = 80, Martinez et al. 1991; Phillips and Rab 2001). In order to follow the tendency toward an increasing number of markers in brown trout genetic studies (Leitwein et al. 2016), a higher density linkage map is needed to increase mapping resolution and provide positional information for genome scans and association studies. The development of a high-density linkage map in brown trout should find at least two direct applications.

First, although the brown trout is the closest relative of the Atlantic salmon (S. salar L. 1758), the two species drastically differ in their number of chromosomes (S. salar 2n = 54–58, Brenna-Hansen et al. 2012; S. trutta 2n = 80, Phillips and Rab 2001), indicative of a high frequency of chromosome rearrangements. For understanding the chromosomal evolution in the Salmo genus, a comparison between the genome architecture of the Atlantic salmon (Lien et al. 2016) and the brown trout, using other salmonid linkage maps as outgroups, is needed. The availability of important genomic resources in salmonids coupled with recent methodological advances in linkage map comparisons (Sutherland et al. 2016) provide favorable conditions for new insights into the recent history of chromosomal rearrangement in the Salmo genus.

Second, another important application of a high-density linkage map in brown trout relates to understanding the consequences of human-mediated introductions of foreign and domestic genotypes in natural populations. The brown trout has been domesticated for decades, and wild populations have been heavily impacted by the stocking of genetically domesticated hatchery strains sometimes originating from evolutionary lineages distinct from the recipient populations (Elliott 1994; Laikre 1999; Berrebi et al. 2000; Bohling et al. 2016). These introductions have often resulted in the introgression of foreign alleles within wild brown trout populations, a generally negatively perceived phenomenon in conservation and management (Largiader and Scholl 1996; Poteaux et al. 1998, 1999; Hansen et al. 2000; Almodóvar et al. 2006; Sanz et al. 2006; Hansen and Mensberg 2009). However, the real evolutionary consequences of hybridization may take multiple facets (e.g., outbreeding depression, heterosis, adaptive introgression, loss of adaptive variation), the individual effects of which remains largely unknown. In order to evaluate those potential fitness outcomes in brown trout, it would be necessary to combine the power of large SNP data sets (Leitwein et al. 2016) with a high-density linkage map and a detailed knowledge of chromosomal variation in recombination rate to perform comprehensive genome scans for introgression in natural populations.

The specific objectives of this study were: (i) to build a high-density linkage map in brown trout based on restriction site-associated DNA (RAD) markers using reciprocal crosses of individuals belonging to two distinct evolutionary lineages (Atlantic and Mediterranean; Bernatchez 2001); (ii) to characterize chromosome morphologies and centromere positions of metacentric chromosomes using the method recently developed in Limborg et al. (2015a); (iii) to perform map comparisons with the S. salar genome and an outgroup salmonid species to infer the timing of chromosome rearrangements during the recent evolutionary history of the Salmo genus; and (iv) to estimate the genome-wide local variation in recombination rate and assess the consequence of this variation on the genomic landscape of nucleotide diversity in brown trout.

**MATERIALS AND METHODS**

**Experimental crosses**

Two F1 hybrid families, each comprising two parents and 150 offspring, were used for the linkage map analysis. Both were obtained by crossing one parent of Atlantic origin with one parent of Mediterranean origin (Bernatchez 2001). The F0 progenitors were either from a domestic Mediterranean strain lineage originally bred in the Babeau hatchery [described in Bohling et al. (2016)] and a domestic Atlantic strain lineage originating from the Cauterets hatchery and independently reared from the Mediterranean strain at the Babeau hatchery. The Cauterets strain is one of the common, internationally distributed Atlantic strains (comATL category; Bohling et al. 2016). Crosses were performed in December 2015 at the Babeau hatchery as follows: FAMILY 1, one Atlantic female × one Mediterranean male; and FAMILY 2, one Atlantic male × one Mediterranean female. Progenies were killed in February 2016, after full resorption of the yolk sac (fish size ~2 cm).

**DNA extraction, library preparation, and sequencing**

Whole genomic DNA was extracted from caudal fin clips for the four parents of the two crosses and from tail clips of 150 offspring of each family, using the commercial Thermo Scientific KingFisher Flex Cell and Tissue DNA kit. Individual DNA concentration was quantified using both a NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific) and Qubit 1.0 Fluorometer (Invitrogen, Thermo Fisher Scientific). Double digest RAD sequencing library preparation was performed following Leitwein et al. (2016). Briefly, DNA was digested with two
restriction enzymes (EcoRI-HF and MspI) and the digested genomic DNA was then ligated to adapters and barcodes. Ligated samples with unique barcodes were pooled in equimolar proportions and CleanPCR beads (GC Biotech, Alphen aan den Rijn, The Netherlands) were used to select fragments between 200 and 700 bp. The size selected fragments were amplified by PCR and tagged with a unique index specifically designed for Illumina sequencing (Peterson et al. 2012). For progenies, PCR products were pooled in equimolar ratios into four pools. Each pool was sequenced in a single Illumina HiSeq2500 (i.e., 75 F1 progeny per lane), producing 125-bp paired-end reads. The four parents were sequenced together in a single lane in order to obtain a higher coverage depth.

Genotyping pipeline
Bioinformatic analyses followed the same procedure as in Leitwein et al. (2016). Briefly, raw reads that passed an Illumina purity filter and were of sufficient FastQC quality (> 28) were demultiplexed, cleaned, and trimmed with process_radtags.pl implemented in Stacks v1.35 (Catchen et al. 2013). The Atlantic salmon genome (GenBank accession number: GCA_000233375.4_ICSASG_v2; Lien et al. 2016) was used to determine individual genotypes at RAD markers using a reference mapping approach. First, reads were aligned to the S. salar genome with the BWA-mem program (Li and Durbin 2010). Alignment result files were processed into pstacks to build loci and call haplotypes for each individual (using \( m = 3 \) and \( \alpha = 0.05 \)). Individuals with a minimum mean coverage depth under 7x were removed, resulting in a data set of 147 offspring in FAMILY 1 and 149 offspring in FAMILY 2. Hereafter, a RAD locus is defined as a sequence of 120 bp, and thus can contain more than one SNP defining different haplotypes. A reference catalog of RAD loci was built with cstacks from the four parents of the two crosses (FAMILY 1 and FAMILY 2). Each individual offspring and parent were then matched against the catalog with sstacks. Then, for each family independently, the genotypes module was executed to identify mappable makers that were genotyped in at least 130 offspring in each family, and with a minimum sequencing depth of five reads per allele. The correction option (\(-c\)) in the genotypes module was used for automated correction of false-negative heterozygote calls. The map type “cross CP” for heterogeneously heterozygous populations was selected to export haplotypic genotypes in the JoinMap 4.0 format (Van Ooijen 2006). For importation into JoinMap, only the loci shared between FAMILY 1 and FAMILY 2 were kept, leaving a total of 7680 mappable loci in both families.

Linkage mapping
Both family data sets were filtered into JoinMap 4.0 (Van Ooijen 2006) to remove (i) loci presenting with significant segregation distortion, (ii) loci in perfect linkage (i.e., similarity of loci = 1), and (iii) individuals with high rates of missing genotypes (>25%). After this first filtering step, 5635 and 5031 loci remained in FAMILY 1 and FAMILY 2, respectively. Only those that were shared by the two families were kept, resulting in a subset of 4270 filtered informative loci. A random subset of 4000 loci common to both families were finally selected, which corresponds to the maximum number of loci handled by JoinMap 4.0. We then discarded four offspring in FAMILY 1 and six offspring in FAMILY 2 due to high rates of missing genotypes (>20%). Markers were then grouped independently within each family using the “independence LOD” parameter in JoinMap, with a minimum LOD value of 10. Unassigned markers were second assigned using the strongest cross-linkage option with a LOD threshold of 5. Marker ordering was finally estimated within each group using the regression mapping algorithm and three rounds of ordering. The Kosambi mapping function was used to estimate genetic distances between markers in centimorgan. Finally, loci with undetermined linkage were removed and a consensus map was produced between families using the map integration function after identifying homologous LGs between FAMILY 1 and FAMILY 2.

Estimation of centromere location and chromosome type
The method recently developed by Limborg et al. (2015a) was used to identify chromosome types (acrocentric, metacentric) and to determine the approximate centromere position of metacentric chromosomes.

Figure 1 The brown trout (Salmo trutta) high-density linkage map. Horizontal black lines within each of the 40 LGs represent mapped RAD markers (n = 3977). The most probable chromosome type determined with the RFm method (Limborg et al. 2015a) is indicated above each LG. Green boxes indicate the approximate position of the centromere for metacentric LGs. Red dots represent the position of 89 additional markers that were transferred from the previous generation linkage map (see Table S2 for details). A, acrocentric; M, metacentric; U, undetermined.
<table>
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<th>Brown Trout LG</th>
<th>Distance (cM)</th>
<th>Number of SNP Markers Mapped to Each LG</th>
<th>Density (markers/cM)</th>
<th>Recombination Rate (cM/Mb)</th>
<th>LG_Map Gharbi</th>
<th>Atlantic Salmon</th>
<th>Brook Charr</th>
<th>Northern Pike</th>
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<td>BC-19</td>
<td>18.1</td>
<td></td>
</tr>
<tr>
<td>BT-40a</td>
<td>15.12</td>
<td>23</td>
<td>1.52</td>
<td>0.58</td>
<td>-</td>
<td>Ssa19qb</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>BT-40b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>Ssa19qa</td>
<td>21.2</td>
<td></td>
</tr>
</tbody>
</table>

The correspondence with LGs of the former generation linkage map established by Gharbi et al. (2006) is reported. The mean recombination rate is reported for each LG, as well as syntenic relationships with homologous chromosome arms from Atlantic salmon (S. salar), the brook charr (Salvelinus fontinalis) used as an outgroup for the Salmo genus, and the northern pike (Esox lucius) used as a nonduplicated outgroup for salmonids. The northern pike chromosomes are listed twice to accommodate the duplicate orthologs in the salmonid species (Sutherland et al. 2016). Boldface corresponds to the seven pairs involved in homeologous pairing (i.e., tetrasomic inheritance) in S. salar (Lien et al. 2016).

aData retrieved from Table 2 of Sutherland et al. (2016).
This method uses phased progeny genotypes to detect individual recombination events in comparison with parental haplotype combinations. For each LG, the cumulated number of recombination events between the first marker and increasingly distant markers was computed from both extremities, using each terminal marker as a reference starting point. When covering a full chromosome arm, the recombination frequency (RFm) values are expected to reach a plateau of ~0.5, thus allowing the determination of chromosome morphology and the approximate centromere location for metacentric chromosomes [see Limborg et al. (2015a) for details]. We used the subset of markers that were found heterozygous only in the female parent to phase progeny haplotypes within each family. We only focused on female informative sites because the lower recombination rate in male salmonids makes them less adequate for inferring centromere locations (Limborg et al. 2015a). Moreover, lower male recombination rates have been reported within the haplotypes within each family. We only focused on female informative sites because the lower recombination rate in male salmonids makes them less adequate for inferring centromere locations (Limborg et al. 2015a). Moreover, lower male recombination rates have been reported in brown trout (Gharbi et al. 2006). The phased genotypes data sets were used to estimate RFm for intervals between markers along each LG. Finally, we plotted the RFm calculated from each LG extremity to determine the chromosome type and the approximate location of the centromere for metacentric chromosomes.

Map comparisons
The microsatellite linkage map established by Gharbi et al. (2006) was compared and anchored to our new high-density linkage map using the MAPCOMP pipeline (available at https://github.com/enormandeau/mapcomp/) (Sutherland et al. 2016). The microsatellite markers’ primer sequences retrieved from Gharbi et al. (2006), as well as the five microsatellites used in Leitwein et al. (2016) and the lactate dehydrogenase (LDH-C1) gene from McMeel et al. (2001) were aligned with BWA_aln (Li and Durbin 2010) to the Atlantic salmon reference genome. Only single hit markers with a quality score (MAPQ) above 10 were retained. The 3977 RAD markers integrated in the high-density S. trutta linkage map (see Results) were also aligned to the S. trutta reference genome with BWA_mem. Microsatellite and LDH markers were anchored onto the RAD linkage map by identifying the closest RAD locus located on the same contig or scaffold. Pairing with closely linked RAD markers enabled us to position markers from the previous generation map onto the newly developed linkage map.

An additional comparison was performed between the S. salar high-density linkage map published by Tsai et al. (2016) and the S. trutta consensus linkage map developed in this study. We ran the MAPCOMP pipeline (Sutherland et al. 2016) to identify blocks of conserved synteny and orthologous chromosome arms between the Atlantic salmon and the brown trout maps, using the Atlantic salmon genome as a reference. Conserved synteny blocks were visualized with the web-based VGSC (Vector Graph toolkit of genome Synteny and Collinearity: http://bio.njfu.edu.cn/vgsc-web/). The brook charr (Salvelinus fontinalis) linkage map published in Sutherland et al. (2016) was used as an outgroup to identify chromosome rearrangements that occurred within the Salmo lineage.

In order to estimate the genome-wide averaged net nucleotide divergence between S. trutta and S. salar (d_w), we used the mean nucleotide diversity within S. trutta (π_w, averaged between Atlantic and Mediterranean lineages) (Leitwein et al. 2016), and the absolute nucleotide divergence between S. trutta and S. salar (π_b, also called d_b). The net divergence was estimated as \( d_w = \pi_b - \pi_w \) by supposing that the mean nucleotide diversity in S. salar (which is not available) was close to the mean nucleotide diversity estimated in S. trutta. To estimate π_b, we used the 3977 consensus sequences of the RAD loci integrated in our S. trutta linkage map, and aligned them with BWA_mem to the Atlantic salmon reference genome. Then, the total number of mismatches divided by the total number of mapped nucleotides was used to provide an estimate of \( \pi_b \).

Estimation of genome-wide recombination rates
To estimate local variation in recombination rate across the genome, we compared our newly constructed genetic map of S. trutta to the physical map of S. salar with MAREYMAP (Rezvoy et al. 2007). In order to reconstruct a collinear reference genome for each brown trout LG, physical positions of brown trout RAD markers on the salmon genome were extracted from the BWA_mem alignment for each block of conserved synteny previously identified with MAPCOMP. The Loess method was used to estimate local recombination rates. It performs a local polynomial regression to fit the relationship between the physical and the genetic distances. The window size, defined as the percentage of the total number of

![Figure 2](https://github.com/enormandeau/mapcomp/). Plots illustrating the recombination frequency estimates (RFm) for intervals between markers along two different LGs. For each LG, RFm was calculated from both chromosomal extremities (right: red circles; left: blue circles), using each of the two terminal markers as a reference starting point. The RFm plot of (A) LG BT-22 illustrates a classical acrocentric pattern, the centromere position remains undetermined with regard to which LG extremity, while (B) LG BT-28 displays a classical metacentric pattern with a centromere position ~70 cM. The RFm plots of all brown trout LGs obtained in the two families are illustrated in Figure S1.
markers to take into account for fitting the local polynomial curve, was set to 0.9 to account for local uncertainties due to the high rate of chromosomal rearrangements between species. A recombination rate equal to the weighted mean recombination rate of the two closest markers on the genetic map was assigned to the markers that were not used for fitting.

Correlation between recombination rate and nucleotide diversity
In order to test for a correlation between local recombination rate and nucleotide diversity in brown trout, we used the estimate of nucleotide diversity (\( \pi \)) in the Atlantic lineage (based on 20 individuals). These estimates of nucleotide diversity were taken from Leitwein et al. (2016), in which brown trout populations and hatchery strains of different origins showed highly correlated genome-wide variation patterns in nucleotide diversity. Markers with a recombination rate value \( > 2 \) cM/Mb or \( \pi < 0.008 \) were considered as outliers and were therefore removed from the analysis. This resulted in a data set containing 3038 markers for which both recombination rate and nucleotide diversity information were available.

Data availability
Supplemental Material, Table S1 contains RAD markers IDs, consensus sequences, mapping position on the brown trout linkage map, physical position on the Atlantic salmon genome, and estimation of local recombination rate. Table S2 contains correspondence between the microsatellite and the new RAD linkage map. Figure S1 shows RFm plots obtained for each female haploid data set. Figure S2 shows MapComp Oxford plot comparing the brown trout to the Atlantic salmon linkage map, with markers paired through the Atlantic salmon genome. Figure S3 shows distribution of female and male marker positions along each LG for FAMILY 1. File S1 is an archive containing the .loc and .map files from both families. Demultiplexed sequence reads aligned against the Atlantic salmon genome (in Bam file format) are available at NCBI Short Read Archive under accession number PRJNA371687.

RESULTS
Construction of a high-density linkage map in S. trutta
On average, 35M of filtered paired-end reads were obtained for each of the four parents (two families), and 5M paired-end reads for each of the 300 F1 (150 progenies per family) analyzed in this study. The retained
subset of 7680 informative markers common to both families had a mean individual sequencing depth of 112× for the parents and 15× for the progenies, ensuring a good genotype calling quality in both families. Among these, a subset of 4000 markers showing no segregation distortion in both families was retained to generate a consensus map. The resulting integrated map thus provides sex- and lineage-averaged genetic distances, which is an important feature considering the reported differences in recombination rates between male and female in salmonids (Allenford et al. 2015; Tsi et al. 2015), and the existence of divergent geographic lineages in brown trout (Bernatchez 2001).

A total of 3977 markers were assigned to 40 S. trutta (BT) LGs on the integrated map (Figure 1), corresponding to the expected number of chromosomes in this species (Phillips and Ráb 2001). The total map length was 1403 cM, and individual LGs ranged from 14.6 cM (BT-03; 47 markers) to 84.9 cM (BT-37; 63 markers). The number of markers per LG ranged from 23 for BT-40 (15.2 cM) to 252 for BT-01 (46.3 cM). Detailed information for each LG including the density of markers per centimorgan is reported in Table 1. Additional details concerning RAD markers IDs, consensus sequences, and mapping position on the salmon genome are reported in Table S1.

**Chromosome morphology and centromere location**

Individual LG RFm plots obtained using estimates of recombination frequencies in progeny were generally similar between FAMILY 1 and FAMILY 2 (Figure S1). Two examples of RFm plots obtained in FAMILY 1 are reported in Figure 2 for BT-22 and BT-28. Following Limborg et al. (2015a), the pattern observed for BT-22 typically reflects an acrocentric chromosome type (paired straight lines; Figure 2A), whereas BT-28 illustrates a metacentric pattern (mirrored hockey stick shapes; Figure 2B) with the centromere located at ~70 cM. The total number of probable acrocentric chromosomes (Figure 1; type A, n = 32) largely exceeded the number of metacentric chromosomes (Figure 1; type M, n = 3). The approximate position of the centromere was determined for each of the three metacentric LGs (Figure 1; green boxes). The chromosome type remained undetermined for five LGs (Figure 1; type U) due to low resolution of the RFm plots (Figure S1).

**Linkage map comparisons**

A total of 87 microsatellite markers (82 out of 160 from Gharbi et al. 2006) were successfully mapped to the Atlantic salmon genome, and anchored to the new brown trout linkage map using their pairing with RAD markers, following the method developed in Sutherland et al. (2016) (Figure 1; red dots). The correspondence between the microsatellite linkage map and the new RAD linkage map is provided in Table S2.

The identification of orthologous chromosomes arms between S. trutta (40 LGs) and S. salar (29 LGs) revealed numerous differences, including fusions and fissions between the two species (Figure 3 and Table 1). We found 15 one-to-one ortholog pairs (i.e., one brown trout LG corresponds to a single Atlantic salmon LG, as for example Ssa6 and BT-5, or Ssa28 and BT-14; Figure 3 and Table 1), 12 cases where Atlantic salmon chromosomes correspond to either two or three brown trout LGs (e.g., Ssa1, Ssa2; Figure 3), and only two cases where brown trout LGs correspond to two Atlantic salmon chromosomes (BT-1 and BT-15; Figure 3). Between-species intrachromosomal inversions were also observed, as for example an inversion flanked by noninverted regions between S. salar Ssa6 and S. trutta BT-5 (Figure S2). Apart from these inter- and intrachromosomal rearrangements, a strongly conserved synteny was observed between the brown trout and the Atlantic salmon genomes. We estimated the average net nucleotide divergence between S. trutta and S. salar to dN = 0.0187 (with πb = 0.0228, and πw = 0.0041).

The identification of orthologous genomic regions between the brook charr, the brown trout, and the Atlantic salmon (Table 1) allowed inference of ancestral and derived chromosomes structures in the *Salmo* genus. We found eight chromosomal rearrangements that occurred in the ancestral lineage of the *Salmo* genus before speciation between *S. trutta* and *S. salar*. This includes five fusion events and three fission events (Figure 4 and Table 1). While no species-specific rearrangement was detected in the *S. trutta* branch, the Atlantic salmon branch contained two fission events and 13 fusion events (Figure 4). Among these fusions, the one between BT-17 and BT-39 corresponds to Ssa15, which is known to contain the salmonid sex-determining gene sdY in the Atlantic salmon (Li et al. 2011; Yano et al. 2013). Since the microsatellite locus OmyRT5TUF was consistently shown to be linked to sdY in the brown trout (Gharbi et al. 2006; Li et al. 2011), our results indicate that LG BT-39 might contain the sex-determining gene in brown trout (Table S2), which will need to be investigated further.

**Estimations of genome-wide and local recombination rates**

The brown trout genome-wide recombination rate averaged across the 3721 markers anchored on the Atlantic salmon reference genome (RAD markers mapped to ungrouped scaffolds were discarded) was estimated at 0.88 ± 0.55 cM/Mb, and ranged from 0.21 cM/Mb (BT-27) to 4.10 cM/Mb (BT-37) across LGs (Table 1 and Table S1). Chromosomal variation in local recombination rate was observed for most chromosomes (e.g., BT-9 and BT-23, Figure 5). A significant positive correlation between local recombination rate and population nucleotide diversity was detected (R² = 0.0598; p-value < 0.001; Figure 6).

**DISCUSSION**

This study describes the first high-density linkage map in the brown trout *S. trutta*, which extends the microsatellite linkage map already available in this species (Gharbi et al. 2006). The map covers 1403 cM and encompasses 3977 nondistorted markers distributed across 40 LGs, the centromere location of which was determined for 35 LGs. Results showed consistent chromosome number and morphologies compared to species karyotype (2n = 80, NF = 100 chromosome arms; Phillips and Ráb 2001). High-resolution mapping (average density of 3.15 markers per cM) was insured by the use of two mapping families, each containing 150 offspring, and the number of informative markers in both families was close to the limit over which saturation is expected (i.e., 4200 markers). The homogeneous distribution of makers across the genome, and the estimation of averaged recombination distances...
between both evolutionary lineages and sexes provide a highly valuable resource for future genomic, aquaculture, conservation, and ecologically studies in brown trout. Beyond these general interests, this new linkage map also reveals strikingly different rates of chromosomal rearrangements that happened between the brown trout and the Atlantic salmon since their recent divergence.

**A new generation RAD linkage map in brown trout**

Comparison between the newly developed linkage map and the previous microsatellite map was made possible by the integration of 82 microsatellite markers from the former generation map (Gharbi *et al.* 2006). This enabled us to identify the correspondence between homologous LGs from the two maps, and to detect cases of LG merging (eight out of 40) and LG splitting (nine out of 40) in the microsatellite map compared to the RAD linkage map. Consistent with the reported overrepresentation of acrocentric compared to metacentric chromosomes in the brook char (i.e., eight metacentric and 34 acrocentric, Sutherland *et al.* 2016), we found three metacentric and 32 acrocentric chromosomes in the brown trout. The morphology of five chromosomes could not be determined from the analysis of RfM plots alone. However, BT-17 was likely a metacentric chromosome based on its correspondence with the Atlantic salmon LG Ssa5 (Figure 3), and BT-17, BT-27, and BT-37 were likely acrocentric chromosomes as were single correspondences with acrocentric chromosomes of the Atlantic salmon and brook char linkage maps (Table 1). This represents a total number of chromosome arms ranging from 88 to 90, which is lower but quite close to the 96–104 interval previously estimated in *S. trutta* (Martinez *et al.* 1991; Phillips and Ráb 2001). Our assessment of chromosome type did not perfectly match the results from Gharbi *et al.* (2006), since the three metacentric chromosomes identified here were found to be either acrocentric or undetermined in their study. However, the metacentric chromosomes BT-2 and BT-5 identified here had more than a single matching LG in the former microsatellite map, which suggests that these homologs represent the two telericomic region of a larger metacentric LG that has been reconstructed in our study. On the contrary, the three metacentric chromosomes identified in Gharbi *et al.* (2006) appeared to be acrocentric using the phase information available from our data set. This could be the consequence of pseudolinkage in the microsatellite map built using males of hybrid origin, in which the preferential pairing of conspecific homologs followed by alternate disjunction may have generated an excess of nonparental gametes (Morrison 1970; Wright *et al.* 1983; Allendorf *et al.* 2015). In our study, the possible effect of pseudolinkage was avoided by using nonhybrid parents with a single lineage ancestry, thus possibly explaining the observed differences with the previous generation map.

Besides pseudolinkage, another broader consequence of past whole genome duplication in salmonids is the existence of duplicated loci that share the same alleles due to homeologous recombination in telomeric regions (Wright *et al.* 1983; Allendorf and Danzmann 1997; Allendorf *et al.* 2015). These residual tetrasomic regions challenge traditional linkage map reconstruction methods since duplicated loci (isochromosomes) often show Mendelian segregation distortion, and are therefore frequently excluded from linkage mapping analyses. Alternative approaches can be used to detect homeologous regions in experimental crosses (Lien *et al.* 2011; Briéuc *et al.* 2014; Staples *et al.* 2016). In brown trout, the location of duplicated regions have been preliminarily established from genome-wide patterns of RAD markers density and nucleotide diversity projected on the Atlantic salmon reference genome (Leitwein *et al.* 2016). Although our new RAD linkage map is based on non-Mendelian markers and therefore probably displays a reduced marker density in regions exhibiting tetrasomic inheritance, it does not completely exclude such regions. This is illustrated by the mapping of multiple RAD loci within the telomorphic region of the seven pairs of homeolog chromosome arms that retain residual tetrasomy in the Atlantic salmon genome (Lien *et al.* 2016) (Figure 3). Except for three of these 14 regions that received relatively few markers (Ssa3q, Ssa11q4, and Ssa17q4 on the salmon map; Figure 3), the remaining arms were well covered in our RAD linkage map, indicating that the filtering of non-Mendelian markers did not completely exclude the telomorphic regions affected by homeologous crossovers. This feature of our RAD linkage map should insure a more even recombination rate estimation between sexes (Lien *et al.* 2011), since the commonly observed lower recombination rate in males compared to females in salmonids could be partly due to a poor characterization of telomorphic regions in mapping studies (Allendorf *et al.* 2015). In order to evaluate the extent to which male and female informative markers tend to map to distinct regions, we compared their positions along each LG in FAMILY 1 that received the highest number of mapped markers (Figure S3). We observed that on average, informative markers in the male parent were less evenly distributed across LGs and tended to aggregate in regions containing lower densities of female informative markers. Although this might also reflect a poor positioning of male markers due to low recombination rates in males (Sutherland *et al.* 2016), the sex-averaged recombination distances in our consensus linkage map should provide relevant estimates.

![Figure 5](image_url) Estimates of local recombination rate \( \rho \) in centimorgan per megabase for markers located along two *Salmo trutta* linkage groups: (A) LG BT-9 and (B) LG BT-23. The average recombination rate (mean \( \rho \)) for each LG is given at the top left side of each panel. Local recombination rate estimates for other *S. trutta* LGs are reported in Table S1 and the average recombination rate of each LG is provided in Table 1.
of recombination rate along chromosomes for future eco-evolutionary studies. For instance, understanding genome-wide introgression patterns in nature needs to integrate information from both male and female recombination rate.

Following the same idea, integrating maps between parents of different lineage origins (Atlantic and Mediterranean) allowed us to generate a consensus linkage map in which the potential differences in recombination rates between these two allopatric lineages are smoothed. Our results suggest that no fusion polymorphism occurs between the two brown trout lineages. This is consistent with the lack of species-specific chromosomal rearrangement following speciation with the Atlantic salmon (Figure 4), and the stable chromosome number previously observed in brown trout (Hartley and Horne 1984; Martinez et al. 1991; Phillips and Ráb 2001). Therefore, the lineage-averaged recombination distances provided here are probably not affected by potential interchromosomal rearrangements within the *S. trutta* species. The lineage-averaged consensus map will therefore be valuable to future conservation genomic studies, considering the extensive human-mediated admixture between Atlantic and Mediterranean brown trout populations in southern Europe (Berrebi et al. 2000; Sanz et al. 2006; Thaulow et al. 2014; Berrebi 2015).

**The recent evolution of chromosomal rearrangements in the Salmo genus**

Salmonids have been undergoing a rediploidization process since a whole genome duplication event that occurred ~60 MYA (Crête-Lafrenière et al. 2012; Nair et al. 2012; Macqueen and Johnston 2014; Lien et al. 2016). This process, also described as functional diploidization by Ohno (1970), was accompanied by numerous chromosomal rearrangements within the salmonid family, making linkage maps of prime importance for studying the complex history of chromosomal evolution in salmonids (Sutherland et al. 2016). Several studies have pointed out a particularly high number of chromosomal rearrangements in the lineage leading to the Atlantic salmon, involving mainly chromosome fusions that reduced the number of chromosomes to 29 in this species (Allendorf and Thorgaard 1984; Lien et al. 2016; Sutherland et al. 2016). However, the timing of these rearrangements remains largely unknown due to the lack of a closely related species in previous studies. Because *S. trutta* is the most closely related species to *S. salar* among salmonids, the new brown trout RAD linkage map allowed for inferring the phylogenetic positions of the chromosome arm rearrangements that occurred within the *Salmo* genus. The ancestral state of *Salmo* chromosome structure was further resolved using the brook charr as an outgroup within salmonids, as well as other salmonid species (e.g., the lake whitefish *Coregonus clupeaformis*) when necessary (Sutherland et al. 2016). This enabled us to identify five fusion events and three fission events (Table 1) that occurred in the *Salmo* ancestral lineage before the Atlantic salmon/brown trout speciation. This is relatively small compared to the total number of chromosomal rearrangements that occurred within the whole *Salmo* branch. Thus, most of the fission and fusion events that were observed in the Atlantic salmon genome (Sutherland et al. 2016) most probably occurred after the divergence between the two *Salmo* species. The net nucleotide divergence between *S. salar* and *S. trutta* was here estimated to 1.87%, which indicates a relatively recent divergence time between these two sister species. Surprisingly, all of the interchromosomal rearrangements that occurred after the salmon/trout speciation happened in *S. salar*. Indeed, we did not observe any fusion/fission events within *S. trutta* branch (Figure 4), whereas 15 rearrangements mostly involving fusions (13 fusions and two fissions) specifically occurred in the Atlantic salmon branch. These Robertsonian translocations explain the increased number of metacentric chromosomes in the Atlantic salmon compared to the brown trout, which were produced by the recent fusion of acrocentric chromosomes (Ssa1 to Ssa4; Figure 3 and Table 1). However, some fusion events have also likely resulted in the formation of acrocentric chromosomes in the Atlantic salmon (e.g., Ssa15; Figure 3 and Table 1), which could partly explain the apparent loss of chromosomes arms in this species (Allendorf and Thorgaard 1984). Finally, intrachromosomal rearrangements also occurred along the *S. salar* branch, as illustrated by the inversion detected within Ssa6, the homologous LG to BT-5 (Figure 3 and Figure S2). Altogether, our results support the previous views that many chromosomal rearrangements have occurred within the *Salmo* lineage.

**Figure 6** Genome-wide positive correlation between the mean nucleotide diversity in the Atlantic brown trout lineage (r) and the local recombination rate (ρ, in centimorgan per megabase), based on 3038 SNP markers ($R^2 = 0.058$; p-value $< 0.001$).
lineage (Allendorf and Thorgaard 1984; Sutherland et al. 2016), but also demonstrate that most of them recently happened in the Atlantic salmon branch, while at the same time, *S. trutta* has retained a chromosomal architecture close to the ancestral form of the *Salmo* genus. The reasons for such a contrasted evolution of chromosome structure between these two species remain to be investigated.

**Recombination rate variation across the brown trout genome**

Despite extensive chromosomal rearrangements in the *S. salar* lineage, we could identify highly conserved syntenic blocks within orthologous chromosome arms to reconstitute local alignments between the brown trout linkage map and the salmon physical map. One of our main objectives was to use these comparisons between maps to estimate local recombination rate variations along each LG in *S. trutta* (Figure 5). The average genome-wide recombination rate was estimated at 0.88 cM/Mb, with strong heterogeneity being found across the genome. Highly recombining regions were observed at the extremities of some LGs (e.g., BT-9; Figure 5), and genomic regions associated with very low recombination rates were also observed (e.g., BT-23; Figure 5). In order to provide an indirect assessment of this local recombination rate estimation for future eco-evolutionary studies, we verified that the frequently observed positive correlation between local recombination rate and nucleotide diversity (Corbett-Detig et al. 2015) also occurs in brown trout. In a comparative study across a wide range of species, Corbett-Detig et al. (2015) suggested that the strength of this correlation is highly variable among species, being generally stronger in species with large vs. small census population size [but see Coop (2016)]. Although brown trout populations are usually considered to have relatively small census sizes (Jorde and Ryman 1996; Palm et al. 2003; Jensen et al. 2005; Serbezov et al. 2012), a significantly positive correlation was found between local recombination rate and nucleotide diversity using polymorphism data from the Atlantic lineage. Thus, recombination rate variation across the brown trout genome shapes the chromosomal landscape of neutral genetic diversity by modulating the efficiency of selection (both positive and negative) at linked neutral sites.

Variation in local recombination rate across genomic regions not only affects genome-wide variation patterns of polymorphism, but also has a profound influence on a range of evolutionary mechanisms of prime importance in brown trout. This recombination map should therefore have beneficial outcomes for our understanding of inbreeding depression, local adaptation, and the historical demography of natural populations (Rezvoy et al. 2007; Dukić et al. 2016; Racimo et al. 2015). It will also be of precious help for understanding genome-wide introgression patterns in natural populations that have been restocked with individuals from a different lineage. Finally, a detailed picture of recombination rate variation in brown trout will help for choosing SNPs for the design of genotyping arrays in future association mapping experiments (e.g., Bourret et al. 2013; Houston et al. 2014).

**Conclusions**

A high-density sex- and lineage-averaged consensus linkage map for *S. trutta* was developed and the morphology (acrocentric or metacentric) of most chromosomes was identified. Interspecies comparisons with the Atlantic salmon allowed identifying genomic regions of highly conserved synteny within chromosome arms and contribute toward a better understanding of recent genome evolution in the genus *Salmo*. A recent and strong acceleration in the rate of chromosomal rearrangements was detected in the *S. salar* branch. These results should stimulate further research toward understanding the contrasted evolution of chromosome structure in sister species with <2% net nucleotide divergence. Recombination rate variation was found to influence genome-wide nucleotide diversity patterns in brown trout. These new genomic resources will provide important tools for future genome scans, QTL mapping, and genome-wide association studies in brown trout. It also paves the way to enable a new generation of conservation genomics approaches that will look at the fitness consequences of introgression of foreign alleles into wild populations at the haplotype level.

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