1 **Sex-specific differences in the synaptonemal complex in the**

2 **genus *Oreochromis* (Cichlidae)**

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20 **Running title**: Sex and the *Oreochromis* synaptonemal complex

21

22 **Abstract**

23 Total synaptonemal complex (SC) lengths were estimated from

24 *Oreochromis aureus* Steindachner (which has a WZ/ZZ sex determination

25 system), *O. mossambicus* Peters and *O. niloticus* L. (both of which have

26 XX/XY sex determination systems). The total SC length in oocytes was

27 greater than that in spermatocytes in all three species (194±30 μm and

28 134±13 μm, 187±22 μm and 127±17 μm, 193±37 μm and 144±19 μm,

29 respectively). These sex-specific differences did not appear to be influenced

30 by the type of sex determination system (the female/male total SC length

31 ratio was 1.45 in *O. aureus*, 1.47 in *O. mossambicus* and 1.34 in *O.*

32 *niloticus*) and do not correlate with the lack of any overall sex-specific

33 length differences in the current *Oreochromis* linkage map. Although based

34 on data from relatively few species, there appears to be no consistent

35 relationship between sex-specific SC lengths and linkage map lengths in

36 fish. Neomale (hormonally masculinized genetic female) *O. aureus* and *O.*

37 *mossambicus* had total SC lengths of 138±13 μm and 146±13 μm

38 respectively, more similar to normal males than to normal females. These

39 findings agree with data from other vertebrate species that suggest that

40 phenotypic sex, rather than genotype, determines traits such as total SC

41 length, chiasmata position and recombination pattern, at least for the

42 autosomes.

43

44

45   **Keywords**: tilapia, *Oreochromis*, synaptonemal complex, sex-specific

46   differences, recombination, linkage

47

48   **Abbreviations:**

49   ANOVA = analysis of variance

50   ET = 17 $\propto$-Ethynyltestosterone

51   Male-T = genetic males treated with ET or MT

52   MT = 17$\propto$-Methyltestosterone

53   SC = synaptonemal complex

54   SCTL = synaptonemal complex total length

55   TSD = temperature sex determination

56

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.

81   Sex-specific differences in recombination rates in fish vary from being

82   much higher in females (e.g. Atlantic salmon, *Salmo salar*: Danzmann et al.,

83   2005) to much higher in males (e.g. Japanese flounder, *Paralichthys*

84   *olivaceous*: Coimbra et al., 2003). There have been very few studies on SC

85   lengths in female and male fish, but these have also shown variation in

86   female:male ratios. We examined the synaptonemal complexes, and in

87   particular SC lengths in both sexes, in three species of tilapia (*Oreochromis*,

88   Cichlidae), two with primarily XX/XY sex determination (*O. mossambicus*

89   and *O. niloticus*) and one with primarily WZ/ZZ sex determination (*O.*

90   *aureus*) (reviewed by Penman and McAndrew, 2000). All of these species

91   show SC pairing anomalies in the heterogametic sex in early pachytene

92   (Carrasco et al., 1999; Campos-Ramos et al., 2001, 2003) and sex-linked

93   markers have been developed (Lee et al., 2003, 2004; Ezaz et al., 2004).

94

95   We use the results from the present study and data from the literature to

96   compare the relationship between SC lengths and recombination rates in

97   male and female fish. No clear overall pattern emerges in fish, although

98   there may be taxon-specific patterns. We also present SC lengths from

99   neomale tilapia (hormonally masculinised genetic females), which are more

100 similar to normal males than to normal females, although there also appears

101 to be an effect of the hormone treatment.

102

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 $\propto$-Ethynyltestosterone

128   (ET) or 17∝-Methyltestosterone (MT) (Sigma-Aldrich) at 50 mg/kg, during

129   60 days from first feeding. The treated *O. mossambicus* males were crossed

130   with normal (XX) females to check if the progeny sex ratio was 1:0

131   female:male that would indicate a putative neomale (XX) or 1:1 that would

132   indicate a normal male (XY). Treated *O. aureus* males were crossed with a

133   ZZ neofemale previously sex reversed with 17 ∝ Ethynylestradiol (Sigma-

134   Aldrich) at 150 mg/kg during 35 days (Melard, 1995), to compare to an

135   expected sex ratio of 1:1 female:male that would indicate a putative

136   neomale (WZ) or 0:1 that would indicate a normal male (ZZ). Some eggs

137   from the ZZ neofemale *O. aureus* where also crossed to a test control ZZ

138   male, which was expected to give a sex-ratio of 0:1 female:male. The

139   number of cells analysed was as described above for normal males. The best

140   three or four images at pachytene stage from each individual (N = 20 in the

141   blue tilapia, and N = 21 in the Mozambique) were measured for SC length

142   analyses.

143   *Males exposed to MT or ET hormone (males-T)*: six progeny tested genetic

144   males of *O. mossambicus* and five progeny tested genetic males of *O.*

145   *aureus* from the groups treated with the hormones MT or ET were analysed.

146   The best four images at pachytene stage from each individual of the blue

147   tilapia (N = 20) were measured, while only N = 13 nuclei among the six

148   specimens of the Mozambique tilapia were suitable for the SC measurement

149   analyses.

150

151   *Preparation of SC spreads (transmission electron microscopy)*

152 Fish were killed with anaesthesia (0.01% benzocaine solution) followed by

153 destruction of the brain before dissection. Preparation of fish SC spreads for

154 the observation in the transmission electron microscope and posterior

155 analysis were made according to Campos-Ramos et al. (2001).

156

157 *Statistical analysis of Synaptonemal Complex total length*

158 In each pachytene nucleus, the maximum and minimum axes of the area

159 occupied by the SC and the bivalent lengths were measured, using the

160 software Image Pro Plus 4.0 (Media Cybernetics). The mean of the two axes

161 was calculated, as an indication of the diameter of the nucleus at this stage.

162 The bivalent lengths were added for each cell to obtain the SC total length

163 (SCTL). Packing density (equal to 1C DNA content (pg)/one

164 chromatid/micrometer of SC) was calculated based on values for 1C DNA

165 contents from Majumdar and McAndrew (1986): *O. niloticus* = 0.95 pg; *O.*

166 *mossambicus* = 1.00 pg; and *O. aureus* = 1.21 pg.

167

168 SCTL and nucleus diameter were compared between species and sexual

169 genotypes/phenotypes through analysis of variance (ANOVA), assessing

170 normality by the Kolmogorov-Smirnov test and homogeneity of variance by

171 Bartlett's test. Since the diameter of nuclei and SCTL varied significantly

172 between males and females (see Results), further analyses of SCTL were

173 also carried out with nucleus diameter as a covariate. Correlations between

174 nucleus diameter and SCTL were also tested.

175

176 **Results**

177 *Pachytene stage*

178 At pachytene stage, SC spreads in all three species contained 22 silver-

179 stained bivalents. The lateral elements were well differentiated and they

180 span the bivalents from telomere to telomere with a distinctly stained plaque

181 without clearly revealing the central region. Kinetochores were usually not

182 observed. Fig. 1 shows images from males and females of the three species

183 of tilapias at pachytene stage (these SCs do not show the sex-associated

184 pairing anomalies referred to in the Introduction, which are normally

185 observed only in early pachytene and have been described in detail

186 elsewhere).

187
188 *Length and packing density of synaptonemal complexes*

189 The SC total length for the different species and genotypes is shown in

190 Table 1. In all three species females had significantly longer SCs than

191 males. Neomale *O. aureus* and *O. mossambicus* had SCs that were

192 significantly shorter than those of normal females. In *O. aureus* the SC total

193 lengths of males and neomales were not significantly different, while in *O.*

194 *mossambicus* the neomale SC total lengths were significantly longer than

195 those of normal males. SC lengths in males-T in both species were not

196 significantly different from neomales. Packing densities for the different

197 genotypes/phenotypes within each species reflected total SC lengths, while

198 at the interspecific level *O. aureus* had the highest packing density and *O.*

199 *niloticus* the lowest.

200

9

201   Mean nucleus diameters for females were 23.0 μm (*O. mossambicus*), 24.1

202   μm (*O. aureus*) and 25.5 (*O. niloticus*), while the means for males were

203   19.9, 21.1 and 21.8 μm respectively. There was no significant effect of

204   species on nucleus diameter but there was a highly significant effect of sex

205   ($F = 20.084$, $P < 0.001$). The effect of sex on SCTL was still highly

206   significant if nucleus diameter was included in the analysis of variance as a

207   cofactor ($F = 83.542$, $P < 0.001$). There was no significant correlation

208   between nucleus spread and SCTL for either sex.

209

210 **Discussion**

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

212 *recombination*

213 In placental mammals, SC length is clearly related to recombination

214 frequency (e.g. Lynn et al., 2002). It might thus be expected that sex-

215 specific differences in SC length in fish might also be correlated with sex-

216 specific differences in recombination frequency. However, although total

217 SC lengths were greater in females than in males for all three tilapia species

218 analysed (Table 1), the most comprehensive linkage mapping data on tilapia

219 suggests that overall female and male map lengths are very similar (Lee,

220 2004). Table 2 summarises the limited data that is available on fish species,

221 including results from the present study. There is considerable variation in

222 female:male ratios for both total SC length and recombination frequency.

223 There are only a few studies where both male and female SC lengths have

224 been measured, and in most cases the number of oocytes studied was low

225 (due to technical difficulties). There was also a large difference between the

226 estimates of total SC length in male rainbow trout in two different studies,

227 and apparently also large differences in SC length in different stages of

228 pachytene in the zebrafish. The extremely high female:male recombination

229 ratios in salmonids appear to be influenced by pseudolinkage due to

230 tetraploid ancestry (Danzmann et al., 2005). Even allowing for these

231 qualifications, it seems that there is no clear relationship between sex-

232 specific differences in total SC length and recombination frequency in fish.

233

234 There is also no apparent association between sex determination system and

235 either SC length or recombination differences between the sexes. Species

236 with XX/XY systems show longer SC complements in males (stickleback,

237 turbot, rainbow trout) or in females (Nile and Mozambique tilapias), and

238 more recombination in males (Japanese flounder) or females (salmonids,

239 threespine stickleback, channel catfish). Within the genus *Oreochromis*,

240 species showing different sex determining mechanisms (XX/XY or WZ/ZZ)

241 show similar patterns of SC length (approximately 1.4:1 F:M). Species

242 without strong genetic sex determination show longer female SC

243 complement (zebrafish – unknown sex determination) and/or more

244 recombination in females than males (zebrafish; sea bass – largely TSD;

245 gilthead sea bream – protandrous hermaphrodite).

246

247 *Does phenotypic or genotypic sex determine SC length?*

248 The total SC length of females was significantly longer than that of males.

249 In neomales (through MT or ET treatment), the SC total length was

250 significantly reduced compared to that of normal females and in males-T it

251 had a tendency to increase compared to control males (Table 1). Thus,

252 androgen treatment has a small effect on total SC length but the major

253 influence is phenotypic sex (i.e. not genotypic sex). Furthermore, WZ males

254 (neomales) of *O. aureus* and XX males (neomales) of *O. mossambicus*

255 showed one or two nucleoli of the same size as normal males, rather than

256 having one larger nucleolus. Therefore, a larger nucleolus in females is also

257 due to phenotypic sex rather than genotypic sex. In contrast, pairing

258 anomalies in the putative sex chromosomes (bivalent 1 and a small bivalent)

12

259   were associated with genotypic sex (heterogamety) rather than phenotypic

260   sex (Carrasco et al., 1999; Campos-Ramos et al., 2001, 2003).

261

262   There were significant differences between the sexes for both nucleus

263   diameter and SC total length. While it could be argued that this indicates

264   that greater nucleus spreading in females led to longer SC total lengths (and

265   thus the difference between the sexes is in a sense an artefact), it could also

266   be argued that longer SC total lengths in females led to a larger nucleus (a

267   comparison could be made to variation in nucleus size according to DNA

268   content, e.g. triploids have a nucleus that is about 1.5x the volume of

269   diploids of the same species). The absence of any correlation between

270   nucleus diameter and SC total length is probably an argument against the

271   former hypothesis.

272

273   There have been relatively few other studies using sex-reversed animals to

274   look at the influence of phenotypic sex on SC length, chiasmata position,

275   recombination, etc. Such studies do support a consistent association of sex-

276   specific differences in such traits with phenotypic sex, at least for the

277   autosomes. XY neofemale crested newts, *Triturus cristatus carnifex*,

278   showed chiasmata distributions typical of normal females (Wallace et al.,

279   1997). Lynn et al. (2005) showed that XY female mice had rates and

280   patterns of autosomal recombination (MLH1 foci) typical of normal XX

281   females. Franch et al. (2006) showed a difference in recombination rates

282   between female and male gilthead sea bream (*Sparus aurata*), although this

283   species is a protandrous hermaphrodite with no genetic sex determination.

284

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).

305

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

14

309 should also be associated with the heterogametic genetic sex. Unfortunately,

310 Lynn et al. (2005) only analysed the autosomes of XY female mice and did

311 not examine MLH1 foci in the XY bivalent (Dr T. Hassold, pers. comm.).

312 We are not aware of any other studies on sex-reversed animals from species

313 with clearly differentiated sex chromosomes.

314

315 **Conclusion**

316 There is a clear influence of phenotypic sex on traits related to meiosis (SC

317 length and recombination) in fish, as in other vertebrates. However, in fish

318 there is no apparent relationship between sex-specific differences in SC

319 length and recombination, as is the case in placental mammals. Further

320 studies may be able to determine if, for instance, there are taxon (e.g. order)-

321 specific patterns within this very diverse group.

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324

332   **References**

333

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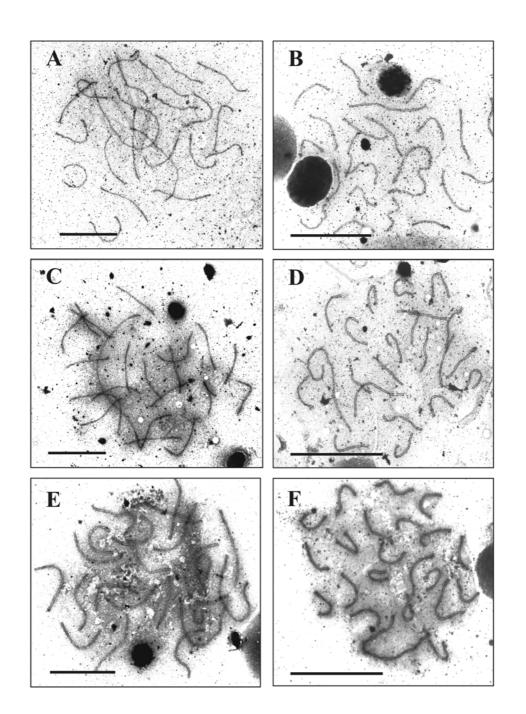
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456   **Figure 1**. Three species of tilapia showing 22 bivalents at pachytene stage;

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

23

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 ($\underline{P}<0.05$).

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).