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Production of YY Male in the Guppy *Poecilia reticulata* by Endocrine Sex Reversal and Progeny Testing

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Abstract

Gravid *Poecilia reticulata* were orally administered estrogens, β-estradiol (200-400 mg·kg^{-1} food), diethylstilbestrol (100-1,000 mg·kg^{-1} food), 17 α-ethynylestradiol (100-500 mg·kg^{-1} food) and 3-benzoate estradiol (200-800 mg·kg^{-1} food) from days 15 to 20 after the previous parturition until the termination of gestation. Treatment with 400 mg β-estradiol per kg food, 300 mg diethylstilbestrol per kg food and 200 mg 17 α-ethynylestradiol per kg food, initiated 5-10 days prior to parturition, caused the birth of 100% females. However, 3-benzoate estradiol, even if administered at high dosage levels, failed to induce 100% sex reversal. Sex-reversed females were functional and their genotype was identified by progeny testing. Sex-reversed females (XY) were mated with normal males (XY) to produce F_2 progeny. Among 21 F_2 males, each tested individually by mating with a normal female (XX), two males were identified to have YY genotype and both of them sired all-male offspring. A program for the mass production of YY broodstock is discussed.

Introduction

Variability in color and shape particularly in males has rendered the guppy *Poecilia reticulata* not only a commercially important ornamental fish but also a valuable object for genetic observation. The genetic sex determining mechanism prevailing in the guppy is that females are homogametic (XX) and males heterogametic (XY) (Winge 1923). However, a heterogametic female can be produced by administering estrogen to young fish at the time of gonadal sex differentiation (Takahashi 1975). The dosage of androgen and duration of treatment are critical to successful sex reversal and these vary from species to species (Yamamoto and

Matsudha 1963; Yamazaki 1983). Females of genotype XY have arisen spontaneously in *P. reticulata* (Winge 1930). When these exceptional females were mated with normal XY males, 25% of the progeny were YY males. YY males are viable only if they are heterozygous for certain Y-linked pigment genes (Winge and Ditlevsen 1938; Yamamoto 1964). In *Xiphophorus maculatus*, sporadic instances of atypical sex determination occur by the interaction of an autosomal factor with a specific sex chromosome (Kallman 1984). In such cases, XY individuals develop into functional females. When these XY females are mated with XY males, the YY offspring may differentiate into either male or female. In 1955, Yamamoto produced YY medaka (*Oryzias latipes*) for the first time using the technique of hormonal reversal and selective breeding. Subsequently, YY goldfish *Carassius auratus* (Yamamoto 1975) and YY tilapias *Oreochromis niloticus* (Scott et al. 1989) and *O. mossambicus* (Varadaraj and Pandian 1989) were produced.

The ultimate objective of this study is to produce all-male populations in *P. reticulata* by mating YY males with normal females (XX). If a large number of YY males are to be produced, one cannot always rely on spontaneous sex reversal for producing XY females. Therefore, we have resorted to an endocrine sex reversal technique for the production of 100% functional XY females and subsequent progeny testing and breeding for producing YY males.

Materials and Methods

The guppies *P. reticulata* used in this study were obtained from breeding stock maintained in the laboratory and originally purchased from a local fish farmer. Fish were kept at 25±3°C under a 16:8 hour light-dark cycle. Gravid females were individually maintained until the delivery of their brood. The young ones were isolated from the mother on the day of birth and served as controls. The gravid females were fed on steroid supplemented diet commencing from different days after parturition until the succeeding parturition.

Twenty-three steroid-treated feeds were prepared using the alcohol evaporation method (Guerrero 1975). A stock solution of hormone was prepared by dissolving the steroid in ethanol or acetone at a concentration of 1 $mg \cdot ml^{-1}$. The stock solution was diluted to the desired level in 95% distilled ethanol and sprayed over a

weighed amount of feed. Even distribution of the chosen hormone was ensured by stirring thoroughly during spraying. Synthetic hormones 17 α-ethynylestradiol, 3-benzoate estradiol, diethylstilbestrol, and a natural hormone, β-estradiol (Sigma Co., USA) were selected for the experiment. Gravid females were fed on feed supplemented with the selected hormones at concentrations of 100-1,000 $mg \cdot kg^{-1}$ feed for 5-10 days before parturition. The number of F_1 progeny in each brood was noted and they were fed on normal pelleted feed.

At the age of three months, sex of the progeny was determined by external examination using caudal, dorsal and anal fins as markers. In additions, some were sexed by examining gonads using the squash technique (Guerrero and Shelton 1974).

Some females from the minimal optimum treatment groups for β-estradiol, diethylstilbestrol and 17 α-ethynylestradiol were retained for progeny testing. Heterogametic females were identified by mating them with normal males. Sex ratio of their progeny indicated the genotype of the female parent, i.e., normal (XX) females gave 1F:1M sex ratio; the sex-reversed (XY) females gave 1F:3M sex ratio. YY males were identified by observing the sex ratio of the progeny of F_2 males (derived from sex-reversed females) mated with normal XX females (Fig. 1).

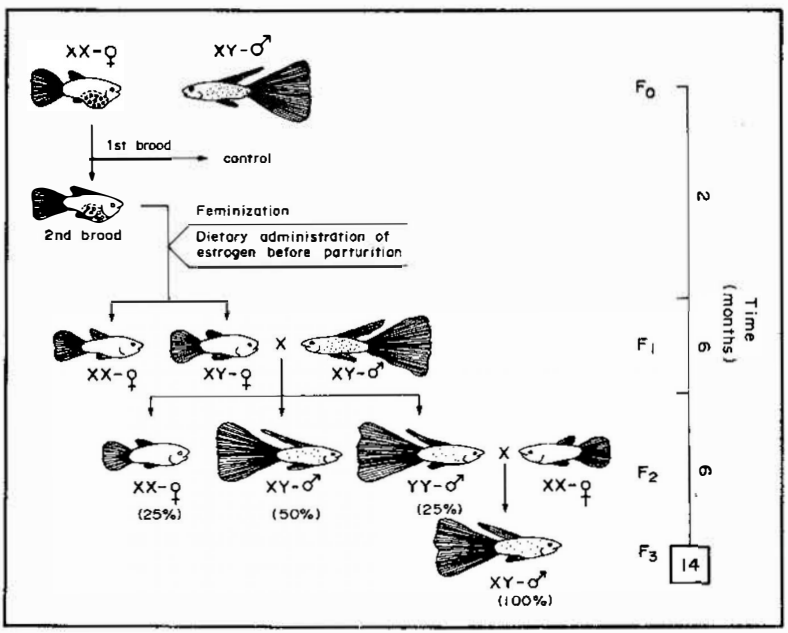

Fig. 1. Schematic diagram showing the production of YY male *P. reticulata* by endocrine sex-reversal and progeny testing.

Results

In the first series of experiments, the minimum dose of β-estradiol and the treatment duration for sex reversal were determined (Table 1). An almost equal number of males and females were present in the control. Administration of 400 mg β-estradiol to gravid females for six days from the 19th day after parturition resulted in 100% feminization of the progeny.

Effects of diethylstilbestrol, 17 α-ethynylestradiol and 3-benzoate estradiol were then determined. Administration of diethylstilbestrol at doses of 300-500 $mg \cdot kg^{-1}$ diet for 4-10 days before parturition significantly altered the normal sex ratio of the

Table 1. Sex distribution among the progenies of female *P. reticulata* fed with a diet containing β-estradiol during gestation.

Dose ($mg \cdot kg^{-1}$ food)	No. born	Sex distribution in control		Commencement of treatment (day after previous parturition)	Succeeding parturition day	Treatment duration (days)	No. born	Sex distribution		
		M	F					M	MF	F
200	43	18	21	16	25	10	36	11	0	16
	37	16	14	17	24	8	41	17	0	13
	31	9	11	18	26	9	38	11	0	14
	42	20	15	19	25	7	40	16	0	22
	44	18	13	20	26	7	44	17	0	21
250	36	11	16	15	26	12	28	8	0	11
	35	11	15	16	27	12	33	11	0	15
	29	9	13	17	24	8	40	21	0	14
	41	21	16	18	25	7	37	17	0	14
	39	20	16	19	24	6	29	14	0	13
	33	14	15	20	25	6	41	17	0	19
300	33	14	11	15	26	12	37	5	0	24
	33	14	17	16	26	12	35	7	0	22
	28	10	12	17	27	11	44	0	3	30
	37	9	17	18	24	7	42	6	0	21
	39	11	14	19	24	6	38	14	0	20
	40	-	-	20	25	6	46	11	0	29
350	21	10	8	15	24	10	45	4	3	31
	-	-	-	16	25	12	33	0	2	21
	26	11	9	17	26	10	39	1	1	26
	39	17	14	18	25	8	44	4	0	31
	43	19	20	19	26	8	40	3	0	29
	40	20	14	20	24	10	40	2	2	30
400	38	17	13	15	24	10	40	0	0	32
	40	18	14	16	25	10	38	0	0	29
	40	16	17	17	26	10	37	0	0	28
	33	19	11	18	24	7	41	0	0	36
	37	18	14	19	24	6	38	0	0	32
	35	16	14	20	25	6	41	0	0	37

M-male; F-female; MF-intersex.

progeny (Table 2). The minimum dose for 100% feminization was 300 mg diethylstilbestrol per kg diet. Treatment with 17 α-ethynylestradiol (200 mg·kg^{-1} food) for 7-10 days from 20th postpartal day resulted in 100% feminization (Table 3) with the exception of one intersex progeny.

Table 2. Sex distribution among the progenies of *P. reticulata* fed with a diet containing diethylstilbestrol during gestation.

Dose (mg·kg^{-1} food)	No. born	Sex distribution in control		Commencement of treatment (day after previous parturition)	Succeeding parturition day	Treatment duration (days)	No. born	Sex distribution		
		M	F					M	MF	F
100	33	14	12	17	28	12	39	19	0	27
	27	12	11	18	27	10	31	18	0	13
	41	21	17	19	29	11	36	17	0	16
200	36	13	18	17	24	8	31	17	0	12
	41	20	17	18	28	11	36	22	0	11
	39	15	19	19	30	12	32	13	0	17
250	37	13	12	17	25	9	33	8	2	23
	27	11	9	18	28	11	36	7	0	29
	28	12	13	19	27	9	27	4	1	20
	19	8	7	20	30	11	38	5	3	27
300	24	7	10	17	26	10	27	0	0	24
	29	14	11	18	24	7	33	0	0	31
	30	13	13	19	25	7	28	0	0	24
	34	12	20	20	24	5	32	0	0	23
400	42	19	16	17	25	9	29	0	0	27
	33	13	17	18	24	7	35	1	0	29
	16	8	6	19	24	6	22	0	0	19
	28	15	13	20	25	6	37	0	0	32
500	40	20	16	17	23	7	22	0	0	19
	29	10	17	18	24	7	18	0	0	13
	34	14	16	19	23	4	16	0	0	14
1,000	38	19	16	17	-	20	-	-	-	-
	28	10	11	18	-	20	-	-	-	-
	32	14	11	19	-	30	-	-	-	-
	41	17	20	20	-	26	-	-	-	-

In the fourth series of experiments, administration of 3-benzoate estradiol at doses of 200-600 mg·kg^{-1} food for 6-13 days before parturition did not alter the sex ratio of the progenies (Table 4). Treatment of 800 mg·kg^{-1} resulted in a decrease in the number of offspring produced. None of these progeny survived. Certain doses (400-500 mg·kg^{-1} food) also resulted in production of intersexes.

Table 3. Sex distribution among the progenies of *P. reticulata* fed with a diet containing 17 α-ethynylestradiol during gestation.

Dose ($mg \cdot kg^{-1}$ food)	No. born	Sex distribution in control		Commencement of treatment (day after previous parturition)	Succeeding parturition day	Treatment duration (days)	No. born	Sex distribution		
		M	F					M	MF	F
100	33	13	18	18	24	7	19	6	0	8
	24	13	10	19	28	10	38	13	0	18
	26	14	11	20	27	8	25	9	2	12
200	15	7	8	19	26	8	30	2	4	23
	41	22	17	19	27	9	25	2	2	21
	27	14	11	19	25	7	23	4	3	16
	25	10	15	20	29	10	29	0	0	26
	27	11	16	20	27	8	39	0	1	38
	31	13	17	20	26	7	34	0	0	31
300	29	14	14	18	25	8	25	0	0	24
	32	13	15	19	26	8	34	0	0	32
	40	18	22	20	24	5	33	0	0	23
400	36	20	15	18	24	7	29	0	0	27
	30	16	12	19	23	5	32	0	0	31
	37	17	18	20	24	5	28	0	0	28
500	34	16	15	18	24	7	22	0	0	7
	41	11	19	19	24	6	26	0	0	8
	35	13	17	18	23	6	18	0	0	4
	29	16	11	19	24	6	20	0	9	0

Table 4. Sex distribution among the progenies of *P. reticulata* fed with a diet containing 3-benzoate estradiol during gestation.

Dose ($mg \cdot kg^{-1}$ food)	No. born	Sex distribution in control		Commencement of treatment (day after previous parturition)	Succeeding parturition day	Treatment duration (days)	No. born	Sex distribution		
		M	F					M	MF	F
200	39	14	16	17	24	8	41	21	0	17
	25	20	14	18	26	9	27	14	0	9
	49	19	17	19	27	9	28	11	0	17
300	33	13	16	17	26	10	26	4	0	7
	29	14	11	18	27	10	37	16	0	19
	32	15	17	19	25	7	27	13	0	10
400	30	13	14	17	29	13	44	22	4	14
	14	6	5	18	30	13	29	7	3	16
	26	10	14	19	26	8	27	6	5	13
500	34	13	18	17	26	10	28	4	2	17
	41	16	19	18	24	7	25	3	0	19
	23	11	11	19	26	8	19	2	2	14
600	33	16	12	17	23	7	17	0	3	11
	41	15	20	18	27	10	11	0	1	9
	28	13	9	19	27	9	18	0	1	15
800	34	16	15	17	22	8	6	0	0	2
	42	18	21	18	25	8	4	-	-	-
	33	14	17	19	24	6	8	-	-	-

Estrogen-treated females were fertile and produced offspring when mated with normal males. Of the 50 matings, 31 yielded progenies in the ratio of 1F:1M. The remaining produced progenies in the ratio of 1F:2.2-3.0M indicating that the genotype of female parents was XY (Tables 5-7). There were differences in the XY and XX females. The number of offspring produced at first parturition was less for XY females than for XX females.

Of the F_2 progeny produced by mating sex-reversed XY females with XY males, 50% of the F_2 males will have XY genotype and 25% YY genotype. F_2 males from XY females were mated with normal females to identify their genotype (Table 8). Two F_2 males were actually identified to have YY genotype and both of them sired all male offspring.

Table 5. Progeny testing of hormone-treated *P. reticulata* mated with normal XY male. Females designated as PC1-PC16 were randomly selected from the group (see Table 2) treated with β-estradiol (400 mg·kg^{-1} food).

Female parent	Progeny produced (No.)	Progeny sexed (No.)	Sex ratio F : M	X^2	Genotype of female parent
PC1	41	32	1 : 1.3	0.50	XX
PC2	27	22	1 : 1.2	0.18	XX
PC3	19	15	1 : 2.7	3.26	XY[a]
PC4	12	10	1 : 2.3*	1.60	XY[a]
PC5	37	31	1 : 0.8	0.29	XX
PC6	27	24	1 : 1.4	0.67	XX
PC7	22	17	1 : 0.7	0.53	XX
PC8	38	29	1 : 0.7	0.86	XX
PC9	10	7	1 : 2.5	1.28	XY[a]
PC10	39	25	1 : 1.5	1.00	XX
PC11	18	12	1 : 3.0	3.00	XY[a]
PC12	29	22	1 : 0.7	0.36	XX
PC13	36	31	1 : 0.8	0.29	XX
PC14	12	11	1 : 2.7*	2.27	XY[a]
PC15	33	24	1 : 1.4	0.67	XX
PC16	35	31	1 : 0.8	0.29	XX

*These randomly selected males were used for further progeny testing (see Table 8).

[a]Based on Chi-square test, genotype of the mother is XX. The small number of progeny sexed (consequent to the smaller family size) rendered the expected ratio not statistically significant. As the ratios deviate far from the expected 1F:1M ratio and are closer to 1F:3M ratio, the genotype of the mother was determined as XY.

Table 6. Progeny testing of hormone-treated *P. reticulata* mated with normal XY male. Females designated as PA1-PA16 were randomly selected from the group (see Table 2) treated with diethylstilbestrol (300 mg·kg^{-1} food).

Female parent	Progeny produced (No.)	Progeny sexed (No.)	Sex ratio F	:	M	X^2	Genotype of female parent
PA1	37	31	1	:	0.8	0.29	XX
PA2	41	35	1	:	1.5	1.40	XX
PA3	16	14	1	:	2.4	2.56	XY[a]
PA4	18	15	1	:	2.7**	3.26	XY[a]
PA5	29	21	1	:	1.3	0.43	XX
PA6	27	24	1	:	0.6	1.50	XX
PA7	33	27	1	:	0.8	0.33	XX
PA8	19	14	1	:	2.5	2.56	XY[a]
PA9	10	8	1	:	3.1	2.00	XY[a]
PA10	17	13	1	:	2.3	1.92	XY[a]
PA11	40	31	1	:	1.2	0.14	XX
PA12	14	11	1	:	2.7**	2.27	XY[a]
PA13	29	17	1	:	1.1	0.06	XX
PA14	16	13	1	:	2.2	2.00	XY[a]
PA15	27	14	1	:	0.7	0.28	XX
PA16	31	21	1	:	1.1	0.04	XX

**These randomly selected males were used for further progeny testing (see Table 8).
[a] As in Table 5.

Table 7. Progeny testing of hormone-treated *P. reticulata* mated with normal XY male. Females designated as PB1-PB18 were randomly selected from the group (see Table 3) treated with 17 α-ethynylestradiol (200 mg·kg^{-1} food).

Female parent	Progeny produced (No.)	Progeny sexed (No.)	Sex ratio F	:	M	X^2	Genotype of female parent
PB1	13	11	1	:	2.6	2.27	XY[a]
PB2	16	10	1	:	2.3	2.00	XY[a]
PB3	24	16	1	:	0.6	1.00	XX
PB4	28	21	1	:	0.9	0.05	XX
PB5	33	29	1	:	1.6	1.68	XX
PB6	41	30	1	:	1.3	0.53	XX
PB7	39	29	1	:	1.4	0.43	XX
PB8	20	18	1	:	2.6***	3.55	XY[a]
PB9	29	22	1	:	0.8	0.18	XX
PB10	22	15	1	:	2.8	3.26	XY[a]
PB11	17	14	1	:	2.5	2.57	XY[a]
PB12	15	11	1	:	2.7	2.22	XY[a]
PB13	41	31	1	:	0.8	0.29	XX
PB14	36	29	1	:	1.4	0.86	XX
PB15	36	24	1	:	1.4	0.66	XX
PB16	27	19	1	:	0.9	0.05	XX
PB17	26	21	1	:	0.6	0.59	XX
PB18	19	15	1	:	2.7***	3.26	XY[a]

***These randomly selected males were used for further progeny testing (see Table 8).
[a] As in Table 5.

Table 8. Sex ratio of F_3 progenies obtained by test crossing F_2 males with normal females. F_2 males derived from sex-reversed XY females PC4 and PC14 (see Table 5), PA4 and PA12 (see Table 6), and PB10 (see Table 7) were randomly selected for progeny testing.

Male	Progeny produced (No.)	Sex distribution (no.) M	F	Genotype of male parent
PC4a	39	17	14	XY
PC4b	44	16	18	XY
PC4c	37	11	17	XX
PC4d	44	16	20	XY
PC4e	41	19	15	XY
PC14a	38	26	0	YY
PC14b	42	18	13	XY
PC14c	45	21	16	XY
PC14d	51	21	16	XY
PA4a	39	18	13	XX
PA4b	38	15	11	XY
PA12a	35	14	13	XY
PA12b	32	10	15	XY
PA12c	40	21	16	XY
PA12d	36	14	12	XY
PA12e	34	13	14	XY
PB18a	39	31	0	YY
PB18b	43	17	11	XY
PB18c	33	10	21	XY
PB18d	27	9	13	XY
PB18e	30	13	14	XY

PC4a-PC4e and PC14a-PC14d are sons of PC4 and PC14, respectively; PA4a-PA4b and PA12a-PA12e are sons of PA4 and PA12, respectively; PB18a-PB18e are sons of PB18.

Discussion

Our results demonstrate that the oral administration of estrogen to gravid *P. reticulata* can result in 100% sex reversal. The functional equality of the sex-reversed individuals was confirmed by back crossing them with normal males. Furthermore the critical doses of the four estrogens administered to ensure 100% feminization were ascertained. Among the four estrogens tested, 17 α-ethynylestradiol was most effective at the lowest dose (200 $mg \cdot kg^{-1}$ food), whereas 3-benzoate estradiol was not effective even at higher doses (600-800

$mg \cdot kg^{-1}$ food). It is difficult to determine precisely the period of sex differentiation as the brood interval may vary with different times of birth in the guppy as shown in Tables 1-4.

The sex distribution of progenies listed in Tables 5-7 confirms the efficacies of hormone treatments. Minor variations from the expected sex ratio of the progeny suggest that there are some genetic or environmental factors which have modifying influence on sex ratio. For instance, the sex-reversing potency of the selected hormone can be modified by temperature (e.g., in *Oreochromis* spp., Mair et al. 1987) and sex-reversing hormone carrying capacity of solvent used (e.g., in *O. mossambicus*, Varadaraj and Pandian 1991). It is also shown in the present study that the sex-reversed XY female guppies sired fewer fry owing to the considerably reduced viability of YY males. This may explain why the overall sex ratio of the progenies of the putative XY female was only in the ratio of 1F:2.6M instead of the expected ratio of 1F:3M. Yet the viability of two YY male guppies suggests that the sex chromosomes of *P. reticulata* are poorly differentiated as in the goldfish (Yamamoto 1975) and *O. niloticus* (Scott et al. 1989).

There are several published reports on the production of YY males in fish (Winge 1934; Yamamoto 1955, 1975; Scott et al. 1989). However, the survival of YY males has been shown to be very low in all these studies. For instance, a recessive lethal gene is demonstrated in *Lebistes* located in the Y chromosome containing the gene *Ma* (Winge and Ditlevsen 1938); and the YY males are viable only when the *Ma* gene exists in heterozygous ($Y^{ma}Y^{pa}$) condition. Yamamoto (1955) reported the rare occurrence of the YY *Oryzias latipes*; the actual ratio of XY to YY significantly deviated from the expected ratio of 2:1. Of 57 males individually tested by mating, only two proved to be viable YY males. A single YY *O. niloticus* male was identified in a fairly large population and the supermale sired all male offspring (Scott et al. 1989). A careful analysis of the mortality data of hormonally sex-reversed *P. reticulata* and fighting fish (*Betta splendens*) indicated a greater mortality among YY males and YY females than among XX males and XX females, respectively: this observation suggested that the presence of more than one Y chromosome renders the male or female less viable (Pandian and Kavumpurath, in press). The results presented in this paper demonstrate that YY male guppies were viable in fewer number; they sired 100% males when crossed with normal females.

Varadaraj and Pandian (1989) devised techniques to produce 100% males for mass production of YY males by integrating sex reversal and gynogenesis in *O. mossambicus*. However, gynogenesis cannot be achieved in the viviparous guppy. Endocrine sex reversal and progeny testing is a laborious technique to distinguish XY from YY males. There was no externally observable trait in YY males. The main problem is that of generating large numbers of YY males. If, however, a YY female could be produced, there exists the potential for the production of YY male broods in sufficient number. Indeed, it has been possible for us to produce one YY female guppy. One of the advantages of this system is that YY male and YY female can be kept together in the same pond to mass produce YY broodstock.

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References

Guerrero, R.D. 1975. Use of androgens for the production of all-male *Tilapia aurea*. Trans. Am. Fish. Soc. 104:342-348.

Guerrero, R.D. and W.L. Shelton. 1974. An aceto-carmine squash method for sexing juvenile fishes. Prog. Fish-Cult. 36:56.

Kallman, K.D. 1984. A new look at sex determination in *Poecilid* fishes, p. 95-590. *In* B.J. Turner (ed.) Evolutionary genetics of fishes. Plenum, New York.

Mair, G.C., D.J. Penman, A. Scott and D.O.F. Skibinski. 1987. Hormonal sex reversal and the mechanism of sex determination in *Oreochromis*, p. 301-302. *In* K. Tiews (ed.) Proceedings of the World Symposium on Selection, Hybridization and Genetic Engineering in Aquaculture, Heenemann Ver Lagsgesellschaft mbH, Berlin.

Pandian, T.J. and S. Kavumpurath. Y-chromosome dependent mortality in fishes. Curr. Sci. (In press).

Scott, A.G., D.J. Penman, J.A. Beardmore and D.O.F. Skibinski. 1989. The "YY" supermale in *Oreochromis niloticus* (L) and its potential in aquaculture. Aquaculture 28:237-251.

Takahashi, H. 1975. Masculinization of the gonad of juvenile guppy, *Poecilia reticulata*, induced by 11-ketotestosterone. Bull. Fac. Fish. Hokkaido Univ. 26:223-234.

Varadaraj, K. and T.J. Pandian. 1989. First report on production of supermale tilapia by integrating endocrine sex reversal with gynogenetic technic. Curr. Sci. 58:434-441.

Varadaraj, K. and T.J. Pandian. 1991. Effect of solubilizing 17 α-ethynyltestosterone in three different solvents on sex reversal of Mozambique tilapia. Prog. Fish-Cult. 53:67-71.

Winge, O. 1923. Crossing-over between the X- and the Y-chromosomes in *Lebistes*. J. Genet. 13:201.

Winge, O. 1930. On the occurrence of XX males in the *Lebistes*, with some remarks on Aida's so-called "Non-disjunctional" males in *Aplocheilus*. J. Genet. 23:69-76.

Winge, O. 1934. The experimental alteration of sex chromosome into autosome and vice versa, as illustrated by *Lebistes*. C.R. Trav. Lab. Carlsberg. Ser. Physiol. 21:1-49.

Winge, O. and E. Ditlevsen. 1938. A lethal gene in the Y chromosome of *Lebistes*. C.R. Trav. Lab. Carlsberg. Ser. Physiol. 22:203-210.

Yamamoto, T. 1955. Progeny of artificially induced reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY male. Genet. 40:406-419.

Yamamoto, T. 1964. The problem of viability of YY zygotes in the medaka, *Oryzias latipes*. Genet. 50:45-58.

Yamamoto, T. 1975. A YY goldfish from mating estrone-induced XY-female and normal male. J. Hered. 66:2-4.

Yamamoto, T. and N. Matsudha. 1963. Effects of estradiol stilbestrol and some alkylcarbonyl androsterones upon sex differentiation in the medaka, *Oryzias latipes*. Gen. Comp. Endocrinol. 3:101-110.

Yamazaki, F. 1983. Sex control and manipulation in fish. Aquaculture 33:329-354.

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