Sex-specific differences in the synaptonemal complex in the genus *Oreochromis* (Cichlidae)

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Running title: Sex and the *Oreochromis* synaptonemal complex

Abstract

Total synaptonemal complex (SC) lengths were estimated from *Oreochromis aureus* Steindachner (which has a WZ/ZZ sex determination system), *O. mossambicus* Peters and *O. niloticus* L. (both of which have XX/XY sex determination systems). The total SC length in oocytes was greater than that in spermatocytes in all three species (194±30 μm and 134±13 μm, 187±22 μm and 127±17 μm, 193±37 μm and 144±19 μm, respectively). These sex-specific differences did not appear to be influenced by the type of sex determination system (the female/male total SC length ratio was 1.45 in *O. aureus*, 1.47 in *O. mossambicus* and 1.34 in *O. niloticus*) and do not correlate with the lack of any overall sex-specific length differences in the current *Oreochromis* linkage map. Although based on data from relatively few species, there appears to be no consistent relationship between sex-specific SC lengths and linkage map lengths in fish. Neomale (hormonally masculinized genetic female) *O. aureus* and *O. mossambicus* had total SC lengths of 138±13 μm and 146±13 μm respectively, more similar to normal males than to normal females. These findings agree with data from other vertebrate species that suggest that phenotypic sex, rather than genotype, determines traits such as total SC length, chiasmata position and recombination pattern, at least for the autosomes.

Keywords: tilapia, *Oreochromis*, synaptonemal complex, sex-specific differences, recombination, linkage

Abbreviations:

ANOVA = analysis of variance

ET = 17 ∝-Ethynyltestosterone

Male-T = genetic males treated with ET or MT

MT = 17∝-Methyltestosterone

SC = synaptonemal complex

SCTL = synaptonemal complex total length

TSD = temperature sex determination

Introduction

In eutherian mammals, the synaptonemal complex length has been shown to reflect the rate of recombination rather than the DNA content of chromosomes (Lynn et al., 2002). Sun et al. (2004) showed that there was a very strong correlation in human males between the mean length of each autosomal bivalent and the number of recombination foci per bivalent. Tease and Hulten (2004) showed that human spermatocytes had shorter synaptonemal complexes and less crossovers per cell than oocytes, and that when the so-called obligate chiasma was eliminated from the analysis, both sexes had essentially identical rates of recombination per unit length of SC. Tease and Hulten (2004) made a general case for a positive correlation between inter-sex differences in SC lengths and inter-sex differences in recombination rates, based on a range of species.

While the general eutherian pattern is for recombination rates to be similar between the sexes or greater in females, the few species of marsupials that have been studied show reduced chiasma frequency and recombination rates in females (Samollow et al., 2004). It has been suggested that birds do not show sex-specific differences in recombination (Calderon and Pigozzi, 2006), unlike mammals. Lorch (2005) reviewed sex differences in recombination and suggested that sexual selection, rather than the sex determination system or differences in metabolic rates between the sexes, might explain differences in recombination rates between the sexes.

Sex-specific differences in recombination rates in fish vary from being much higher in females (e.g. Atlantic salmon, *Salmo salar*: Danzmann et al., 2005) to much higher in males (e.g. Japanese flounder, *Paralichthys olivaceous*: Coimbra et al., 2003). There have been very few studies on SC lengths in female and male fish, but these have also shown variation in female:male ratios. We examined the synaptonemal complexes, and in particular SC lengths in both sexes, in three species of tilapia (*Oreochromis*, Cichlidae), two with primarily XX/XY sex determination (*O. mossambicus* and *O. niloticus*) and one with primarily WZ/ZZ sex determination (*O. aureus*) (reviewed by Penman and McAndrew, 2000). All of these species show SC pairing anomalies in the heterogametic sex in early pachytene (Carrasco et al., 1999; Campos-Ramos et al., 2001, 2003) and sex-linked markers have been developed (Lee et al., 2003, 2004; Ezaz et al., 2004).

We use the results from the present study and data from the literature to compare the relationship between SC lengths and recombination rates in male and female fish. No clear overall pattern emerges in fish, although there may be taxon-specific patterns. We also present SC lengths from neomale tilapia (hormonally masculinised genetic females), which are more similar to normal males than to normal females, although there also appears to be an effect of the hormone treatment.

Materials and methods

Tilapia stocks

The fish used in this study came from populations derived from the River Zambezi, Zimbabwe (*O. mossambicus*) and Lake Manzala, Egypt (*O. niloticus* and *O. aureus*), and held in the Tilapia Reference Collection at the Institute of Aquaculture, University of Stirling.

Fish used for SC analysis

Males: one *O. niloticus*, six *O. mossambicus* and six *O. aureus* males between 30 and 50 g body weight were studied. They had been previously crossed with normal females and the progeny were sexed to ensure that the sex ratio fitted with the expected 1:1 ratio. For each male, 10 spermatocytes at pachytene stage were analysed. The best three images from each individual (N = 18 in the blue tilapia, and N= 18 in the Mozambique tilapia) were measured for SC length analyses. In the Nile tilapia, the ten nuclei at pachytene stage came from the single specimen studied.

Females: six females of each of the three species were studied at 70 to 90 days after hatching. These could not be progeny tested (SC spreads in females can only be prepared from pre-vitellogenic ovaries, about 3 months after hatch) but it was assumed that they were they were XX (*O. niloticus* and *O. mossambicus*) or ZW (*O. aureus*). For each female, at least two oocytes at pachytene stage were analysed. Thus, 12 nuclei at pachytene stage were measured for SC length analyses for each of the three species.

Neomales: six XX neomales (*O. mossambicus*) and six WZ neomales (*O. aureus*) were studied. Fry were sex-reversed with 17 ∝-Ethynyltestosterone

(ET) or 17∝-Methyltestosterone (MT) (Sigma-Aldrich) at 50 mg/kg, during 60 days from first feeding. The treated *O. mossambicus* males were crossed with normal (XX) females to check if the progeny sex ratio was 1:0 female:male that would indicate a putative neomale (XX) or 1:1 that would indicate a normal male (XY). Treated *O. aureus* males were crossed with a ZZ neofemale previously sex reversed with 17 ∝ Ethynylestradiol (Sigma-Aldrich) at 150 mg/kg during 35 days (Melard, 1995), to compare to an expected sex ratio of 1:1 female:male that would indicate a putative neomale (WZ) or 0:1 that would indicate a normal male (ZZ). Some eggs from the ZZ neofemale *O. aureus* where also crossed to a test control ZZ male, which was expected to give a sex-ratio of 0:1 female:male. The number of cells analysed was as described above for normal males. The best three or four images at pachytene stage from each individual (N = 20 in the blue tilapia, and N = 21 in the Mozambique) were measured for SC length analyses.

Males exposed to MT or ET hormone (males-T): six progeny tested genetic males of *O. mossambicus*_and five progeny tested genetic males of *O. aureus* from the groups treated with the hormones MT or ET were analysed. The best four images at pachytene stage from each individual of the blue tilapia (N = 20) were measured, while only N = 13 nuclei among the six specimens of the Mozambique tilapia were suitable for the SC measurement analyses.

Preparation of SC spreads (transmission electron microscopy)

Fish were killed with anaesthesia (0.01% benzocaine solution) followed by destruction of the brain before dissection. Preparation of fish SC spreads for the observation in the transmission electron microscope and posterior analysis were made according to Campos-Ramos et al. (2001).

Statistical analysis of Synaptonemal Complex total length

In each pachytene nucleus, the maximum and minimum axes of the area occupied by the SC and the bivalent lengths were measured, using the software Image Pro Plus 4.0 (Media Cybernetics). The mean of the two axes was calculated, as an indication of the diameter of the nucleus at this stage. The bivalent lengths were added for each cell to obtain the SC total length (SCTL). Packing density (equal to 1C DNA content (pg)/one chromatid/micrometer of SC) was calculated based on values for 1C DNA contents from Majumdar and McAndrew (1986): *O. niloticus* = 0.95 pg; *O. mossambicus* = 1.00 pg; and *O. aureus* = 1.21 pg.

SCTL and nucleus diameter were compared between species and sexual genotypes/phenotypes through analysis of variance (ANOVA), assessing normality by the Kolmogorov-Smirnov test and homogeneity of variance by Bartlett's test. Since the diameter of nuclei and SCTL varied significantly between males and females (see Results), further analyses of SCTL were also carried out with nucleus diameter as a covariate. Correlations between nucleus diameter and SCTL were also tested.

Results

Pachytene stage

At pachytene stage, SC spreads in all three species contained 22 silver-stained bivalents. The lateral elements were well differentiated and they span the bivalents from telomere to telomere with a distinctly stained plaque without clearly revealing the central region. Kinetochores were usually not observed. Fig. 1 shows images from males and females of the three species of tilapias at pachytene stage (these SCs do not show the sex-associated pairing anomalies referred to in the Introduction, which are normally observed only in early pachytene and have been described in detail elsewhere).

Length and packing density of synaptonemal complexes

The SC total length for the different species and genotypes is shown in Table 1. In all three species females had significantly longer SCs than males. Neomale *O. aureus* and *O. mossambicus* had SCs that were significantly shorter than those of normal females. In *O. aureus* the SC total lengths of males and neomales were not significantly different, while in *O. mossambicus* the neomale SC total lengths were significantly longer than those of normal males. SC lengths in males-T in both species were not significantly different from neomales. Packing densities for the different genotypes/phenotypes within each species reflected total SC lengths, while at the interspecific level *O. aureus* had the highest packing density and *O. niloticus* the lowest.

Mean nucleus diameters for females were 23.0 μm (*O. mossambicus*), 24.1 μm (*O. aureus*) and 25.5 (*O. niloticus*), while the means for males were 19.9, 21.1 and 21.8 μm respectively. There was no significant effect of species on nucleus diameter but there was a highly significant effect of sex ($F = 20.084$, $P < 0.001$). The effect of sex on SCTL was still highly significant if nucleus diameter was included in the analysis of variance as a cofactor ($F = 83.542$, $P < 0.001$). There was no significant correlation between nucleus spread and SCTL for either sex.

Discussion

Sex-specific differences in SC total length, and relationship to recombination

In placental mammals, SC length is clearly related to recombination frequency (e.g. Lynn et al., 2002). It might thus be expected that sex-specific differences in SC length in fish might also be correlated with sex-specific differences in recombination frequency. However, although total SC lengths were greater in females than in males for all three tilapia species analysed (Table 1), the most comprehensive linkage mapping data on tilapia suggests that overall female and male map lengths are very similar (Lee, 2004). Table 2 summarises the limited data that is available on fish species, including results from the present study. There is considerable variation in female:male ratios for both total SC length and recombination frequency. There are only a few studies where both male and female SC lengths have been measured, and in most cases the number of oocytes studied was low (due to technical difficulties). There was also a large difference between the estimates of total SC length in male rainbow trout in two different studies, and apparently also large differences in SC length in different stages of pachytene in the zebrafish. The extremely high female:male recombination ratios in salmonids appear to be influenced by pseudolinkage due to tetraploid ancestry (Danzmann et al., 2005). Even allowing for these qualifications, it seems that there is no clear relationship between sex-specific differences in total SC length and recombination frequency in fish.

There is also no apparent association between sex determination system and either SC length or recombination differences between the sexes. Species with XX/XY systems show longer SC complements in males (stickleback, turbot, rainbow trout) or in females (Nile and Mozambique tilapias), and more recombination in males (Japanese flounder) or females (salmonids, threespine stickleback, channel catfish). Within the genus *Oreochromis*, species showing different sex determining mechanisms (XX/XY or WZ/ZZ) show similar patterns of SC length (approximately 1.4:1 F:M). Species without strong genetic sex determination show longer female SC complement (zebrafish – unknown sex determination) and/or more recombination in females than males (zebrafish; sea bass – largely TSD; gilthead sea bream – protandrous hermaphrodite).

Does phenotypic or genotypic sex determine SC length?

The total SC length of females was significantly longer than that of males. In neomales (through MT or ET treatment), the SC total length was significantly reduced compared to that of normal females and in males-T it had a tendency to increase compared to control males (Table 1). Thus, androgen treatment has a small effect on total SC length but the major influence is phenotypic sex (i.e. not genotypic sex). Furthermore, WZ males (neomales) of *O. aureus* and XX males (neomales) of *O. mossambicus* showed one or two nucleoli of the same size as normal males, rather than having one larger nucleolus. Therefore, a larger nucleolus in females is also due to phenotypic sex rather than genotypic sex. In contrast, pairing anomalies in the putative sex chromosomes (bivalent 1 and a small bivalent)

were associated with genotypic sex (heterogamety) rather than phenotypic sex (Carrasco et al., 1999; Campos-Ramos et al., 2001, 2003).

There were significant differences between the sexes for both nucleus diameter and SC total length. While it could be argued that this indicates that greater nucleus spreading in females led to longer SC total lengths (and thus the difference between the sexes is in a sense an artefact), it could also be argued that longer SC total lengths in females led to a larger nucleus (a comparison could be made to variation in nucleus size according to DNA content, e.g. triploids have a nucleus that is about 1.5x the volume of diploids of the same species). The absence of any correlation between nucleus diameter and SC total length is probably an argument against the former hypothesis.

There have been relatively few other studies using sex-reversed animals to look at the influence of phenotypic sex on SC length, chiasmata position, recombination, etc. Such studies do support a consistent association of sex-specific differences in such traits with phenotypic sex, at least for the autosomes. XY neofemale crested newts, *Triturus cristatus carnifex*, showed chiasmata distributions typical of normal females (Wallace et al., 1997). Lynn et al. (2005) showed that XY female mice had rates and patterns of autosomal recombination (MLH1 foci) typical of normal XX females. Franch et al. (2006) showed a difference in recombination rates between female and male gilthead sea bream (*Sparus aurata*), although this species is a protandrous hermaphrodite with no genetic sex determination.

In the medaka (*Oryzias latipes*), there are differences between the sexes in recombination patterns in the sex chromosomes (Kondo et al., 2001) and XY neofemale medaka show a pattern of recombination in the sex chromosomes that is typical of XX females rather than XY males. The medaka sex chromosomes showed a pattern typical of autosomes in that male recombination was suppressed near the centromere and female recombination was suppressed near the telomeres, so it is not clear from these studies whether the sex chromosomes actually show any differences in sex-specific recombination pattern from the autosomes. The female:male ratio of the map length of this chromosome is very similar to the ratio of the total map lengths from female and male derived medaka linkage maps (both are around 1.3:1 - Kondo et al., 2001 and references therein). Sex determination in medaka is now known to be primarily determined by the *DMY* gene, located in a duplicated region of autosomal origin present only in the Y chromosome (Matsuda et al., 2002; Kondo et al., 2006), and no cytologically detectable differences between the sex chromosomes have been detected (Kondo et al., 2001). Full pairing of the XY bivalent has been reported in medaka (Iwai et al, 2006), with the SYCP1 structural component located along the entire bivalent (unlike in mammals where this is only found in the region corresponding to the pseudoautosomal region).

The association of pairing anomalies in putative tilapia sex chromosomes with the genotypic (heterogametic) sex, rather than phenotypic sex, suggests that pairing anomalies in species with more differentiated sex chromosomes

should also be associated with the heterogametic genetic sex. Unfortunately, Lynn et al. (2005) only analysed the autosomes of XY female mice and did not examine MLH1 foci in the XY bivalent (Dr T. Hassold, pers. comm.). We are not aware of any other studies on sex-reversed animals from species with clearly differentiated sex chromosomes.

Conclusion

There is a clear influence of phenotypic sex on traits related to meiosis (SC length and recombination) in fish, as in other vertebrates. However, in fish there is no apparent relationship between sex-specific differences in SC length and recombination, as is the case in placental mammals. Further studies may be able to determine if, for instance, there are taxon (e.g. order)-specific patterns within this very diverse group.

Acknowledgements

RCR was supported by a PhD grant from Consejo Nacional de Ciencia y Tecnologia (CONACYT) and CIBNOR, Mexico. SCH was supported by a research grant from BBSRC, awarded to DJP and colleagues. We would like to thank several colleagues for provision of unpublished information (as indicated) and comments on the Discussion, and Mr. Keith Ranson and Mr. William Hamilton for assistance with maintenance of fish stocks.

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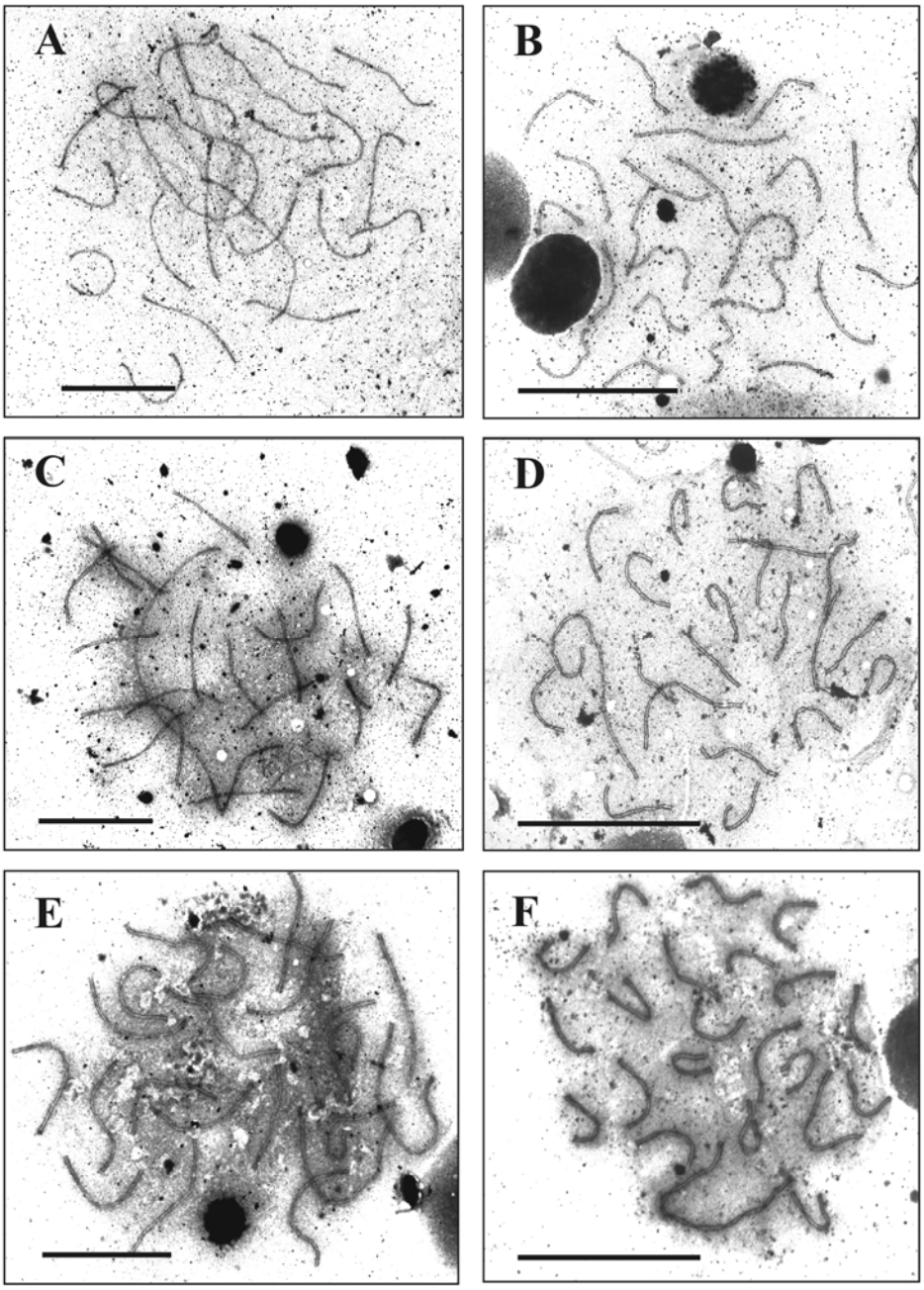
456 **Figure 1**. Three species of tilapia showing 22 bivalents at pachytene stage;

457 *O. niloticus*, (A and B), *O.aureus* (C and D), and *O. mossambicus* (E and F);

458 females (A, C, and E) and males (B, D, F). Bar represents10 μm.



459

460 **Table 1**. Synaptonemal complex length and packing density in three species

461 of tilapia.

462

Species; Sexual genotype/ phenotype	N	SCTL ± SD (μm)	Max (μm)	Min (μm)	Range	Packing Density*
O. aureus						
Female	12	194 ± 30(a)	242	152	1.59x	6.2×10^{-3}
Male	18	134 ± 13(b)	156	109	1.43x	9.0×10^{-3}
Neomale	20	138 ± 13(b)	171	113	1.51x	8.7×10^{-3}
Male-T	20	141 ± 14(b)	168	121	1.38x	8.5×10^{-3}
O. mossambicus						
Female	12	187 ± 22(a)	219	151	1.45x	5.3×10^{-3}
Male	18	127 ± 17(c)	162	96	1.68x	7.8×10^{-3}
Neomale	21	146 ± 13(b)	160	117	1.36x	6.8×10^{-3}
Male-T	13	145 ± 19(b)	191	116	1.64x	6.8×10^{-3}
O. niloticus						
Female	12	193 ± 37(a)	233	121	1.92x	4.9×10^{-3}
Male	10	144 ± 19(b)	164	106	1.54x	6.5×10^{-3}

463

464 SCTL = Mean synaptonemal complex complement total length; SD =

465 standard deviation; Max = maximum value observed; Min = minimum value

466 observed; * units = pg DNA/one chromatid/micrometer SC. Different letter

467 superscripts for SCTL values within each species indicate significant

468 differences ($P<0.05$).

469

Table 2. Synaptonemal complex (SC) lengths and recombination in male and female fish.

Order Family Species	Primary sex determining mechanism	Total SC length		F:M ratio	Linkage map length		F:M ratio*	Comments	References
		Female (mean ± SD, µm) (n)	Male (mean ± SD, µm) (n)		Female (cM)	Male (cM)			
Cypriniformes: Cyprinidae									
Zebrafish *(Danio rerio)*	Unknown	199 ± 18 (4) 324 ± 64 (6)	174 ± 12 (8) 198 ± 28 (9)	1.14:1 1.64:1	2583	943	2.74:1	SC lengths from earlier (upper) and later (lower) pachytene	1,2,3
Salmoniformes: Salmonidae									
Rainbow trout (*Oncorhynchus mykiss)*	XX/XY p	223 ± 34 (2)	259 ± 49 (10) 144 ± 16 (10)	1:1.16	2276	1104	4.31:1	Single estimate for SC length in males from 5 is much lower than that from 4	4,5,6
Atlantic salmon (*Salar salar*)	XX/XY				901	103	16.81:1	Map lengths from ref. 8, F:M ratio from ref. 6	7,8,6
Brown trout (*S. trutta*)	XX/XY				913	346	6.4:1		9
Perciformes: Cichlidae									
Blue tilapia (*Oreochromis aureus*)	WZ/ZZ p	194 ± 30 (12)	134 ± 13 (18)	1.45:1	2394	2451	1:1.02	Map based on F2 hybrid between *O. aureus* and *O. niloticus* (ratios for individual LGs vary from 3.86:1 – 1:3.39)	10,11,12
Nile tilapia (*O. niloticus*)	XX/XY p	193 ± 37 (12)	144 ± 19 (10)	1.34:1					
Mozambique tilapia (*O. mossambicus*)	XX/XY p	187 ± 22 (12)	127 ± 17 (18)	1.47:1					
Moronidae									
European sea bass	Largely TSD				906	567	1.48:1		13

(*Dicentrarchus labrax*)									
Sparidae									
Gilthead sea bream (*Sparus aurata*)	Protandrous hermaphrodite				1452	1171	1.10		14
Pleuronectiformes: Scophthalmidae									
Turbot (*Scophthalmus maximus*)	XX/XY?	172 ± 29 (10)	205 ± 12 (32)	1:1.17					15,16
Paralichthyidae									
Japanese flounder (*Paralichthys olivaceous*)	XX/XY				670	741	1:7.4		17
Gasterosteiformes: Gasterosteidae									
Threespine stickleback (*Gasterosteus aculeatus*)	XX/XY	143 ± 12 (7)	150 ± 18 (33)	1:1.05	1010	757	1.33:1		18,19,20
Siluriformes: Ictaluridae									
Channel catfish (*Ictalurus punctatus*)	XX/XY				-	-	3.18:1	Pairwise F:M recombination ratios ranged from 0.07:1 – 23.5:1	21

n = no of meiotic cells analysed; **p** = SC pairing anomalies seen in the heterogametic sex; **TSD** = temperature sex determination; ***** F:M ratio generally calculated on comparable regions/markers, and may differ from ratio of map lengths if some markers were non-informative in one sex or if identification of linkage groups differed between sexes.

References: 1 Traut and Winking (2001); 2 Wallace and Wallace (2003); 3 Singer et al (2002); 4 Oliveira et al (1995); 5 Peterson et al (1994); 6 Danzmann et al (2005); 7 Artieri et al (2006); 8 Moen et al (2004); 9 Gharbi et al (2006); 10 Penman and McAndrew (2000); 11 present study; 12 Lee (2004); 13 Chistiakov et al (2005); 14 Franch et al (2006); 15 Cal et al (2006); 16 Cunado et al (2001); 17 Coimbra et al (2003); 18 Peichel et al (2004); 19 Cunado et al (2002); 20 Dr CL Peichel (pers. comm.); 21 Waldbieser et al (2001).