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EVALUATION OF AUTOSOMAL AND CHROMOSOMAL INFLUENCE ON TILAPIA SEX DETERMINATION

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TERMINAL REPORT

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INTRODUCTION

The study of genetic mechanisms of sex determination in tilapias is of both practical and theoretical interest because of the possibility to produce of all-male and all-female broods for culture and for hatchery purposes.

The first all male broods were produced by crossing Oreochromis mossambicus with O. hornorum male while reciprocal crosses yielded 1:3 sex ratios (Hickling, 1960). Hickling explained his results by adapting the chromosomal sex-determining mechanism of the platyfish Xiphophorus maculatus (Gordon, 1947) where a dual system of sex-determining chromosomes exist in different species. The model suggested for mouthbrooding tilapias involved 4 sex chromosomes X,Y,W,Z exhibiting both male and female heterogamety in different species.

Two further investigations were carried out by Chen (1969) and Jalabert et al. (1971). In both of them some crosses resulted in predicted sex ratios, whereas other had unexpected results. Hence, this simple dual system of sex determination in tilapias could not explain all the results obtained from crosses in subsequent studies, particularly the non-Mendelian segregation for sex, and variation in the sex ratio of individual pair crosses, such as between O. niloticus and O. aureus (Pruginin et al,1975).

Autosomal influence on the sex determination process has been postulated by a number of authors in different fishes, e.g. in Xiphophorus maculatus (Anders and Anders, 1963; Kosswig, 1964; Kallman, 1968), Lebistes reticulatus (Winge, 1934) and Oryzias latipes (Yamamoto, 1969). The possible involvement of autosomal genes in tilapias was suggested by Jalabert et al. (1971), and a model with three sex chromosomes and autosomal gene influence was proposed by Avtalion and Hammerman (1978) and Hammerman and Avtalion (1979). This model was proposed to explain sex ratios among hybrids of different species of Oreochromis (Tilapia). According to this model, each species is homozygous for a certain allele at the autosomal locus, but different alleles have become fixed in different species. Those species with male heterogamety were assigned the genotype XXAA oo; XYAA oo and the species with female heterogamety, the genotype WYaa oo and YYaa oo. Interspecific crosses of XXAA oo with YYaa oo should result in all-male progeny, XYAa, but the reciprocal crosses in a ratio of one female to three males (WXAa ♀, WYAa ♂, XYAa ♂, YYAa ♂). The Y chromosomes of the different tilapias appear to be identical. The factor A appears to be involved in suppressing the female-determining action of the W chromosomes, hence the male differentiation of WYAA, WYAa and WWAA individuals. Based upon the sex ratio of subsequent crosses (F2, backcross generations) they worked out the theoretical sex phenotypes of the 18 possible genotypes.

The present project was aimed at validating Hammerman and Avtalion's model by (1) analyzing the outcomes of their proposed crosses (2) sex inversion studies (3) sex ratios in gynogenetic tilapias (3) sex-linked markers (electrophoretic and chromosomal) to enable the interpretation of results obtained from the planned crosses.

I. Research Work Accomplished from July 1987 - November 1988.

A. Intraspecific crosses (section 5.1 of proposal).

The resulting sex-ratios in progenies of intraspecific crosses were as follows:

Ten successful spawns of intraspecific crosses of *Oreochromis hornorum* (H X H), gave a sex ratio average of 52.7 : 47.3 female to males which is closed to the expected 1:1 (X^2=17.72; P= 0.05) (cross A, Table 1).

Almost similar results were obtained in *O. mossambicus* (M x M) (average sex ratio of 48.6 : 51.4) also close to 1:1 as expected, though this result was not significant (X^2 =7.48; P= 0.25) (cross A Table 1). The sex ratio variations can be explained by the high mortality of the progeny until sexing. The few spawns which reached the sexing age in *O. aureus* (A X A) (6 groups) and *O. niloticus* (N X N) (2 groups) gave unexpected sex ratio averages (1:2 and 1:3, respectively) (crosses A3 and A4, Table 1).

B. Interspecific crosses (sections 6.1, 6.2 of proposal)

All 10 batches of spawns of the *O. mossambicus* x *O. hornorum* cross gave 100% all-male progenies (cross B1, Table 2).

In the reciprocal 11 crosses of *O. hornorum* x *O. mossambicus* (B4, Table 2) the average sex ratio was 26.5:73.5, close to 1:3 as expected (X^2=21.38; P= 0.025).

Similar results (100% males) were obtained in all 8 spawns of the *O. niloticus* X^2 *O. aureus* (cross B3, Table 2). Only 2 spawns of reciprocal *O. aureus* x *O. niloticus* crosses (B2), were determined as of present time. The observed results seem to conform with the expected 1:3 sex ratio.

C. F2 crosses (section 6.3).

Fifteen different males from F1 progeny of cross 6.2 were each crossed with 7-8 F1 females of the same cross, to test if the sex ratio would be closed to 5:3, 9:7 and 1:3 predicted by the autosomal theory (Avtalion and Hammerman, 1987) or 3:1, 1:1 and 1:3 predicted by the WXYZ theory (Hickling, 1960). Sex ratio results of various males, as well as of repeated spawns of the same male, were highly variable, and could not be fitted with statistical significance to any of the predicted ratios (Table 3). Some males gave surplus of male offspring, due presumably to the YYAa genotype that should have produced 1:3 sex ratio (under both models). No male could be identified with confidence as carrying the WYAa genotype, whose offsprings were required for sections 6.4 and 6.5.

D. Sex inversion (section 3.1 in the proposal). (An M.Sc. student was employed to take care of this part of the project).

This study was carried out in order to produce the required fish for the experiments (listed in sections 3.1, 7.1 and 7.2 of the proposal) proposed for the production of a supermale as predicted by the autosomal model (Hammerman and Avtalion, 1979).

Feminization of tilapia fry using sex steroids (estrogens) have met with very little success so far. Jensen and Shelton (1979) reported on very low success rate. Hopkins et al (1979) did not

succeed much better, in spite the use of methalibure and cyproterone acetate (both known as androgen-blocking compounds). Their conclusion was that testis differentiate earlier than ovaries, and thus when the feminizing hormone is orally administered (as the androgen treatment is done) from day 10 post hatching onwards, the critical time for inversion is missed. Recently several papers on feminization of salmonids using estrogen applied to the developing eggs and embryos by immersion, with (Goetz et al, 1979) or without (Hunter et al, 1986) further treatment with estrogen treated food, suggested that the critical period for sex inversion of males to females occurs indeed at earlier stages of development. Furthermore, Van den Hurk and Slof (1981) suggested that progesterone is probably the inducer of onset of ovarian development in rainbow trout.

The work plan adopted to study this subject included the use of the following hormones: 17α-estradiol, 17β-estradiol together with flutamide (a testosterone blocker), progesterone, and progesterone together with flutamide.

The following treatments were planned to be applied:

a) Low dose (0.1-10 ug/l) bath to developing eggs and fry for periods up to 30 days.
b) High dose (200-1500 µg/l) bath to developing eggs until yolk-sac absorption.
c) Combination of bath treatment and oral administration of hormone-treated feed.

All experiments were planned to be applied to the all-male hybrid offspring of O. mossambicus x O. hornorum. This should enable easy detection of feminization effect and will also produce the required fish to perform section 3.1 in the proposal. However, due to lack of enough spawns treatment was also applied to pure strain O.mossambicus offspring.

Hormonal treatment was applied to eggs in Erlenmayer bottles on a laboratory shacker, the hormone solution being replaced every other day.

The results, listed in Table 4, showed no feminization influence of 17 -estradiol (E-17α), flutamide and progesterone given by bath treatment during different periods of time on developing embryos. In contrast, oral administration of E-17β and diethylstilbestrol (DES) was proven very successful, yielding high rates of feminization in both O.mossambicus x O. hornorum and pure strain O. mossambicus offsprings (Table 5). Sex inversed (phenotypically females) O. mossambicus x O. hornorum offsprings were grown to maturity, and recently mated to normal O. mossambicus males (according to section 7.1 in the proposal) and O. hornorum males (according to section 7.2 in the proposal). Offspring from these backcrosses are now being grown up.

E. Diploid gynogenetic Tilapias .

Some few efforts have been made by many investigators to study the sex determination mechanism by chromosomal manipulations leading to polyploidy and gynogenesis, (Penman et al., 1987). This approach was

used in different fishes and amphibians as a tool for understanding the sex determination mechanism (Thorgaard & Gall, 1979; Colombelli et al., 1984; Nakamura, 1988).

We have studied the sex ratio results obtained in three generations of diploid gynogenetic O.aureus which were produced in our laboratory in the frame of a separate scientific program (Don and Avtalion, 1988).

Eighteen gynogenetic offspring produced from three normal O.aureus females showed a sex ratio of 13:5 (female:male) progenies. All five F1 males dyed before sexual maturity and were not used for test-crosses. Four of the above 13 F1 females were used to produce F2 gynogenetic generation. Three of these 4 females produced both female and male progenies (type 1) while the fourth female produced only female offspring in two different spawnings (type 2). A third gynogenetic generation (F3) of 12 females and 5 males, was produced by a F2 female of the first type (Table 6).

Analysis of the sex-ratios results obtained in F1, F2 and F3 gynogenetic offspring, where only female (20:0, in F2b) or more females than males (13:5, 20:9 and 12:5, in F1, F2a and F3, respectively) were produced (Table 6). showed a significant deviation from the expected 1:1 sex ratio, and the probability of correlation to the 1:1 sex ratio is considerably smaller in comparison to the control crosses (Table 7). These findings might be considered as statistically significant although they are based on small numbers of fish (Gynogenetic offspring showed high mortality rates in comparison to normal, Don & Avtalion, 1988a).

However, the occurrence of male offspring, in F2 and F3 gynogenetic generations cannot be explained by neither the WXYZ and the autosomal models suggesting heterogamety for O.aureus female (WZ or aaWY, respectively). An explanation, based on the occurrence of a recombination mechanism was suggested by several authors on the basis of their works in Xenopus laevis (Nakamura, T. 1988) and in F1 gynogenetic O.aureus (Penman et al. 1987).

A such recombination model could be described in details and graphically illustrated (Fig.1) on the basis of our above F2 and F3 sex ratio results. According to this model F1 females with the aaWY combination can produce a second gynogenetic generation in which, again, the three parental genotypic combinations (aaWY and aaWW females and aaYY male) are expected. Therefore, our F1 females (1, 8 and 15) and F2 female (5) are assumed to possess the WY combination. Similarly the F1 female 7 and the F2 female 12 that gave a sex ratio of 1:1 when crossed with normal or with gynogenetic males (Table 2) should also be considered as WY. However, F1 female 2 might be considered as possessing the WW combination because all-female offspring were produced. To strengthen this assumption we attend to cross this female and/or its progenies with a normal male in order to obtain an expected sex-ratio of 1:0.

This model of sex determining gene recombination suggest that sex ratio of the next gynogenetic generation depends on the recombination frequency between these genes. When a single crossover takes place, between the centromeres and the sex determining genes, crossing over would result in a WY combination, and the higher this single crossover frequency is the higher the tendency towards females will be. On the other hand gametes in which a double crossover took place,

or gametes in which crossing over did not occur, WW or YY combinations will be performed (Fig.1). Therefore, variations in sex ratios in Oreochromis seems to be determined by differences in recombination frequencies proper to these genus.

The relative high frequency of crossing over between sex determining genes and the centromere and the high degree of homozygocity that seems to characterize these gynogenetic fish (Avtalion et al 1988; Don and Avtalion 1988a; Timan and Avtalion, in preparation), make the long subthelocentric chromosomes of O.aureus preferred candidates on which these sex determining genes are located. This speculation is supported by the fact that all the other chromosomes in O.aureus are much smaller (Fig 2).

F. Chromosomal markers

The experiments to culture tilapia lymphocytes in 2% pooled carp serum in MEM (modified Eagle's medium) + glutamide at 28 C pH 7 were successful. Karyotypes have been obtained with Giemsa and silver nitrate combining and modifying the techniques by Hovell and Black (1980) and Bloom and Goodpasture (1976). Preliminary results pointed out similarities in the number of NORs (nucleolus organizer regions) among males and females of O.mossambicus and O.hornorum and their hybrids. These similarities may have resulted from loss or chromosome overlap during preparation. Efforts to optimize the conditions to obtain early metaphases were successful which may show some promise in locating heteromorphic sex chromosomes in tilapias. A technique of acridin orange fluorescent staining was developed in our laboratory (Fig. 2).

G. Male sex proteins (MSP)

Male specific protein bands (MSP) were found previously in the electropherogram of sera from Sarotherodon galilaeus. These bands which were located in the prealbumin region of the electropherogram, are the fastest moving proteins in the 7% polyacrylamide gel, and their relative position were found to be sex-specific. Other tilapia species tested were not as rich as S. galilaeus. Immunization of the rabbits against electrophoretically purified MSP was carried out.

Using immunoelectrophoresis, the anti-total tilapia serum revealed the existence of 2 different precipitin lines located in the anodic part of the electropherogram. However, a single band was apparently obtained when this material was tested using PAGE (Fig. 2). This result suggests that eluted MSP solution contains two different antigens existing in both male and female sera. These were tested using the analytical rocket immunoelectrophoresis technique. It was shown that the MSP antigen, while present in high quantities in males it could be found in tiny amounts in females. In order to test whether this product is sex linked, the occurrence of MSP in sex inversed female and males would be tested in the near future (Fig.3).

DISCUSSION, CONCLUSIONS, LIMITATIONS OF RESEARCH AND PERSPECTIVES

Results on intraspecific crosses in O. hornorum and O. mossambicus showed expected 1:1 sex ratios but the few crosses carried out with O.aureus and O.niloticus showed severe deviations from the normal (a mean of 1:2 in 6 different females). Such a deviation from the normal 1:1 ratio was also reported by others in the same fish (Majumdar and McAndrew, 1983; Penman et al., 1987). This phenomenon also emerged from a particular sex ratio (85% instead of 100%) obtained in crosses of sex inversed O. niloticus males with their sibling females (Calhoun and Shelton, 1982). While this finding is difficult to explain, it might speculated that it is resulting from the influence of heterozygocity in sex-regulating autosomal genes. In fact, our sex ratio results obtained in sibling F2 gynogenetic O.aureus were regularly close to 1:1 (table 7).

Interspecific crosses of O. mossambicus female x O. hornorum male and of reciprocal crosses gave 0:1 and 1:3 sex ratios, respectively, as expected. Similar results (100% males) were obtained in all 8 spawns of the O. niloticus X O. aureus. Only 2 spawns of reciprocal O. aureus x O. niloticus crosses (B2), were determined as of present time. The observed results seem to conform with the expected 1:3 sex ratio.

The sib-matings of F1 hybrids from reciprocal cross yielded different sex ratios. The sex ratios of 9:7 (shown in 3 different males, each tested in crosses with 3-5 females) and of 5:3 (shown in 2 different males tested in crosses with 8 and 4 females) predicted by the autosomal theory, were obtained. These ratios were not consistent with the sex determination based on the simple WXYZ chromosome mechanism. However non of the above theories could explain the sex ratio of 7:9 obtained in offspring of 4 different males. This result is difficult to explain, mainly because of the small samples of surviving fish from each group, and possible differential mortality. However the above speculation that heterozygocity in sex-regulating autosomal genes might have strong influence on sex determination should be taken into account here too.

Hence, the control of sex determination in tilapias seems to be more complicated and the present sex-ratio data in not enough consistent in volume to be conclusive. The breeding program should be continued and sections 6.3, 6.4, 6.6 and 7 of the proposal must be completed. However, the experimental difficulties and statistical problems in analyzing small number of spawns proved that the breeding approach alone would not offer a sufficient tool to explain the sex-determination mechanisms.

The use of gynogenetic fish produced from heterogametic tilapias females offered a new outlook on this problem. A model of recombination between sex-determining genes emerged from the analysis of sex ratio results obtained in 3 generations of O.aureus. This model seems to be complementary and does not necessarily contradict the basic idea of our model of autosomal influence on sex determination in tilapias (Avtalion and Hammerman, 1978 and Hammerman and Avtalion 1979) which could explain the experimental sex ratio results obtained by Chen (1969) and Jalabert et al (1971).

Three major findings emerged from the results of this last study:

1) Existence of gynogenetic males among first, second and third generations of gynogenetic tilapias.
2) the sex ratio results observed amongst gynogenetic offspring reflect two genetically different maternal types. The first gave rise to both gynogenetic males and females and the second type only to females. Females of the first type produced more females than males.
3) A genetic recombination model between sex determining genes and the centromere is suggested to explain these results.
4) A reduced variability of sex ratios in crosses of gynogenetic females with gynogenetic males or even with normal males (table 2) was shown.

This last finding could be attributed to the increased degree of inbreeding which minimize the autosomal influence as suggested above.

In conclusion, it seems to us that some few autosomal loci (more than one) wich recombine between them, in addition to recombinations occuring in the sex determining genes are the factors which dominate the the sex determining mechanism, control variations from the expected 1:1 sex ratio in normal intraspecific crosses as discussed above.

II. Ms. Galman's doctorate

Ms. Galman has successfully defended her Ph. D. dissertation on a French government scholarship in France (Ecole Nationale Superieure Agronomique de Toulouse) in collaboration with Bar-Ilan University (see attached dissertation). A part of her previous work in Israel was incorporated in her dissertation. Prof. Avtalion served as member of her board of examiners. She presented 2 papers in the last International Symposium on Tilapia in Aquaculture in Bangkok, Thailand on May 16-20,1987. She went back to Israel in May after reporting to her institute in the Philippines and continued this project. She will be leaving for France to attend a training course on cellular genetics where she will learn genetic mapping and chromosome banding. She hopes to apply these techniques in studying further the sex-determination mechanisms in tilapia.

III. Publications (Includes data which result from this scientific program and acknowledgment to USAID-Israel CRD fund.

1. O.Galman and R.R. Avtalion. (1989) Further study on the embryonic development of Oreochromis niloticus (Cichlidae, Teleostei) using scanning electron microscopy. J. Fish Biol. (in press).

2. O.Galman, J. Moreau and R.R. Avtalion.(1989) Breeding characteristics, growth performance and implications for aquaculture of Philippine red tilapia. ISTA (International Symposium on Tilapia in Aquaculture) II Proceedings. ICLARM (in press).

3. O. Galman, J. Moreau, G. Hulata and R. Avtalion. (1989). The use of electrophoresis as a technique for the identification and control of breeding stocks in Israel. ISTA II Proceedings (in press).

4. O. Galman. The Philippine Red Tilapia- Study of morphological, biological and genetic characteristics. (Ph.D. Thesis) 142 p. 1987.

5. R.R. Avtalion and J. Don (198-). Sex determination in tilapias. Recombination of sex determining genes emerging from sex ratios obtained in three generations of diploid gynogenetic Oreochromis aureus (submitted).

6. O.Galman, G. Hulata and R. Avtalion. The significance of sex-ratio results obtained in offspring of F1 hybrids of the cross Oreochromis mossambicus oo x O.hornorum oo and of its reciprocal. (In preparation).

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Table 1. Summary of intraspecific crosses of Oreochromis hornorum (H), O. mossambicus (M), O. aureus (A) and O. niloticus (N).

Cross No.	Species crossed ♀ x ♂	Spawning date	Larvae, initial No.	Surviving fish at sexing %♀♀	%♂♂	Sex Ratios %♀♀	%♂♂	Close to
A1 - H x H								
1.	H(20) x H(25)	8-01-85	-	23	19	55	45	
2.	-do-	25-12-85	214	32	21	60	40	
3.	-do-	18-05-87	-	47	48	49	51	
4.	H(19) x H(25)	29-01-86	332	22	40	36	64	
5.	H(20) x H(20)	28-01-86	562	47	48	49	51	1:1
6.	H(19) x H	30-06-85	89	48	15	76	24	
7.	-do-	9-08-85	-	60	40	60	40	
8.	H(17) x H	9-08-85	-	12	16	43	57	
9.	H(4) x H	22-11-85	-	22	17	56	44	
10.	H(25) x H(31)	24-08-86	126	12	26	43	57	
				Average:		52.7	47.3	
				SD =		11.4	11.6	
				X^2=17.72	P=0.05			
A2 - M x M								
1.	M(57) x M(51)	14-07-85	188	30	27	53	47	
2.	M(57) x M(51)	14-07-85	199	30	25	55	45	
3.	M(54) x M(51)	14-05-85	81	36	26	58	42	
4.	M(54) x M(51)	2-06-85	-	11	14	39	61	1:1
5.	M(56) x M(51)	13-06-85	140	40	42	49	51	
6.	M(56) x M(51)	25-05-85	188	29	62	32	68	
7.	M(53) x M(51)	31-07-85	-	38	33	54	46	
				Average:		48.6	51.4	
				SD =		9.5	9.5	
				X^2= 7.48	P = 0.25		N.S.	
A3 - A x A								
1.	A(117) x A(6)	27-05-86	315	32	27	54	46	
2.	A(117) x A(6)	3-08-86	1306	33	64	34	66	
3.	A(118) x A(6)	27-06-86	319	12	41	23	77	
4.	A(118) x A(6)	-	587	8	17	32	68	
5.	A(117) x A(6)	3-08-86	-	13	22	37	63	
6.	A(Nile)x A(Nile)	10-6-88	-	37	26	58	42	
7.	-do-	**	-	99	126	27	73	
				Average:		36 :	64	
				SD		=11.3	11.3	
A4 - N x N								
1.	N(5) x N(12)	15-11-85	145	6	23	21	79	
2.	N(G x N(*)	4-09-86	230	43	106	29	71	

In the brackets, Tag number of individual fish; G, Ghana strain; *,Ein Hamifratz; ** 2 spawns; - data not available

Table 2. Summary of Two ways interspecific crosses between O. niloticus and O. aureus (A), and O. mossambicus (M) and O. hornorum (H), and the corresponding sex ratios of their progeny.

Cross No.	Species crossed ♀ x ♂	Spowning date	Larvae, initial No.	Surviving fish at sexing %♀♀	%♂♂	Sex Ratios %♀♀	%♂♂	Close to
B1 - M x H (6.1)								
1.	M(57) x H(25)	12-01-86	217	0	29	0	100	
2.	-do-	5-01-87	340	7	211	3	97	
3.	-do-	27-07-87	265	0	229	0	100	
4.	M(49) x H(25)	22-12-86	215	0	73	0	100	
5.	M(52) x H(25)	22-06-86	266	0	21	0	100	
6.	M(49) x H(22)	13-08-86	288	0	34	0	100	0:1
7.	M(52) x H(29)	1-12-86	382	0	28	0	100	
8.	-do-	20-08-86	118	0	67	0	100	
9.	M(62) x H(19)	5-01-87	357	0	255	0	100	
10.	M(?) x H(?)	5-02-87	-	0	77	0	100	
B2 - H x M (6.2)								
1.	M(29) x H(51)	12-07-86	172	20	62	24	76	
2.	-do-	30-07-86	516	44	166	21	79	
3.	-do-	12-11-86	-	4	27	13	87	
4.	M(29) x H(2)	5-12-86	415	10	34	29	71	
5.	M(23) x H(51)	18-05-86	189	57	80	42	58	
6.	M(25) x H(51)	6-01-87	400	13	42	24	76	1:3
7.	M(25) x H(31)	24-08-86	121	12	26	32	68	
8.	M(191 x H(42)	20-02-87	139	32	90	26	74	
9.	M(41) x H(42)	17-06-87	80	12	29	24	76	
10.	M(?) x H(27)	27-05-87	84	12	21	36	64	
11.	M x H *	5-12-86	-	9	23	28	72	
				Average:		26.50 : 73.50		
				SD		=7.9		
				X^2=21.38		P = 0.025		
B3 - N x A (cross 6.1a)								
1.	N(G) x A	25-06-86	36	0	29	0	100	
2.	N(5p) x A(55)	15-07-85	-	0	61	0	100	
3.	N(108) x A(11)	24-04-85	-	0	9	0	100	
4.	-do-	29-05-86	487	0	145	0	100	
5.	N (?) x A(33)	20-12-86	-	0	24	0	100	
6.	N(108) x A**	31-03-86	436	0	150	0	100	
7.	-do-	25-06-86	1042	0	75	0	100	
8.	N(G) x A(113)	-	-	0	13	0	100	
-9.	N(G31)x(A34)	25-07-87	310	4	102	4	96	
B4 - A x N (6.2a)								
1.	A(31)x N(24)	25-07-87	226	6	16	27	73	
2.	A(*) x N(G)	4-09-86	230	43	106	29	71	
3.	A(130)xN(G)	10-09-86	271	64	106	38	62	

* mixture of 2 unidentified females offspring;
** Crosses B3 and B4, while not figured in the proposal, have been carried out in parallel to crosses B1 & B2 in order to test whether the suggested model holds for other species.
G, Ghana; p, pink No.5; in the brackets, tag no. of fish.

Table 3. Results obtained by crossing F1 hybrid males from 6.2 crosses with females of 6.2 crosses.

Cross No.	Female No.	Spawning date	Larvae, initial No.	Surviving fish at sexing ♀♀	♂♂	Sex Ratios ♀♀	♂♂	Close to
F1 (H x M) x F1 (H x M)								
C1 - Male no. 238								
♀♀ 1.	230	20-02-87	230	68	53	56	44	9:7
2.	230	15-03-87	230	42	27	61	39	5:3
3.	230	10-04-87	222	90	51	64	36	5:3
4.	231	5-03-87	70	25	21	56	44	9:7
5.	231	30-03-87	216	93	49	66	34	5:3
6.	232	-	214	96	53	64	36	5:3
7.	233	5-03-87	128	66	49	57	43	9:7
8.	233	10-04-87	277	121	60	67	33	5:3
					Mean:	61	39	5:3
C2 - Male no. 295								
1.	231	22-04-87	231	124	71	64	36	5:3
2.	232	27-04-87	231	51	16	76	24	3:1
3.	233	8-05-87	346	46	38	55	45	9:7
4.	230	11-05-87	560*	42	41	50	50	1:1
					Mean:	61	39	5:3
C3 - Male no. 297								
1.	293	11-06-87	61	24	18	57	43	9:7
2.	231	11-06-87	283	10	12	52	48	9:7
3.	230	10-06-87	147	45	29	61	39	5:3
4.	233	5-06-87	290*	78	47	62	38	5:3
					Mean:	58	42	9:7
C4 - Male no. 2311								
1.	232	25-06-87	362*	64	98	40	60	3:5
2.	294	5-06-87	**	27	31	47	53	7:9
3.	230	21-06-87	450	15	20	43	57	7:9
4.	230	13-07-87	146	63	79	44	56	7:9
5.	293	24-06-87	279	45	59	43	57	7:9
					Mean:	43	57	7:9
C5 -Male no. 2513								
1.	255	13-04-88	88	31	33	48	52	8:9
2.	253	3-05-88	521	59	118	33	67	1:2
3.	257	6-04-88	264	44	64	41	59	7:9
4.	254	3-05-88	136	38	38	50	50	1:1
					Mean:	43	57	7:9
C6 -Male no. 299								

Table 3,

1.	230	6-04-88	178	38	13	74	26	3:1	
2.	291	6-06-88	104	48	37	56	44	9:7	
3.	231	6-04-88	103	55	45	55	45	9:7	
4.	232	6-04-88	228	66	57	54	46	9:7	
5.	293	6-04-88	275	48	95	36	64	3:5	
					Mean:	55	45		9:7
C7 -Male no. 298									
1.	296	6-04-88	330	117	35	76	24	3:1	
2.	290	17-12-88	156	37	7	84	16	5:1	
3.	293	14-01-88	299	41	34	55	45	9:7	
4.	294	17-12-88	257	41	11	79	21	4:1	
					Mean	74	26		3:1
C8-Male no. 2511									
1.	257	3-12-88	247	44	38	54	46	9:7	
2.	257	18-01-88	389	35	44	39	61	3:5	
3.	256	18-01-88	198	31	35	47	53	7:9	
4.	256	28-02-88	192	14	32	29	71	1:3	
5.	254	18-02-88	152	13	13	50	50	1:1	
					Mean	44	56		7:9
C9-Male no. 2901									
1.	293	5-05-88	401	25	30	45	54	7:9	
2.	291	5-05-88	285	108	85	56	44	9:7	
3.	230	5-05-88	362	99	176	36	64	3:5	
4.	231	5-05-88	302	44	86	34	66	3:5	
						43	57		7:9
C10-Male no. 2902									
1.	233	19-05-88	650	108	85	56	44	9:7	
2.	293	9-07-88	131	15	9	65	35	5:3	
3.	233	7-06-88	625	182	139	57	43	9:7	
					Mean	59	41		9:7
C11- Male no. 296									
1.	292	27-08-87	131	34	61	36	64	1:2	
2.	232	1-06-87	368	13	24	35	65	1:2	
						35.5	64.5		1:2
C12-Male no. 258									
1.	251	10-09-87	277	27	15	64	36	2:1	
C13-male no. 294									
1.	2902	19-05-88	445	17	5	77	23	4:1	
C14-Male no. 254									
1	2903	19-05-88	-	10	9	52	48	1:1	
F1 (A x N) x F1 (A x N)									
Male no. 7									
1.	12	13-09-88	295	39	73	35	65	3:5	

* unsexed, ** unsexed and uncounted.

Table 4. Influence of immersion treatments of developing embryos on sex inversion.

A. Oreochromis mossambicus ♂ O. hornorum offsprings

Hormone / Dose	Duration of bath treatment (days)	No. eggs/fry at beginning of treatment	survival at sexing: males	females	intersex or unidentified	sex ratio (% females)
E-17β						
1 µg/l	0 (control)	50	29	0	0	0
	10	50	27	0	0	0
	17	50	18	0	1	0
	23	50	9	0	0	0
	30	50	6	0	1	0
3 µg/l	0 (control)	50	38	0	1	0
	7	50	25	0	0	0
500 µg/l	0 (control)	50	37	0	0	0
	9	50	33	0	3	0
Flu						
	0 (control)	75	63	0	0	0
10 µg/l	12	75	56	0	5	0
100 µg/l	12	75	11	0	1	0
1000 µg/l	12	75	24	0	0	0
Progest.						
	0 (control)	60	48	0	0	0
5000 µg/l	7	60	53	0	0	0

Table 4 (continue)

B. O. mossambicus X O. mossambicus offsprings

Hormone / Dose	Duration of bath treatment (days)	No. eggs/fry at beginning of treatment	Survival at sexing: males	females	intersex or unidentified	sex ratio (% females)
E-17β						
	0 (control)	60	34	19	0	36*
500 µg/l	3 hrs	60	21	23	0	52
	6 hrs	60	28	23	2	43
	22 hrs	60	27	20	0	43
	28 hrs	60	20	6	3	21
750 µg/l	43 hrs	60	38	17	4	29
1500 µg/l	43 hrs	60	16	16	1	48
5000 µg/l	13	100	26	30	0	54
Flu						
	0 (control)	75	29	27	0	48
5 µg/l	10	75	29	29	1	50
10 µg/l	10	75	28	34	0	54
100 µg/l	10	100	34	43	0	56
200 µg/l	10	100	48	36	2	44
Progest.						
	0 (control)	75	19	6	0	24*
5 µg/l	12	75	26	25	0	50
10 µg/l	12	75	35	27	2	42
100 µg/l	12	75	18	24	1	53
500 µg/l	12	75	30	22	1	42

*) Unexplained deviation from expected sex ratio.

Table 5. Influence of oral administration of various estrogens to fry, starting from termination of yolk sac absorption, on sex inversion.

A. Oreochromis mossambicus I O. hornorum offsprings

Hormone / Dose	Duration of treatment (days)	No. eggs/fry at beginning of treatment	Survival at sexing: males	Survival at sexing: females	Survival at sexing: intersex or unidentified	sex ratio (% females)
E-17β						
	0 (control)	50	43	0	3	0
75 μg/g	41	50	0	30	4	88
DES						
	0 (control)	60	49	0	8	0
100 μg/g	33	60	0	46	0	100
125 μg/g	33	60	0	51	3	94

B. O. mossambicus I O. mossambicus offsprings

DES						
	0 (control)	100	43	38	3	45
75 μg/g	32	100	0	66	1	98
75 μg/g	32	50	0	28	4	88
100 μg/g	32	100	0	70	0	100
100 μg/g	32	50	0	28	0	100
	0 (control)	60	23	22	0	50
75 μg/g	14	100	0	69	4	95
75 μg/g	32	100	0	50	0	100
100 μg/g	14	100	0	84	3	97
100 μg/g	32	100	1	42	2	93
	0 control	60	24	25	2	49
50 μg/g	31	100	1	70	4	93
75 μg/g	31	100	2	37	1	93
125 μg/g	31	100	0	28	0	100

Table 6. Sex ratios obtained in offspring of three succecive gynogenetic generations of O. aureus.

Generation	Tag No. of mother	Offspring females	Offspring males	Correlation to 1:1 ratio (a)
F1	20	1	1	P=1
	25	9	3	0.1> P > 0.05
	30	3	1	0.5> P > 0.3
	total	13	5	0.1> P > 0.05
F2,a	1	5	5	P=1
	8	5	2	0.2> P > 0.1
	15	10	2	0.05> P > 0.02
	total	20	9	0.05> P > 0.02
F2,b	2	8	0	0.02> P > 0.01
		12	0	0.01> P > 0.001
F3	5	12	5	0.1> P > 0.05

(a), probability of correlation to 1:1 sex ratio calculated by the x^2 test.

Table 7. Sex ratio results obtained in control-crosses of normal O.aureus females with normal O.aureus males (a-c), of F1 gynogenetic female with a normal O.aureus male (d) and of F2 gynogenetic females with a normal male (e) or with a gynogenetic F2 male (f-g)

Cross	Tag No. of female	Tag No. of male	Offspring females	Offspring males	Correlation to 1:1 ratio (a)
a	25	18	117	127	0.7> P > 0.5
b	43	36	54	46	0.5> P > 0.3
c	44	32	58	42	0.2> P > 0.1
total			229	215	0.7> P > 0.5
d	F1, 7	10	5	6	0.7> P > 0.5
e	F2, 5	18	48	52	0.7> P > 0.5
f	F2, 5	F2, 6	59	56	0.9> P > 0.8
g	F2,12	F2,11	20	21	0.9> P > 0.8

(a), probability of correlation to 1:1 sex ratio calculated by the x^2 test.

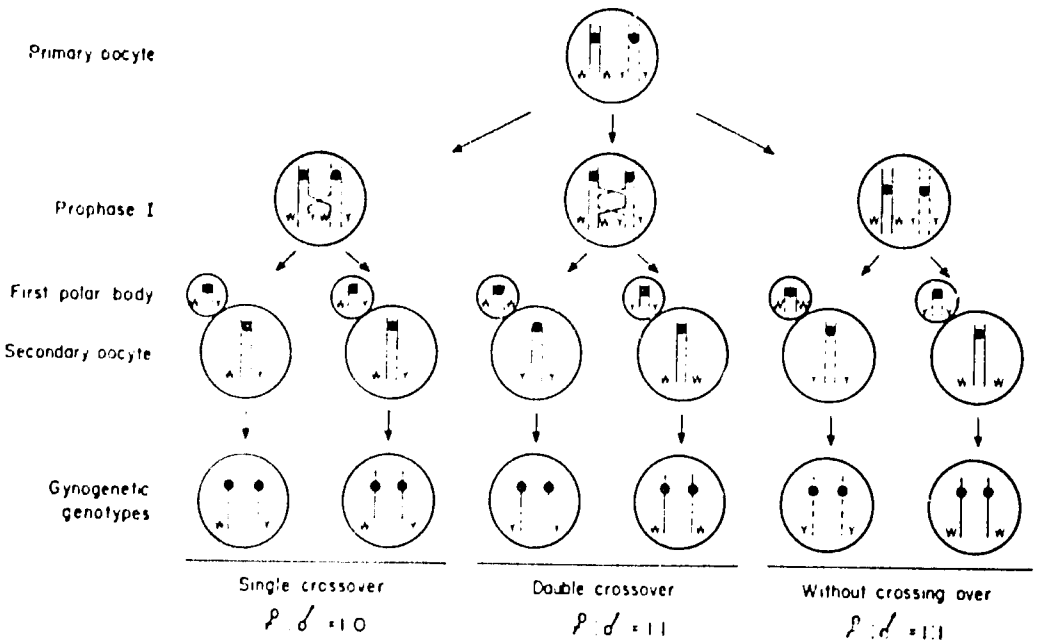

Fig.1. Schematic illustration of the suggested model in which crossing over between sex determining genes (W,Y) and the centromere enables 3 different genotypes among gynogenetic offspring (WW, WY, YY). Gynogenesis is obtained by extrusion of second polar body after haploid activation of eggs. From gammets in which a single crossover occurs, only WY females would be obtained. From gammets in which double crossover occurs or in which crossing over does not occur WW females and YY males would be produced in 1:1 ratio.

a

b

Fig. 2. Caryotypes of tilapia; a, acridin orange staining; b, Ag nitrate staining.

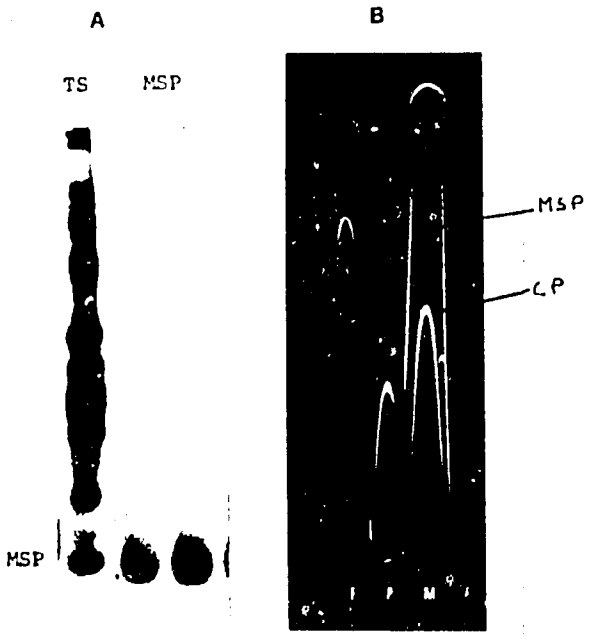

Fig. 3. Polyacrylamide gel electrophoresis (A) and rocket electrophoresis (B) of electrophoretically purified male specific protein (MSP) and of total serum of male (TS). F, female serum; P, MSP; M, male serum; CP, contaminating protein.