

# Characterization of a Chromosomal Rearrangement Responsible for Producing “Apparent” XY-Female Fall-Run Chinook Salmon in California

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## Abstract

Fluorescence in situ hybridization (FISH) was used to identify the X and Y chromosomes of offspring produced by normal and “apparent” XY-female fall-run Chinook salmon (*Oncorhynchus tshawytscha*) from California. FISH experiments were performed using probes to 2 sex-linked loci, growth hormone pseudogene (GH- $\Psi$ ), and OtY1, as well as a probe to a sex-linked microsatellite (Omy7INRA). Comparison of FISH staining patterns between the offspring produced by normal and apparent XY-females revealed that the apparent XY-female examined transmitted a “Y-like” chromosome with an attenuated OtY1 and GH- $\Psi$  signal to half of its offspring. Segregation analysis of microsatellites derived from rainbow trout (*Oncorhynchus mykiss*) with respect to phenotypic sex was carried out for 2 normal and 2 apparent XY-female Chinook salmon families. Inheritance patterns of Omy7INRA were consistent with this locus being closely linked to GH- $\Psi$  in males and in apparent XY-females carrying the Y-like chromosome. Another microsatellite locus (Omm1077) was closely linked to the primary sex-determining locus (*SEX*) in males but not to GH- $\Psi$ /OtY1 in apparent XY-females. The FISH analyses suggest that apparent XY-female fall-run Chinook salmon in California are not the product of a Y chromosome to autosome translocation. Despite the combined FISH and inheritance analyses, we were unable to differentiate between 2 alternative explanations for apparent XY-females, namely, recombination of markers between the sex chromosomes, or a Y chromosome with a dysfunctional or missing sex-determining region.

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Multiple studies have provided evidence that sex is chromosomally determined in salmonids (Thorgaard 1977; Thorgaard and Gall 1979; Ueda and Ojima 1984a, 1984b) and follows a XY pattern of inheritance (Donaldson and Hunter 1982; Allendorf and Thorgaard 1984). Identification of the primary sex-determining locus (*SEX*), however, has not been successful. Phillips et al. (2005) showed that sex chromosomes are not conserved between Chinook salmon and closely related coho salmon and rainbow trout (*Oncorhynchus kisutch* and *Oncorhynchus mykiss*, respectively). Either the sex chromosome pair including *SEX* evolved independently in different salmonid species or a small male-specific region including *SEX* has transposed or translocated to a different chromosome in each species (Phillips et al. 2005). Two male-specific markers, OtY1 (Devlin et al. 1991, 1994) and growth hormone pseudogene (Du et al. 1993), are tightly linked to each other and *SEX* in Chinook

salmon. Both OtY1 and growth hormone pseudogene (GH- $\Psi$ ) have been used to identify the Chinook salmon Y chromosome via fluorescence in situ hybridization (FISH) assays. Stein et al. (2001) and Phillips et al. (2005) have used FISH to locate OtY1 and GH- $\Psi$ , respectively, on the short arm of the acrocentric Y chromosome. As is common with most fishes that have sex chromosomes those of salmonids have a small sex-specific region and large pseudoautosomal regions (Devlin and Nagahama 2002). Phillips et al. (2005) demonstrated that the rainbow trout derived microsatellite locus Omy7INRA (Gharbi K, Guyomard R, personal communication, INRA, France) is located in the pseudoautosomal region on the X and Y chromosomes of Chinook salmon. The location of these 3 markers relative to *SEX* on the Chinook salmon Y chromosome has not yet been clearly resolved.

Chromosomal rearrangements may disrupt associations between genetic markers and specific phenotypic traits.

Salmonids have experienced whole arm Robertsonian fusions and fissions during their evolutionary history (Hartely 1987). Linkage analyses in several salmonid species (Wright et al. 1983; Allendorf and Thorgaard 1984; Danzmann et al. 2005) suggest that this has been the predominant mode of chromosomal evolution within salmonids. The sex chromosomes in salmonids are pseudoautosomal and are still evolving differences between each other. It is possible that rearrangements involving the sex chromosomes have disrupted linkage between the genetic sex markers and *SEX*.

Salmonid populations that appear to deviate from the otherwise strict chromosomal determination of sex may provide a fundamental system within which to study the evolution of salmonid sex chromosomes. Insights learned from this system can be applied to the understanding of sex chromosome evolution in other taxa (Stein et al. 2001). Incongruent genotypic and phenotypic sex has been documented in Chinook salmon in the Pacific Northwest (Nagler et al. 2001; Devlin et al. 2005) and California (Williamson and May 2002). "Apparent" XY-female fish (phenotypic females that have a male genotype according to OtY1 and GH-Ψ) have been identified in California Chinook salmon (Williamson and May 2005, 2007). Cytogenetic examination of California fall-run Chinook salmon that have incongruent genotypic and phenotypic sex may provide insight regarding mechanisms of sex chromosome evolution and/or how chromosomal rearrangements disrupt relationships between genetic markers and phenotypic traits in salmonids or other taxa.

Three genetic mechanisms have been proposed for the observed incongruent genotypic and phenotypic sex in these fish (Williamson and May 2005). First, a Y to X chromosome/autosome translocation involving both *SEX* and Y chromosome markers may result in the creation of a new sex chromosome in Chinook salmon. Although rare, XX-male sex reversal has been documented in humans (de la Chapelle et al. 1964; Tomomasa et al. 1999). Sex reversal in most cases results from a Y chromosome fragment, containing the testis-determining factor (TDF) region, exchanging with the X chromosome (Page et al. 1987; Kolon et al. 1998). Second, unequal crossing over may have occurred only within Y-specific marker copies (not *SEX* itself). Third, a mutational event may have occurred that functionally inactivated or deleted *SEX* from the Y chromosome. For example, deletion mutations observed in the human and mouse (*Mus musculus*) (Gubbay et al. 1990; Jager et al. 1990, respectively) *SRY* gene result in a frameshift that presumably leads to a nonfunctional TDF protein. Loss of TDF function by mutation or entire deletion of *SRY* from the Y chromosome results in sex-inverted (XY-female) individuals. The experimental crosses performed by Williamson and May (2005), however, did not identify which genetic mechanism is more likely to be responsible for producing apparent XY-female Chinook salmon. The development of the FISH method now provides a potential means to detect if chromosomal rearrangements have occurred involving the Y chromosome and an autosome.

Phillips et al. (2005) indicated 100% sex linkage for the rainbow trout derived microsatellite Omy7INRA in a single

Chinook family. This suggests that the other rainbow trout linkage group 7 (LG7) microsatellites (appendix to Danzmann et al. 2005) may also be sex linked in Chinook salmon. A contemporary linkage mapping study in Chinook salmon using the rainbow trout LG7 markers has subsequently confirmed that they are sex linked in Chinook (Naish K, personal communication).

In this study, we examine evidence for a possible Y-autosomal translocation as an explanation for incongruent genotypic and phenotypic sex observed in California Chinook salmon. We compare FISH staining patterns with probes for Omy7INRA, OtY1, and GH-Ψ and inheritance patterns of LG7 microsatellites between the offspring of normal and apparent XY-female fall-run Chinook salmon. Observable linkage differences between normal and apparent XY-female families may provide additional clues regarding whether apparent XY-female fish are the result of an intra- or interchromosomal rearrangement involving *SEX* and the LG7 loci.

## Methods

Gamete collection for artificial crosses, genetic screening to detect apparent XY-female fish, breeding experiments, and fish rearing were conducted as described in Williamson and May (2005). Fin clips and gametes were sampled from adult fall-run Chinook salmon at the Merced River Fish Hatchery in November 2004. The selection criterion for sets of gametes to be used in artificial crosses was based on the sexual genotype of putative parents as determined by polymerase chain reaction (PCR) assays for both GH-Ψ and OtY1 loci. Eggs from selected apparent XY-females and normal females were separately fertilized with sperm from different males. Fertilized eggs and hatchlings from individual families were incubated for approximately 45 days (just before swim-up stage) in Heath trays before being transferred to separate larger rearing tanks. Juvenile fish were raised for another 188 days until they had grown large enough (>12 cm fork length) so that phenotypic sex (gross gonad morphology) could be easily determined by necropsy and approximately 1.0 ml of whole blood could be sampled from each individual to establish lymphocyte cultures for FISH analysis. Fin clips, used for analysis of genotypic sex as performed for the parents, were collected immediately after blood was sampled from euthanized juveniles according to the methods described in Williamson and May (2005). Making 2 incisions in the anterior ventral portion of the body cavity exposed the heart. The pericardial area was irrigated with ~50 μl of sterile heparin solution (1000 U/ml; USB Corporation, Cleveland, OH) to prevent clotting. Blood was drawn from the dorsal vascular sinus using a sterile 1-ml syringe loaded with a 25-gauge needle that was previously charged with ~20 μl of heparin solution. Separate blood samples were transferred to blood collection vacuum tubes containing sodium heparin (Becton, Dickinson, and Company, Franklin Lakes, NJ) and immediately shipped overnight for FISH analysis.

Lymphocyte cultures were established using whole blood taken from phenotypic male and female offspring, and chromosome preparations were obtained by standard methods (Phillips et al. 2004; Phillips 2005). The Y chromosome was cytogenetically identified using either a plasmid clone harboring the male-specific 8-kb repeat (OtY8) that contains the OtY1 locus (Devlin et al. 1998) or a cosmid clone (provided by R. Devlin, Fisheries and Oceans Canada) containing the GH-Ψ locus. To avoid confusion, the OtY8 FISH probe will hereafter be referred to as the OtY1 probe because OtY8 contains the OtY1 locus. Phillips et al. (2005) previously isolated an Omy7INRA microsatellite containing FISH probe. The Omy7INRA probe was obtained from a bacterial artificial chromosome library prepared from DNA from the Swanson rainbow trout YY clonal line (Palti et al. 2002). Probes were labeled using nick translation with either Spectrum Orange (red signal; Abbott Laboratories, Abbott, IL) or digoxigenin (green signal; F. Hoffmann-La Roche Ltd., Basel, Switzerland). FISH analysis was performed as described in Stein et al. (2001) with minor modifications. Blocking repetitive sequence (Cot1 DNA prepared from rainbow trout) was added to the probe, and the probe and chromosomal DNA were denatured at 72 °C for 10 min. The denatured probe was added to the slide and hybridized overnight under controlled stringency. After a series of posthybridization washes, slides were counterstained with 4',6'-diamidino-2-phenylindole (DAPI) and viewed under fluorescent illumination. For digoxigenin-labeled probes, antibodies to digoxigenin (Roche, Inc.) diluted in phosphate-buffered saline were added to the slides after the posthybridization washes. The slides were then incubated for 45 min before a final series of washes and application of the DAPI counter stain. Fluorescent images were captured separately for each fluorophor with an Olympus BX60 microscope and Sensys digital camera and combined using Cytovision Genus image analysis software (Applied Imaging, Inc.). Staining intensities of each probe can be estimated using this software.

The Mendelian inheritance of rainbow trout derived microsatellites Omm1077 (Rexroad et al. 2002), Omm1318 (Palti et al. 2002), and Omy7INRA was tested in the crosses used for cytogenetic analysis as well as in other normal and apparent XY-female produced families used in a previous study (Williamson and May 2005). Alleles were amplified by PCR assays using 15 ng of genomic DNA, 1.5, 5.0, and 2.5 mM MgCl<sub>2</sub> (Omm1077, Omm1318, and Omy7INRA, respectively), 0.2 mM each deoxynucleoside triphosphate, 0.4 μM of each PCR primer, 0.25 units of *Taq* DNA polymerase (Promega Biosciences, San Luis Obispo, CA), 20 mM Tris (pH 8.5), and 50 mM KCl in 10 μl volumes. The forward primer of each PCR primer pair was labeled with a fluorescent phosphoramidite (FAM, NED, or VIC). PTC100 thermal cyclers (MJ Research, San Francisco, CA) were programed with the following conditions: one denaturation cycle at 95 °C for 180 s; 40 amplification cycles of 94 °C for 30 s; 58, 54, and 59 °C annealing temperature (Omm1077, Omm1318, and Omy7INRA, respectively) for 30 s; and 72 °C for 30 s. Final extension cycles for Omy7INRA and the other LG7 loci were 60 °C for 20 min

and 72 °C for 10 min, respectively. Amplification products and size standards (GeneScan 400 and 500) were resolved on a 5.5% acrylamide–7 M urea gel and imaged by an MJ Research BaseStation (MJ Research). Individual genotypes were scored using Cartographer software as well as manually verified for every individual genotyped.

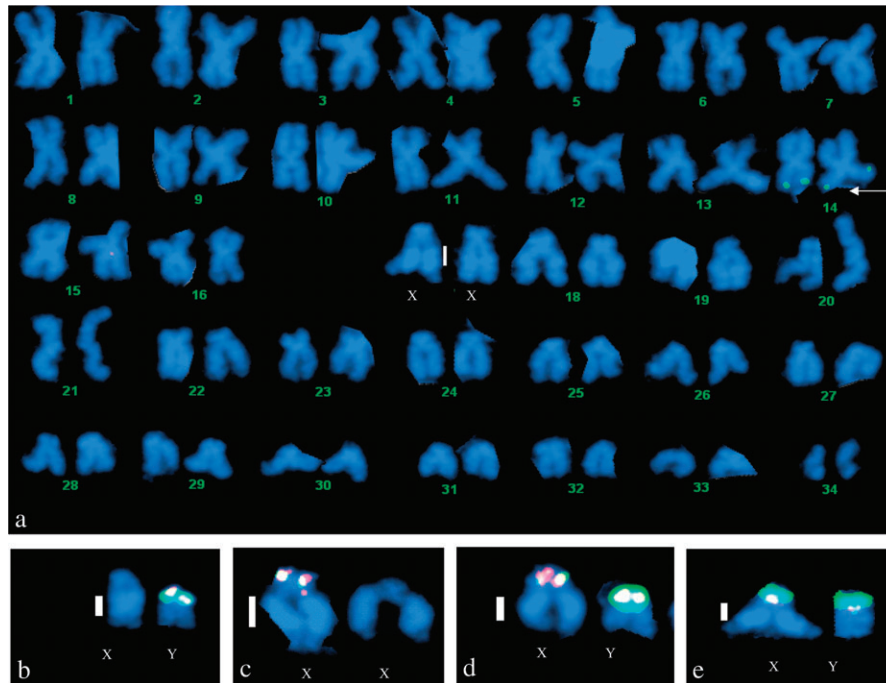
Linkage between each locus and *SEX* was compared in families produced by normal and apparent XY-female fish. Linkage in male and apparent XY-female parents was quantified by calculating the recombination fraction (*r*) of alleles inherited in a sex-specific fashion and the phenotypic sex of offspring in each family. Linkage in the male parent was calculated by dividing the number of recombinant genotypes by the total number of recombinant plus parental genotypes observed in male, female, and apparent XY-female (if present) offspring. Linkage in the apparent XY-female parent was calculated by dividing the number of recombinant genotypes by the total number of recombinant plus parental genotypes observed in female and apparent XY-female (if present) offspring. For the apparent XY-female parents, recombination values can be calculated between GH-Ψ/OtY1 and each of the microsatellite loci because the derived (Y\*) chromosome contains GH-Ψ/OtY1. These can be compared with recombination values between *SEX* and the microsatellite loci in the male because GH-Ψ is closely linked to *SEX* in Chinook salmon (Devlin et al. 2001). Because one cannot tell which X chromosome that either male or female offspring receive from their normal female parent, recombination values between *SEX* and the microsatellite loci cannot be calculated for normal female parents in these crosses. Fisher's exact test (Fisher 1934) as implemented in JMP-in version 4.0.3 (SAS Institute Inc., Cary, NC) was performed to test the statistical significance of recombination values.

## Results

### Identification of Sex Chromosomes in Offspring of Normal and Apparent XY-Female Chinook Salmon

No hybridization signals with either the Oty1 or GH-Ψ probes were present on the sex chromosomes (Figure 1a) of phenotypic female offspring from the control cross of a normal female and male Chinook salmon. The weak, nonspecific (sex) OtY1 signal observed on a medium-sized metacentric pair of autosomes is consistent with data previously obtained for female Chinook salmon (Devlin et al. 1994, 2005; Williamson and May 2002). Strong hybridization signals for OtY1 and GH-Ψ probes were visualized on the telomere of the short arm of the Y chromosome of male offspring in the control cross (Figure 1b). These results are similar to what has been previously observed for OtY1 (Stein et al. 2001) and GH-Ψ (Phillips et al. 2005) in male Chinook salmon.

Fluorescent staining of chromosomes of offspring produced by the experimental cross of an apparent XY-female and male Chinook salmon indicated different patterns compared those observed in the control cross.



**Figure 1.** (a) Karyotype of normal female Chinook salmon offspring from the control cross. The OtY1 (green) signal is shown on the long arm of medium-sized metacentric chromosomes 14 (arrow). There was no detectable signal from the male-linked GH-Ψ. The subteloentic X chromosomes can be identified by the large interstitial block of heterochromatin in the middle of the long arm (DAPI positive band indicated by a white bar). (b) XY sex chromosomes of normal male Chinook salmon offspring from the control cross. Locations of the OtY1 (red) and GH-Ψ (green) signals appear on the telomere of the small acrocentric Y chromosome. Due to the overlap of separately acquired camera images for each probe, the combined images of probe signals appear gold/white. (c) Sex chromosomes of an apparent XY-female offspring from an apparent XY-female parent. The OtY1 (red) and GH-Ψ (green) signals are present on the short arm of the X chromosome. Here, both signals are reduced in size about 75% compared with that observed in male offspring of normal female Chinook (Figure 1b). (d) Sex chromosomes of a male offspring from an apparent XY-female parent showing the location of OtY1 (red) and GH-Ψ (green) signals on both sex chromosomes. The X chromosome inherited from the dam (left homolog) has reduced probe signal compared with the Y inherited from the sire (right homolog). (e) Sex chromosomes of male offspring from apparent XY-female parent showing the location of Omy7INRA (red) and GH-Ψ (green) signals.

Analysis of phenotypic female offspring positive for OtY1 and GH-Ψ revealed reduced signals on the short arm of one member of the sex chromosome pair (Figure 1c). The size (area of chromosome to which probe bound) of the signal from each probe was reduced about 75% compared with that observed in male offspring of the normal female Chinook (Figure 1b). A large interstitial block of heterochromatin (light blue DAPI band indicated by a white bar) located in the middle of the long arm is also apparent on the fluorescently stained chromosome. This band is variable in size but usually found on both the X and Y chromosomes (Phillips et al. 1985; Stein et al. 2001). Due to limited throughput capacity to process samples, only 3 phenotypic male offspring produced by the apparent XY-female parent were examined cytogenetically. Two phenotypic male offspring had OtY1 and GH-Ψ signals on each sex chromosome (Figure 1d). The chromosome inherited from the dam (indicated by reduced signal from each probe) and that inherited from the sire (large signal from each probe

and a small DAPI band on the short and long chromosome arms, respectively) are clearly recognizable. The third phenotypic male offspring exhibited a fluorescent staining pattern similar to that of male offspring produced in the control cross (data not shown).

The Omy7INRA probe was used in tandem with GH-Ψ to visualize the X and Y chromosomes of male offspring produced in the XY-female cross. Again, 2 chromosomes indicated signals from both probes. On both chromosomes, the GH-Ψ signal was localized to the telomere region and the Omy7INRA probe hybridized to the proximal portion of the long arm (Figure 1e).

#### Segregation Analysis of Sex-Linked Microsatellites in Normal and Apparent XY-Female Chinook

Segregation analysis of Omy7INRA in normal female families revealed tight linkage with *SEX* in the male parents (Table 1). Segregation analysis of Omy7INRA in apparent

**Table 1.** Inheritance pattern of rainbow trout derived microsatellite loci in families produced by normal female fall-run Chinook salmon

Locus	Control cross of normal family I12 × D										Control cross of normal family 84 × B											
	Genotypes <sup>a</sup>		Observed no. of progeny genotypes <sup>b</sup>			Linkage from parents					Genotypes <sup>a</sup>		Observed no. of progeny genotypes <sup>b</sup>			Segregation analysis						
	Parental	Progeny	PFGF	PFGM	PMGM	N	r value			P		Parental	Progeny	PFGF	PFGM	PMGM	N	r value			P	
							Female	Male	Female	Male	Female							Male	Female	Male		
Omm1077	312/316	312/384	12	—	—	46	—	0.043	—	<0.0001	349/404	349/345	9	—	43	—	0.023	—	<0.0001			
	<b>384/420</b>	312/420	1	9	<b>345/404</b>						349/404	—	15									
		316/384	12	—							404/345	11	1									
Omm1318	444/484	444/352	5	4	—	46	—	NS	—	0.83	364/368	364/null	2	24	46	—	—	—	—			
	<b>352/456</b>	444/456	6	3	<b>Null/null</b>						368/null	9	11									
		484/352	7	7							—	—	—									
		484/456	8	6							—	—	—									
Omy71NRA	262/284	262/284	12	—	—	48	—	0.021	—	<0.0001	292/312	292/262	—	15	46	—	0.022	—	<0.0001			
	<b>284/290</b>	262/290	1	14	<b>262/290</b>						292/290	10	1									
		284/284	13	—							312/262	—	9									
		284/290	—	8							312/290	11	—									

The total number (*N*) of progeny examined/family for each locus is shown. Statistical significance (nonsignificant = NS) of recombination (*r*) values was assessed using Fisher's exact test (*P*).

<sup>a</sup> Paternally derived alleles are in bold.

<sup>b</sup> Observed genotypes are for phenotypic female/genotypic female or male (PFGF or PFGM, respectively) and phenotypically male/genotypic male (PMGM) progeny.

**Table 2.** Inheritance pattern of rainbow trout derived microsatellite loci in families produced by apparent XY-female (XYF) fall-run Chinook salmon

Locus	Experimental cross of apparent XY-female family I81 × A											Experimental cross of apparent XY-female family 93 × A										
	Genotypes <sup>a</sup>		Observed no. of progeny genotypes <sup>b</sup>			Linkage from parents					Genotypes <sup>a</sup>		Observed no. of progeny genotypes <sup>b</sup>			Segregation analysis						
	Parental	Progeny	PFGF	PFGM	PMGM	N	r value			P		Parental	Progeny	PFGF	PFGM	PMGM	N	r value			P	
							XYF	Male	XYF	Male	XYF							Male	XYF	Male	XYF	Male
Omm1077	400/424	400/392	4	8	2	62	NS	0.048	0.44	<0.0001	357/373	357/341	2	5	—	41	NS	0.073	0.51	<0.0001		
	<b>392/404</b>	400/404	—	—	18						<b>341/357</b>	357/357	—	—	9							
		424/392	9	9	—							373/341	4	5	—							
Omm 1318	360/null	360/352	4	3	11	64	NS	0.359	0.62	0.21	316/332	316/352	3	2	1	40	NS	NS	0.75	1.00		
	<b>352/null</b>	360/null	5	5	4						<b>352/396</b>	316/396	1	3	11							
		Null/352	1	6	10							332/352	1	2	6							
		Null/null	4	5	6							332/396	4	4	2							
Omy71NRA	282/280	282/284	1	17	2	64	0.065	0.047	<0.0001	<0.0001	268/320	268/262	—	—	11	46	0.048	0.022	0.001	<0.0001		
	<b>284/260</b>	282/260	—	1	12						<b>262/282</b>	268/282	7	1	—							
		280/284	13	1	—							320/262	—	1	13							
		280/260	—	—	17							320/282	—	13	—							

The total number (*N*) of progeny examined/family for each locus is shown. Statistical significance (nonsignificant = NS) of recombination (*r*) values was assessed using Fisher's exact test (*P*).

<sup>a</sup> Paternally derived alleles are in bold.

<sup>b</sup> Observed genotypes are for phenotypic female/genotypic female or male (PFGF or PFGM, respectively) and phenotypically male/genotypic male (PMGM) progeny.

XY-female families revealed linkage with OtY1/GH-Ψ in both female and male parents. Just as male offspring were more likely to receive one particular allele from the sire, female and apparent XY-female offspring were more likely to receive one particular allele from the XY dam (Table 2). Very similar results were obtained for Omy7INRA in multiple apparent XY-female families produced in separate years. Out of 238 meioses (across 5 families), 8 recombinatorial events at the Omy7INRA locus within sires were observed. Out of the 8 recombinant offspring, 8 had also recombined at the Omm1077 locus.

Two other microsatellites, Omm1077 and Omm1318, had consistent segregation patterns in both types of families. Significant ( $P < 0.0001$ ) male parent linkage was detected between Omm1077 and *SEX* in both normal and apparent XY-female families (Tables 1 and 2). No apparent XY-female parent linkage was detected between either the Omm1077 or the Omm1318 locus and GH-Ψ/OtY1 in apparent XY-female families (Table 2). Similarly, no linkage was detected between Omm1318 and *SEX* in the male parents of either normal or apparent XY-female families. Very similar results were obtained overall for these loci in 2 separate sampling years. The male parent for family 84 × B was not variable for locus Omm1318 (Table 1). Hence, this male parent could not be tested for sex linkage at Omm1318. We do note, however, an apparent significant result ( $r = 0.28$ ,  $P = 0.05$ ) for the female parent of cross 84 × B. This is not a viable test as the male is the heterogametic sex. We view this result as simply a coincidence, and the result was not included in Table 1 in order to avoid confusion regarding how  $r$  values were calculated.

## Discussion

The FISH analyses suggest that apparent XY-female fall-run Chinook salmon in California are not the result of a Y chromosome to autosome whole-arm translocation. The FISH data alone, or in combination with the inheritance data, however, do not clearly discriminate between the other alternative explanations for apparent XY-females, namely, recombination of Y-specific markers between the sex chromosomes, or a Y chromosome with a dysfunctional or missing sex-determining region. If a chromosomal rearrangement involving the X and Y has occurred, this would be difficult to distinguish from a mutation because the sex chromosomes differ only in a small region containing OtY1 and GH-Ψ in males. Degradation of the telomere region of the Chinook salmon Y chromosome may have resulted in the partial or whole loss of *SEX*. Devlin et al. (2001) present evidence that suggests *SEX* is distal to GH-Ψ and OtY1 on the Y chromosome. If the sex-determining region resided within the degraded telomere region, it may have been partially or wholly lost from the Y while the Y-specific markers remained. Further, telomere degradation could also be responsible for reduced copy number of OtY1 on the Y chromosome. The fluorescently stained chromosome of apparent XY-female offspring

produced by the apparent XY-female (Figure 1c) may be due to recombination between the sex chromosomes that have transferred to the X chromosome several of the many hundred copies of GH-Ψ and OtY1 present on the Y chromosome (Chowen and Nagler 2005; Williamson and May 2005). The OtY1 locus is part of a larger 8-kb DNA sequence (OtY8) that is present approximately 300 times and organized as clusters of direct tandem repeats that span a 3.7-Mb region of the Chinook salmon Y chromosome (Devlin et al. 1998). Differentiation of the sex chromosomes in salmonids is presumed to be limited to the region immediately adjacent to the sex-determining locus, and the remainder of the chromosomes remains pseudoautosomal with sufficient homology for recombination to occur (May et al. 1989; Allendorf et al. 1994). The pseudoautosomal character of Chinook salmon sex chromosomes and the high-copy number of OtY1 present the opportunity for recombination involving the OtY1 locus to occur. Recombination of markers between the sex chromosomes may also explain the relatively equal amounts of staining observed on the 2 chromosomes of male offspring produced by the apparent XY-female (Figure 1c). Yet, the observed inheritance pattern for the sex-linked microsatellite Omy7INRA in apparent XY-female produced families suggests that recombination of GH-Ψ and OtY1 between the sex chromosomes may not be the sole explanation for apparent XY-females.

It is interesting to note that the inheritance pattern of Omy7INRA in apparent XY-female families revealed both female and male parent linkage (Table 2). Given the large differences in recombination rates between female and male salmonids (Sakamoto et al. 2000; Nichols et al. 2003; Danzmann et al. 2005), we expect, in general, reduced recombination on the Y compared with the X. Therefore, it is not surprising that linkage was detected in the male parent between Omm1077 and *SEX* but not between Omm1077 and GH-Ψ/OtY1 in the apparent XY-female parent. Surprisingly, recombination rates between Omy7INRA and *SEX* or GH-Ψ were nearly as low in apparent XY-females as was observed in male parents (Table 2). No linkage was detected between *SEX* or GH-Ψ/OtY1 and Omm1318 suggesting that if this locus is present on the Chinook salmon sex chromosome, it is distal to Omm1077 unlike the case in rainbow trout. According to the linkage map of rainbow trout LG7 (appendices to Danzmann et al. 2005), Omm1077 and Omm1318 are located ~37 and ~20 map units, respectively, from Omy7INRA and presumably further from *SEX*. Linkage between LG7 microsatellite loci and GH-Ψ/OtY1 in the apparent XY-female parent suggests that the fluorescently stained chromosome transmitted (Figure 1c) may be a Y that no longer confers genetic information necessary for development as a phenotypic male.

Another alternative explanation for apparent XY-female fall Chinook includes XX/XY mosaicism. A postembryonic mutation of *SEX* in the germ cells of a male Chinook salmon could lead to the creation of an individual that is a *SEX*+/*SEX*- mosaic. Reproduction with a normal (XX)

female would produce a 1:1, normal female to XY-female, offspring sex ratio. Subsequent reproduction by the XY-female ( $XY^{SEX-}$ ) offspring with a normal (XY) male would in turn produce 1:1, male to female, phenotypic and 3:1, male to female, genotypic sex ratios. Similar genotypic and phenotypic sex ratio patterns have been observed in offspring produced by apparent XY-female fall-run Chinook salmon (Williamson and May 2005). Hines et al. (1997) found evidence in humans of a somatic and germ-line mosaicism for *SRY*. In this case, a postembryonic missense mutation within *SRY* carried by a sexually mature male gave rise to mosaicism for 2 distinct cell populations. Wild-type *SRY* was present in the somatic cell line, whereas the nonfunctional mutant *SRY* was present in the germ cell line. Because the mutated *SRY* was only present in the germ line, the male carrier was phenotypically normal. Reproduction by the male produced 2 XY sex-reversed offspring. By itself mosaicism is an unlikely explanation for the cause of apparent XY-female Chinook salmon. Mosaicism is rare whereas the incidence of apparent XY-females is moderately high (2–33%) within California Rivers (Williamson and May 2002, 2007). Currently, available sex-specific markers for Chinook salmon would be unable to distinguish a *SEX+*/*SEX-* mosaic from an otherwise genetically normal individual, so the possibility of mosaicism as an explanation for apparent XY-females cannot be overlooked.

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## References

Allendorf FW, Gellman WA, Thorgaard GH. 1994. Sex-linkage of two enzyme loci in *Oncorhynchus mykiss* (rainbow trout). *Heredity*. 72:498–507.

Allendorf FW, Thorgaard GH. 1984. Tetraploidy and the evolution of salmonid fishes. In: Turner BJ, editor. *Evolutionary genetics of fishes*. New York: Plenum Press. p. 1–46.

Chowen TR, Nagler JJ. 2005. Lack of sex specificity for growth hormone pseudogene in fall-run Chinook salmon from the Columbia River. *Trans Am Fish Soc*. 134:279–282.

Danzmann RG, Cairney M, Davidson WS, Ferguson MM, Garhbi K, Guyomard R, Holm LE, Leder E, Okamoto N, Ozaki A, et al. 2005. A comparative analysis of the rainbow trout genome with 2 other species of

fish (Arctic charr and Atlantic salmon) within the tetraploid derivative Salmonidae family (subfamily: Salmoninae). *Genome*. 48:1037–1051. Appendix data available at: [www.uoguelph.ca/~rdanzman/appendices/](http://www.uoguelph.ca/~rdanzman/appendices/). [Last accessed on Oct. 12, 2007].

de la Chapelle A, Horting H, Niemi M, Wennstrom J. 1964. XX sex chromosomes in a human male: first case. *Acta Med Scand*. 175:25–28.

Devlin RH, Biagi CA, Smalil DE. 2001. Genetic mapping of Y-chromosomal markers in Pacific Salmon. *Genetica*. 111:43–58.

Devlin RH, McNeil BK, Groves TD, Donaldson EM. 1991. Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in Chinook salmon (*Oncorhynchus tshawytscha*). *Can J Fish Aquat Sci*. 48:1606–1612.

Devlin RH, McNeil BK, Salar II, Donaldson EM. 1994. A rapid PCR-based test for Y-chromosomal DNA allows simple production of all-female strains of Chinook salmon. *Aquaculture*. 128:211–220.

Devlin RH, Nagahama Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture*. 208:91–364.

Devlin RH, Park L, Sakhrani DM, Baker JD, Marshall AR, LaHood E, Kolesar SE, Mayo MR, Biagi CA, Uh M. 2005. Variation of Y-chromosomal DNA markers in Chinook salmon (*Oncorhynchus tshawytscha*) populations. *Can J Fish Aquat Sci*. 62:1386–1399.

Devlin RH, Stone GW, Smalil DE. 1998. Extensive direct-tandem organization of a long repeat DNA sequence on the Y chromosome of Chinook salmon. *J Mol Evol*. 46:277–287.

Donaldson EM, Hunter GA. 1982. Sex control in fish with particular reference to salmonids. *Can J Fish Aquat Sci*. 33:99–110.

Du SJ, Devlin RH, Hew CL. 1993. Genomic structure of growth hormone genes in Chinook salmon (*Oncorhynchus tshawytscha*): presence of two functional genes, GH-I and GH-II, and a male-specific pseudogene, GH-PSI. *DNA Cell Biol*. 12:739–751.

Fisher RA. 1934. *Statistical methods for research workers*. 5th ed. Edinburgh (Scotland): Oliver and Boyd.

Gubbay J, Collignon J, Koopman P, Capel B, Economou A, Münsterberg A, Vivian N, Goodfellow P, Lovell-Badge R. 1990. A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes. *Nature*. 346:245–250.

Hartely SE. 1987. The chromosomes of salmonid fishes. *Biol Rev Camb Philos Soc*. 62:197–214.

Hines RS, Tho SP, Zhang YY, Plouffe L Jr, Hansen KA, Khan I, McDonough PG. 1997. Paternal somatic and germ-line mosaicism for a sex-determining region on Y (*SRY*) missense mutation leading to recurrent 46, XY sex reversal. *Fertil Steril*. 67:675–679.

Jager RJ, Anvret M, Hall K, Scherer G. 1990. A human XY-female with a frame shift mutation in the candidate testis-determining gene *SRY*. *Nature*. 348:452–453.

Kolon TF, Ferrer FA, McKenna PH. 1998. Clinical and molecular analysis of XX sex reversed patients. *J Urol*. 160:1169–1172.

May B, Johnson KR, Wright JE Jr. 1989. Sex linkage in salmonids: evidence from a hybridized genome of brook trout and arctic char. *Biochem Genet*. 27:291–301.

Nagler JJ, Bouma J, Thorgaard GH, Dauble DD. 2001. High incidence of a male-specific genetic marker in phenotypic female Chinook salmon from the Columbia River. *Environ Health Perspect*. 109:67–69.

Nichols KM, Young WP, Danzmann RG, Robison BD, Rexroad C, Noakes M, Phillips RB, Bentzen P, Spies I, Knudsen K, et al. 2003. A consolidated linkage map for rainbow trout (*Oncorhynchus mykiss*). *Animal Genet*. 34:102–115.

Page DC, Brown LG, de la Chapelle A. 1987. Exchange of terminal portions of X- and Y-chromosomal short arms in human XX males. *Nature*. 328:437–440.

- Palti Y, Fincham R, Rexroad CE III. 2002. Characterization of 38 polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Mol Ecol Notes. 2:449–452.
- Phillips RB. 2005. Chromosome morphology. In: Friedland K, Waldman J, Cadrin S, editors. Stock identification methods. Burlington, MA: Academic Press. p. 273.
- Phillips RB, Morasch MR, Park LK, Naish KA, Devlin RH. 2005. Identification of the sex chromosome pair in coho salmon (*Oncorhynchus kisutch*): lack of conservation of the sex-linkage group with Chinook salmon (*Oncorhynchus tshawytscha*). Cytogenet Genome Res. 111:166–170.
- Phillips RB, Noakes MA, Morasch MR, Felip A, Thorgaard GH. 2004. Does differential selection on the 5S rDNA explain why the rainbow trout sex chromosome heteromorphism is not linked to the *SEX* locus? Cytogenet Genome Res. 105:122–125.
- Phillips RB, Zajicek KD, Utter FM. 1985. Q band chromosomal polymorphisms in Chinook salmon (*Oncorhynchus tshawytscha*). Copeia. 2:273–278.
- Rexroad CE III, Coleman RL, Hershberger WK, Killefer J. 2002. Rapid communication: thirty-eight polymorphic microsatellite markers for mapping in rainbow trout. J Animal Sci. 80:541–542.
- Sakamoto T, Danzmann RG, Gharbi K, Howard P, Ozaki A, Khoo SK, Woram RA, Okamoto N, Ferguson MM, Holms L-E, et al. 2000. A microsatellite linkage map of rainbow trout (*O. mykiss*) characterized by large sex-specific differences in recombination rates. Genetics. 155:1331–1345.
- Stein JD, Phillips RB, Devlin RH. 2001. Identification of sex chromosomes in Chinook salmon (*Oncorhynchus tshawytscha*). Cytogenet Cell Genet. 92:108–110.
- Thorgaard GH. 1977. Heteromorphic sex chromosomes in male rainbow trout. Science. 196:900–902.
- Thorgaard GH, Gall GAE. 1979. Adult triploids in a rainbow trout family. Genetics. 93:961–973.
- Tomomasa H, Adachi Y, Iwabuchi M, Tohyama Y, Yotsukura M, Oshio S, Umeda T, Takano T, Yamanouchi Y, Nakahori Y. 1999. XX-male syndrome bearing the sex-determining region Y. Arch Androl. 42:89–96.
- Ueda T, Ojima Y. 1984a. Sex chromosomes in the rainbow trout *Salmo gairdneri*. Bull Jpn Soc Sci Fish. 50:1499–1504.
- Ueda T, Ojima Y. 1984b. Sex chromosomes in the kokanee salmon, *Oncorhynchus nerka*. Bull Jpn Soc Sci Fish. 50:1495–1498.
- Williamson KS, May B. 2002. Incidence of phenotypic female Chinook salmon (*Oncorhynchus tshawytscha*) positive for the male Y-chromosome specific marker OtY1 in the Central Valley, California, U.S.A. J Aquat Anim Health. 14:176–183.
- Williamson KS, May B. 2005. Inheritance studies implicate a genetic mechanism for apparent sex-reversal in Chinook salmon. Trans Am Fish Soc. 134:1253–1261.
- Williamson KS, May B. 2007. Mitochondrial DNA haplotype diversity in ‘apparent’ XY-female fall- and spring-run Chinook salmon in California’s Central Valley. Trans Am Fish Soc. Forthcoming. Trans Am Fish Soc: 136:1480–1486.
- Wright JE, Johnson K, Hollister A, May B. 1983. Meiotic models to explain classical linkage, pseudolinkage, and chromosomal pairing in tetraploid derivative salmonid genomes. Isozymes Curr Top Biol Med Res. 10:239–260.

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