Study of sex ratio in progeny of a complex *Oreochromis* hybrid,

the Florida red tilapia

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Abstract

This paper reports a study on the progeny sex-ratio distribution in the Florida red tilapia. This hybrid originated as a cross between *O. mossambicus* male and *O. urolepis hornorum* female, which was then crossed with *O. niloticus* and *O. aureus* in order to improve its growth rate and its resistance to low temperature. We have studied the sex-ratio of 111 progenies from 46 females and 12 males, where the male percentage varied between 5 to 89 %. The analysis of distribution showed three groups characterised by a mean male percentage of 26.7 ± 9.8; 50.0 ± 8.9 and 70.3 ± 5.5 %. The stability of sex ratios in repeated single pair matings and the parental influence on progeny sex ratios obtained are in agreement with a

polygenic basis of sex determination in this complex hybrid.

1. Introduction

The model of sex determination has been intensively studied in the genus *Oreochromis*, where both male homogamety (ZZ / WZ) and female homogamety (XX / XY) have been proposed, based on experimental sex-ratios from inter- and intra-specific crosses, involving normal or sex-reversed parents, and also from chromosome-set manipulations (triploidisation and gynogenesis) (Jalabert et al., 1971; Mair et al., 1991 a,b; Trombka and Avtalion, 1993; Müller-Belecke and Hörstgen-Schwark, 1995). Observation of significant deviations from predicted sex ratios suggests the influence of additional factor(s) (environmental or genetic) other than sex chromosomes on the sex determination in this genus (Avtalion and Hammerman, 1978; Majumdar and MacAndrew, 1983; Mair et al., 1991 a,b; Wohlfarth and Wedekind, 1991; Baroiller et D'Cotta, 2001; Desprez et al., 2003a). The main interest in the model of sex determination in tilapia is motivated by the practical and commercial implications in the production of monosex male populations for aquaculture. Monosex male populations, usually preferred to optimise the production system (males grow faster than females; decreased reproduction; reduction of sexual/territorial behaviour; achievement of higher average growth rate), are generally produced on a large scale by hormonal sex inversion or by genetic approaches (interspecific hybridisation ; YY super male or ZZ pseudofemale technology, respectively in *O. niloticus* and *O. aureus*) (Guerrero and Guerrero, 1988; Wohlfarth, 1994; Mair et al., 1997; Desprez et al., 2003 a).

Apart from manual sexing, sex reversal with androgen is the only process used to produce populations of high male percentage in Florida red tilapia (Desprez et al., 2003 b).

This hybrid, supposedly resulting from hybridisation of four species (*O. niloticus* and *O. mossambicus* with XX/XY model ; *O. hornorum urolepis* and *O. aureus* with ZZ/ZW model), is one of the most commercially important tilapia strains, notably in Latin America, Asia, Caribbean and in Reunion Island. Studies on interspecific crosses suggested that a complex model or a polygenic system of sex determination exists in hybrids (Pruginin et al., 1975; Avtalion and Hammerman, 1978; Majumdar and McAndrew, 1983). However, results on hybridisation and sex determination in hybrids were restricted to the F_1 to F_3 generations (Chen, 1969; Jalabert et al., 1971). The sex ratio distribution in a complex hybrid after many generations since the 1970s is little known. The purpose of this paper was therefore to investigate the progeny sex-ratio in Florida red tilapia. Investigations of the possible model of sex determination in this hybrid are a preliminary stage, before a possible genetic approach for the production of monosex male population in red tilapia hybrids.

2. Materials and methods

2.1. Origin of fish

This study was carried out at the Freshwater Aquaculture Centre (A.R.D.A.) in Reunion Island (Indian Ocean). The experimental fish were a commercial red tilapia strain known as Florida red tilapia. The Florida red tilapia originated from a cross between a red mutant male of *O. mossambicus* and a female of *O. urolepis hornorum* (Behrends et al., 1982). In order to improve its growth rate and its resistance to low temperature, this hybrid was crossed with *O. niloticus* and *O. aureus* (Behrends and Smitherman, 1984). Two commercial Florida red tilapia strains, imported from Martinique (1990) and Jamaica (1991), were mixed in 1991/1992 to give the Florida red tilapia strain, used at present in Reunion Island. Florida red tilapia males and females tested in present study were taken from the broodstock of the Freshwater Aquaculture Centre.

2.2. Progeny testing

Florida red tilapia males and females were maintained in a series of 400-l thermo-regulated aquaria (27 °C ± 1 °C) in a recirculating system (one male and 3-4 females per aquarium). All tested males and females were individually tagged with passive integrated transponder tags (PIT tag system / Destron Fearing Corporation, USA). The reproductive status was checked twice a day by detection of female mouthbrooding behaviour. Mouthbrooding females were isolated in their respective aquaria with a movable partition. Fry were removed from the mouth of female 9-10 days after spawning. The mean number of fry reared per progeny was 369 ± 278, ranging from 135 to 1694. Each progeny was identified by male and female tag numbers and was reared in a 100-l tank within a recirculating system. The minimum and maximum temperatures for these experiments were 26.8 and 31.2 °C, respectively. After a period of 60 to 90 days or on attaining an average weight of > 3 g, a sample of 100 to 200 fish from each progeny group was sexed by the aceto-carmine squash method (Guerrero and Shelton, 1974). At this stage of development, oocytes are easily identifiable in their auxocytosis or previtellogenesis stages and a typical lobular configuration is observed in the testes. The sex ratios of progenies were compared using a 2 x 2 contingency χ^2 test.

3. Results

Eighty four single pair-mating, involving 12 males and 46 females of broodstock, were produced in the present study: twenty-three of these crosses had two replicates and two had three replicates. Thus a total of 111 progenies were reared with a mean survival rate of 54.8, ranging from 21.8 to 96.4 % (Table 1).

Data from repeated crosses of single pair-mating that were found to be homogenous (χ^2 test), were pooled (Figure 1 and Table 2). There was a large variability in sex ratios of Florida red tilapia progeny groups. The male percentage ranged from 5.0 to 89.0 % with a mean of 51.3 %.

Figure 1 shows the distribution of sex ratio among progeny groups (sexed sample size ≥ 100). Analysis of this distribution into Gaussian components (Bhattacharya, 1967) showed three groups (Figure 1). A group was characterised by a mean male percentage ± standard deviation, the minimum and maximum limits and the percentage of single pair-mating included in the group. The main group (58 % of the single pair-matings) had a mean male percentage of 50.0 ± 8.9 %. The minimum and the maximum limits of this group were 41.1 and 58.9 % of male, respectively. A second group (22 % of single pair-matings) had a lower male percentage (26.7 ± 9.8 %) with a minimum and a maximum of 16.9 and 36.6 %, respectively. The last group had a mean male percentage of 70.3 ± 5.5 %, contained between 64.8 and 75.8 %. Three single pair-matings, producing respectively 5, 11 and 89 % male, were not included in the three groups given by the analysis of the distribution into Gaussian components (Bhattacharya, 1967) (Figure 1).

Progeny sex-ratios from repeated pair-mating (n = 25 pair-matings) were not significantly different in the successive crossings, except for four pair-matings presenting heterogeneous results (Table 2). The two crosses of male "71e" x female "e1b" produced sex ratios that were not significantly different from 1:1. In contrast, among the crosses between male "240" x "245", two sex ratios were not significantly different from 1:1 (χ^2 = 0.32 and 2.45 ; df = 1 ; P < 0.05) and the other one was not significantly different from 1:3 (χ^2 = 1.20 ; df = 1 ; P < 0.05). Similar results were observed with the pair-mating "07d" x "642" and "b47" x "a7c" (Table 2). The difference in survival rate in successive crossings of a pair-mating ranged from 0.7 % ("212" x "119" with a survival rate of 21.8 and 22.5 %,

respectively) to 51.9 % ("213" x "222" with 19.9 and 71.8 %). In the four pair-matings, presenting significantly heterogeneous sex-ratios, the variation of the survival rate in the successive crossings was higher in cross "71e" x "e1b" (25.9 and 69.6 %) than in the three others (48.5 to 68.5 % in cross "240" x "245" ; 74.1 and 88.4 % in cross "07d" x "642" ; 81.7 and 92.3 % in cross "B47" x "a7c").

Table 3 shows the sex ratio resulting of crosses of 3 males with 16 females. The sex ratios obtained from crosses of male "225" with seven different females were skewed towards female (72 to 95 % of females). On the other hand, crosses of male "213" with four females gave balanced sex ratios (44 to 51 % of males). In contrast, the crosses of male "212" with nine different females produced balanced sex ratios or skewed towards male sex ratios (Table 3). Crossing females "222" and "248", the percentage of males in progeny involving males "212" and "213" (48 to 56 %) was significantly higher than with male "225" (5 and 11 %) (χ^2 test ; $\chi^2 > 35.9$; $df = 1$; $P > 0.05$) (Table 3).

4. Discussion

The frequency distribution of sex ratios, with three peaks at 27, 50 and 70 % of males suggests a polygenic sex determination model in the hybrid Florida red tilapia. The stability of sex ratio in most repeated crosses and the observed parental influence on progeny sex ratios are in agreement with a polygenic basis of sex determination in this complex Florida red tilapia hybrid. The significant differences, observed between the sex ratio in the few repeated crosses ("240" x "245"; "07d" x "642"; "B47" x "a7c"; "71e" x "e1b") are unexplained. Differential survival of the sexes is not sufficient to explain these variations: the results in the present study show that the progeny sex ratios in the successive crossings from the repeated pair-matings is not influenced by the survival rate. The effect of rearing temperature on sex determination is well known in *Oreochromis niloticus* (Baroiller et al.,

1995). In the present study, it is unlikely that temperature was involved because the rearing temperature (26.8 to 31.2 °C) of Florida red tilapia progenies was below the minimum level (34 °C) for temperature influence determined by Baroiller et al. (1995) *in O. niloticus*. However, other environmental factors (pH, day length….) could influence the sex determination and could induce slight variations of sex ratio, explaining the differences in repeated crossings in Florida red tilapia.

To explain unpredicted results in *Oreochromis*, which are not consistent with a monofactorial sex chromosomes model, a more complex system was proposed by adding a further genetic sex determining / regulating locus, the autosomal recessive gene (F,f) to the major "sex chromosomes" (Wohlfarth and Wedekind, 1991; Mair et al., 1991 a,b; Trombka and Avtalion, 1993; Desprez et al., 2003a). Crossing-over and recombination of sex determining genes could also account for these unpredicted sex-ratios (Mair et al., 1991 a,b; Müller-Belecke and Hörstgen-Schwark, 1995). The complexity of sex determination model in the pure species of *Oreochromis* is encountered in its hybrids involving species with two opposing "sex chromosomes" models (XX/XY in *O. niloticus* and *O. mossambicus* and WZ/ZZ in *O. aureus* and *O. urolepis hornorum*). The four "sex chromosomes" (W, X, Y, Z) model also failed to explain all sex ratio results obtained in hybrids (Chen, 1969; Jalabert et al., 1971; Pruginin et al., 1975) and Avtalion and Hammerman (1978) proposed an autosomal theory, assuming that sex is determined by "sex chromosomes" and a pair of autosomes. Stating that Z and Y chromosomes seem to be identical, the autosomal theory would be based on three gonosomes (X, Y, W) and each genotype would have a complement of two gonosomes (XX, XY, XW, WY, WW or YY) and a pair of autosomes (AA, Aa or aa). This autosomal theory predicts eight different sex ratios (1:0 ; 3:1 ; 5:3 ; 1:1 ; 7:9 ; 3:5 ; 1:3 ; 0:1). Three of them (5:3 ; 7:9 ; 3:5), that were obtained by Chen (1969) and Jalabert et al. (1971), were not predicted by the four sex chromosomes model (W, X, Y, Z). Application of the

autosomal theory to the results obtained in Florida red tilapia showed noteworthy differences (Table 4). First, monosex male or female ratios have never been observed in Florida red tilapia whereas the expected frequencies of these sex-ratios should be respectively 17.5 and 10.0 % according to the autosomal theory. Second, the observed percentage of crosses giving the sex-ratios of 5:3 ; 7:9 and 3:5 is higher in Florida Red tilapia than expected according to the above theory. In contrast, the balanced sex-ratio (1:1) is theoretically expected more frequently than was actually observed in Florida red tilapia. Finally, the sex-ratios observed in Florida red tilapia were less scattered than those expected under the autosomal theory proposed by Avtalion and Hammerman (1978). Data from the Florida red tilapia suggest that sex ratios cannot be predicted by a simple monofactorial model and by the autosomal theory proposed by Avtalion and Hammerman (1978). The successive programmes of improvement of colour, growth rate and resistance to low temperature applied since the 1970s to the Florida red tilapia (Behrends and Smitherman, 1984), may have selected certain genotypes and thus contributed to the discrepancies between observed results and those predicted by the autosomal theory. The hypothesis of a differential mortality of certain genotypes, suggested by Jalabert et al. (1971) and Chen (1969), cannot be excluded. Moreover, as demonstrated by Shirak et al. (2002) in an inbred line of tilapia *O. aureus*, deleterious alleles could induce a sex selective mortality and could distort the sex-ratios. Not all sex determining factors originating from the four initial species are unlikely to have been conserved, this perhaps also explaining the difference with the autosomal theory. Globally, the results in Florida red tilapia, suggesting a polygenic sex determination model, show that a balanced sex ratio is maintained (51.3 % of males in all crosses – Table 1) even in a population derived from a complex hybrid of unknown origin.

Production of genetically monosex population is well developed in *O. aureus* and *O. niloticus* (Mair et al., 1997; Desprez et al., 2003a). These recent studies have demonstrated

that male genetic sex determining factors (sex chromosomes, autosomal factors) can be fixed in a line of breeders. Owing to the stability of sex ratio in most repeated crosses of single pair mating and the observation of skewed sex-ratio towards male in some progeny groups of Florida red tilapia, the selection of pairs with high percentages of male progeny may bring about a high proportion of male in their progenies. This process may be repeated in successive generations to progressively increase the male percentage. Of course, in a complex hybrid as Florida red tilapia, there should be much more potential for altering sex ratios through selective breeding than in *O. niloticus* and *O. aureus*. It is clear that developing this method to increase the male percentage in progenies of Florida red tilapia will be long and laborious, since their colour must be also conserved, but it may well be an alternative to using the hormonal sex inversion method to produce a population with high male percentage.

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Table 1. Summary of sex ratios from single-pair mating of Florida red tilapia

	All crosses
Male broodstock	12
Female broodstock	46
Progeny groups	111
Fry sexed	20,577
Fry survival rate (%)	54.8
Minimum % male	5
Maximum % male	89
Mean % male	51.3

Table 2. Progeny sex-ratios (:) from repeated pair mating (two or three replicates) in Florida red tilapia

Cross (x)	1 % male	2 % male	3 % male	Cross (x)	1 % male	2 % male
240 x 245*	32	46	39	212 x 119	59	61
023 x 87d	38	32	26	239 x 119	40	53
023 x 41c	35	48		225 x 201	19	18
07d x 512	68	61		240 x 205	47	41
068 x 614	54	60		240 x 220	53	51
B47 x 642	61	56		212 x 222	50	61
07d x 642*	48	33		213 x 222	55	45
068 x 818	47	50		221 x 233	62	66
023 x a7c	43	38		212 x 248	60	48
B47 x a7c*	68	48		213 x 248	46	50
64f x b2c	53	59		221 x 266	52	64
71e x e1b*	46	61		239 x 860	63	65
07d x f1f	53	53				

* = Sex-ratio significantly different (χ^2 test ; df = 1 ; $P < 0.05$) from one or other results in successive crosses

Table 3. Sex-ratios (:) from crosses of three males and sixteen females in Florida red tilapia

Female	Male (no.)		
(no.)	212	213[a]	225
119	60:40		
201			16:84
209		44:56	
211	72:28		
220			28:72
222*	56:44	50:50	11:89
227			18:82
232	46:54		
234	61:39		
236			16:84
241		51:49	
242			24:76
243	71:29		
248*	54:46	48:52	5:95
859	68:32		
860	63:37		

* = Sex-ratio significantly different (χ^2 test ; $\chi^2 > 35.90$; $df = 1$; $P < 0.05$) from result in cross of male "225"

a = Sex-ratio not significantly different (χ^2 test ; $\chi^2 < 2.00$; $df = 1$; $P < 0.05$) from one or other results in successive crosses

Table 4. Number and percentage of crosses giving each sex-ratio (:) in theory of Avtalion and Hammerman (1978) and observed in Florida red tilapia (present study).

Sex-ratio (:)	1:0	3:1	5:3	1:1	7:9	3:5	1:3	0:1
% males	100	75	62.5	50	43.8	37.5	25	0

Avtalion and Hammerman's theory

No. of crosses giving the sex-ratio	14	11	2	33	1	4	7	8
% of crosses giving the sex-ratio	17.5	13.75	2.5	41.25	1.25	5	8.75	10

Results in Florida red tilapia

No. of crosses giving the sex-ratio	0	11	27	32	12	14	11	0
% of crosses giving the sex-ratio	0	10.3	25.2	29.9	11.2	13.1	10.3	0

Figure 1. Sex-ratio distribution of progeny groups from single-pair mating of Florida red tilapia as determined using the Gaussian distribution analysis of Bhattachariya (1967).