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Sex determination in the Genus *Oreochromis*

2. Sex reversal, hybridisation, gynogenesis and triploidy in *O. aureus* Steindachner

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Summary. Sex ratios from 62 single-pair matings of normal broodstock *O. aureus* were highly heterogeneous with an overall deficit of males (41.4%). Peaks in the sex ratio frequency distribution occurred at 1:1, 3:5 and 1:3 (male:female). Hybridisation of *O. aureus* with *O. mossambicus, O. spilurus* and *O. niloticus* produced highly variable sex ratios, suggesting a complexity of hybrid sex determination. Few valid inferences could be made regarding intraspecific sex determination from these hybrid data. Sex ratios from progeny testing of sex-reversed males (1:3) and most sex-reversed females (1:0) provide evidence for female heterogamety in *O. aureus*. Several aberrant ratios observed suggest Mendelian inheritance of an autosomal recessive gene (F,f), epistatic to the major sex-determining gene (W,Z). Sex ratios of triploids and gynogens support the hypothesis of recombination between the centromere and the major sex-determining locus. Progeny testing of a female mitogyne demonstrated the viability of a novel WW "superfemale", which gave only female offspring. Not all data could be explained by a two-factor model of sex determination. Further exceptional sex ratios may be accounted for by rare autosomal or environmental sex-modifying factors. It is concluded that *O. aureus* has a multifactorial mechanism of sex determination with the underlying primary mechanism of female heterogamety.

Key words: Sex determination – *Oreochromis aureus* – Gynogenesis – Sex reversal – Hybridisation

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Introduction

Research on sex determination in *Oreochromis aureus* (Steindachner) largely parallels that in *O. niloticus* (reviewed by Mair et al. 1991), with the important exception that genetic evidence points toward female heterogamety of the former, and male heterogamety of the latter species.

Many authors have studied sex ratios in progeny from interspecific crosses on the assumption that a male *O. aureus* crossed with a female, of a species possessing female homogamety, will yield all-male progeny. Several authors have observed all-male hybrids produced in crosses of male *O. aureus* with female *O. niloticus* (Fishelson 1962; Pruginin et al. 1975; Hsiao 1980; Hulata et al. 1983), with reciprocal crosses giving the expected 3:1 ratios, conforming with the theory of hybrid sex determination proposed by Chen (1969) and Jalabert et al. (1971). However, a number of authors have observed highly variable proportions of males (52–99%) in matings of male *O. aureus* with female *O. niloticus* and *O. mossambicus* (Pruginin et al. 1975; Mires 1977; Majumdar and McAndrew 1983; Hulata et al. 1983). These results are not fully explained by the theory of autosomal influence proposed by Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979). On the basis of these and their own studies, Majumdar and McAndrew (1983) suggested that a polygenic system of sex determination exists in tilapia.

In an extensive study of intraspecific pair spawnings in *O. aureus*, Shelton et al. (1983) observed sex ratios varying from 0 to 100% male in 126 progenies; 20% of these had ratios significantly different from the expected 1:1, although the overall mean proportion of males (49.4%) was not significantly different from 1:1. The authors suggested that females exert a greater influence

on progeny sex ratio, particularly in the production of all-female (four broods) and all-male (two broods) progenies, and concluded that sex determination in tilapia requires a more complex explanation than that described by a simple sex chromosome model.

Studies involving hormonal sex reversal and intraspecific progeny testing of treated fish have been more revealing. Several authors have successfully induced sex reversal to male (98–100%) in *O. aureus* by oral administration of the androgens ethynyltestosterone and methyltestosterone (Guerrero 1975; Sanico 1975; Shelton et al. 1981). Although rates of reversal are lower (60–90%), sex reversal to female has been achieved in this specis using β-estradiol (Mair et al. 1987a) and, more effectively, ethynylestradiol in combination with the pituitary blocker, methallibure (Hopkins 1977; Hopkins et al. 1979; Mair et al. 1987a). Progeny testing of androgen-treated males (Guerrero 1975) produced a number of ratios not significantly different from 1:3 (1:2.0–1:3.1), which indicates female heterogamety in this species. Confirmatory results were obtained by Liu (1977).

Progeny testing of estrogen-treated fish has yielded variable results. Liu (1977) progeny tested estrogen-treated females from a brood in which the sex ratio had not been altered from 1:1 by hormone treatment (Sanico 1975). One of these females, however, produced two successive broods of all-male offspring. Hopkins (1979) tested a total of 41 estrogen-treated females, of which five gave all-male progeny, one gave 95% male and one an all-female progeny. The remaining females produced ratios not significantly different from 1:1. In a series of similar experiments, we previously progeny tested a total of 27 estrogen-treated fish (Mair et al. 1987a). Seven presumptive sex-reversed females produced sex ratios of 87.5–100% male, of which only two crosses produced the 100% male progeny expected, assuming male homogamety. Sex ratios from these 27 females were significantly heterogeneous and we tentatively suggested that the treated fish could be divided into four groups producing 1:2, 1:1, 3:1 and 1:0 (or close to) sex ratios. These results are not predicted by any of the current theories on sex determination in this species and present a confusing picture. We concluded from this preliminary study that *O. aureus* has a largely monofactorial sex-determining mechanism with occasional deviations from predicted ratios, possibly caused by rare combinations of autosomal sex factors and/or by environmental influences.

The objective of the study reported here was to utilise established techniques of genetic manipulation, including hybridisation, sex reversal, gynogenesis and triploidisation, to elucidate further the sex-determining mechanism in *O. aureus*.

Materials and methods

The strain of *O. aureus* used in this investigation originated from Lake Manzala, Egypt, where it occurs sympatrically with *O. niloticus*. Fish from the latter *O. niloticus* strain were used in hybrid crosses. *O. spilurus* material came from the river Tana, Kenya, and *O. mossambicus* from an inbred aquarist's stock of unknown origin. Founder stocks for all strains were obtained from the Institute of Aquaculture, University of Stirling, Scotland, in 1981/82. Electrophoretic and morphometric analyses of stocks provided no evidence of hybrid introgression of any one species with another (unpublished data).

The fish were maintained, spawned and further grown as described for *O. niloticus* (Mair et al. 1991). Fry from progeny testing were grown for a minimum period of 3 months prior to sexing, using the gonad squash technique of Guerrero and Shelton (1974). Hybrids were produced using artificial fertilisation.

A number of experiments was performed to investigate the hormonal induction of sex reversal to male or female (Mair et al. 1987a; Penman 1989). Effective treatment for induction of sex reversal to male was found to be oral administration of 17α-methyltestosterone at 60 or 80 $mg.kg^{-1}$ of feed. Ethynylestradiol plus methallibure, each at 100 $mg.kg^{-1}$ of feed (EE100 + ME100), gave the highest rate of sex reversal to female (95.3%), but some sex reversal was induced by oral application of β-estradiol at 100 (βE100) and 200 (βE200) $mg.g^{-1}$ of feed. Four testosterone-treated males were progeny tested by crossing with a single female (009), and a fifth was tested by crossing with a known sex-reversed female. A total of 55 estrogen-treated females was progeny tested by crossing with 20 related and unrelated males. Sex-reversed fish are described according to the nomenclature proposed by Mair et al. (1987a), the delta (Δ) prefix denoting functional sex reversal (i.e. a Δ♀ is a genetic male, sex-reversed to female).

A second generation of hormone treatment was applied to putative monosex male progeny from three known Δ♀♀, and subsequently four of these second generation hormone-treated females were progeny tested. In addition, three females arising spontaneously in putative monosex male broods from a known Δ♀ were progeny tested by crossing with normal males.

The induction of gynogenesis and of triploidy was performed as for *O. niloticus* (Mair et al. 1991), using techniques described by Penman et al. (1987) and Mair et al. (1987b). Triploids were induced by heat shocking fertilized eggs 5 min post fertilization (NoUV HS_5). Gynogens were produced by applying the same heat shock to eggs fertilized with UV-irradiated sperm 5 min post fertilization (meiogynes-UV HS_5) and 30–35 min post fertilization (mitogynes-UV LHS).

A total of 94 triploid, 132 meiogyne and 3 mitogyne progeny were sexed. One male and one female mitogyne were progeny tested and their progeny were sexed.

Results

Table 1 shows a summary of sex ratios from single-pair matings of normal broodstock. Data from repeat spawnings were all found to be homogeneous and were pooled. The data are significantly heterogeneous ($P<0.01$) and the overall sex ratio is significantly different from 1:1 ($P<0.001$), with a deficit of males (41.4%). Figure 1 shows the frequency distribution of progeny sex ratio (sample size ≥ 25; mean sample size = 58.3). The distribution is skewed towards female and two peaks are appar-

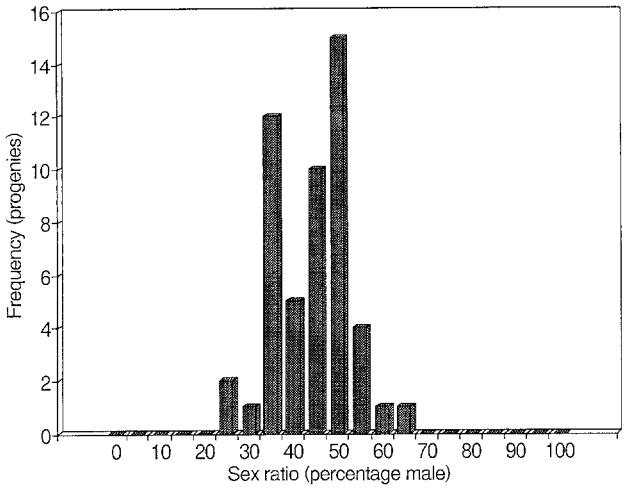

Fig. 1. Sex ratio frequency distribution of progeny from single-pair matings of normal broodstock *O. aureus* (sample size ≥ 25)

Table 1. Summary of sex ratios from single-pair matings of *O. aureus*. Chi-squared values are for comparisons with the expected sex ratio of 1:1

Male broodstock	20
Female broodstock	44
Progenies	62
Progeny sexed	3,147
Total % male	41.40
Mean % male	40.89
Total χ^2	$181.60_{[62]}$***
Pooled χ^2	$93.00_{[1]}$***
Heterogeneity χ^2	$88.59_{[61]}$**

** $P < 0.01$
*** $P < 0.001$

Table 2. Sex ratios from crosses of Female 007 with seven males to determine the degree of paternal influence on sex ratio

Male	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (1:1)	$\chi^2_{[1]}$ (3:5)
196	20:40	33.3	6.67**	0.44
005	20:40	33.3	6.67**	0.44
206	18:36	33.3	6.00*	0.40
151	28:56	33.3	9.33**	0.62
171	27:51	34.6	7.38**	0.28
201	15:19	44.1	0.47	0.63
203	39:44	47.0	0.30	3.19

* $P < 0.05$
** $P < 0.01$

ent at 50 and 35% male with, possibly, a third peak at 25% male. This suggests that progeny sex ratios can be divided into two or three types, a result not in accordance with a monofactorial mechanism of sex determination.

Sex ratios from repeat crosses of a single female (007) with seven males (Table 2) produced five ratios significantly different from 1:1 ($P<0.05$). These same five ratios were found to be not significantly different from 3:5, the importance of which was not recognised until later in the study. The remaining two crosses produced 1:1 ratios. Although this data was homogeneous ($\chi^2_{het} = 5.14_{[6]}$), the occurrence of two apparently different ratios suggests a specific paternal effect on sex ratio. No assessment was made of the degree of maternal influence on sex ratio.

Table 3. Sex ratios from interspecific crosses of *O. aureus* with *O. mossambicus*, *O. spilurus* and *O. niloticus*

Female	Male	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (1:1)	$\chi^2_{[1]}$ (3:1)
O. aur	*O. moss*				
008	128	86:52	62.3	8.38**	11.83***
Δ228	B36	39: 0	100.0	39.00***	13.00**
O. aur	*O. spil*				
008	127	119:67	64.0	14.54***	12.05***
O. aur	*O. nil*				
T21	208	18:25	41.9	1.14	25.19***
T21	179	15:18	45.4	0.27	15.36***
007	092	57:39	59.4	3.37	12.50***
D12	208	53:18	74.6	17.25***	0.00
299	208	45:13	77.6	17.65***	0.21
Δ297	g1	56:47	54.4	0.79	N/A
Δ261	208	43:14	75.4	14.75***	N/A
Δ228	208	19: 0	100.0	19.00***	N/A
Δ198	283[a]	41: 0	100.0	41.00***	N/A
311	283[a]	45: 0	100.0	45.00***	N/A
O. moss	*O. aur*				
130	005	111:14	88.8	75.27***	N/A
O. spil	*O. aur*				
126	006	126:14	90.0	89.60***	N/A
O. nil	*O. aur*				
T17	313	10:19	34.5	2.79	N/A
199	196	27:37	42.2	1.56	N/A
A2	211	46:51	47.4	0.26	N/A
A1	211	23:16	59.0	1.26	N/A
T17	181	23:13	63.9	2.78	N/A
T17	T16	39: 0	100.0	39.00***	N/A

[a] YY supermale produced by gynogenesis on Δ♀ (see Mair et al. 1991)
N/A Not applicable
** $P < 0.01$
*** $P < 0.001$

Table 3 shows the sex ratios resulting from crosses of *O. aureus* with *O. mossambicus*, *O. spilurus* and *O. niloticus*. In reciprocal crosses with *O. aureus*, *O. mossambicus* and *O. spilurus* gave similar sex ratios, suggesting that these two species have similar sex-determining mechanisms. In crosses with male *O. aureus*, the ratios fell short of the 1:0 ratio predicted if *O. mossambicus* and *O. spilurus* exhibit female homogamety (XX) and *O. aureus* male homogamety (ZZ).

There was a large degree of variability in sex ratios of hybrid crosses with *O. niloticus*. Even tests utilising Δ♀ *O.*

Table 4. Sex ratios from progeny testing of 17α-methyltestosterone (80 mg.kg^{-1} feed) treated male *O. aureus*

Female	Male	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (Control)	$\chi^2_{[1]}$ (1:1)	$\chi^2_{[1]}$ (1:3)
009 (WZ)	005 (ZZ) (control)	22:28	44.0	–	0.72	9.63**
009 (WZ)	1 (WZ)	17:37	31.5	1.74	7.41**	1.21
009 (WZ)	2 (WZ)	12:31	27.9	2.58	8.39**	0.19
009 (WZ)	3 (ZZ)	26:26	50.0	0.37	0.00	17.33***
009 (WZ)	4 (ZZ)	18:28	39.1	0.23	2.17	4.90*
Δ297 (ZZ)	211 (ZZ) (control)	40:16	71.4	–	10.29**	
Δ297 (ZZ)	1 (WZ)	46:50	47.9	7.96**	0.17	

Hypothetical genotypes given in parentheses
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Table 5. Results from repeat spawnings of estrogen-treated female *O. aureus*

Treated female	Male	Sex ratio ♂ :♀	% Male	χ^2 (Contingency)[b]
1 EE100+ME	211	9:15	37.5	
1 EE100+ME	201	7: 6	53.8	$0.93_{[1.0]}$
2 βE200	281	58:39	59.8	
2 βE200	211	40:16	71.4	$2.10_{[1.0]}$
3 βE100	171	56:14	80.0	
3 βE100	156	40: 3	93.0	$3.65_{[1.0]}$
4 βE200	211	14: 7	66.7	
4 βE200	146	26: 1[a]	96.3	$8.03_{[1.1]}$**
5 EE100+ME	181	20: 7	74.1	
5 EE100+ME	145	88: 1	98.9	$20.51_{[1.0]}$***
6 βE200	171	22:12	64.7	
6 βE200	201	45: 0	100.0	$19.68_{[1.1]}$***
7 EE100+ME	171	82: 4	95.3	
7 EE100+ME	181	15: 0	100.0	
7 EE100+ME	196	27: 0	100.0	$1.86_{[1.9]}$
8 EE100+ME	196	21: 1	95.4	
8 EE100+ME	171	26: 1[a]	96.3	
8 EE100+ME	172	79: 1	98.7	
8 EE100+ME	182	43: 0	100.0	
8 EE100+ME	4	43: 0	100.0	$14.92_{[10.7]}$
9 EE100+ME	211	48:10	82.8	
9 EE100+ME	146	29: 0	100.0	$5.99_{[1.1]}$*
10 βE200	166	50: 0	100.0	
10 βE200	175	52: 0	100.0	$0.00_{[1.0]}$
11 βE200	171	6: 0	100.0	
11 βE200	201	54: 0	100.0	$0.00_{[1.0]}$
12 EE100+ME	196	7: 0	100.0	
12 EE100+ME	181	12: 0	100.0	$0.00_{[1.0]}$

[a] Single intersex classed as female
[b] Chi-squared values calculated according to Nass (1959) due to some expected values <5
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

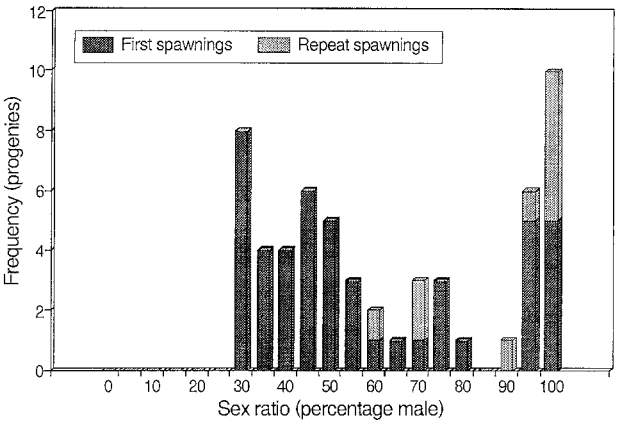

Fig. 2. Sex ratio frequency distribution of progeny from single-pair matings of 55 estrogen-treated female *O. aureus* (sample size ≥25)

aureus gave variable results (54–100%), but YY supermale *O. niloticus* did give all-male progeny when crossed with normal or Δ♀ *O. aureus*. Crosses of female *O. niloticus* with male *O. aureus* commonly yield 95–100% male progeny (Pruginin et al. 1975; Hulata et al. 1983). However, in this study results from six crosses were highly variable (37–100% male).

Results from progeny testing of testosterone-treated male *O. aureus* are shown in Table 4. Four fish were tested in crosses with a single female, and the resulting sex ratios of progenies compared with expectations derived from the control (untreated male), 1:1 and 1:3 ratios, respectively. None of the progenies had sex ratios significantly different from the control, but two of these males (nos. 1 and 2) gave ratios significantly different from 1:1 ($P<0.01$) but not from 1:3, suggesting they were indeed sex reversed (Δ♂♂). One of the treated males (no 1) was crossed to a Δ♀ and gave a ratio significantly different from the control but not from 1:1, confirming it as a Δ♂. In this latter progeny test, the control did not produce the expected all-male sex ratio; this phenomenon was observed in other Δ♀♀ and will be discussed later. These results are nevertheless in accordance with the theory of female heterogamety in this species and agree with those of Guerrero (1975).

Figure 2 shows the frequency distribution of sex ratios (sample size ≥25; mean sample size=49.6) derived from progeny testing of 55 estrogen-treated females. Mair et al. (1987a) gave details of hormone treatments applied and some preliminary results from progeny testing of treated females. Four peaks can be observed at approximately 30%, 45–50%, 70–75% and 95–100% male progeny. It is assumed that the first two peaks relate to those in Fig. 1 and that the treated fish producing these ratios are genetic females. The progenies with sex ratio 90–100% male are likely to be derived from Δ♀♀ having undergone functional sex reversal. Table 5 shows that, in the case of repeat tests of putative Δ♀♀, a number

Table 6. Sex ratios of progeny from delta female *O. aureus*, receiving hormone treatments (EE100+ME100). Chi-squared values are based on contingency tests with control ratios

Female male (Code)	Treatment	Duration (days)	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (control)
Δ198×172	Control	46	40: 1	97.6	
(A)	EE100[a]	46	21:19	52.5	22.11***
Δ198×182	Control	77	43: 0	100.0	
(B)	EE100[a]	77	42: 4	91.3	3.91*
Δ228×196	Control	44	27: 0	100.0	
(C)	EE100[a]	44	2:26	7.1	47.55***
Δ197×171	Control	70	16: 6	72.7	
(D)	EE100[a]	70	4:57[b]	6.6	38.71***

[a] All treatments include methallibure at 100 mg.kg^{-1}
[b] Three intersexes classed as females
* $P<0.05$
*** $P<0.001$

Table 7. Sex ratios from progeny testing of second generation hormone – treated female *O. aureus* (letters in parentheses refer to treatment group – see Table 6)

Female	Male	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (1:1)
1 (A)	016	8: 0	100.0	8.00*
2 (C)	034	78: 0	100.0	78.00***
3 (D)	181	42:15	73.7	12.79***
4 (D)	181	16:15	51.6	0.03

* $P<0.05$
*** $P<0.001$

Table 8. Sex ratios from progeny testing of "rare" females arising from a cross Δ♀197×♂171

Female	Male	Sex ratio ♂ :♀	% Male	$\chi^2_{[1]}$ (1:1)
336	016	11:0	100.0	11.00***
	181	5:7	41.7	0.33
339	016	141:0	100.0	141.00***
340	016	28:0	100.0	28.00***
	313	6:0	100.0	6.00*

* $P<0.05$
*** $P<0.001$

of different sex ratios were produced when a female was crossed with different males. Treated fish 3, 4, 5, 6 and 9, which gave intermediate ratios (64.7–82.8% male) when crossed with one male, gave 100% male progeny (or close to) when crossed with another male. It seems therefore that females producing 3:1 ratios are likely to be sex reversed. It may be noted that 3:1 ratios were produced only when males 171, 181, 211 and 281 were used in progeny tests, suggesting a genetic basis for these unusual ratios. A model to account for genetic difference between males is given in the discussion.

Hormone treatments (EE100+ME100) applied to putative monosex male progeny (Table 6) produced a significant increase ($P<0.05$) in the proportion of females, over those in the control, in all experiments; however, the results were variable. There was no evidence to suggest that increased duration of hormone administration (70,77 from 44,46 days) improved the efficacy of treatments.

Four second-generation Δ♀♀ were progeny tested with three different males (Table 7). Females derived from crosses A and C gave the expected all-male progeny but two females from cross D, tested with ♂181, gave 3:1 and 1:1 sex ratios not predicted by a monofactorial model of sex determination. Furthermore, the control ratio for cross D (Table 6) approximated to 3:1, suggesting that heritable factors are responsible for modifying sex ratios from those predicted by a simple theory. Further evidence for this was gained from progeny testing of 3 "rare" females arising from the cross Δ♀197×171, which produced a 16:6 sex ratio (Table 6). All three produced monosex male progeny in tests with ♂016 or ♂313, conforming to what would be expected of genetic males which have undergone some form of "natural sex reversal" (Table 8). Female 336, when crossed to 181, however, gave a 1:1 sex ratio, confirming the genetic differences between males noted above.

Sex ratios of triploid and gynogenetic fish are shown in Table 9. Under the theory of female heterogamety in this species, it is predicted that triploids will occur in a 1:1 ratio of WWZ and ZZZ genotypes. Given dominance of the W element it would be expected that triploids would have a 1:1 sex ratio. Similarly, gynogens would be expected to occur in a 1:1 ratio of male to female, assuming viability of the novel WW female genotype. If, however, there is crossing-over between the centromere and the sex-determining element during meiosis, recombinants will be female (WZ in meiogynes, WZZ in triploids). Sex ratios of triploids and meiogynes were not significantly different from each other, but both were significantly different from 1:1 ($P<0.001$) with a large excess of females. Although only three mitogynes were produced, the occurrence of a single male suggests that the ratio may approximate to 1:1, lending support to the hypothesis, first proposed by Penman et al. (1987), that significant numbers of female recombinants occur in triploid and meiogyne progeny. Meiogynes derived from Δ♀♀ were all male.

Progeny testing of a single male and a single female mitogyne produced the predicted results (Table 10). The male gynogen sired progeny with sex ratio 1:1, confirming its normal male genotype (ZZ). The female mitogyne gave only female progeny in tests with three males, confirming it as a WW "superfemale".

Table 9. Sex ratio of control, triploid and gynogenetic *O. aureus*

Female parent	Male parent	Control NoUV NoHS ♂ : ♀	% Male	Triploid NoUV HS_5 ♂:♀	% Male	Meiogyne UV HS_5 ♂ : ♀	% Male	Mitogyne UV LHS ♂:♀	% Male
007	005+006 (2)	12: 19	38.7	1:13	7.1	4: 64	5.9		
	151 (2)	4: 18	18.2			1: 40	2.4		
	196	20: 40	33.3			1: 5	16.7	1:1	50.0
009	006 (2)	15: 18	45.4			0: 6[a]	0.0		
010	005	13: 12	52.0	3:22	12.0				
	006	27: 30	47.4	2:53	3.6	1: 2	33.3		
170	169 (2)	31: 34	47.7			0: 2	0.0		
	196	6: 19	24.0			0: 6[b]	0.0	0:1	0.0
Total		128:190	40.2	6:88	6.4	7:125	5.3	1:2	33.3
Δ−4	146	26: 0[c]	100.0			18: 0	100.0		
Δ223	211	34: 17	66.7			21: 0	100.0		
		60: 17	77.9			39: 0	100.0		

Numbers in parentheses represent number of experiments pooled
[a] Three intersexes (possessing both ovarian and testicular tissue) were also observed
[b] UV HS_{10}
[c] One intersex observed

Table 10. Sex ratios in the progeny of two mitogynes

Mitogyne parent	Normal parent	Sex ratio ♂:♀	% Male	$\chi^2_{[1]}$ (1:1)
♂ 328	♀ 311	6:10	37.5	1.00
♀ 322	♂ 181	0:10	0.0	10.00***
	♂ 014	0:36	0.0	36.00***
	♂ 328[a]	0:30	0.0	30.00***

[a] Also a mitogyne
*** $P < 0.001$

Conclusions and discussion

The large between-family sex ratio variance and the specific paternal influence on progeny sex ratio can both be used as evidence for a polygenic mechanism of sex determination (Bull 1983). Analysis of sex ratios of the interspecific hybrids revealed a large degree of variation. In the case of crosses with *O. niloticus* this variation was greater than that observed by other authors. This suggests considerable complexity of interspecific sex determination and it is our conclusion that few valid inferences can be made regarding intraspecific sex determination from these hybrid sex ratio data.

Analysis of sex ratios produced by testosterone- and estrogen-treated fish provides evidence for an underlying monofactorial mechanism of sex determination in *O. aureus*. Sex-reversed males gave 1:3 ratios and sex-reversed females, in most cases, produced all (or nearly all) male progeny. The occurrence of small proportions (1.2–7%), and in some cases large proportions (18.3–40.2%), of

Table 11. Genotypes and predicted sex ratios (♂:♀) from crosses of normal and sex-reversed female *O. aureus*, incorporating an autosomal recessive allele "*f*" inducing femaleness

Female	Male	
	♂ZZFF	♂ZZFf
♀WZFF	ZZFF : WZFF	ZZFF : WZFF ZZFf : WZFf
	1:1	1:1
♀WZff	ZZFf : WZFf	ZZFf : WZFf WZff ZZff
	1:1	1:3
♀WZFf	ZZFF : WZFF ZZFf : WZFf	ZZFF : WZFF ZZFf : WZFf ZZFf : WZFf WZff ZZff
	1:1	3:5
Δ♀ZZFF	ZZFF	ZZFF ZZFf
	1:0	1:0
Δ♀ZZFf	ZZFF ZZFf	ZZFF : ZZff ZZFf ZZFf
	1:0	3:1
Δ♀ZZff	ZZFf	ZZFf : ZZff
	1:0	1:1

females in progeny from ♂♀♀ indicates the presence of modifying factors altering sex ratios from those predicted by a monofactorial model, further supporting the hypothesis of polygenic sex determination.

Closer analysis of these data, however, shows that many of these observed sex ratios, not predicted by a simple monofactorial model of sex determination, are nevertheless strongly suggestive of Mendelian ratios. The 1:3 and 3:5 ratios produced in control crosses and the 3:1 ratio in progeny testing of estrogen-treated females would confirm this view.

We hypothesize the existence of an autosomal recessive gene, epistatic to the major sex-determining factors W and Z, which, when homozygous for the recessive allele, causes the fish to develop as female. Such a gene can be assigned alleles "*F*" and "*f*". Table 11 shows the sex ratios that would result from independent segregation of these alleles in crosses between the different parental genotypes. A cross between two heterozygotes at this locus would then yield a 3:5 sex ratio. A high proportion of heterozygotes at the *F* locus in the Swansea subpopulation would explain the significant number of progenies with sex ratio 35–40% male (see Fig. 1). Similarly, a female homozygous for the recessive *f* allele, crossed to a male heterozygote, would produce a 1:3 sex ratio, which could explain the secondary peak at 25% male shown in Fig. 1.

Sex-reversed females (♂♀♀), heterozygous at this sex-modifying locus, would give 3:1 sex ratios in crosses with heterozygous males, providing an explanation for the peak at 70–80% male in Fig. 2.

It can be conjectured further that males 171, 181, 211 and 281 are heterozygous at this locus and so, correspondingly, are ♂♀♀ 2, 3, 4, 5, 6 and 9 (from Table 5). It was noted that the four males referred to above frequently produced 3:5 ratios in crosses with normal females (as noted in Table 2, plus data presented by Mair 1988). On this basis, cross D (Table 6) is between two heterozygotes and therefore the two fish that were progeny tested with ♂181 (Table 7, nos. 3 and 4) could be of three genotypes: ZZFF, ZZFf or "naturally sex reversed" ZZff. The 3:1 ratio produced by female 3 suggests that this individual is ZZFf, whilst the 1:1 ratio from female 4 suggests that this is homozygous recessive ZZff.

Progeny testing of "rare" females (those not receiving hormone treatment) from cross D (Tables 6 and 8) further supports this hypothesis. It is expected that these "naturally sex-reversed" fish would be ZZff and would give 1:1 sex ratios in crosses with males heterozygous at the sex-modifying locus. Such a ratio was produced when one of these females was crossed with ♂181. Males 016 and 313 are likely to be ZZFF.

Some of the data presented here could alternatively be explained by the presence of a third, codominant allele (female) at the major sex-determining locus. However, this hypothesis does not explain the 3:5 ratios observed in control crosses and the 1:0 ratios observed in the progeny testing of "rare" females. Similarly, some of the data could have been explained by a sex-linked (on the Z-bearing chromosome), sex-modifying gene, but again this fails to explain the 3:5 ratios observed in control crosses.

The presence of a second-sex-determining factor clearly complicates the analysis of triploid and gynogenetic sex ratios. In the case of gynogenesis applied to a female heterozygous at the *F* locus, it would be expected that 50% of the progeny would be homozygous for *f* (WWff or ZZff) in the absence of crossing-over between the centromere and this sex-modifying locus. A sex ratio of 1:3 would thus be predicted. In the unlikely event that all females used were heterozygous at the *F* locus (it is thought that none were homozygous recessive), the expected ratio would be 1:3, however, there is a significantly greater proportion of females in the gynogenetic progeny ($P<0.001$), suggesting that there is recombination between the major sex-determining locus and the centromere, producing an excess of recombinant female triploid and meiogyne progeny. This phenomenon has been reported in amphibians and is analogous to the recombination between centromere and sex-determining locus observed in *O. niloticus* (Mair et al. 1991). The putative sex-modifying gene makes it difficult to estimate the recombination frequency between the centromere and the major sex-determining locus; however, it is clear that the majority of meiogynes and triploids are female recombinants.

Progeny testing of a single female mitogyne proved the viability of WW "superfemales" previously unknown in fish, although there is a report of viable WW females in the amphibian *Xenopus laevis* (Colombelli et al. 1984), produced by gynogenesis.

To conclude, evidence from this comprehensive study of sex ratios of hormonally sex-reversed, hybrid, gynogenetic and triploid *O. aureus* suggests that this species has a multifactorial mechanism of sex determination. Most sex ratios can be explained by an underlying monofactorial mechanism of female heterogamety (WZ). Most aberrant sex ratios can be explained by the presence of an autosomal, recessive sex-modifying gene, epistatic to the major sex-determining factor. Homozygous recessive fish develop as female. Not all results can be explained by this two-factor model (e.g. the 90–98% male ratios produced by progeny testing of Δ♀♀) and some other factor or factors must operate to produce these aberrant ratios. These factors could be autosomal, although evidence of temperature-modulated sex determination in this species (Mair et al. 1990) suggests that these aberrations may be environmentally induced.

As a final observation, the identification of individuals carrying a second sex-determining gene would enable

selection for or against this gene. Selection for this sex-modifying gene in hormonally sex-reversed females (homogametic, ZZ) could produce a strain with sex-determining mechanisms opposite to the normal mechanism of female heterogamety and analogous to that in the closely related *O. niloticus*. Presence of opposing sex-determining mechanisms in such closely related congeneric species is perhaps a unique phenomenon in the animal kingdom. Given that *O. aureus* possesses the genetic variation for a sex-determining mechanism with male heterogamety/female homogamety, it is possible that a switch in sex-determining mechanism may have played a role in the development of reproductive barriers between, and ultimately evolutionary divergence of, *O. aureus* and *O. niloticus*.

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