The Sex Determining Loci and Sex Chromosomes in the Family Salmonidae

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Abstract

Salmonids are descended from a common ancestor that underwent an autotrapolidization event. After a whole genome duplication species could deal with sex determination by deleting one copy of *SEX*, the sex determining locus, or by recruiting a duplicated transcription factor to become a novel sex determining gene. It is not known which if any of these strategies salmonids adopted, but it appears that they all have primarily a genetic mechanism of sex determination with male heterogamety. The sharing of sex linked markers on the X and Y chromosomes and the difficulty in identifying Y specific markers indicate that X and Y chromosomes in salmonids have a large pseudoautosomal region and a small sex determining region. Linkage analyses suggest that either *SEX* differs in different lineages or else has remained the same and moved by transposition to different chromosomes. The identification of the sex chromosomes in salmonid species has not resolved this issue. It is clear that salmonids are at an early stage in sex chromosome differentiation and therefore provide a wonderful opportunity to study the evolution of sex determination. The availability of a reference salmonid genome sequence would provide an important resource for research in this area.

The family Salmonidae comprises three sub-families: Coregoninae (whitefishes and ciscos), Thymallinae (grayling), and Salmoninae (salmon, trout, and charr). These fish are further classified into twelve genera and 72 species that are more than 92% similar at the DNA level (Nelson, 2006; Koop et al., 2008) (fig. 1). Many members of the Salmoninae, which includes Atlantic salmon (*Salmo salar*), Pacific salmon and rainbow trout (*Oncorhynchus sp.*) and Arctic charr (*Salvelinus alpinus*), are important for aquaculture, wild stock fisheries and recreational sport fisheries. Besides their great economic and societal importance, the salmonids are also of considerable scientific importance in such fields as evolutionary biology, ecology, physiology, genetics, immunology, nutrition and environmental toxicology (Thorgaard et al., 2002). Indeed, no other group of fish receives such comprehensive commercial and scientific attention. There is a large body of work on salmonid sex determination and sex manipulation, fueled in part by the desire to produce all female stocks for aquaculture.

Salmonid Genome Duplication

The radiation of teleost species appears to have coincided with a genome duplication event. This is supported by the observation that ray-finned fishes, including teleosts, have two paralogous copies of many genes, while tetrapods only have one (Hoegg et al., 2004; Christoffels et al., 2004; Taylor and Raes, 2004). The common ancestor of salmonids underwent an additional genome duplication sometime within the early Tertiary to the late Cretaceous periods (Allendorf and Thorgaard, 1984). The evidence for a salmonid-specific autotetraploidization comes from observations of

salmonid DNA and chromosomes. Salmonids have about twice as much DNA per cell as their closest relatives, the Esociformes and Osmeriformes (Gregory et al., 2007), and also have twice as many chromosome arms. The modal range of chromosome arms in salmonids is 96-104 (Phillips and Rab, 2001), whereas in most teleost species, especially freshwater groups such as the Esociformes, it is 48-52 (Mank and Avise, 2006). Recent genetic maps and studies on members of duplicated gene families reveal homeologous chromosomal segments that are located on unlinked chromosome arms (Nichols et al., 2003; Moghadam et al., 2005; Gharbi et al., 2006; Danzmann et al., 2008). Furthermore, quadrivalent meiotic configurations are often observed within male-specific meioses. These pairings always involve metacentric chromosomes that may or may not include an acrocentric pair (Wright et al., 1983). Segregation ratios consistent with tetrasomic inheritance or partial tetrasomic ratios are also observed following male meioses (Allendorf and Danzmann, 1997) indicating that salmonid genomes have not completed the rediploidization process that leads to a stable diploid state (Wolfe, 2001).

One of the major problems facing an organism that has undergone a whole genome duplication is how to deal with sex determination. A potential solution would be to delete one of the sex determining loci as soon as possible so that the original master switch gene returns to the diploid state. An alternative strategy would be for a novel sex determining master switch to take control of the sex determining pathway. This novel gene could arise by subfunctionalization or neofunctionalization (Force et al., 1999) of any one of a number of duplicated transcriptional factors. It is quite reasonable to expect that different lineages would adopt different novel master switches, which would

in turn promote speciation. This may very well be one of the ways that a genome duplication event leads to a species radiation.

Recent genome studies have targeted Ostariophysi (e.g., zebrafish), or Acanthopterygii (e.g., pufferfish, medaka, stickleback), euteleostei lineages that have been separated from Protacanthopterygii (e.g., salmonids) for 200~300 million years (Inoue et al., 2003; Steinke et al., 2006). Salmonids, with their genome duplication and wealth of biological data, are excellent model organisms for studying the rediploidization process, the fates of duplicate genes and the genetic architecture of complex phenotypes such as sex determination. Here we review what is known about sex determining loci and sex chromosomes in the Salmonidae. In particular, we will review the evidence for multiple sex determining genes in this group of fish.

Salmonid Males are the Heterogametic Sex

Several salmonid species have been shown to possess a genetic mechanism of sex determination characterized by an XY chromosomal system (for review see Devlin and Nagahama, 2002), although evidence has been presented that environmental factors such as temperature (Craig et al., 1996; Azuma et al., 2004) or other sex modulating loci (Quillet et al., 2002) may influence the expression of the sex phenotype. Salmonids can be sex reversed via methyltestosterone (leading to males) or estradiol-17ß (leading to females) treatment in early development. Subsequently, when sex reversed female salmonids (being phenotypically male while genotypically female) are

crossed with normal females (phenotypically and genotypically female) all offspring are female (Johnstone et al., 1979; Okada et al., 1979; Hunter et al., 1982, 1983; Olito and Brock, 1991; Devlin et al., 2001). Similarly, when normal males (being phenotypically and genetically male) are crossed with sex reversed males (being phenotypically female while genotypically male) there is a 3:1 ratio of male to female progeny. It follows then that females must be homogametic (XX) and males heterogametic (XY) (Johnstone et al., 1979; Hunter et al., 1982, 1983; Johnstone and Youngson, 1984).

Linkage Analysis and Genetic Maps

Linkage analysis using allozyme markers showed that the phenotypic sex determining locus, Sex-1 (hereafter referred to as *SEX*), was linked to Ldh-1, Aat-5 and Gpi-3 in Arctic char (May et al., 1989). Moreover, these markers were not associated with *SEX* in lake trout (*S. namaycush*), brook trout (*S. fontinalis*) or rainbow trout (*O. mykiss*). Allendorf et al. (1994) found *SEX* to be associated with HEX-2 (bGLUA) and sSod-1 in rainbow trout. Allele frequency differences and an excess of heterozygotes in males suggested that *PEPB-1* is sex linked in chinook salmon (*O. tshawytscha*), and it was shown that there is less than 1% recombination between *PEPB-1* and *SEX* (Marshall et al., 2004). Within the genus *Salmo*, a minisatellite known to be in tight association with *SEX* in brown trout (*S. trutta*) showed no association with *SEX* in Atlantic salmon (Taggart et al., 1995). These observations suggested that there might be different sex determining linkage groups in salmonids. More extensive linkage analysis has been carried out using microsatellite markers to construct genetic maps.

Genetic maps that include *SEX* have been constructed for rainbow trout (Young et al., 1998; Sakamoto et al., 2000; Nichols et al., 2003), brown trout (Gharbi et al., 2006), Atlantic salmon (Gilbey et al., 2004; Moen et al., 2004, 2008; Danzmann et al., 2008), Arctic charr (Woram et al., 2004), coho salmon (*O. kisutch*) (McClelland and Naish, 2008) and chinook salmon (K. Naish, personal communication). Linkage data provide no evidence for the retention of a duplicated sex determining region, or for any degree of polygenic sex determination in salmonids. Rather, as shown in fig. 2, the linkage data indicate that there is a lack of conservation of synteny of *SEX* and microsatellite markers in these species (Woram et al., 2003; McClelland and Naish, 2008) with the exception of rainbow trout and Yellowstone cutthroat trout (*O. clarki bouvieri*) which appear to be conserved (Alfaqih et al., 2008). In fact, *SEX* linked microsatellite markers with the ability to amplify across salmonids, map to autosomal, homologous linkage groups in other species (Woram et al., 2003).

The genetic maps show that *SEX* is located at the end of a linkage group in Atlantic salmon, brown trout, Arctic charr, coho salmon and chinook salmon, and therefore it is implied that *SEX* is proximal to the telomeric region of their respective sex chromosomes. The assumed telomeric placement of *SEX* in these species is consistent with the hypothesis that there is a single sex determining locus in salmonids and that it is transposing within the genomes of some salmonids without relocating adjacent markers, thereby causing disruption of sex linkage among species (Phillips et al., 2001). This hypothesis is supported by the finding of Moghadam et al. (2007) of two distinct

sex linkage classes in five families of the Fraser strain of Arctic charr. However, it could also be argued that these observations are equally supportive of the hypothesis that these closely related species have evolved different sex determination master switches. In rainbow trout, *SEX* was initially mapped to a distal position on one of the linkage groups (Young et al., 1998). The consolidation of the Young et al. (1998) linkage map with that of Sakamoto et al. (2000) also placed *SEX* at the end of a linkage group (Nichols et al., 2003). Although the rainbow trout linkage map constructed by Guyomard et al. (2006) used doubled haploid gynogenetic lines and thus *SEX* could not be mapped, this map is also consistent with the previously described sex linked markers being located at the distal end of a linkage group and close to a centromere. It is interesting to note that five markers linked to *SEX* in rainbow trout are also sex linked in the very closely related cutthroat trout and consistent with *SEX* being at the end of this linkage group (Alfiqih et al., 2008). However, the identification of two additional sex linked microsatellite markers and six AFLP markers strongly supported a more intercalary location for the sex determining locus in this species (Woram et al., 2003). It has been suggested that the sex chromosome in rainbow trout was created by a translocation of an ancestral sex chromosomal segment to an autosome (Woram et al., 2003) or that an inversion might have been the initial process in the Y chromosome differentiation from an ancestral pair of morphologically identical chromosomes (Iturra et al., 2001a; Thorgaard et al., 2002).

Genetic Markers Unique to Male Salmonids

Although some of the genetic markers used to construct the genetic maps of various salmonids are considered sex linked, these markers usually have alleles that are found in both sexes. Therefore, unless the phase of transmission of the alleles at these loci is known, it is not generally possible to use these markers to identify the gender of an immature fish. There have been extensive searches to find genetic markers that are unique to the males within a salmonid species. Among the unsuccessful attempts was the use of bulked segregant analysis with pools of DNA from Atlantic salmon males and females to look for randomly amplified polymorphic DNA (RAPD) markers that are sex specific (McGowan and Davidson, 1998). However, not all attempts ended in failure. It was possible to use RAPD screening and bulked segregant analysis to identify an oligonucleotide, OP-P9, that amplified a 390 bp fragment specifically from male DNA in some strains of rainbow trout (Iturra et al., 1998), and to convert it into a SCAR (sequence characterized amplified region), designated *OmyP9* (Iturra et al., 2001b). An RsaI polymorphism in *OmyP9* is sex linked in several families and strains of rainbow trout, but fluorescent in situ hybridization (FISH) analysis revealed that similar sequences are found on both the X and the Y chromosome (Iturra et al., 2001a, 2001b).

Subtractive hybridization enabled a male specific fragment of DNA to be cloned from chinook salmon (Devlin et al., 1991). When this marker, OtY1, was used as a probe in Southern blots of chinook DNA, it hybridized to an 8 Kb BamHI fragment from males and gave little or no hybridization with DNA from female fish. Subsequent characterization of OtY1 revealed that it is part of an 8 Kb tandemly repeated sequence

(OtY8) that is present in at least six clusters containing approximately 12 to 250 copies, and comprising ~2.4 Mb of Y chromosomal DNA in the genome of chinook salmon (Devlin et al., 1998). Although OtY8 related sequences are present in other members of the Pacific salmon group and to a lesser extent in Atlantic salmon, the extensive amplification of a sex linked repeat structure is unique to chinook salmon and does not occur in coho, its sister species which is thought to have diverged from chinook less than 10 MYA (Devlin et al., 1998).

A growth hormone pseudogene (GH-ψY), derived from one of the duplicated salmonid growth hormone genes (GH-2), is sex linked in coho, chinook, chum (*O. keta*) and pink salmon (*O. gorbuscha*), but it is absent from sockeye salmon (*O. nerka*) and rainbow trout (Du et al., 1993; Forbes et al., 1994). Sequence comparisons suggest that GH-ψY arose from GH-2 after the divergence of *Oncorhynchus* from *Salmo* and *Salvelinus* but prior to the radiation of *Oncorhynchus* species (fig. 3). This interpretation implies that GH-ψY was subsequently lost from the genomes of sockeye and rainbow trout (Devlin et al., 2001). A study of the closely related Japanese masu salmon (*O. masou*) and amago salmon (*O. rhodurus*) initially indicated that GH-ψY was present in the masu and absent in the amago (Nakayama et al., 1999). A subsequent study of the *O. masou* complex, that comprises masu (*O. masou masou*), amago (*O. masou ishikawae*) and Biwa salmon (*O. masou subsp.*), confirmed the absence of GH-ψY in the amago, and the general presence of this pseudogene in male masu and Biwa (Zhang et al., 2001). However, approximately 6% of phenotypic males lacked this marker whereas the same proportion of phenotypic females was positive for GH-ψY.

The authors suggest that this mismatch between phenotypic sex and the expected sex linked marker is a result of sex reversion rather than crossing over between *SEX* and GH-ψY as there would be no apparent sex specificity of GH-ψY in masu or Biwa even if it happened at a low rate (Zhang et al., 2001).

It was reported that some phenotypic female chinook salmon from the Columbia River (Nagler et al., 2001) and from California (Williamson and May, 2002) were positive for OtY1. A more extensive survey involving 2478 individuals from 55 chinook populations found that 96.7% showed the expected sex linkage with OtY1 and GH-ψY, with only 5 males lacking these markers and 14 females being positive for them (Devlin et al., 2005). A recent study that tried to resolve the question of the “apparent” XY female chinook salmon in California was unable to differentiate between this phenomenon being the result of recombination between *SEX* and OtY1 and GH-ψY or the possibility of a Y chromosome with a dysfunctional or missing sex determining region (Williamson et al., 2008).

Amplified fragment length polymorphism (AFLP) analysis of DNA purified from gynogenetic male (YY) and female (XX) chinook salmon identified another marker, OtY2, that was amplified in male chinook salmon but not female chinook (Brunelli and Thorgaard, 2004). OtY2 also gave male specific amplification products in coho, chum and sockeye salmon, but not in pink salmon or rainbow trout. Characterization of a 12.5 Kb fragment of the chinook Y chromosome flanking OtY2 and its comparison with a 21 Kb segment of the rainbow trout genome revealed approximately 10 Kb of chinook /

rainbow trout Y chromosome sequence similarity (Brunelli et al., 2008). The sequence of one of the primers that was used to amplify the OtY2 male specific marker is shared between these species, but the other primer is in a sequence that is similar to a non-long terminal repeat (LTR) retrotransposon / non-LTR retrovirus reverse transcriptase, “similar to ReO_6” found in zebrafish (*Danio rerio*) that does not occur in this location in the rainbow trout genome. This explains why OtY2 primers do not amplify a male specific product in rainbow trout. Of particular interest was the identification of a minisatellite, OtY3, that gave a male specific amplification product in chinook and coho, but failed to show sex linked profiles for rainbow trout, chum and sockeye salmon despite successful sex typing of chinook, coho, chum and sockeye salmon with the OtY2 primers. Moreover, a pair of primers was found in this region in the rainbow trout sequence that amplified a fragment, OmyY1, which could be used to identify males with 96.5% accuracy. Fig. 3 summarizes the distribution of male specific markers in *Oncorhynchus* sp. The finding that the chinook / rainbow trout Y chromosome sequence similarity ends abruptly at the remnant of a retrotransposon is intriguing. It raises the possibility that *SEX* is indeed the same in all *Oncorhynchus* sp., and that it has moved to different linkage groups in these species via a mechanism involving repetitive elements.

Heteromorphic Salmon SEX Chromosomes

While the Y chromosome determines maleness, morphologically distinct sex chromosomes are rare in salmonid species (Phillips and Ráb, 2001). Heteromorphic sex

chromosomes have been identified in rainbow trout, sockeye salmon, lake trout and brook trout and are suspected in least cisco (*Coregonus sardinella*) and vendace (*C. albula*). In rainbow trout size differences between a homologous pair of chromosomes in males and females was observed (Thorgaard, 1977); however, rainbow trout populations lacking heteromorphisms have also been observed, indicating that chromosomal rearrangements differentiating the sex chromosomes are still in the process of fixation (Thorgaard, 1983; Felip et al., 2004). In sockeye salmon there is a Y-autosome fusion resulting in a diploid chromosome number of 57 in males and 58 in females (Thorgaard, 1978). Both lake trout and brook trout have a specific heterochromatin block at the end of the short arms of the largest pair of submetacentric chromosomes, thus identifying them as the sex chromosome pair in these closely related species (Phillips and Ihssen, 1985; Phillips et al., 2002). Variation in the number of chromosomes has been reported for least cisco and vendace, a closely related species that may be a conspecific. It has been suggested that females have a 2n of 80 whereas males have a 2n of 81, the extra chromosome being a metacentric in *C. sardinella* but an acrocentric in *C. albula* (Frolov, 1990; Jankun et al., 1991). As heteromorphic chromosome formation often includes addition of heterochromatin, it is surprising that heteromorphic sex chromosomes have not been observed in chinook salmon given the 2.4 Mb of repetitive DNA (OtY8) that is male specific, but see below.

Salmon Sex Chromosomes Identified by Fluorescent in situ Hybridization

The application of FISH has enabled major advances in the characterization of salmonid chromosomes (Phillips and Reed, 1996). When OtY1 was used as the probe, a single hybridization signal was observed on the short arm of an acrocentric chromosome in male chinook salmon whereas no signal was seen in chromosomes from females (Stein et al., 2001). A probe from the male specific growth hormone pseudogene confirmed the identification of the Y chromosome in chinook, and showed that *SEX* is also located on the short arm of a large acrocentric chromosome in coho salmon (Phillips et al., 2005). Although the sex chromosome pairs appear to be morphologically similar in chinook and coho salmon, the short arms of these acrocentric chromosomes differ in that only chinook contains 5S rDNA. In addition, the long arms do not appear to be homologous in these closely related species or to the sex chromosome of rainbow trout based on differential hybridization of probes that contain sex linked markers. The GH-ψY probe has also been used to identify the sex chromosomes of pink and chum salmon (Phillips et al., 2007). *SEX* resides on one member of the smallest metacentric chromosome pair in pink salmon and on an acrocentric chromosome in chum salmon. The sex chromosome pairs and the locations of 5S rDNA appear to differ in the North American species of Pacific salmon. However, the finding that these species share male specific markers adjacent to *SEX* and that they are located at the telomere of the short arms of the Y chromosome in each case supports the hypothesis that the sex determining gene is identical in these species and that transpositions involving *SEX* have occurred along different lineages (Phillips et al., 2007).

Microdissection of the short arm of the morphologically distinct Y chromosome of lake trout led to the production of Yp FISH probes (Reed et al., 1995). The Yp probes hybridized to both the X and the Y chromosomes of lake trout. Cloning of these fragments generated a microsatellite containing clone, SnaYp136. Linkage analysis suggests that the microsatellite marker Yp136 and *SEX* are genetically linked at a distance of 37 cM in lake trout. Results from gynogenetic crosses places Yp136 19 cM from the centromere, and this in turn indicates that *SEX* is likely to be located near the telomere of the short arm of the Y chromosome (Stein et al., 2002). There is no evidence that Yp136 is linked to *SEX* in Arctic charr (Woram et al., 2003). The results of FISH analysis of five North American species in the genus *Salvelinus* with Yp probes suggested that there are at least two distinct groups with respect to Y chromosome. One includes lake trout and brook trout and another contains Dolly Varden charr (*S. malma*) and Arctic charr (Phillips et al., 2002).

By integrating data from linkage mapping, physical mapping and FISH analysis Artieri et al. (2006) were able to position *SEX* on the long arm of chromosome 2, the second largest metacentric chromosome in Atlantic salmon. Oligonucleotide probes, designed from sex linked microsatellite markers on linkage group 1, were used to screen a BAC (bacterial artificial chromosome) library (Thorsen et al., 2005; Ng et al., 2005), and six BACs were selected for FISH. These results made it possible to align linkage group 1 and chromosome 2 and suggested that *SEX* is located between a block of heterochromatin at the telomere of the q arm and the microsatellite marker Ssa202DU.

Candidate Sex Determining Genes in Atlantic Salmon

Positional cloning and sequence analysis was used to identify *DMY* as the sex determining gene in the medaka (*Oryzias latipes*) (Matsuda et al., 2002). This strategy has been adopted as a means of characterizing the sex determining regions of several other fish species, including blue tilapia *(Oreochromis aureus)* (Lee et al., 2004), threespine stickleback *(Gasterosteus aculeatus)* (Peichel et al., 2004), platyfish (*Xiphophorous maculatus*) (Schulthies et al., 2006) and tiger pufferfish (*Takifugu rubripes*) (Kikuchi et al., 2007). However, to date no master sex determining genes have been identified in these organisms, in spite of the availability of genomic sequences for the threespine stickleback and tiger pufferfish (see Volff et al., 2007 for review).

As of August 2008, Atlantic salmon linkage group 1 contained 38 microsatellite markers and 5 single nucleotide polymorphisms (SNPs) (Danzmann et al., 2008; www.asalbase.org). Thirty one of these markers have been integrated with the physical map based on BAC fingerprints. As many of the BACs have been end-sequenced, it was possible to carry out comparisons with fish whose genomes have been sequenced. It appears that the long arm of Atlantic salmon chromosome 2 is homologous to linkage group IX of stickleback and linkage group 1 of medaka (Danzmann et al., 2008; Huang et al., 2008). We identified two contigs comprising 28 BACs that cover four microsatellite loci (Sal1UoG, Ssa202DU, Ssa55BSFU and Ssa233BSFU) in the sex determining region of the Atlantic salmon genome. Nine of the BACs that make up two minimum tiling paths in these contigs were sequenced and annotated, and a couple of

genes encoding putative DNA binding motifs caught our attention. One is similar to a U1-like zinc finger and the other is similar to a ligand dependent nuclear co-repressor. The former is expressed most strongly in heart whereas the latter is expressed predominantly in testis, making this gene worthy of further study as a candidate for *SEX* (Fujiki et al., 2008).

An alternative approach for identifying sex determining genes in fish is to screen for candidate genes based on physiology and what is known about sex determining pathways in other organisms. This method was used to identify *dmr1ib*Y as the sex determining gene in the medaka independently of the positional cloning approach (Nanda et al., 2002). Although the genome of the zebrafish has been sequenced, the primary genetic mechanism behind sexual differentiation has not been elucidated. However, a model based on what is known from the sex determining pathway in mammals has been proposed (von Hofsten and Olsson, 2005). The list of proteins considered as possible master sex determining regulators in zebrafish, and by extension other teleosts, includes homologues of the following: Sox9, AMH (anti Mullerian hormone) also known as Mullerian inhibiting substance (MIS), WT1 (Wilms Tumour suppressor), FTZ-F1 (*fushi tarazu* factor-1) now classified as NR5A, aromatase (Cyp19), SF-1 (steroidogenic factor), GATA4, Dax, and Dmrt1 or DMY, even though Kondo et al. (2003) showed that *dmrt1b*Y is unlikely to be the male sex-determining gene in fishes other than medaka. The strategy that we are using is to position candidate genes on the Atlantic salmon genetic map or chromosomes. Candidates are rejected if they do not map to the sex determining region on linkage group 1 or if FISH

analysis does not position them on the long arm of chromosome 2. To date, based on this approach we have been able to exclude Dax-1 and Dax-2, a potential homologue of DMY and AMH (MIS) as sex determining genes in this species (von Schalburg et al., in preparation). Having a reference salmonid genome sequence would undoubtedly help this research activity.

Questions to be Addressed and Future Prospects

The sharing of sex linked markers on the X and Y chromosomes and the limited number of Y specific markers that have been discovered suggest that the X and Y chromosomes in salmonids still retain similar gene complements. This is supported by the observation that YY males are both viable and fertile (Chevassus et al., 1988). It follows that salmonid Y chromosomes should have a large pseudoautosomal region and a very small sex determining region. There can be no doubt that salmonids are at an early stage in sex chromosome differentiation, and therefore, they provide a wonderful opportunity to study the evolution of sex determination.

Much has been learned to date with regard to sex determination in salmonids, but many questions remain. It is still not clear how many sex determining genes there are in the Salmonidae; however, there is strong evidence that the sex factors are different in *Salmo*, *Salvelinus* and *Oncorhynchus*. Within *Salmo* it is not clear if Atlantic salmon and brown trout share the same sex determining gene. Indeed, it is not known what chromosome in brown trout carries *SEX*. Among *Salvelinus* sp. the sex

chromosome has only been identified in lake trout, and although there is supporting evidence for it to be shared with brook trout, there is good reason to believe that it is different in the Arctic charr complex. The sharing of genetic markers that are very closely linked to *SEX* in Pacific salmon and rainbow trout indicate that these species share a common sex determining gene that appears to have moved by transposition so that it now lies on different linkage groups.

It is important to complete the identification of sex chromosomes in all species in the Salmoninae and to expand this to include representatives of the Coregoninae and Thymallinae. What may be equally important is to carry out a comparison of the organization of the X and Y chromosomes in one or more species. With the low recombination rate in male salmonids and the general reduction of recombination in Y chromosomes in general, it is not easy to compare the male linkage data that contains *SEX* with the equivalent female linkage group. For example, it was assumed by Artieri et al. (2005) that Atlantic salmon linkage group 1 is collinear in males and females. The limited FISH data are in agreement with this hypothesis, but the resolution may not have been sufficient to identify rearrangements of the type that have recently been described in the threespine stickleback (Ross and Peichel, 2008). The availability of BAC clones containing microsatellite markers along the length of the sex linkage group now makes this type of analysis possible; however, it would benefit from improved methods for preparing chromosomes that are stretched out.

The speculation about the number of sex determining genes in salmonids will continue until *SEX* has been identified in one species and it can be tested as a candidate in conspecifics and other more distantly related species. It is important to keep an open mind when searching for a sex determining factor. The most obvious choice may be a DNA binding protein or transcription factor based on what is known in most mammals and the medaka. However, we must be willing to consider other possibilities. For example, given that treatment with estradiol-17ß in early development of salmonids yields sex reversed males and that exposing fish to an aromatase inhibitor during the period of gonadal development results in testicular differentiation (Rashid et al., 2007; Fenske and Segner, 2004), a gene on a nascent Y chromosome that produced a protein that could inhibit aromatase activity could potentially become a master sex determining factor. The gene product need not be an inhibitor of transcription of the aromatase gene. It would be equally effective if it were involved in the splicing of its mRNA or acted as an inhibitor of translation via anti-sense RNA or inhibited the aromatase enzyme activity directly at the protein level.

When a candidate factor has been identified, the question then becomes how to test it. Some form of functional genomics will be necessary to determine if it is really the master switch. Assuming that the master sex determining gene produces males, then this will probably involve making a transgenic fish of known chromosomal content (an XX female) and waiting to see if it produces a male phenotype. Given the time required for salmonids to reach sexual maturity, knockout experiments using RNAi are unlikely to provide reliable results.

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Legends to Figures

Fig. 1 Phylogenetic tree of Salmonid fishes. The phylogenetic relationships among the three subfamilies of the Salmonidae family are shown. There are twelve genera and 72 species in this monophyletic group (Nelson, 2006). The genera and some representative species mentioned in this review are shown.

Fig. 2 Comparison of *SEX* containing linkage groups in salmonid species. Ssa-1 (*Salmo salar*) (www.asalbase.org), Str-28 (*Salmo trutta*) (Gharbi et al., 2006), Sal-4 (*Salvelinus alpinus*) (Woram et al., 2004), Oki-23 (*Oncorhynchus kisutsch*) (McLelland and Naish, 2008), Ots-1 (*Oncorhynchus tshawytscha)* (K. Naish, personal communication) and Ocl-1 (*Oncorhynchus clarki*) (Alfaqih et al., 2008). Two linkage groups are given for rainbow trout (*Oncorhynchus mykiss*) as there appears to be some dispute concerning whether *SEX* is distal (Omy-1(18); Nichols et al., 2003) or intercalary (Omy-18; Woram et al., 2003).

Fig. 3 Phylogenetic tree adapted from McKay et al. (1996) showing gain (+) and loss (-) of Y specific markers in *Oncorhynchus* sp. Ogo (*Oncorhynchus gorbuscha*), Oke (*Oncorhynchus keta*), One (*Oncorhynchus nerka*), Ots (*Oncorhynchus tshawytscha*), Oki (*Oncorhynchus kisutsch*), Orh (*Oncorhynchus rhodurus*), Oma (*Oncorhynchus masou*) and Omy (*Oncorhynchus mykiss*). OtY1 (Devlin et al., 1991), GHψY (Du et al., 1993), OtY2 (Brunelli and Thorgaard, 2004), OtY3 and OmyY1 (Brunelli et al., 2008).

Fig. 1

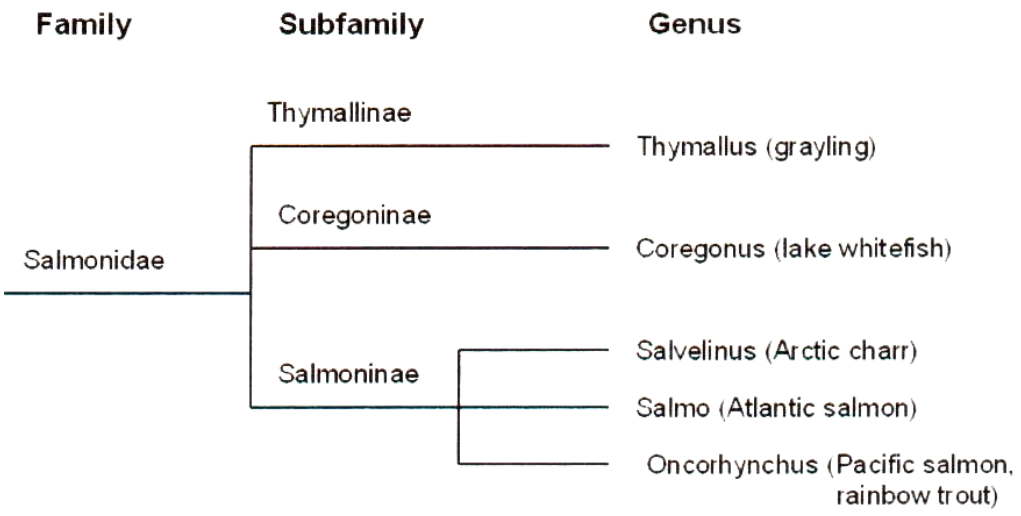

Fig. 2

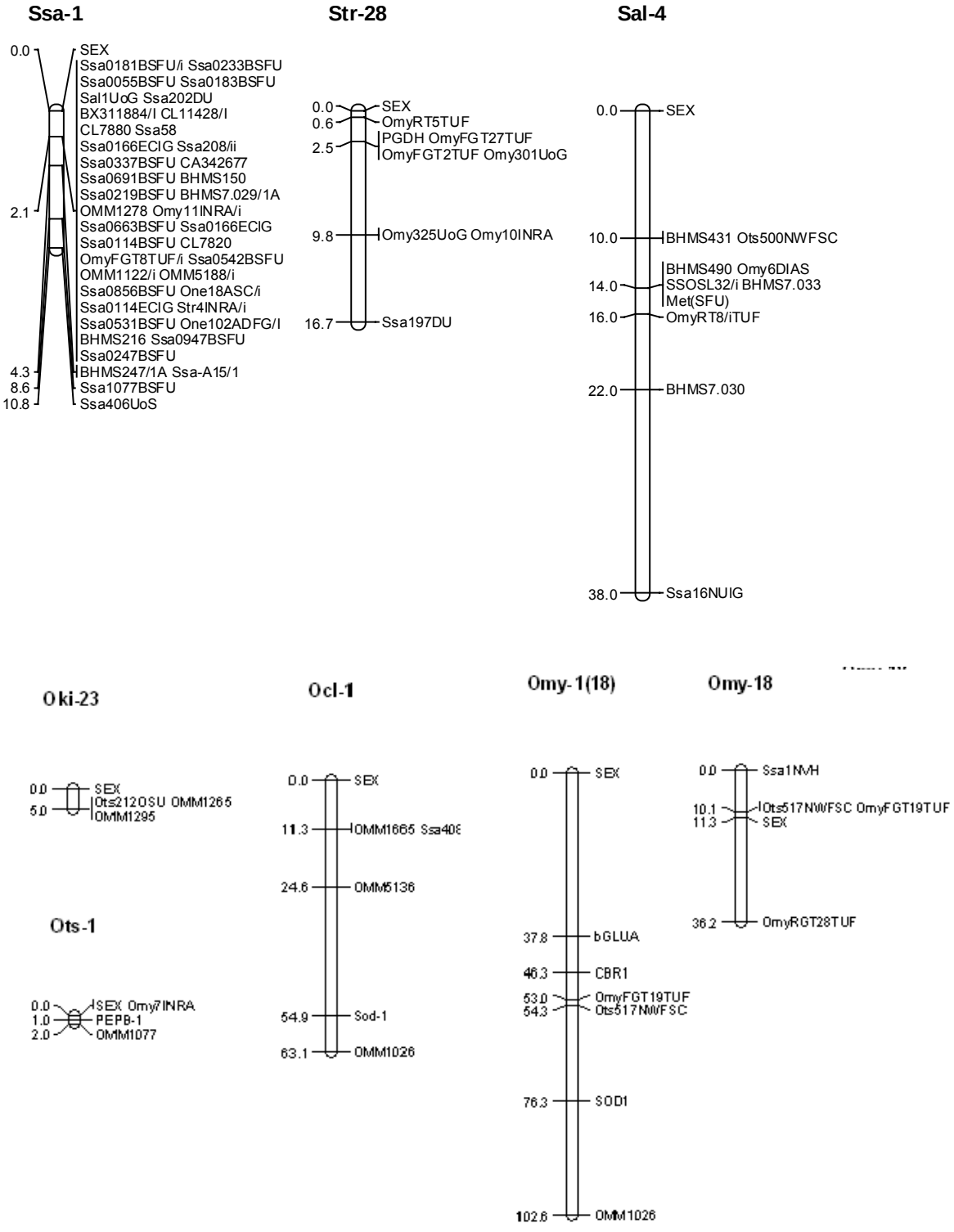

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Fig. 3