Identification of the Sex Chromosomes of Brown Trout (*Salmo trutta*) and Their Comparison with the Corresponding Chromosomes in Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*Oncorhynchus mykiss*)

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Key Words

Atlantic salmon • Brown trout • Rainbow trout • Salmonids • Sex chromosomes • Sex linkage

Abstract

Males are the heterogametic sex in salmonid fishes. In brown trout (*Salmo trutta*) the sex-determining locus, *SEX*, has been mapped to the end of linkage group BT-28, which corresponds to linkage group AS-8 and chromosome SSA15 in Atlantic salmon (*Salmo salar*). We set out to identify the sex chromosomes in brown trout. We isolated Atlantic salmon BAC clones containing microsatellite markers that are on BT-28 and also on AS-8, and used these BACs as probes for fluorescent in situ hybridization (FISH) analysis. *SEX* is located on the short arm of a small subtelocentric/acrocentric chromosome in brown trout, which is consistent with linkage analysis. The acrocentric chromosome SSA15 in Atlantic salmon appears to have arisen by a centric fusion of 2 small acrocentric chromosomes in the common ancestor of *Salmo* sp. We speculate that the fusion process that produced Atlantic salmon chromosome SSA15 disrupted the ancestral sex-determining locus in the Atlantic salmon lineage, providing the impetus either for the relocation of *SEX* or selection pressure

for a novel sex-determining gene to arise in this species. Thus, the sex-determining genes may differ in Atlantic salmon and brown trout.

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Fish exhibit a remarkably wide array of sex-determining mechanisms [Devlin and Nagahama, 2002; Mank and Avise, 2006, 2009]. Thus, fish species provide tremendous resources for studying sex determination and the evolution of sex chromosomes [Schultheis et al., 2009]. The plasticity of teleost sex-determining systems is clearly illustrated from studies in the genus *Oryzias* [Kondo et al., 2009]. *DMY/dmrt1bY* has been identified as the sex-determining gene in medaka (*O. latipes*) [Matsuda et al., 2002; Nanda et al., 2002], and this is also the master switch in *O. curvinotus*. This gene is absent in the closely related species, *O. luzonensis*, and indeed the sex-determining gene resides on a different linkage group in this species. Moreover, although these *Oryzias* spp. have a strictly genetic sex-determining system in which the male is the heterogametic sex, in other *Oryzias* spp. (e.g. *O. hubbsi* and *O. javanicus*) there is a ZZ/ZW heterogametic system. Similar transitions between male and fe-

male heterogamety are observed in the tilapiine cichlids [Cnaani et al., 2008] and *Xiphophorus* spp. [Schultheis et al., 2009].

A therian-centric view of biology emphasizes the importance of sex chromosomes in sex determination [Marshall Graves and Peichel, 2010]. Sex chromosomes appear to have evolved independently in the avian ancestor and probably have done so several times in different fish lineages [Marshall Graves, 2008]. Models have been proposed that describe how karyotypically distinct sex chromosomes emerge after a mutation in an autosomal gene leads to a novel sex-determining master gene [Charlesworth et al., 2005]; however, it is interesting to note that less than 10 percent of cytogenetically characterized fish species have distinct sex chromosomes [Devlin and Nagahama, 2002]. This makes it all more important to identify fish chromosomes that carry sex-determining gene(s), especially if there is no evidence of gross heteromorphism from standard karyotypic analysis.

The family Salmonidae contains 11 genera and includes the salmon, trout, charr, grayling and freshwater whitefish [Nelson, 2006]. Many of these species, including Atlantic salmon and brown trout, are economically important for aquaculture, wild stock fisheries and recreational sports fisheries. Salmon and trout are sentinel species for monitoring the aquatic environment, and therefore they are used extensively for eco-toxicology studies. The salmonid research community is extensive, and as a result more is known about the biology of salmonids than any other fish group [Thorgaard et al., 2002; Davidson et al., 2010]. There are also some fundamental scientific questions that can be explored using their genomes. The common ancestor of salmonid fishes underwent an autotetraploidization event 50–120 million years ago [Allendorf and Thorgaard, 1984]. A major problem facing an organism that has undergone a whole genome duplication is how to deal with sex determination [Ohno, 1967]. A potential solution is to delete one of the duplicated sex-determining genes, and different duplicates could be deleted in different species. An alternative strategy is to recruit a novel sex-determining master gene from among the duplicated genes encoding transcription factors. Either strategy could potentially lead to a species radiation [Davidson et al., 2009].

Salmonid species possess a genetic mechanism of sex determination characterized by an XX/XY heterogametic system [Devlin and Nagahama, 2002], although evidence has been presented that environmental factors such as temperature [Craig et al., 1996; Azuma et al., 2004; Magerhans and Hörstgen-Schwark, 2010] or other

sex-modulating loci [Quillet et al., 2002] may influence the sex phenotype. The construction of genetic maps that include the sex-determining locus, *SEX* (defined as the male phenotype), for several salmonid species revealed that there is a lack of conservation of synteny of *SEX* and microsatellite markers in Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), Arctic charr (*Salvelinus alpinus*), coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*Oncorhynchus mykiss*) [Woram et al., 2003; Davidson et al., 2009]. These genetic maps place *SEX* at the end of a linkage group in these salmonids, and on this basis it has been assumed that *SEX* is located close to the telomeric regions in their respective chromosomes. The main question concerning sex determination in salmonids is whether the sex-determining gene is the same in all species, or at least within a genus, and has ‘jumped’ from one chromosome to another in different lineages without relocating adjacent markers [as has been suggested by Phillips et al., 2001] or whether *SEX* has evolved independently in several salmonid species as a result of the whole genome duplication that occurred in the common ancestor of extant salmonids [Allendorf and Thorgaard, 1984].

Over the past decade, many genomic resources have been developed for Atlantic salmon. These include a Bacterial Artificial Chromosome (BAC) library [Thorsen et al., 2005], a BAC-based physical map [Ng et al., 2005] and ~200,000 BAC end sequences, which provide a rich source of genetic markers for the construction of genetic maps [Danzmann et al., 2008; Phillips et al., 2009; Lorenz et al., 2010]. The integration of the linkage and physical maps and the assignment of linkage groups to specific chromosomes were accomplished using BACs known to contain specific genetic markers that had been placed on the Atlantic salmon genetic map [Phillips et al., 2009]. The Atlantic salmon has been chosen as a representative salmonid for genomics studies, and an international collaboration to sequence the Atlantic salmon genome has been established [Davidson et al., 2010].

The sex-determining locus in Atlantic salmon resides near a block of repetitive DNA at the end of the long arm of chromosome 2, a large metacentric chromosome [Artieri et al., 2006]. The brown trout is the only other species in the genus *Salmo*, although various taxa previously synonymized with *Salmo trutta* have recently been recognized as separate species in Italy [Kottelat and Freyhof, 2007]. The karyotype of brown trout has been well characterized [Hartley and Horne, 1987; Phillips and Ráb, 2001; Caputo et al., 2009], but no sex chromosomes have been identified. Given the close phylogenetic relation-

Table 1. Oligonucleotide sequences used for linkage analysis and BAC library screening

Name	Sequence
OmyRT5TUF-F	TGTAAAACGACGGCCAGTTCTAATCTCCATCAACACTGGC
OmyRT5TUF-R	GTTAAATGGCATTAGGTCTGCT
OmyFGT27TUF-F	TGTAAAACGACGGCCAGTAATATATCCCCCTCTCACCACC
OmyFGT27TUF-R	TACCCTTGTCAGTAGGTCACCA
OmyFGT2TUF-F	TGTAAAACGACGGCCAGTGTGTGGAACCCAACAGGC
OmyFGT2TUF-R	GAAACACGTCTGGGGTCAGT
Omy325UoG-F	TGTAAAACGACGGCCAGTTGTGAGACTGTCAGATTTTGC
Omy325UoG-R	CGGAGTCCGTATCCTTCCC
Omy10INRA-F	TGTAAAACGACGGCCAGTGATCCTCATACTGTACTTCTCC
Omy10INRA-R	CTCTTAGGCCAAACAAGGG
Ssa197DU-F	TGTAAAACGACGGCCAGTGGGTTGAGTAGGGAGGCTTG
Ssa197DU-R	TGGCAGGGATTTGACATAAC
OmyRT5TUF AS Probe	ACTGGCTGATAAAGAGCTTGCCTGCCTCTGTCTTTGCACT
OmyRT5TUF AS R	ATGGCATTAGGTCTGCTCT
Omy301UoG-F	TGTAAAACGACGGCCAGTACTTAAGACTGGCAACCTT
Omy301UoG-R	CTACACGGCCTTCGGGTGAGA

ships among the salmonids and the observation that on average their DNA sequences are greater than 90% identical [Rise et al., 2004; Leong et al., 2010], it is to be expected that genomic resources developed for 1 salmonid can be used to investigate the genome of another. As no comparable genomic resources are available for brown trout, we used Atlantic salmon BACs to identify the sex chromosomes of brown trout and then compared them with the syntenic chromosomes in Atlantic salmon and rainbow trout.

Materials and Methods

Linkage Group Analysis

Linkage analysis was carried out using the 2 Atlantic salmon SALMAP mapping families as previously described [Danzmann et al., 2008; Phillips et al., 2009]. The 5' end of the forward microsatellite primer has the 21M13 sequence, 5'-TGTTAAACGACGGCCAGT-3', added to it as part of the genotyping reaction. The PCR reaction mixture used for microsatellite mapping contained the following: 1 µl of 10× PCR buffer containing MgCl₂ (QIAGEN Inc., Mississauga, Ont., Canada), 0.2 µl of 10 mM dNTPs, 0.2 µl of 10 µM forward primer, 0.5 µl of 10 µM reverse primer, 0.5 µl of 10 µM 21M13-labeled primer (either FAM- or HEX-labeled), 0.1 µl of Taq DNA polymerase (QIAGEN Inc.), 0.5 µl of genomic DNA (15 ng) and 7 µl of dH₂O. The touchdown PCR conditions comprised 94°C for 2 min followed by 21 cycles of 94°C for 30 s, 60°C for 30 s, -0.5°C/cycle and 72°C for 30 s, and then an additional 14 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 30 s, and then 72°C for 10 min. Loading dye (formamide; 2.5 µl) was added to 2 µl of the PCR reaction mixture, heated for 4 min and then immediately put on ice. Two microliters of the cold

mixture was loaded onto a polyacrylamide gel made with following recipe: 11.6 g of urea, 13.2 ml of dH₂O, 3.85 ml of Long Range Buffer, and 2.75 ml of 10× TBE with 150 µl of 10% ammonium persulphate and 25 µl of TEMED, and run on an ABI 377 automated sequencer. The gel result was revealed by GeneScan® 3.1.2. Linkage analysis was carried out using LINKMFEX (<http://www.uoguelph.ca/~rdanzman/software/LINKMFEX/>) [Danzmann and Gharbi, 2001].

Identification of Atlantic Salmon BACs Containing Brown Trout Sex-Linked Microsatellites Probes

The reverse primer (20-mer) for microsatellite marker Omy301UoG was used directly as a probe to screen the Atlantic salmon BAC library. For microsatellite marker OmyRT5TUF, PCR using the touchdown conditions described above, was carried out with Atlantic salmon genomic DNA as the template, and the PCR product band of ~200 bp was cut from an agarose gel, cloned and sequenced. The sequence of the PCR product was used as the input to design an oligonucleotide probe (40-mer) and a corresponding complementary primer (20-mer) using Primer3 (v. 0.4.0) online software [Rozen and Skaletsky, 2000]. The oligonucleotides were designed such that they had a GC content of 50% or higher, with a T_m of the probe of at least 55°C. The probes and reverse primers are listed in table 1.

Screening the CHORI-214 BAC Library

We screened the CHORI-214 Atlantic salmon BAC library [Thorsen et al., 2005] with probes for the microsatellite markers. The 40-mers were end-labeled with ³²PγATP using T4 polynucleotide kinase and hybridized to 6 BAC filters at a time as described by Johnstone et al. [2008]. Briefly, pre-hybridization was carried out in 5× saline-sodium citrate buffer (SSC), 0.5% sodium dodecyl sulfate (SDS) and 5× Denhardt's solution at 65°C for at least 2 h, and then the ³²P-labeled probes were added and incubated overnight. The filters were washed 3 times, each for 1 h at 50°C,

in 1× SSC and 0.1% SDS. The filters were wrapped in Saran™ wrap and exposed to phosphor screens that were subsequently scanned using the Typhoon Imaging System and visualized using ImageQuant software, giving an image of the ³²P-labeled hybridization-positive BACs containing the microsatellite markers.

Confirmation of Hybridization-Positive BACs by PCR

The hybridization-positive BAC clones were picked from the Atlantic salmon BAC library, cultured in 5 ml LB media containing chloramphenicol (12.5 µg/ml) overnight at 37°C with shaking at 250 rpm. Glycerol stocks were made for subsequent PCR confirmation that these BACs did indeed contain the appropriate microsatellite marker. The PCR reaction mixture contained: 1 µl of 10× PCR buffer containing MgCl₂ (QIAGEN Inc.), 1 µl of 2 mM dNTPs, 0.5 µl of 10 µM 40-mer probe, 0.5 µl of 10 µM complementary reverse primer, 0.15 µl of Taq DNA polymerase (QIAGEN Inc.) and 6.8 µl of dH₂O. A small amount of BAC clone glycerol stock was added into the PCR reaction mix as template. The PCR conditions comprised 95°C for 5 min followed by 35 cycles of 95°C for 45 s, 65°C for 45 s and 72°C for 2 min, and then 72°C for 10 min. PCR products were separated by electrophoresis through a 1.3% agarose gel with 1× TBE, stained with ethidium bromide and visualized using a UV trans-illuminator (Alpha Innotech).

Isolation of BAC DNA for FISH Analysis

A single colony of each of the Atlantic salmon BAC clones picked for FISH analysis was obtained by streaking the glycerol stock on an LB agar plate containing chloramphenicol (12.5 µg/ml) and incubating at 37°C for 16 h. Eight single colonies were picked up and each was incubated in 5 ml of LB medium containing chloramphenicol (12.5 µg/ml), shaken at 250 rpm for 16 h at 37°C. The 8 colony cultures were tested by PCR with the primer sets specific for each markers using either touchdown PCR or regular PCR profile, and the PCR products were visualized as described previously. A PCR-positive colony was selected and prepared for QIAGEN Large Construct starter culture by inoculating in 5 ml LB medium containing chloramphenicol (12.5 µg/ml) and grown at 37°C for 8 h. The starter culture was diluted and grown in 500 ml LB medium containing chloramphenicol (12.5 µg/ml), with shaking at 250 rpm for 16 h at 37°C. The cells were harvested by centrifugation at 6,000 g for 15 min at 4°C. The cell pellet was then used to extract Atlantic salmon BAC DNA. The BAC DNA extraction exactly followed the QIAGEN Large Construct Kit Protocol (QIAGEN Inc.).

Fluorescent *in situ* Hybridization of BACs on Brown Trout Chromosomes

Blood was cultured from brown trout using standard methods [Reed and Phillips, 1995]. Atlantic salmon BAC DNA was labeled with Spectrum Orange using a nick translation kit (Abbott Molecular). Human placental DNA (2 µg) and Cot-1 DNA (1 µg, prepared from Atlantic salmon) or Cot-1 DNA (1 µg, prepared from rainbow trout) were added to the probe mixture for blocking. Hybridizations were carried out at 37°C overnight and post-hybridization washes were as recommended by the manufacturer (Abbott Molecular) with minor modifications [Phillips and Reed, 2000]. Antibodies to Spectrum Orange (Molecular Probes) were used to amplify the signal. Slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI) at a concentration of 125 ng

DAPI in 1 ml antifade solution. Images were captured with a Jai camera and analyzed with Cytovision Genus (Applied Imaging, Inc.) software. Chromosomes were arranged according to size within the metacentric/submetacentric and acrocentric groups.

Results and Discussion

Comparative Linkage Analysis

A linkage map for brown trout has been constructed with *SEX* on linkage group BT-28 [Gharbi et al., 2006]. In addition to *SEX*, this linkage group contains 7 microsatellite markers, OmyRT5TUF, OmyFGT27TUF, OmyFGT2TUF, Omy301UoG, Omy325UoG, Omy10INRA and Ssa197DU and an allozyme marker, PGDH. It should be noted that 6 of the microsatellite markers were first identified in rainbow trout (designated by the prefix Omy), whereas the seventh was isolated from Atlantic salmon (designated by the prefix Ssa). It is common that primers designed to amplify a microsatellite in 1 salmonid species are capable of hybridizing accurately to the DNA of other salmonids and amplifying the orthologous loci [Presa and Guyomard, 1996]. A comparative sex linkage group analysis by Woram et al. [2003] showed that 2 of the brown trout sex-linked markers (Omy301UoG and Ssa197DU) on BT-28 mapped to Atlantic salmon linkage group AS-8 and rainbow trout linkage group RT-21. We attempted to map the other 5 sex-linked microsatellite markers in brown trout in the Br5 and Br6 SALMAP Atlantic salmon mapping families, which were used to construct the most comprehensive Atlantic salmon genetic map to date [Danzmann et al., 2008; Phillips et al., 2009; www.AsalBase.org]. Neither OmyFGT27TUF nor OmyFGT2TUF was informative in the parents of Br5 or Br6 and so could not be mapped. Omy325UoG and Omy10INRA mapped to AS-8 in the male and the female genetic maps at a LOD score of 4 and 3, respectively. Note that it is important to construct independent male and female maps in Atlantic salmon because of the difference in recombination rates between the sexes [Moen et al., 2004]. OmyRT5TUF was only informative in the male parent of family Br6 and mapped to the middle of AS-8. A comparison of the male BT-28 and AS-8m and AS-8f is shown in figure 1. It is interesting to note that these markers reside in the bottom half of AS-8f and RT-21, which corresponds to the long arm of rainbow trout chromosome 9 (OMY9) [Phillips et al., 2006].

Identification of the Brown Trout Sex Chromosomes

An integration of the Atlantic salmon genetic map with its karyotype assigned linkage groups to specific chromo-

Fig. 1. Comparison of male brown trout linkage group 28 (BT-28m) and the male and female Atlantic salmon linkage group 8, AS-8m and AS-8f, respectively. OmyRT5TUF was only informative in the male parent of Br6 and thus is only on AS-8m. The microsatellite markers in Atlantic salmon AS-8f are more spread out than in AS-8m because Atlantic salmon females have a higher recombination rate than males [Moen et al., 2004]. Note that the markers in BT-28m that were mapped in AS-8f are located in the bottom half of this linkage group. The black dots correspond to the bars on the linkage groups closest to them and indicate the positions in cM where markers mapped in brown trout and Atlantic salmon are located.

somes using fluorescence in situ hybridization (FISH) with BAC probes containing genetic markers mapped to each linkage group [Phillips et al., 2009]. This study showed that AS-8 corresponds to chromosome 15 (SSA15). SSA15 is a large acrocentric chromosome with a small block of repetitive DNA in the middle of the arm, which is thought to represent the site of a chromosome fusion [Phillips et al., 2009]. As the sex-linked markers from brown trout mapped to the lower region of AS-8, potentially corresponding to the section between the block of repetitive DNA and the telomere in SSA15, we wondered what the corresponding chromosome would be in brown trout.

Atlantic salmon BACs containing the microsatellites OmyRT5TUF (S0059C22), Omy301UoG (S0164H12) or Ssa197DU (S0121A09) were chosen for FISH analysis as they are located at the top, middle and bottom of BT-28, respectively [Gharbi et al., 2006]. The result of FISH with S0121A09 is shown in figure 2A. This Atlantic salmon BAC hybridized to the telomeric regions of a pair of small subtelocentric chromosomes, which is consistent with the microsatellite it contains (Ssa197DU) mapping to the end of BT-28. S0164H12 hybridized to the middle of the same chromosome pair (fig. 2B), again consistent with the location of Omy301UoG in BT-28. Initially no hy-

Fig. 2. FISH analysis of brown trout chromosomes with Atlantic salmon BAC clones containing microsatellite markers Ssa197DU (**A**), Omy301UoG (**B**) and OmyRT5TUF (**C**). The white arrows in **A** indicate the chromosomes hybridized with the Ssa197DU containing BAC. The chromosome pairs are arranged by size with the chromosomes that hybridize to the BACs containing Omy301UoG and OmyRT5TUF shown at the bottom right hand corner in **B** and **C**, respectively.

bridization was seen with S0059C22, which contains the microsatellite most closely linked to *SEX* in brown trout (i.e. OmyRT5TUF). However, when rainbow trout Cot-1 DNA was used as the blocking agent in place of Atlantic salmon Cot-1 DNA in the FISH analysis, S0059C22 hybridized to the tip of the short arms of the same chromosome pair (fig. 2C). There was no difference in the FISH patterns between chromosomes prepared from blood of male and female brown trout. The results of the FISH analysis show that the brown trout sex chromosomes are homomorphic, and are a pair of small subtelocentric chromosomes. The FISH analysis strongly indicates that the location of the sex-determining locus is predicted to be at the end of the short arm of the subtelocentric chromosome. Centromere mapping by Gharbi et al. [2006] localized OmyRT5TUF to the opposite side of the cen-

tromere than the other microsatellite markers on BT-28, which supports the interpretation of the FISH analysis.

Comparison of the Brown Trout Sex Chromosomes and Atlantic Salmon AS-8 and SSA15

Atlantic salmon linkage group AS-8 has been assigned to a large acrocentric chromosome, SSA15 [Phillips et al., 2009], which contains an internal block of repetitive DNA that splits the chromosome into 2 ancestral salmonid chromosome arms corresponding to rainbow trout chromosomes OMY8p and OMY9q. The karyotype of Atlantic salmon is the most derived among the salmonids, with fewer apparent chromosome arms than other species such as brown trout or rainbow trout [Phillips and Ráb, 2001]. Comparison of genetic maps of rainbow trout and Atlantic salmon strongly suggests that SSA15 is the result of a fu-

Fig. 3. Comparison of Atlantic salmon linkage group AS-8f with brown trout linkage groups, BT-19 and BT-28, that share conserved syntenic blocks of genetic markers. The locations of the centromeres, shown in red, were determined by centromere mapping in the case of BT-19f and BT-28f [Gharbi et al., 2006] and inferred from cytogenetic analysis in the case of AS-8f [Phillips et al., 2009]. The black dots correspond to the bars on the linkage groups closest to them and indicate the positions in cM where markers mapped in brown trout and Atlantic salmon are located.

sion between 2 acrocentric chromosomes that were present in the common ancestor of the genus *Salmo* [Phillips et al., 2009]. Brown trout linkage group BT-19 contains markers that are colinear in the top portion of Atlantic salmon linkage group AS-8 (fig. 3). As the locations of the centromeres have been mapped in BT-19 and BT-28 [Gharbi et al., 2006],

we can see from figure 3 that the simplest explanation to account for the production of AS-8 is a fusion of BT-19 and BT-28 through their centromeres (shown in red in fig. 3). Such a fusion would explain the internal block of repetitive DNA seen in SSA15, but it would require another rearrangement to yield the centromere of the acrocentric

SSA15 [Phillips et al., 2009]. It appears that either the sex-determining locus was lost, inactivated, or relocated as a result of the fusion in the Atlantic salmon lineage. If it was lost, a novel sex-determining gene was recruited, which is now located on AS-1 (SSA2) [Artieri et al., 2006].

Is the Sex-Determining Gene the Same in Different Salmonids?

It is clear that genetic markers linked to the sex-determining locus are not conserved in salmonids except in the cases of 2 closely related pairs of species: rainbow trout and cutthroat trout (*Oncorhynchus clarkii*) [Alfaqi et al., 2008] and brook trout and lake trout [Phillips et al., 2002]. However, this does not necessarily imply that the sex-determining gene is different in each salmonid species. Phillips et al. [2001] have suggested that the sex-determining gene may have jumped from one chromosome to another in different salmonid lineages without relocating adjacent markers. This would be easy to imagine if the sex-determining gene were located at the end of the linkage group in subtelomeric chromosomal regions, which it is in every case. The North American species of the genus *Oncorhynchus* (Pacific trout and salmon) share a common male-specific marker, OmyY1 [Brunelli et al., 2008] and the Pacific salmon possesses a couple of other male-specific markers: Oty2 and GH-Y. This is strong evidence for these species sharing a common sex-determining gene. The GH-Y gene has not been found in species of the genera *Salmo* or *Salvelinus* and appears to have diverged from GH2 after *Oncorhynchus* diverged from the other 2 genera [Devlin et al., 2001]. Therefore, it is possible that there is a unique sex-determining gene found only in Pacific salmon and trout.

Gharbi et al. [2006] found that the brown trout *SEX*-containing linkage group BT-28 has a block of syntenic

genetic markers that are conserved in rainbow trout linkage group RT-21 and Atlantic salmon linkage group AS-8, neither of which contains *SEX*. The linkage group RT-21 has been assigned to a metacentric chromosome, OMY9 [Phillips et al., 2006], and the markers on BT-28 are on the long arm. This is supported by centromere mapping, which places OmyRT5TUF on the same side of the centromere as Omy301UoG, Omy10INRA and Ssa197DU [Danzmann et al., 2005]. Moreover, OmyRT5TUF maps to the middle of AS-8m in Atlantic salmon (fig. 1), suggesting that it could be located just below the DAPI band. It seems likely that an inversion in the brown trout sex chromosome occurred following the divergence with Atlantic salmon, which placed OmyRT5TUF on the short arm of the brown trout sex chromosome. Similarly, a small inversion in the Yellowstone cutthroat sex chromosome has relocated microsatellite Omm1665 to the short arm, while it remains on the long arm of the sex chromosome in rainbow trout [Alfaqi et al., 2008]. In any case, there is strong support for a centric fusion between the long arms of BT-19 and BT-28 to form AS-8. Following this event, the sex-determining gene could have either relocated to AS-1 or been lost and a new sex-determining gene recruited in Atlantic salmon. Thus, we predict that the sex-determining gene may be different in brown trout and Atlantic salmon.

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