FIRST REPORT ON PRODUCTION OF SUPERMALE TILAPIA* BY INTEGRATING ENDOCRINE SEX REVERSAL WITH GYNOGENETIC TECHNIQUE†

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ABSTRACT

Supermale (YY−♂) *Oreochromis mossambicus* was produced for the first time by judiciously combining the endocrine sex reversal technique with selective breeding or gynogenetic technique. The combination of endocrine and gynogenetic techniques increased supermale production from 25 to 50% and reduced the time cost of production from 22 to 8 months. A programme for commercial production and maintenance of YY broodstock for large-scale generation of monosex male populations is presented.

INTRODUCTION

FOR a given unit of food energy, tilapias are known to produce the maximum protein with high quality flesh[1]. The major drawback in world-wide culture of tilapias is their ability to quickly overpopulate aquatic systems[2]. The most widely used technique to eliminate unwanted reproduction is the production of monosex populations. All-male populations are often preferred because of the faster growth rate exhibited by males. Monosex populations may be obtained by: (i) manual sexing of fingerlings and separating the sexes[3], (ii) hybridization[4], and (iii) sex-reversal by hormone treatment[5–7].

Manual sexing is laborious and requires some skill; the major disadvantages of this method are human error in sexing and the wastage of females[1]. Interspecific and intergeneric hybridizations are known to produce all-male progeny[6]. However, this technique has very limited scope for the following reasons: (i) difficulty in maintaining pure parental stocks that consistently produce 100% male offspring[8], (ii) poor spawning success[9], and (iii) incompatibility of breeders resulting in low fertility[10].

A monosex male population can be produced by feeding the fry with androgen-supplemented diet, which brings about sex reversal by interfering with the sex-determining mechanism in females. This is the most widely used technique but many workers have achieved only 90–98% males in a given population. They adopted a feeding regime in which some fry in the last peck of the hierarchy order do not receive the effective minimum hormone-treated diet to ensure complete sex reversal; in such individuals the gonadal differentiation proceeds only up to a point resulting in the production of intersexes or even females[11]. Although the hormonal sex reversal technique is effective, it is now not preferred for the following reasons: (i) inconsistency in producing a cent per cent monosex population, and (ii) consumer's concern for carcinogenicity and sex interference of the treated androgen and its residues. Whether it is manual sexing or endocrine sex reversing technique, one female inadvertently introduced into a pond of males can undo all the efforts undertaken to establish an all-male population.

An alternative technique for commercial production of all-male populations has been sought for a long time. Sperm from supermale (YY) fish could theoretically be used[12]. Using suitable combinations of endocrine sex reversal and selective breeding techniques Yamamoto[13] produced the first set of goldfish (*Carassius auratus*) supermales. Briefly, the technique combines the endocrine sex reversal of F_1 generation and selective breeding at F_2 to produce 25% supermales, which could be distinguished through progenies of F_3. Since its first description in 1975, this useful technique has not been extended to other species, including tilapias. This paper describes an alternative procedure: a combination of endocrine sex reversal in F_1 and chromosome manipulation in F_2 for producing 50% supermale tilapias,

*Patent pending.
†Dedicated to Prof. Otto Kinne (West Germany) on his 66th birthday.

which can easily be recognized at F_2 itself; hence it eliminates the need for distinguishing homogametic from heterogametic males of F_2 from the progenies of F_3.

MATERIALS AND METHODS

Rearing and breeding procedures for *O. mossambicus* used in the present study were essentially the

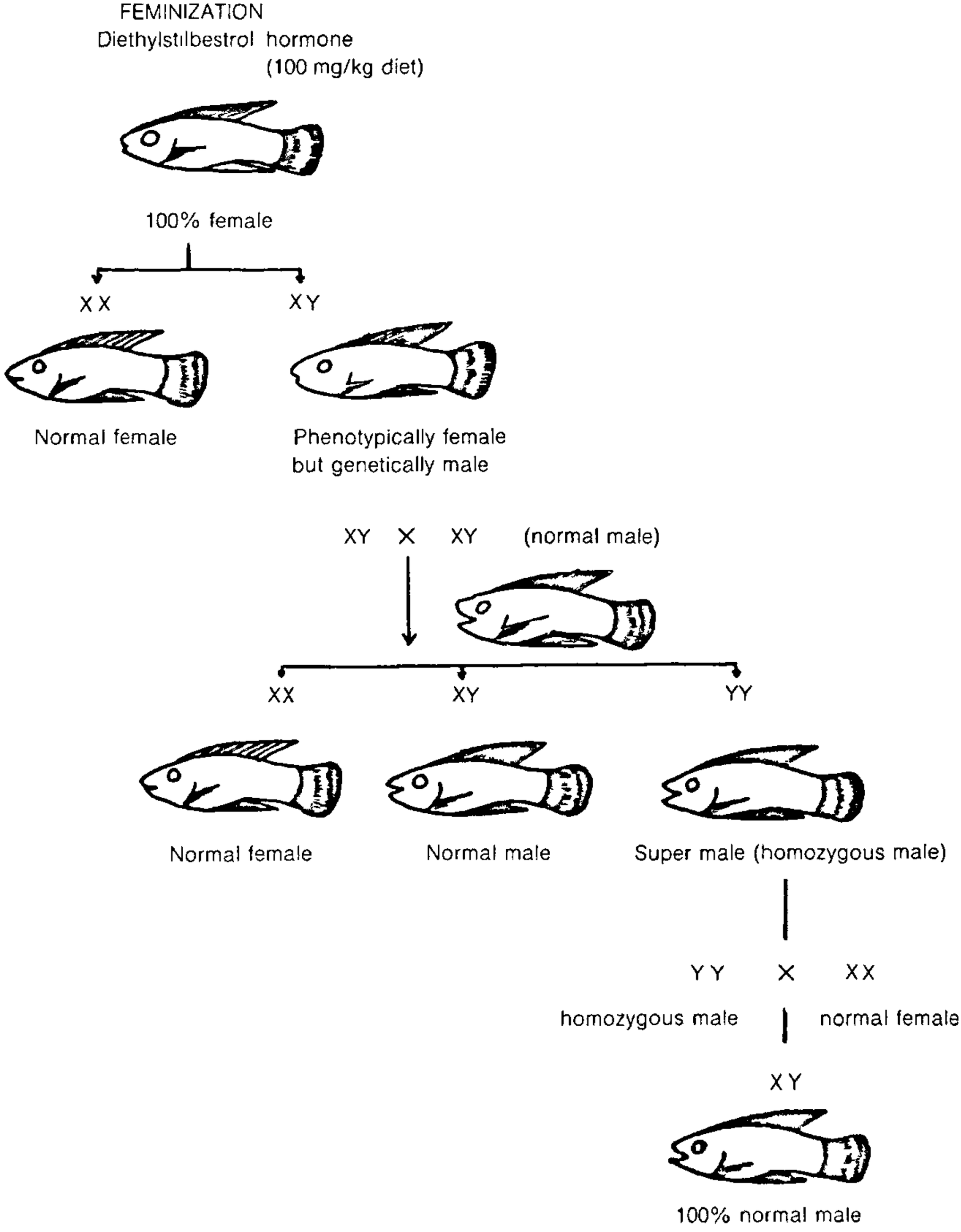

Figure 1. Schematic diagram showing supermale production by integrating endocrine and selective breeding techniques.

same as those used by Rothbard and Pruginin[14]. As sexually active tilapias are brightly coloured[5], the prospective parents in a given population and the possible time of spawning could be easily known. The published and newly described procedures for supermale production are described in figures 1 and 2. We have established that: (i) an *ad libitum* feeding of 9-day-old fry with diethylstilbestrol-supplemented diet (100 mg/g diet for 11 days) resulted in all-female population (F_1)[15], and (ii) a thermal shocking of

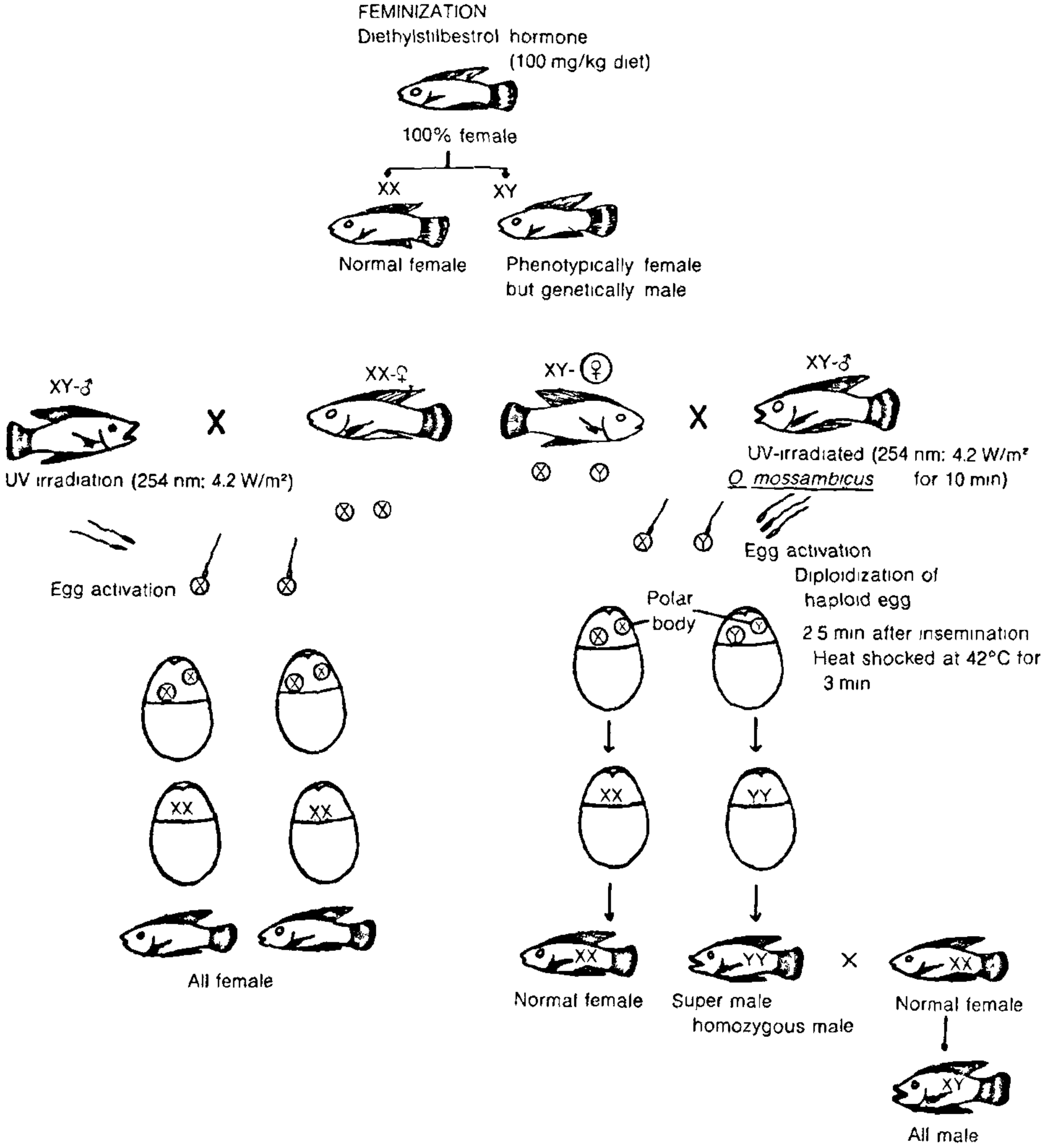

Figure 2. Schematic diagram showing supermale production by integrating endocrine and gynogenetic techniques.

2.5-min-old (post-insemination) eggs at 42°C for 3 min ensured the production of 100% gynogens (Varadaraj, unpublished results); these eggs were activated by irradiated sperm.

Experiment I

Sexually active pairs were separately bred and progenies (F_1) were subjected to hormone treatment for feminization. As heterogametic and homogametic females could not be externally distinguished, all F_1 progenies were separately reared and individually bred with suitable males. Using the sex ratio at F_2 generation, the prospective heterogametic females of F_2 generation were identified. The supermale and all

Table 1 *Results of progeny testing of sex-reversed females in Oreochromis mossambicus*

F_1 ♀♀	Mated with normal male	Progeny tested in 2 spawnings	Sex ratio ♀ : ♂	Total ♂♂	Genotype of tested F_1 ♀♀
1	1	126	0.25:0.75	95	XY
	2	193	0.26:0.74	144	
2	1	97	0.47:0.53	51	XX
	2	135	0.4:0.56	75	
3	1	162	0.24:0.76	123	XY
	2	199	0.27:0.73	146	
4	1	83	0.46:0.54	45	XX
	2	116	0.47:0.53	61	
5	1	168	0.22:0.77	131	XY
	2	150	0.23:0.77	116	
6	1	113	0.23:0.77	87	XY
	2	174	0.21:0.79	138	
7	1	88	0.44:0.56	49	XX
	2	123	0.50:0.50	62	
8	1	104	0.20:0.80	83	XY
	2	117	0.22:0.78	91	
9	1	66	0.44:0.56	37	XX
	2	82	0.40:0.60	49	
10	1	79	0.19:0.81	64	XY
	2	123	0.21:0.79	97	
11	1	56	0.27:0.73	41	XY
	2	81	0.21:0.79	64	
12	1	136	0.21:0.79	107	XY
	2	111	0.20:0.80	89	
13	1	94	0.46:0.54	51	XX
	2	158	0.47:0.53	84	
14	1	142	0.47:0.53	75	XX
	2	167	0.47:0.53	89	
15	1	179	0.30:0.70	126	XY
	2	192	0.21:0.79	151	
16	1	107	0.48:0.52	56	XX
	2	85	0.45:0.55	47	
17	1	61	0.43:0.57	35	XY
	2	88	0.44:0.56	49	
18	1	212	0.33:0.67	141	XY
	2	176	0.23:0.77	136	
19	1	94	0.18:0.82	77	XY
	2	121	0.21:0.79	96	
20	1	158	0.49:0.51	81	XX
	2	193	0.47:0.53	103	

other males of F_2 generation were individually bred and from the sex ratio obtained in F_3, all the supermales were identified (figure 1).

Experiment II

In the new procedure for supermale production through gynogenesis, five sex-reversed heterogametic females from experiment I were used. All the eggs obtained from the identified heterogametic females were subjected to chromosome manipulation to ensure the production of 100% diploid gynogens.

RESULTS

Among the diethylstilbestrol-treated females, 20 matured, sex-reversed females (F_1) were subjected to progeny testing by mating them individually with three normal males during August 1986. Of these, 9 individuals produced offspring (F_2) in the ratio of 1♀:1♂, indicating that their sex genotype was XX (table 1). The remaining 11 produced progenies (F_2) in the ratio of 1♀:3♂♂, suggesting that their genotype might have been XY. Hence, it is very likely that normal females of *O. mossambicus* are homogametic (AA XX−♀) and normal males heterogametic (AA XY−♂) in nature.

Ten F_2 males from F_1 (table 1, number 8) were crossed with normal females during March 1987 and the results are shown in table 2. Of these, seven F_2 males, when crossed with normal females, produced

Table 2 *Test crosses between F_2 males and normal females (XX−♀♀) for identifying supermales (YY−♂♂)*

F_2 ♂♂	Mated with normal ♀♀	Sex ratio ♂ : ♀	Genotype of tested F_2 ♂♂
1	1	0.55:0.45	XY
	2	0.60:0.40	
	3	0.57:0.43	
2	1	0.59:0.41	XY
	2	0.54:0.46	
	3	0.51:0.49	
3	1	1.00:0.00	YY
	2	1.00:0.00	
	3	1.00:0.00	
4	1	0.57:0.43	XY
	2	0.51:0.49	
	3	0.56:0.44	
5	1	1.00:0.00	YY
	2	1.00.0.00	
	3	1.00:0.00	
6	1	0.54:0.46	XY
	2	0.52:0.48	
	3	0.55:0.45	
7	1	1.00:0.00	YY
	2	1.00:0.00	
	3	1.00:0.00	
8	1	0.59·0.41	XY
	2	0.53:0.47	
	3	0.56:0.44	
9	1	0.49:0.51	XY
	2	0 53:0.47	
	3	0.54:0 46	
10	1	0.57:0 43	XY
	2	0.56:0.44	
	3	0.59:0.41	

F_2 males obtained from F_1 ♀ number 8 in table 1.

Table 3 *Sex ratio of diploid gynogens produced by F_1 females and their genotype*

F_1 ♀♀	Total offspring tested	Sex ratio of gynogens ♂ : ♀	Females (%)	Genotype of tested F_1 ♀♀
1	250	0.58:0 42	42	XY
2	194	0.54:0.46	46	XY
3	260	0.51:0 49	49	XY
4	198	0.52:0.48	48	XY
5	159	0.54:0.46	46	XY
6	223	0.00:1.00	100	XX
7	177	0.00:1.00	100	XX

Females 1–5 are sex-reversed females (XY ♀♀); 6 and 7 are normal females (XX ♀♀).

Table 4 *Test crosses between F_2 male gynogens (YY−♂♂) and normal females (XX−♀♀) for all-male production*

F_2 ♂ gynogens	Mated with normal ♀♀	Sex ratio ♂ : ♀	Genotype of tested F_2 ♂ gynogens
1	1	1:0	YY
	2	1:0	
	3	1:0	
2	1	1:0	YY
	2	1:0	
	3	1:0	
3	1	1:0	YY
	2	1:0	
	3	1:0	
4	1	1:0	YY
	2	1:0	
	3	1:0	
5	1	1:0	YY
	2	1:0	
	3	1:0	

F_2 males 1–3 obtained from F_1 ♀ number 1 in table 3; 4 and 5 from F_1 ♀ number 5 in table 3.

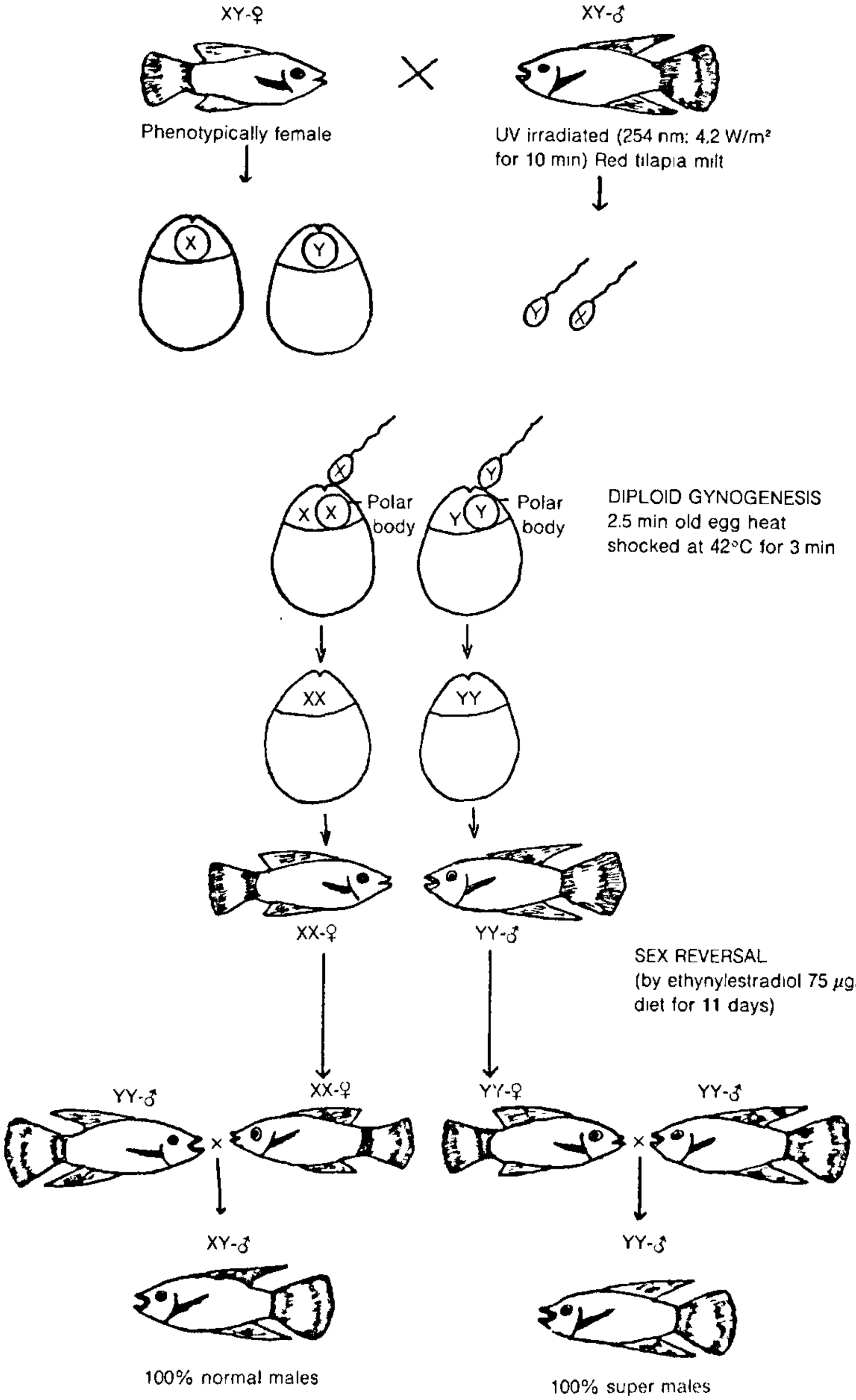

Figure 3. Schematic diagram showing method for consistent production of supermale tilapia.

males and females (F_3), indicating that they had XY sex chromosome complement; but the other three F_2 males produced only males (F_3), suggesting that they were supermales with genotype YY. The time required for the production and identification of supermales was 22 ± 3 months. This is perhaps the first report on the production of supermale (YY) tilapia; the procedure involves combination of endocrine and selective breeding techniques.

Five F_1 sex-reversed females (XY−♀) and two normal females (XX−♀) from the first experiment were subjected to gynogenesis during August 1987 and the results are shown in table 3. All the sex-reversed females produced 1 male : 1 female, whereas the two normal females produced all-female offspring. The male gynogens from XY females were crossed individually with a normal female (XX). Offspring produced from the male gynogens were all males, suggesting that the genotype of the male gynogens might be YY (table 4). The time required for this procedure is 8 ± 2 months only; the supermales can be easily recognized at F_2 itself and hence this procedure eliminates the need for distinguishing homogametic males from heterogametic males through the progenies of F_3. Unlike the procedure described by Yamamoto[13] and in the first half of this paper, this procedure involves a combination of endocrine and gynogenetic techniques.

DISCUSSION

The results presented in this experiment demonstrate the possibility of producing supermale (YY−♂) tilapia. The sperm from these YY males have been shown to produce monosex male (100%) populations when used to fertilize eggs from normal females. Incidentally, our observation of using supermales for all-male (monosex) culture confirms the previous reports (*Oryzias latipes*[16]; *Carassius auratus*[13]; Coho salmon[17]. This is perhaps the first report for tilapias.

The second method for YY male production, through gynogenesis (combining endocrine and chromosome manipulation techniques), is an alternative for all-male tilapia production that allows one to avoid the time-consuming procedure of progeny rearing and pedigree analysis of treated fish at sexual maturity. This technique effectively reduces the time cost of producing YY males from 22 ± 3 to 8 ± 2 months and increases the scope for producing supermales from 25 to 50% at F_2. Thus our results for supermale production will help augment commercial production of all-male tilapias.

Figure 3 is a flow chart of a general plan for mass production of all-male populations and supermales in heterogametic male tilapia species. The present technique has a problem, i.e. the need for a large number of YY males as broodstock in the commercial tilapia production system. In an attempt to produce consistent brood population of super-male tilapia, we have now devised the procedure illustrated in figure 3*. It involves the endocrine sex reversal of YY males to YY phenotypic females. When such YY females are produced, it may be possible for the development and maintenance of broodstock consisting of YY−♂♂ and YY−♀♀, and also for mass culture of YY−♂♂ and all-male (XY−♂♂) populations. We are currently working on this theoretical possibility.

ACKNOWLEDGEMENTS

The authors are thankful to Dr J. Muthukrishnan for a perusal of the manuscript, and to UGC-DBT-CSIR for financial assistance.

13 February 1989

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NEWS

THE IAEA AND FOOD IRRADIATION

Food irradiation is only one of many fields in which the IAEA assists its 113 Member States to apply nuclear energy and related applications for their social, industrial, and economic development. All told, the IAEA is supporting more than 900 projects at the request of its Member States through which expert technical assistance, training, and equipment are provided in nuclear power, nuclear medicine, nuclear physics, and other fields. It further supports nearly 500 research contracts with scientific institutes that co-ordinate global research in specialized areas ranging from plant breeding to isotope hydrology.

In the field of food irradiation, a range of national and regional research and training programmes are supported through the Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agricultural Development.

Global research and training in food irradiation is further supported by the International Facility for Food Irradiation Technology (IFFIT), based in the Netherlands and sponsored by the FAO, IAEA, and the Dutch Ministry of Agriculture and Fisheries. More than 200 food scientists and other specialists from 40 countries have taken part in IFFIT training courses over the past 8 years.

Formed in 1964, the Joint FAO/IAEA Division assists and advises Member States interested in radiation processing of food and related products. Assistance is geared toward technical, economic, and technological aspects of food irradiation. It includes, for example, support for feasibility studies of irradiation facilities, training seminars on good manufacturing practices, and conferences to promote technical information exchange and acceptance of international standards and codes.

The latest conference, convened in Geneva during 12–16 December 1988, was organized in response to food irradiation's growing acceptance by national regulatory authorities and the need to reach global consensus on important aspects of trade and consumer acceptance. Entitled the International Conference on the Acceptance, Control of, and Trade in Irradiated Foods, it was co-sponsored by the FAO, IAEA, WHO, and International Trade Centre (ITC), which is jointly operated by the United Nations Conference on Trade and Development (UNCTAD) and the General Agreement on Tariffs and Trade (GATT). Delegates from about 80 countries registered, including senior officials in areas of legislation, regulation, health, food production, trade, consumer affairs, and food industries. Proceedings of the Conference will be published by the IAEA in 1989. (IAEA News Features, No. 5, December 1988, p. 12; Published by IAEA Division of Public Information, P.O. Box 100, A-1400 Vienna, Austria.)