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PRODUCTION OF ANDROGENETIC NILE TILAPIA, *OREOCHROMIS NILOTICUS* L.: OPTIMIZATION OF HEAT SHOCK DURATION AND APPLICATION TIME TO INDUCE DIPLOIDY

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Abstract

Diploid androgenetic Nile tilapia, *Oreochromis niloticus,* were produced using 5 min UV irradiation at 150 µW/cm and heat shock at 42.5ºC for 3:30 min applied 25 min after fertilization. The survival rates to morula, pigmentation, hatching and yolk sac resorption stages were 42.84±4.73%, 10.69±2.24%, 2.03±0.60% and 0.07±0.07% (relative to controls), respectively. A significant female, but not male, effect on the survival was confirmed. Multilocus DNA fingerprinting produced by hypervariable 33.15 DNA probe verified the all-paternal inheritance in the androgens. The sex ratio of the androgenetic tilapia was not significantly different from the expected ratio of 1:0 (male:female), indicating that *O. niloticus* has a monofactorial sex-determining mechanism with female homogamety and male heterogamety. This study confirmed that the YY genotype is viable and fertile in *O. niloticus*.

Introduction

To induce androgenesis, the female genome is inactivated by gamma rays, X-rays or UV irradiation. The male genome is duplicated by suppressing the first mitotic division of the egg by means of physical shocks such as temperature and pressure or by fertilization of inactivated eggs with diploid spermatozoa from tetraploid males, if the latter can be produced. The first successful production of androgenetic diploids was reported by Gillespie and Armstrong (1981) in the Mexican axolotl, *Ambystoma mexicanum*. Diploid androgenesis has been induced in rainbow trout (*Oncorhynchus mykiss;* Thorgaard *et al.*,

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1990; Scheerer, *et al.*, 1991; Ueda, 1996), brook trout (*Salvelinus fontinalis;* May *et al.*, 1988), amago salmon (*Oncorhynchus masou;* Nagoya *et al.*, 1996), loach (*Misgurnus anguillicaudatus;* Masaoka *et al.*, 1995), carp (*Cyprinus carpio;* Bongers *et al.*,1994; Rothbard *et al.*, 1999), zebra fish (*Danio rerio;* Corley-Smith *et al.*, 1996), Siberian sturgeon (*Acipenser baeri;* Grunina and Neyfakh, 1991), catfish (*Clarias gariepinus;* Bongers *et al.*, 1995) and tilapia species (Myers *et al.*, 1995; Marengoni and Onoue, 1998).

An important use of androgenesis has been to analyze sex-determining mechanisms in fish. Androgenesis should lead to the production of all-male populations in species having male homogamety or populations of 50% YY males and 50% XX females in species having male heterogamety. In the case of *O. niloticus*, the genotype of inbred male androgens would be YY and they can be used to produce all-male progeny in subsequent crosses with ordinary XX females (Myers *et al.*, 1995).

One powerful application of androgenesis is to recover genotypes from cryopreserved sperm, particularly for those which are facing extinction or the threat of contamination by hybridization. Sperm cryopreservation, unlike that of eggs, is becoming relatively routine in some species and its combination with androgenesis offers a invaluable way to conserve genetic resources The other use of androgenesis is rapid production of inbred lines. Production of androgenetic clones has been reported in *Oncorhynchus rhodurus* (Nagoya *et al.*, 1996) and *Oreochromis niloticus* (Sarder, 1998).

When androgenetic diploids are produced, it is important to prove that the eggs did not contribute genetically to the embryo. Proof can be obtained by several methods. Nowadays, DNA markers provide very accurate assessment of parentage. Multilocus DNA fingerprinting is a powerful technique for genetic studies. In aquaculture, DNA fingerprinting can be used for the identification of individuals, construction of pedigrees, population analysis (Hallerman and Beckman, 1988), estimation of inbreeding rates in commercial broodstocks (Eknath and Doyle, 1990) and family identification without using tags, especially for small fish. Another proposed use of DNA fingerprints is to monitor the absence of paternal or maternal genomic contribution in fish (Chourrout, 1986). DNA fingerprinting has been used to verify successful gynogenesis and androgenesis in fish (Takagi *et al.*, 1995; Eenennaam *et al.*, 1996; Nagoya *et al.*, 1996).

In this report we describe the optimal heat shock duration and its time of application to suppress the first cleavage in androgenetic *O. niloticus*, and the production of homozygous red and blond YY males by androgenesis, thus fixing both traits in a single line of *O. niloticus*. Multilocus DNA fingerprinting and recessive color marker were used to confirm paternal inheritance. The sex determination mechanism of *O. niloticus* was also further investigated.

Materials and Methods

Origin and maintenance of fish stock. The red, blond and wild type *O. niloticus* broodstock used in this study descended from an electrophoretically tested pure stock of the Tilapia Reference Collection, maintained at the Institute of Aquaculture at the University of Stirling (McAndrew and Majumdar, 1983). McAndrew *et al.* (1988) reported that the red mutation in the Stirling red *O. niloticus* is an autosomal dominant with two alleles (RR) over the wild type (rr). The blond color pattern in Nile tilapia was first reported by Scott *et al.* (1987). The recessive blond skin pigmentation can be used as a visual marker to indicate the successful production of haploid androgenetic fish.

Fish were reared in recirculating freshwater systems. Lighting in the systems was adjusted by an automatic timer to 12 hours light and 12 hours dark. The water temperature was maintained at 28±1ºC. Individual female broodstock were kept in partitioned glass tanks of 120 x 44 x 30 cm, aerated by airstones coupled to a low-pressure blower unit. Fish were fed commercial trout feed (Trouw Aquaculture Nutrition, Russhive, UK) three times a day *ad libitum*.

Androgenesis. Androgenesis was carried

out according to Myers *et al.* (1995). Eggs were collected in a clean Petri dish by gently stripping ovulated females. Four ml of eggs were measured into a graduated vial, then washed gently with water. For each spawn, individual treatment groups contained 100-300 eggs. The water was poured off and new water was added to bring the total volume of eggs and water to 14 ml. The eggs and water were poured into a 75 mm diameter Petri dish which was then placed on a mechanical egg whirler under a 254 nm UV lamp (Ultra-Violet Products, San Gabriel, USA) and DNA in the egg nucleus was inactivated by UV irradiation at a dose of 150 µW/cm for 5 min. The eggs were then fertilized with normal sperm,

Diploidy. To diploidize the haploid androgenetic zygotes, the first mitotic division was suppressed using heat shock. The incubation temperature was maintained at 28±1ºC throughout the experiment. Eggs were heat-shocked 23, 24, 25, 26 or 27 min after fertilization for 3:30, 3:45 or 4 min, by pouring the eggs into plastic tea strainers immersed in a temperature-controlled water bath at 42.5ºC. Following heat shock, the eggs were returned to the recirculating incubation system.

Controls. A batch of eggs was neither irradiated nor shocked and served as the diploid control. A portion of the eggs was irradiated but not subjected to heat shock and served as the UV-treated haploid control. The haploid controls were fertilized with sperm of the same recessive blond male in each experiment to be able to visualize the successful production of haploid androgenetic fish.

Experimental design. A total of 13 wild type female and 6 homozygous red males were used for the experiments. The same females and males were used several times but in different experiments. Blond males were also used to produce diploid androgenetic *O. niloticus* for preliminary experiments.

Because of the limited number of available eggs, not all treatment parameters could be tested on a single batch of UV-irradiated eggs. Myers *et al.* (1995) reported that the most effective heat shock for inducing diploid androgenetic tilapia was 3-4 min at 42.5ºC, 25-27.5 min after fertilization (a.f.). Therefore, most of the experiments were conducted in these ranges.

The groups were examined at four stages: morula (6-8 hours a.f.), pigmentation (45-50 hours a.f.), hatching (80-90 hours a.f.) and yolk sac resorption (9-11 days a.f.). Survival of the diploid control was calculated as: (number of embryos surviving at a given development stage/total number of eggs) x 100. Survival of the heat shocked groups was calculated relative to the diploid control: survival of the treatment group/survival of the corresponding control.

Progeny testing. The sex of the diploid androgenetic fish (>20-30 g) was determined by checking the urogenital papilla. Progeny testing of putative androgenetic YY males was carried out by crossing them with normal females (XX). The sex ratios of the resultant offspring were determined by the aceto-carmine staining method (Guerrero and Shelton, 1974).

DNA fingerprinting. Ten µl of fresh or thawed pelleted blood cells was added to a mixture of 435 µl of TEN buffer (100 mM Tris-HCl pH 8.0, 10 mM EDTA, 250 mM NaCl) and 10 µl of DNase-free RNase (10 mg/ml) in a 1.5 ml sterile microcentrifuge tube and mixed gently. Fifty µl of 10% (w/v) SDS solution was then added. After 30-60 min incubation at 37ºC, 10 µl of proteinase K (10 mg/ml) was added to each sample. The samples were incubated overnight at 37ºC in a water bath. After two phenol and two chloroform/isoamyl alcohol (24:1 v:v) extractions, DNA was precipitated with 0.6 volumes of isopropanol. The DNA precipitate was washed twice with 70% ethanol, dessicated and resuspended in 100 µl of TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). *Hinf I* (Stratagene) restriction enzyme was used to digest 5 µg of each DNA sample in a total volume of 100 µl. Following one phenol and one chloroform/isoamyl alcohol extraction, restricted DNA was precipitated with 2.5 volumes of cold 100% ethanol and 1/50 volume of 5 M NaCl. The pellets were washed with cold 70% ethanol, dried and resuspended in 6 µl of TE buffer. The restricted DNA was run on 0.7% agarose gel (200 ml) at 1.5 V/cm for 14-16 h, by which time all fragments less than 1.0 kb

had migrated out of the gel. Southern transfer of restricted DNA to Hybond-N membrane (Sartorius Ltd.) was carried out by means of alkaline vacuum blotting (Vacu Gene™, Pharmacia LKB). The membranes were wetted by 250 ml of 1 x SSC solution and placed into a hybridization canister with the DNA side inwards and prewarmed (50ºC) prehybridization buffer (990 ml/l of 0.5 M Na_2HPO_4, 10 ml/l of 10% SDS) was added for each membrane. The canister was replaced into the hybridizer and left for 20 min at 50ºC in a solution containing 18 ml of prehybridization buffer plus 2 ml of casein hammarsten (BDH) along with 5 µl of NICE™ probe 33.15 (Cellmark Diagnostics). The filter was rinsed twice for 10 min each at 50ºC with 50 ml of wash solution 1 (160 ml/l of 0.5 M Na_2HPO_4 pH 7.2, 10 ml/l of 10% SDS) prewarmed to 50ºC. Finally, the membrane was washed twice with 50 ml of wash solution 2 (13.8 g/l of maleic acid $C_4H_3O_4Na$, 8.7 g/l of NaCl pH 7.2) for 5 min at room temperature. The membrane was removed from the canister and placed with the DNA side up on a clean glass plate. Approximately 3-4 ml of Lumi Phos™ 350 (Cellmark Diagnostics) was sprayed evenly over the membrane by means of a spray gun (BDH). The sprayed membrane was then sandwiched between two 21 x 21 cm acetate sheets and placed in an X-ray cassette. A sheet of autoradiography film (Hyperfilm MP™, Amersham) was laid on the cassette in a dark room. The cassette was kept in an incubator at 30ºC for at least 6 h and then developed.

Statistical analyses. Since the egg quality of each spawn varied greatly within and between females, the survival of each treatment was calculated relative to the survival of the corresponding diploid control. When the survival rate of the control group was less than 30%, that particular batch of eggs was not included (Myers *et al.*, 1995). The data applying to the morula stage were arc-sine transformed for statistical analysis and normality was tested by Anderson-Darling Normality test and a test for homogeneity of variance applied (Sokal and Rohlf, 1987). Only the results of the morula stage were tested by one-way ANOVA since they were normally distributed. The data for the pigmentation, hatching and yolk sac resorption stages, which included many zero values, were transformed to square root and tested by the Kruskal-Wallis Test (Sokal and Rohlf, 1987; Gardiner, 1997). Results were presented as means and standard errors of means (±SE). Statistical analyses were performed by Minitab 9.2 software. The female effects on survival were tested by one-way ANOVA. The sex ratios were analyzed by a Chi-square test to see if they statistically differed from the expected 1:1 sex ratio or from the sex ratio in the normal control for that group of fish.

Results

Optimization of heat shock. Due to the limited number of eggs obtained from a single spawn, only 3:30 min heat shocks at 23, 24, 25, 26 and 27 min a.f. were conducted to test fertilization (survival to the morula stage; Table 1). There were no significant differences between groups ($p>0.05$). The highest mean fertilization rate (96.62±2.54%) was obtained in the haploid control while the lowest (84.14±9.41%) was at 24 min a.f. Maximum fertilization rates for all treatments ranged 99.64-100%.

At the pigmentation stage, survival in heat-shocked treatments ranged from zero to 58.27% (Table 2). The mean survivals in treatments where the heat shock was administered for 3:30 min at 25, 26 or 27 min a.f. were significantly ($p<0.05$) higher than those of the other heat-shocked treatments. Although high survival rates were obtained at the pigmentation stage, they decreased sharply by the hatching stage. At the hatching stage, survival remained significantly higher for the above treatments, with the highest survival being in the group shocked for 3:30 min 25 min a.f. By the yolk sac resorption stage, survival rates in these treatments had dropped. They did not differ from survival in the other heat-shocked treatments which, in all cases, was zero. The maximum survival obtained in any group at the yolk sac resorption stage (2.34%) was obtained in one group of the 25 min a.f./3:30 min heat shock treatment. In general, late heat shocks (at 25, 26,

Table 1. Mean (±SE) fertilization rates (relative to the diploid control and expressed as survival to morula stage) of diploid androgenetic Nile tilapia (n=4 for each treatment), produced from eggs exposed to UV irradiation for 5 min and a thermal shock of 42.5ºC for 3:30 min at various times after fertilization (a.f.).

Treatment		*Fertilization rate (%)*
Diploid control (neither irradiated nor shocked)*	Mean	94.22±1.82
	Minimum	89.89
	Maximum	98.29
Haploid control (UV-irradiated but not shocked)	Mean	96.62±2.54
	Minimum	90.54
	Maximum	100
23 min a.f.	Mean	88.28±6.70
	Minimum	76.04
	Maximum	100
24 min a.f.	Mean	84.14±9.41
	Minimum	63.02
	Maximum	100
25 min a.f.	Mean	92.84±4.73
	Minimum	79.63
	Maximum	100
26 min a.f.	Mean	90.73±5.40
	Minimum	78.48
	Maximum	100
27 min a.f.	Mean	85.58±5.36
	Minimum	73.75
	Maximum	99.64

* Rates are actual (not relative).

27 min a.f.) resulted in higher survival rates while earlier heat shocks (at 23 and 24 min a.f.) resulted in no survival.

There was a significant effect of the female parent on survival in all stages ($p<0.05$) but no significant effect of the male parent ($p>0.05$).

Verification of all-paternal inheritance. Multilocus DNA fingerprinting generated by using Jeffrey's 33.15 probe confirmed success in producing diploid androgenetic *O. niloticus*. Fig. 1 shows that all the *O. niloticus* have only paternal bands and no specifically maternal bands (a and b). Some paternal

Table 2. Mean (±SE) survival (relative to the diploid controls) of presumed androgenetic Nile tilapia to pigmentation, hatching and yolk sac stages where eggs were subjected to UV irradiation for 5 min and a thermal shock of 42.5ºC for various durations at various times after fertilization (a.f.).

Treatment	*Heat shock duration (min)*	*n*		*pigmentation stage*	*Survival to: hatching stage*	*yolk sac resorption stage*
Diploid control (neither irradiated nor shocked)*	-	32	Mean	55.47±2.65	46.93±4.53	42.02±4.53
			Minimum	31.45	24.71	24.47
			Maximum	89.97	78.93	60.47
Haploid control (UV-irradiated but not shocked)	-	32	Mean	24.58±3.52	1.67±0.58	0.00
			Minimum	0.00	0.00	0.00
			Maximum	80.35	10.76	0.00
23 min a.f.	3:30	4	Mean	0.39±0.25^{a}	0.00^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	1.04	0.00	0.00
24 min a.f.	3:30	4	Mean	0.69±0.51^{a}	0.00 ^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	2.17	0.00	0.00
25 min a.f.	3:30	32	Mean	10.69±2.24^{b}	2.03±0.60^{b}	0.07±0.07^{a}
			Minimum	0.00	0.00	0.00
			Maximum	50.63	11.71	2.34
25 min a.f.	3:45	13	Mean	2.53±1.48^{a}	0.50±0.50^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	18.96	6.56	0.00
25 min a.f.	4:00	9	Mean	0.80±0.43^{a}	0.10±0.10^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	3.82	0.89	0.00
26 min a.f.	3:30	14	Mean	11.14±4.54^{b}	1.17±0.73^{b}	0.03±0.03^{a}
			Minimum	0.00	0.00	0.00
			Maximum	58.27	10.36	0.35
27 min a.f.	3:30	28	Mean	7.91±1.90b^{b}	1.21±0.53^{b}	0.03±0.03^{a}
			Minimum	0.00	0.00	0.00
			Maximum	32.88	11.86	0.72
27 min a.f.	3:45	9	Mean	0.81±0.50^{a}	0.00 ^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	4.17	0.00	0.00
27 min a.f.	4:00	9	Mean	1.40±0.84^{a}	0.18±0.12^{a}	0.00 ^{a}
			Minimum	0.00	0.00	0.00
			Maximum	7.77	2.34	0.00

Common superscripts in a column indicate means which are not significantly different (p>0.05).
* Rates are actual (not relative).

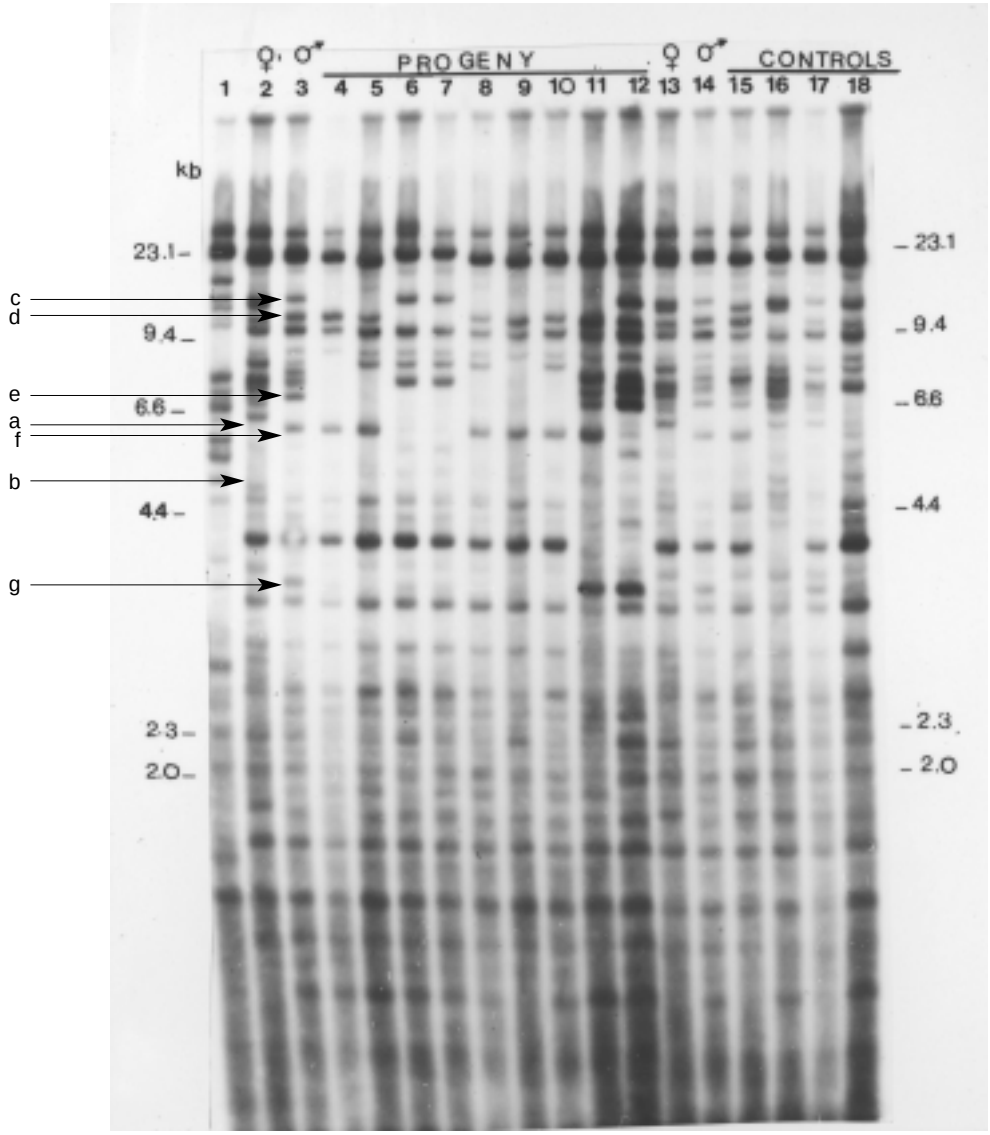

Fig. 1. DNA fingerprinting of tilapia. Lane 1 - unrelated control, lanes 2 and 13 - female parents, lanes 3 and 14 - male parents, lanes 4-12 - androgenetic offspring, lanes 15-18 - controls. For information on bands labeled a-g see Results.

bands are present in some progeny but not in the others (band c in progeny 6, 7 and 12; d in progeny 4, 5, 8, 9, 10, 11 and 12; e in progeny 11 and 12; f in progeny 4, 5, 6, 8, 9, 10, 11 and 12 and g in progeny 11 and 12). The segregation of these paternal bands presumably results from the heterozygosity of the male parent.

Sex ratios in progeny of the androgenetic males. A total of 28 blond and six red andro-

genetic *O. niloticus* were produced. Assessed at four months, the sex ratio was 15 male:19 female, not significantly different from the expected ratio of 1:1 ($p>0.05$). Table 3 shows the results of progeny testing of 14 of the males (11 blond and three red androgenetic males) crossed with normal wild type females (XX). With the exception of one male (013 620 833) which produced 84% male progeny, all the males produced 100% male progeny, indicating that they had the YY genotype. The sex ratios of all the progeny significantly differed from 1:1 while their respective controls did not significantly differ from the expected 1:1 ratio ($p>0.05$).

Discussion

The results demonstrate that androgenetic diploids can be produced in *O. niloticus*, although the percentage was extremely low. Such poor viability was not surprising as it was similar to that of mitotic gynogenetic diploids and tetraploid fish obtained by suppressing the first cleavage by physical shock.

The reason for low survival can be attributed to several factors. First, radiation can induce structural chromosome aberrations which may be transmitted from cell to cell during subsequent cleavage (George *et al.*, 1991; Lin and Dabrowski, 1998). Bongers *et al.* (1995) suggested that UV irradiation of eggs may damage the maternal RNA which is essential for development to the blastula stage, and thereby affect the differentiation process in embryonic development by altering cell fates and lineages. On the other hand, Myers *et al.* (1995) reported that egg mtDNA in *O. niloticus*, analyzed with ultraviolet endonuclease, was not affected by doses of UV irradiation high enough to denucleate the eggs and suggested that UV irradiation had relatively little impact on the eggs beyond nuclear inactivation. Higher survival rates were reported for *Oncorhynchus mykiss* androgenetic diploids produced using spermatozoa of artificial tetraploids (Thorgaard *et al.*, 1990). Thorgaard *et al.* suggested that radiation treatments in androgenesis are not an overwhelming obstacle to good survival. Parsons and Thorgaard (1984) found that androgenetic haploid and gynogenetic haploid *O. mykiss* had similar survival rates, also suggesting that egg irradiation alone may not be responsible for excessive mortality. In the present study, high survival to pigmentation stage in some groups of the haploid control (80.35% after 5 min UV irradiation) was obtained, in agreement with the results of Myers *et al.* (1995) and Thorgaard *et al.* (1990), suggesting that UV irradiation is not the only cause of reduced viability in androgenetic tilapia.

Secondly, low survival might be the result of deleterious effects of the physical shock used to inhibit the first mitotic division (Thorgaard *et al.*, 1990; Masaoka *et al.*, 1995). Chromosomal changes such as terminal deletion, exchange type aberration, inter or intra-arm exchange or interchromosome exchanges through rapid cell cycles caused by hydrostatic pressure treatment at the first cleavage were reported in gynogenetic *O. masou* (Yamazaki and Goodier, 1993) and in gynogenetic *Misgurnus anguillicaudatus* (Masaoka *et al.*, 1995). These changes are similar to those induced by irradiation (Yamazaki and Goodier, 1993), aging or interspecific hybridization (Yamazaki *et al.*, 1989). In the present study, survival rates sharply decreased from the pigmentation stage to the yolk sac stage under optimal conditions (heat shock of 42.5ºC for 3:30 at 25 min a.f.), suggesting that low viability may be a result of heat shock affecting zygote and blastula development rather than karyokinesis. Deleterious effects of heat shock may be more harmful at later development stages.

Thirdly, inbreeding depression arising from homozygosity of deleterious alleles was suggested for the low viability of androgenetic progeny (Bongers *et al.*, 1994) and for mitotic gynogenetic progeny (Yamazaki and Goodier, 1993). Additionally, based on the hypotheses of Markert (1982), homozygosity of androgenetic progeny may cause inviability through the disruption of topographic interaction of chromosomes of the interphase nucleus. However, studies of gynogenesis and androgenesis with inbred parents produce conflicting results. Komen *et al.* (1992a) reported that

Table 3. Sex ratios of progeny of red (RR) and blond (bb) androgenetic homozygous male *O. niloticus* and their respective controls. Androgenetic males (YY) were denoted as those producing progeny with a sex ratio that significantly differed from 1:1. The same letter in the control columns indicates the same control (total 5 control crosses).

Androgenetic male (tag no.)	*Progeny from androgenetic male crossed with normal female*				*Progeny in respective control*				*Proposed genotype of androgenetic parent*
	Male (no.)	*Female (no.)*	*Male (%)*	χ^2	*Male (no.)*	*Female (no.)*	*Male (%)*	χ^2	
010 317 057	20	0	100	20.0***	10	15	40.0	1.00	YY(bb)
013 354 595	20	0	100	20.0***	17(a)	16(a)	51.5(a)	0.03(a)	YY(bb)
014 524 616	18	0	100	18.0***	a	a	a	a	YY(bb)
013 556 535	24	0	100	24.0***	a	a	a	a	YY(bb)
010 554 342	26	0	100	26.0***	a	a	a	a	YY(bb)
014 383 330	14	0	100	14.0***	17(b)	21(b)	44.7(b)	0.42(b)	YY(bb)
010 893 049	22	0	100	22.0***	b	b	b	b	YY(bb)
000 360 821	14	0	100	14.0***	26(c)	24(c)	52.0(c)	0.08(c)	YY(bb)
012 570 379	16	0	100	16.0***	c	c	c	c	YY(bb)
015 286 567	16	0	100	16.0***	c	c	c	c	YY(bb)
013 378 126	24	0	100	24.0***	c	c	c	c	YY(bb)
013 630 048	30	0	100	30.0***	c	c	c	c	YY(RR)
013 620 833	42	8	84	23.1***	c	c	c	c	YY(RR)
013 296 291	21	0	100	21.0***	18	10	64.3	2.29	YY(RR)

*** significantly different from the respective control ($p<0.001$)

homozygotic male parents yielded significantly more normal and fewer deformed gynogenetic *Cyprinus carpio* fry than heterozygous males. Higher survival of androgenetic *C. carpio* was reported using homozygous males (Bongers *et al.*, 1994). On the other hand, Scheerer *et al.* (1991) could not improve the survival of androgenetic *O. mykiss* by using an inbred sperm source.

Finally, specific female effects on the survival rate of androgenetic and mitotic gynogenetic fish were reported by several authors (Bongers *et al.* 1994, 1995; Myers *et al.*, 1995). Quillet *et al.* (1991) argued that genetic factors specific to females could contribute to the viability of their homozygous progenies and thus interfere with the assessment of the efficiency of a treatment as far as survival is concerned. In the present study, a significant female, but not male, effect was found on the survival of the androgens, suggesting that great differences exist between egg batches of different females in their susceptibility to the UV and diploidization treatments. A similar observation was reported by Myers *et al.* (1995) who claimed that suitable broodstock must be chosen to produce "host" eggs under a specific UV exposure protocol.

The absolute time of cytokinesis-1 (or the first mitotic interval) and the timing at which gametes are exposed to late shocks are vital in chromosome-set manipulations. Rubinshtein *et al.* (1997) stated that the late shock (τ_s) can be initiated 50-80% into the cytokinesis-1 (T) duration, which can be predicted according to the embryological age (τ_0) and minimal temperature of reproduction (τ).

Multilocus DNA fingerprinting was successfully used to verify the all-paternal inheritance of the androgens. Jeffreys *et al.* (1985) have shown that the hypervariable 33.15 DNA probe can be used to produce individual-specific DNA fingerprints of tilapia.

The results of our progeny testing supported the viability of YY males in blond and red *O. niloticus*. The percent of male offspring of one of the androgenic males was only 84%. The occurrence of 16% females in this group could be explained by other genetic sex-modifying factors or/and environmental effects on sex determination. Although female homogamety and male heterogamety were proposed by using a gynogenetic technique in *O. niloticus* (Penman *et al.*, 1987), Mair *et al.* (1991) proposed a monofactorial system with rare autosomal recessive genes epistatic to the major sex determining gene. The presence of a rare secondary sex-determining loci (SDL-2) in *O. niloticus* has been suggested by Hussain *et al.* (1994) and Karayucel (1999). A secondary sex-determining gene was described in mitotic gynogenetic *C. carpio* (Komen *et al.*, 1992b). The present study showed that the sex ratios of androgenetic *O. niloticus* did not differ from the expected sex ratio of 50% female and 50% male ($p>0.05$), strongly supporting a homogametic female and heterogametic male system for *O. niloticus* (Penman *et al.*, 1987; Mair *et al.*, 1991).

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