

PROGENY OF ARTIFICIALLY INDUCED SEX-REVERSALS OF MALE
GENOTYPE (XY) IN THE MEDAKA (*ORYZIAS LATIPES*)
WITH SPECIAL REFERENCE TO YY-MALE

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THE genetic mechanism for sex determination in the medaka (*Oryzias latipes*), where the female is homogametic (XX) and the male heterogametic (XY), was established by AIDA (1921). There are a number of color varieties in the medaka. According to AIDA (1921) the various color effects are referred to the action of genes, as indicated by the following formulae when homozygous: brown (wild) *BBRR*, orange-red (called red) *bbRR*, white *bbrr*, variegated orange-red *B'B'RR*, and variegated white *B'B'rr*. The multiple alleles *B*, *B'* and *b* control melanin formation and are autosomal. The alleles *R* and *r* control the formation of orange-red pigment and are linked to the X or Y chromosomes or both.

Since both white (*bbrr*) and red (*bbRR* or *bbRr*) medakas have common recessive genes *bb*, their phenotypes (*br* and *bR*) may be expressed simply as *r* and *R*, respectively, when reference is made to these two varieties only.

When a white female (X^rX^r) is crossed with a heterozygous red male (X^rY^R), the offspring are white females and red males in equal numbers because in this mating system the dominant red gene *R* exists only in the Y chromosome. The father-to-son inheritance of the red condition *R*, was confirmed in my breeding experiments in three consecutive generations by crossing white females (X^rX^r) with heterozygous red males (X^rY^R). Therefore the sex-linked characters *R* and *r* can be used as sex-chromosomal "markers". Previous study has established that the oral administration of estrogens (estrone or stilbestrol) at the time of hatching and for several months thereafter induced complete sex-reversal in the genotypic male (X^rY^R).

All the red (*R*) fish developed into perfect females, and when fully grown these sex-reversals have the functional sex-equipment and reproductive capacity of the female. It was concluded that complete and functional sex-reversal from male to female can be artificially induced under the influence of estrogens (YAMAMOTO 1953).

A genetic study on the offspring of sex-reversals of the male genotype affords us further proof of the genetic constitution of sex-reversals. When sex-reversed XY females are mated with normal XY males, their offspring should be XX females, normal XY males and YY males in the 1:2:1 ratio if YY males are viable. Can YY males be found among F_1 offspring of induced sex-reversals of the male genotype? If this question is answered in the affirmative, we are then provided with further proof that we are correct in the conclusion that sex-reversals induced by estrogens had the sex genotype of the male (XY).

MATERIALS AND METHODS

Genetic analyses were made of two fully grown red (X^rY^R) females, E8 and E9 showing sex-reversal under the action of estrone and two fully grown red (X^rY^R) females, S8 and S9 showing sex-reversal through the influence of stilbestrol, designated as estrone-sex-reversed females and stilbestrol-sex-reversed females, respectively. The two estrone-sex-reversed females were mated with two normal red males (X^rY^R) and the two stilbestrol-sex-reversed females were mated with two normal red males (X^rY^R). The F_1 offspring of sex-reversals were tested by fish of known genotypes. Fish used as testers were derived from consecutive backcrossings between white females (X^rX^r) and heterozygous red males (X^rY^R) and were fifth and sixth generation. These generations are designated as FR_5 and FR_6 (R = backcross to recessive), respectively. Results of progeny testing were examined when fish reached maturity or an age at which the sex could be determined by external sexual characteristics. External determination of the two sexes is easily possible by their secondary sexual characteristics (*cf.* OKA 1931, OKADA and YAMASHITA 1944, YAMAMOTO 1953).

As the medaka is an oviparous cyprinodont, fertilization and development are normally external. In the breeding season, a mature female may produce a batch of eggs daily for a considerable period of time. That internal fertilization may take place in rare cases and that development in the ovarian cavity of the parent may proceed in some degree, was pointed out by AMEMIYA and MURAYAMA (1931). In the present study every care was taken to guard against this possibility when previously mated females were used. The first hatched larvae were usually discarded to avoid a possible mixture of zygotes in which some would have been previously fertilized by an unknown father.

Crossings were carried out indoors in glass jars which contained water hyacinths (*Eichhornia crassipes*). Their long, dense roots are admirable as egg receivers. Shortly before hatching time, water hyacinths, with eggs attached, were removed from the jar-aquaria to outdoor concrete pools measuring 75 cm. square and 18 cm. deep, remaining there until they attained maturity. The fry were given a standard dried food composed of the following ingredients: 60 g. powdered shrimp, 30 g. toasted wheat grains pounded to flour (Japanese "kosen"), 6 g. yeast preparation ("wakamoto") and 4 g. powdered green tea ("macha"). From the young to the adult stage their diet consisted chiefly of a mixture of powdered shrimp and toasted wheat-flour (kosen) to which we occasionally added water fleas (*Moina macrocopa*) and tubificiid worms (*Limnodrilus* and others).

All of our body length measurements were made on fish in an anesthetized state and were taken from the tip of the snout to the end of the caudal fin.

 F_1 OFFSPRING OF ESTRONIZED AND STILBESTROLIZED FEMALES OF FEMALE GENOTYPE

Before proceeding further, a brief account is given of the F_1 offspring of estrogen-administered genotypic females (X^rX^r). These white females were mated with FR_4 red males of the genotype X^rY^R . As noted in the previous paper, they were proved to be fertile. Three estronized white females (X^rX^r) in mating with normal red

males (X^rY^R) produced 60 white (r) daughters, 76 red (R) sons and one exceptional red (R) daughter. This exceptional daughter was tested by crossing with a red male (X^rY^R) but the offspring failed to survive. A stilbestrolized white female (X^rX^r) mated to a normal red male (X^rY^R), produced a few offspring, of which only two white (r) daughters matured.

F₁ OFFSPRING OF INDUCED SEX-REVERSALS

The F₁ offspring produced by mating sex-reversed red (X^rY^R) females with normal heterozygous red (X^rY^R) males were fully grown by the summer of 1952. Based upon the theoretical diagram of the F₁ offspring of sex-reversals, as shown in figure 1, we could expect that phenotypically the F₁ offspring would include white (r) females and red (R) males in the ratio of 1:3, and genotypically white females (X^rX^r), ordinary red males (X^rY^R) and extraordinary red males (Y^RY^R) in the ratio of 1:2:1, if YY zygotes could develop into mature males. Observed F₁ offspring of estrone-sex-reversals and of stilbestrol-sex-reversals are shown in table 1. Out of 74 F₁ offspring of estrone-sex reversals, 21 were white daughters and 51 were red sons with one exceptional white son and one exceptional red daughter. Observed ratio of white females to red males was 1:2.4 while theoretically it is 1:3. Out of 177 F₁ offspring of stilbestrol-sex-reversed females 54 were white daughters and 119 were red sons with three exceptional white sons and one exceptional red daughter. Observed ratio of white females to red males was 1:2.2 instead of the theoretical 1:3 ratio.

By a preponderance of males, the conclusion set forth in the previous paper (YAMAMOTO 1953) is verified, i.e., that sex-reversals must have the genetic constitution X^rY^R . If they had the genetic constitution X^rX^R , as the result of either crossing over between the X^r and Y^R chromosomes in their male parent or mutation from X^r to X^R , the sex-ratio of the F₁ offspring would be 1:1 and white females ($r♀$), red females ($R♀$) and red males ($R♂$) would be produced in the ratio of 1:1:2. The observed ratio (table 1) definitely differs from the assumption that they have the

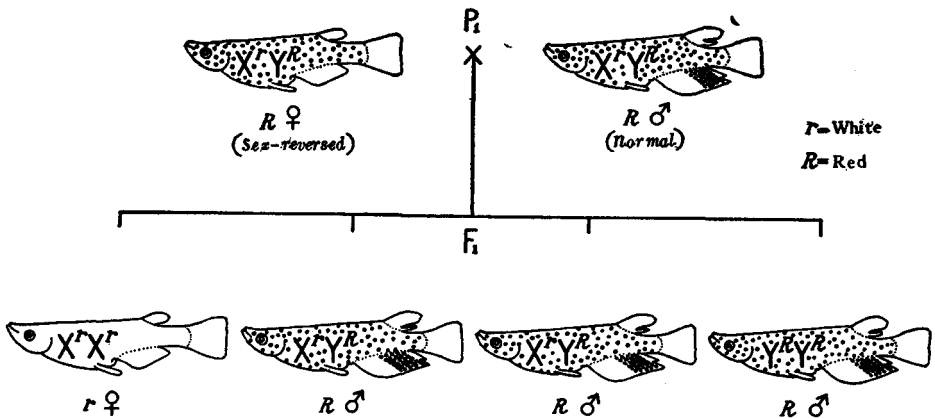


FIGURE 1. Theoretical diagram of F₁ offspring produced by mating a sex-reversed female of the male genotype (X^rY^R) with a normal male (X^rY^R).

TABLE 1

F₁ offspring from estrone- and stilbestrol-treated red females of male genotype (X^rY^R) mated with FR₄ normal red males (X^rY^R)

	Estrone treated		Stilbestrol treated	
	Observed	Expected	Observed	Expected
White females	21	18.5	54	44.3
White males	1	0	3	0
Red females	1	0	1	0
Red males	51	55.5	119	132.7
Total	74	74.0	177	177.0

genetic constitution X^rX^R but it coincides with the assumption that they have the genetic constitution X^rY^R if we assume that Y^RY^R zygotes were fewer in number than the expected value.

GENETIC ANALYSIS OF EXCEPTIONAL WHITE MALES AND EXCEPTIONAL RED FEMALES AMONG F₁ OFFSPRING OF SEX-REVERSALS OF MALE GENOTYPE

Genetic analyses of exceptional white sons and exceptional red daughters are given before passing on to the results of progeny tests of F₁ red sons. One exceptional white son (labeled No. 74) and one exceptional red daughter (No. 73) were yielded among F₁ offspring of estrone-sex-reversed females (table 1). Three exceptional white sons (No. 174, 175, 176) and one exceptional red daughter (No. 177) appeared among F₁ offspring of stilbestrol-sex-reversed females (table 1).

There are three possibilities for the production of exceptional white sons and exceptional red daughters. The first possibility is that they may be the result of crossing over between the X^r and Y^R chromosomes, which produce the X^R and Y^r gametes. If fertilization occurs between the X^R and X^r gametes, or between the Y^r and X^r gametes, both a red daughter (X^rX^R) and a white son (X^rY^r) are produced. A second possibility is that these exceptional fish may be the product of mutation of genes, X^r to X^R or Y^R to Y^r. The third possibility is that an exceptional white son may be a spontaneous sex-reversal of the female genotype (X^rX^r) and an exceptional red daughter may be a spontaneous sex-reversal of the male genotype (X^rY^R).

Progeny tests of exceptionals were explored to determine which possibility is correct. The results are summarized in table 2. An exceptional white son, F₁(E)r No. 74, among F₁ offspring of estrone-sex-reversals was mated with white females of the genetic constitution X^rX^r, with the result that white females and white males were approximately equal numbers among the progeny. If the genetic constitution of F₁(E)r No. 74 had been X^rX^r, an all-female broods could have been expected. The result indicates that the exceptional white son has the genetic constitution X^rY^r. An exceptional red daughter, F₁(E)R No. 73, among F₁ offspring of sex-reversals was mated with a heterozygous red male, FR₅ R♂, pedigreed genetic constitution of which was X^rY^R. Among the offspring of this cross, white females, red females and red males in the 1:1:2 ratio were obtained. Had the exceptional female been a

TABLE 2

Progeny tests of exceptional white (r) males and exceptional red (R) females among F₁ offspring of sex-reversed X^rY^R females. r = white, R = red

Expt. No.	Parents		Offspring				Genotype of exceptional fish
	♀	♂	r♀	r♂	R♀	R♂	
51	r(X ^r X ^r)	F ₁ (E)r No. 74	20	14	0	0	X ^r Y ^r
52	F ₁ (E)R No. 73	FR ₅ R(X ^r Y ^R)	6	0	7	11	X ^r X ^R
151	F ₁ (S)R No. 177	FR ₅ R(X ^r Y ^R)	—	—	—	—	—
152	r(X ^r X ^r)	F ₁ (S)r No. 174	17	22	0	0	X ^r Y ^r
153	r(X ^r X ^r)	F ₁ (S)r No. 175	9	8	0	0	X ^r Y ^r
154	r(X ^r X ^r)	F ₁ (S)r No. 176	65	72	0	0	X ^r Y ^r

spontaneous sex-reversal of the male genotype X^rY^R, then white females and red males in the 1:3 ratio could have been expected and no red females would have occurred. The result indicates that the genetic constitution of the exceptional red daughter was X^rX^R, and that white females (X^rX^r), red females (X^rX^R) and red males (X^rY^R and X^RY^R) in the ratio of 1:1:2 were produced.

To test the genotype of an exceptional red daughter, F₁(S)R No. 177, among F₁ offspring of stilbestrol-sex-reversals of the male genotype, she was mated with a heterozygous red male (FR₅ R♂) of the genetic constitution X^rY^R. Unfortunately she died before she could produce offspring. Exceptional white sons, F₁(S)r No. 174, F₁(S)r No. 175 and F₁(S)r No. 176 among F₁ offspring of stilbestrol-sex-reversals of the male genotype were singly mated with white females, FR₅ r♀, of the constitution X^rX^r. Their offspring were equally white females, and white males. If the exceptional white sons had been spontaneous sex-reversals of the female genotype (X^rX^r), an all-female broods could have been expected. Progeny tests proved that the exceptional white sons had the genetic constitution X^rY^r.

These results indicate that these exceptionals among F₁ offspring of sex-reversals of the male genotype had the genetic constitution either X^rY^r or X^rX^R. This may have resulted from either crossing over between the X^r and Y^R chromosomes or mutations of genes, X^r to X^R and Y^R to Y^r. Judging from the frequency of exceptionals the first alternative appears the most plausible.

PROGENY TESTS OF RED SONS OF SEX-REVERSALS OF MALE GENOTYPE

Table 1 indicates that 51 red sons were produced among F₁ offspring of estrone-sex-reversals and 119 red sons were produced among F₁ offspring of stilbestrol-sex-reversals. Since both parents have the genetic constitution X^rY^R, it might be expected that the ratio of white daughters and red sons would be 1:3, and also that red sons would include X^rY^R and Y^RY^R males in the 2:1 ratio if Y^RY^R zygotes are able to develop to maturity. As mentioned before, the observed ratio of white females to red males was 1:2.4 among F₁ offspring of estrone-sex-reversed females and 1:2.2 among F₁ offspring of stilbestrol-sex-reversed females instead of the theoretical 1:3. This discrepancy suggests the rarity of Y^RY^R males. Since the two genotypes of red males, X^rY^R and Y^RY^R, can not be identified phenotypically,

TABLE 3

Progeny tests of F₁ red sons of estrone- and stilbestrol-sex-reversed females of male genotype (X^rY^R). Sons of estrone-reversed females are designated by F₁(E)R; of stilbestrol-reversed females by F₁(S)R. The total offspring of all matings that yielded offspring of both sexes are pooled

Number of matings	Parents		Offspring				Genotype of ♂ parent
	♀	♂	r♀	r♂	R♀	R♂	
19	r(X ^r X ^r)	F ₁ (E)R	360	1	4	420	X ^r Y ^R
1	r(X ^r X ^r)	F ₁ (E)R	0	0	0	72	Y ^R Y ^R
21	r(X ^r X ^r)	F ₁ (S)R	404	0	12	410	X ^r Y ^R

progeny tests were performed to determine their genotypes. If a red son of the genotype X^rY^R is mated to white females (X^rX^r), 50 percent of his offspring would be white females and 50 percent would be red males. If a red son of the genotype Y^RY^R is mated to white females (X^rX^r), all of his offspring would be red males.

Red sons produced by mating estrone-sex-reversed females with normal red males were designated as F₁(E)R♂ and red sons from matings of stilbestrol-sex-reversed females and normal red males were labeled F₁(S)R♂. Twenty-two F₁(E)R males and twenty-eight F₁(S)R males were chosen at random and submitted to progeny tests by crossing with white females (X^rX^r). Of twenty-two F₁(E)R males tested two died before producing offspring. Of twenty-eight F₁(S)R males tested seven died before yielding offspring. Therefore, only twenty F₁(E)R males and twenty-one F₁(S)R males successfully produced offspring which belonged to the F₂ generation of sex-reversals.

When the offspring were fully grown, they were examined as to sex and phenotypes. The results are shown in table 3. Table 3 indicates that with one exception F₁(E)R males in crosses with white females (X^rX^r) produced white females and red males in approximately equal numbers, indicating that the genotype of the male parent was X^rY^R. One test, however, yielded 72 mature offspring, all of which were red males. This clearly shows that the genotype of this male parent, which was a red son of an estrone-sex-reversed mother was Y^RY^R and the genotype of his offspring was presumably X^rY^R. As is also shown in table 3, all twenty-one F₁(S)R males tested produced white females and red males. All produced nearly equal numbers except one which produced 8 females and 23 males. The genotype of male parents tested must be X^rY^R. No Y^RY^R male was detected among red sons of stilbestrol-sex-reversals. Since only twenty-one out of 119 red sons were successfully submitted to test mating, there remains a possibility that some Y^RY^R males may be among non-tested red sons. Judging from the ratio of 1 white female to 2.2 red males and not of 1:3 among F₁ offspring of the stilbestrol-sex-reversals (table 1), it is expected that Y^RY^R males would be but few among F₁(S)R males.

PROGENY TESTS OF EXCEPTIONALS AMONG F₂ OFFSPRING OF SEX-REVERSALS

A few exceptional red females and one exceptional white male were produced in progeny tests of the red sons of induced sex-reversals (table 3). In the summer of 1953 fifteen exceptionals were tested to determine their genotypes. Exceptional red

TABLE 4

Progeny tests of exceptional red females and an exceptional white male among F₂ offspring of sex-reversals of the male genotype (cf. table 3). r = white, R = red

Expt. No.	Parents		Offspring				Genotype of except. fish
	♀	♂	r♀	r♂	R♀	R♂	
Ex-2	R(104-ex-1)	R(X ^r Y ^R)	59	0	2	127	X ^r Y ^R
Ex-3	R(104-ex-2)	R(X ^r Y ^R)	34	1	0	57	X ^r Y ^R
Ex-6	R(105-ex-1)	R(X ^r Y ^R)	9	0	7	13	X ^r X ^R
Ex-15	r(X ^r X ^r)	r(14-ex-1)	28	33	0	0	X ^r Y ^r

females were singly tested by crossing with red males (X^rY^R) and an exceptional white male was tested by mating with a white female (X^rX^r). Eleven of the fifteen tested either failed to produce mature offspring or those obtained were not sufficient to determine their genotypes. Only four exceptionals produced a sufficient number of offspring to decide their genetic constitutions, the result of which are summarized in table 4.

Two exceptional red females R(104-ex-1) and R(104-ex-2) singly mated to X^rY^R males produced white females and red males in the 1:2 ratio with two exceptional red females and one exceptional white male. These two exceptional red females which were tested might be spontaneous sex-reversals of the male genotype (X^rY^R). If they had the genetic constitution X^rY^R and were mated to X^rY^R males, the expected offspring would be phenotypically white females (r♀) and red males (R♂) in the 1:3 ratio, and genotypically white females (X^rX^r), ordinary red males (X^rY^R) and extraordinary red males (Y^RY^R) in the ratio of 1:2:1. The discrepancy between expected 1:3 and observed 1:2 ratios may be attributed to the rarity of Y^RY^R males.

One exceptional red female R(105-ex-1) among F₂ offspring of induced sex-reversals in mating with a red male (X^rY^R) produced white females, red females and red males in the ratio of approximately 1:1:2, showing she was of the genetic constitution X^rX^R as a result of crossing over. One exceptional white male r(14-ex-1) among F₂ offspring of induced sex-reversals mated with white females (X^rX^r), fathered white females and white males in the 1:1 ratio indicating that he was of the genetic constitution X^rY^r. This exceptional white male is accounted for by crossing over in the sex chromosomes of the male parent.

Table 3 indicates a preponderance of exceptional red females over exceptional white males. This is attributed to the fact that exceptional red females among F₂ offspring of induced sex-reversals include both X^rX^R and X^rY^R females resulting from crossing over and spontaneous sex-reversal of the male genotype, respectively.

F₃ AND F₄ OFFSPRING OF INDUCED SEX-REVERSALS

The offspring of exceptionals among F₂ progeny of induced sex-reversals have already been described (table 4). These offspring belong to the F₃ generation of sex-reversals.

In the summer of 1953, using F₂ offspring of induced sex-reversals, breeding experiments were also performed with normal white females (X^rX^r) and normal red males

(X^rY^R). F_2 white females and F_2 red males of estrone-sex-reversals were inbred. The same breeding experiment was performed with the F_2 offspring of stilbestrol-sex-reversals. Matings of white females (X^rX^r) and red males (X^rY^R) among F_2 offspring of estrone-sex-reversals produced 17 white females and 28 red males. Crossings of white females (X^rX^r) and red males (X^rY^R) among F_2 offspring of stilbestrol-sex-reversals yielded 13 white females and 17 red males. These F_3 white females and red males matured by the summer of 1954 and they produced a vast number of young which are thriving normally in our outdoor concrete pools.

SECONDARY SEXUAL CHARACTERISTICS IN YY MALE

As previously mentioned, a single YY male, $F_1(E)R$ No. 19, was detected among F_1 offspring produced by mating estrone-sex-reversals of the male genotype (X^rY^R) with normal red males (X^rY^R). This male, after it had produced a considerable number of the offspring in cross with white females (X^rX^r) and before it was proved to be YY male by its unisexual male progeny, was fixed with formalin. Figure 2 is a photograph of this male. Its general physical characteristics were similar to those of ordinary (XY) males. Secondary characteristics of the YY male were studied. The most prominent external sexual characteristic *inter alia* in the male of the medaka is the presence of numerous numbers of papillar processes on the anal fin, according to OKA (1931). Papillar processes first appear when the male reaches a length of 22–26 mm (caudal fin included) and they increase in number with age. Usually they are present in pairs on segments of several posterior anal fin-rays. NAGATA (1934, 1936) and OKADA and YAMASHITA (1944) experimentally proved that they are manifestations of the male hormone and OKADA (1952) pointed out that they can be used as a quantitative determination of androgens. Therefore, the total number of papillar processes on the anal fin may be regarded as an outward sign of functional activity of the testis. Counts were made of both numbers of anal fin-rays which carry the processes and the total number of the processes in the YY male and in the ordinary (XY) males, the genotype of which have been determined

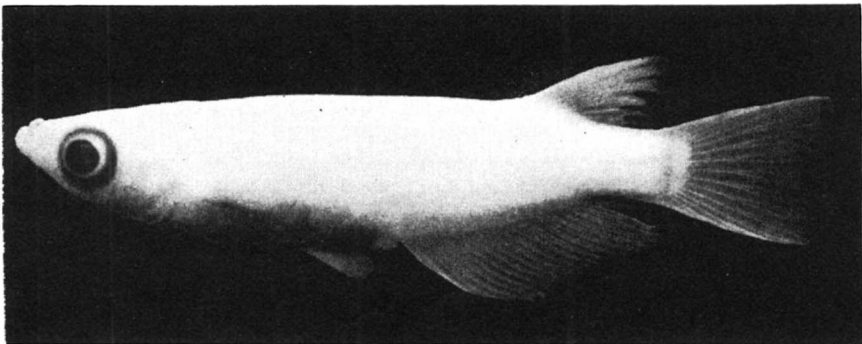


FIGURE 2. A YY male of the medaka (*Oryzias latipes*). An orange-red son, $F_1(E)R$ ♂ No. 19, detected among F_1 offspring produced by mating estrone-sex-reversed females of the male genotype (X^rY^R) with normal heterozygous orange-red males (X^rY^R). This $Y^R Y^R$ male in cross with white females (X^rX^r) fathered all-male broods, a total of 72 orange-red sons.

TABLE 5

Papillar processes (P.P.) on anal fin-rays of normal (XY) males and the YY male. Figures in the first row are average values. Figures in parentheses are ranges of variability. s = Standard deviation. V_c = Coefficient of variability

Genotype of males	Number of fish	Body length in mm.	Number of anal fin-rays	Numb. of P.P.-bearing anal fin-rays	Total numb. of P.P.
X ^r Y ^R	32	36.6 ± 0.3 (31.5 - 42.0) s = ±2.5 V _c = 6.7	20 ± 0.2 (18 - 23) s = ±1.1 V _c = 5.5	8.7 ± 0.1 (6 - 10) s = ±1.0 V _c = 11.5	280 ± 5 (205 - 357) s = ±38 V _c = 13.6
Y ^R Y ^R	1	36.5	20	10	330

from their progenies. The results are tabulated in table 5. There were more papillar processes in the YY male than the average value plus standard deviation of those in XY males. Although there were some XY males which carry more numerous papillar processes, he was a decidedly masculine male. Since this YY male produced very many offspring, his sexual behavior was also supposed to be vigorous.

DISCUSSION

Fish geneticists have often found spontaneous sex-reversals in their breeding experiments. In certain varieties of the guppy (*Lebistes reticulatus*) where the female is homogametic (XX) and the male heterogametic (XY), WINGE (1930) found XX males. By various systems of selective mating, WINGE (1934) obtained XY females and XX males. He was able to produce YY males through mating exceptional XY females to normal XY males.

Among "domesticated" platyfish (*Platypoecilus maculatus*) where the female is heterogametic (WZ) and the male homogametic (ZZ), BREIDER (1942) found an exceptional male of the female genotype (WZ). The mating of this exceptional WZ male to a normal (WZ) female yielded the offspring in the ratio of 1WW:2WZ:1ZZ, or three females to one male. The WW females were identified by their phenotypes. Unfortunately, however, the genetical behavior of the WW females was not analyzed. If WW females had been mated with ordinary ZZ males, all of their offspring would necessarily have been females. BELLAMY and QUEAL (1951) described a series of mating of the platyfish (WZ-ZZ type). They found exceptional males with reference to their phenotypes. Progeny testings of these exceptionals indicated that some exceptional males were ZZ males produced by crossing over of color genes while the others were either sterile or spontaneously sex-reversed males of the female genotype (WZ). Matings of these exceptional WZ males to normal females (WZ) produced the sex ratio of 3 females to 1 male as in BREIDER's study. About one third of the females when mated with normal male (ZZ) breed as though they were WW and mothered all-female broods. GORDON (1946a, 1946b, 1947) reported a case of spontaneous sex-reversal from female to male direction in "wild" platyfish from the Rio Jamapa (Mexico) population, the genetic constitution of which was XX for female and XY for male. GORDON found an exceptional male of the female genotype (XX). When this

exceptional XX male was mated to a normal "wild" XX female, all their offspring were females. Spontaneous sex-reversal in the platyfish from male to female direction has not yet been found.

AIDA (1936) reported spontaneous sex-reversals in the medaka, *Oryzias (Aplocheilus) latipes*, where the female is XX and the male XY. In his strain he found a number of exceptional males of the female genotype (XX). According to his opinion this was due to the low potency of the X chromosome caused by many years' close inbreeding. He also reported two cases of spontaneously sex-reversed females of the male genotype (XY). When these exceptional XY females were mated with normal males (XY), they produced the offspring in the sex ratio of 1 female to 3 males. One out of the three males tested was found to be YY male which sired all-male broods. He reported the rarity of spontaneous sex-reversal from male to female direction in his strain.

Accidental hermaphrodites are frequently found in adult frogs. CREW (1921) described a case in which a female frog (*Rana temporaria*) was assumed to have undergone a process of masculinization transforming into a hermaphrodite with predominant male characteristics. This frog in mating with a normal female functioned as a male and sired an all-female brood, as would be expected if it was a genetic female of the constitution XX. WITSCHI (1923) reported a sporadic hermaphrodite in the same form which developed both functional sperm and ova and produced offspring.

Among the vast numbers of sex-reversals known in birds, only one sporadic case of functional sex-inversion has been reported. CREW (1923) described this case of accidental sex-reversal in which a hen became a cock and fathered two chickens. A post-mortem examination of the abnormal fowl revealed that the ovary had been completely destroyed by tuberculosis. This situation has not been duplicated by experimental means. Sex-reversals in fowls induced by sinistral ovariectomy, rarely exhibit spermatogenesis in the right gonad. DOMM (1930) attempted a breeding experiment with such an inverted ovariectomized fowl but no fertility was found.

The cases before mentioned of functional sex-inversion in fish and frogs are spontaneous sex-reversals in normal breeding experiments and not the result of artificial induction. Despite the studies of many investigators on experimental control of sex differentiation in a series of vertebrates, few breeding experiments with sex-reversals thus produced have been reported. Hence, whether or not sex-reversals have the capacity of the opposite of their genetically determined sex has rarely been verified. A survey of the literature on this subject revealed that only two cases of artificially induced sex-reversals producing offspring, have hitherto been reported. These sex-reversals were induced by means of castration or by implantation of gonad-preprimordium in amphibians. HARMS (1926) and PONSE (1925, 1926, 1931) showed that in castrated toads (*Bufo vulgaris*) Bidder's organs were converted into functional ovaries and that sex-reversals mated with normal males produced offspring. HUMPHREY (1945) published a brilliant study of complete and functional sex-reversal in ambystomid salamanders. He implanted the testis preprimordium of a donor embryo in the right side of a genetic female host embryo. Under the influence of a testis, the out-growth of an implanted gonadal preprimordium, the left prospective ovary-preprimordium of the host developed into a functional testis. Sex-reversals from

female to male direction thus produced were tested as breeders and produced offspring in crosses with normal females.

While there are a great number of reports of experimental inductions of sex-reversal and intersex by means of sex hormones in a series of vertebrates, presumed sex-reversals thus produced were all killed when in very young stages so were not tested as breeders. In the genetically analyzed strain of the medaka (*Oryzias latipes*) YAMAMOTO (1953) showed that complete and functional sex-reversal from male to female can be induced under the influence of estrogens. Sex-linked color genes (R and r) were used as markers of the sex-chromosomes. Genotypic females (X^rX^r) and genotypic males (X^rY^R) taken at hatching and up to the age of eight months were continuously fed estrogens. The results showed that all the genotypic males (X^rY^R) had differentiated into phenotypic females and all the genotypic females (X^rX^r) developed into females. Red females, however, may possibly be produced in ways other than sex-reversal. One way, for instance, by the effect of estrogens on the formation of orange-red pigment in white females (X^rX^r). However, we have no certain evidence of this and this way may be excluded for two reasons. (a) First, estrogenized fish were distinctly identified as white and orange-red, none being of intermediate color. (b) If estrogen-administered red fish had the genotype of X^rX^r , the F_1 offspring from mating with X^rY^R male should show the sex-ratio of 1 $r\text{♀}$: 1 $R\text{♂}$. This condition is in strong contrast to results obtained in the F_1 offspring (table 1).

Red females may also be produced by crossing over between the X^r and Y^R chromosomes in the male parent, resulting in a transfer of the gene R to the X from the Y . If an egg (X^r) is fertilized with an X^R spermatozoon, a red female (X^rX^R) may be produced. Although this possibility cannot be excluded, it should be noted that crossing over between the sex chromosomes X^r and Y^R is of relatively rare occurrence. Since 100 percent of the red fish treated with estrogens turned into females as previously pointed out (YAMAMOTO 1953) it is obvious that a majority, if not all, of the estrogen-treated red females described in the previous paper, might have had the genetic constitution X^rY^R . Furthermore, if these red females had the genetic constitution X^rX^R resulting from crossing over and were mated with X^rY^R males, the F_1 offspring should show the sex ratio of 1 $r\text{♀}$: 1 $R\text{♀}$: 2 $R\text{♂}$, namely the two sexes in equal numbers. This condition also is quite different from the results given in this paper (table 1).

The present progeny testing serves to endorse the fact that estrogens can induce complete and functional sex-reversal in the genotypic males (X^rY^R) of the medaka. When sex-reversed females of the male genotype (X^rY^R) are crossed with normal red males (X^rY^R), the F_1 offspring should show the sex ratio of 1 $r\text{♀}$: 3 $R\text{♂}$,—that is a preponderance of red males. The offspring would include three genotypes in the proportion of 1 $X^rX^r\text{♀}$: 2 $X^rY^R\text{♂}$: 1 $Y^RY^R\text{♂}$, if the zygotes of Y^RY^R genotype develop into viable males. Data presented in table 1 indicate a preponderance of red males over white females. The actual proportion of red males to white females was significantly lower than the theoretical proportion. Data suggest the rarity of YY males. This was confirmed by progeny testing of F_1 red males. Although a number of progeny tests were performed using F_1 red sons produced by mating sex-reversals with normal males for the purpose of determining either X^rY^R or Y^RY^R males, only

one fish was actually determined to be $Y^R Y^R$ male. This indicates that there were only a very few YY zygotes which survived to the time of progeny-test matings. Our $Y^R Y^R$ male mated with white females ($X^r X^r$) fathered all-male broods, a total of seventy-two red sons. This occurrence of a YY male producing unisexual male progeny is a valid proof that the estrone-administered red females described in our previous paper were, in fact, sex-reversed females of the male genotype ($X^r Y^R$). Basing our judgment upon the observed 1:2.4 and 1:2.2 ratios among F_1 offspring of sex-reversals, we might expect to detect more YY males. However the fact that only a single YY male was detected may be due to the high death rate of YY males taking place between the time of our F_1 -examination and the time of progeny-test matings, a period of several months.

The fact that a YY male lived and produced offspring is in striking contrast to the result obtained by BRIDGES (1916) in his study of the fruit-fly (*Drosophila*). He proved that YY zygotes produced by non-disjunctions were not able to survive. In contrast to many genes in the X chromosome, it is generally agreed that the Y chromosome of *Drosophila* contains but few genes. That YY zygotes in fish of the male heterogametic type (XY) are viable and fertile has been shown by WINGE (1934) in *Lebistes* and by AIDA (1936) in *Oryzias*. This indicates the presence of important genes in the Y chromosome of these fish other than those involved in sex determination. Later WINGE and DITLEVSEN (1938, 1947) found that in *Lebistes*, although $Y^{Ma} Y^{Pa}$ males ($Ma = maculatus$, $Pa = pauper$) were viable and fertile, $Y^{Ma} Y^{Ma}$ zygotes were lethal. This fact is attributable to the presence of a recessive lethal gene in the Y chromosome, according to them. Further studies are now in progress to clarify the cause of the rarity of YY males in our experiments and determined whether this is due to the semi-lethality of the YY zygotes or whether it may be attributable to the foreshortened life-span of the YY males brought about by their higher metabolic rate.

It is important to note that the normal sex-determining mechanism, XX for female and XY for male, is quickly reestablished in the F_1 offspring when sex-reversed XY females are crossed with normal XY males. The XX-XY mechanism was also generally retained by the F_2 and F_3 offspring of induced sex-reversals.

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SUMMARY

A study was made of the progeny of functional sex-reversals of the male genotype (XY) in the medaka (*Oryzias latipes*) which were induced artificially through the administration of either estrone or stilbestrol. The observed ratio of white daughters

($r\varphi$) and orange-red sons ($R\sigma^7$) among F_1 offspring produced by estrone-sex-reversed mothers (X^rY^R) mated to normal orange-red males (X^rY^R) was 1:2.4 and the ratio of white daughters ($r\varphi$) and orange-red sons ($R\sigma^7$) among F_1 offspring produced by stilbestrol-sex-reversed mothers (X^rY^R) mated to normal orange-red males (X^rY^R) was 1:2.2, the theoretical ratio of both being 1:3.

A number of orange-red sons were singly submitted to progeny tests by crossing with white females (X^rX^r) for the purpose of determining either X^rY^R or Y^RY^R males which would be included among F_1 offspring if Y^RY^R zygotes were viable. A single orange-red son among F_1 offspring of estrone-sex-reversed mothers was identified as Y^RY^R male because in cross with white females (X^rX^r) he fathered all-male broods, a total of 72 orange-red sons. In all other progeny tests the genotype of orange-red sons was proved to be X^rY^R since they produced white females and orange-red males in the ratio of approximately 1:1.

Exceptional white males and exceptional orange-red females among F_1 and F_2 offspring were submitted to progeny testings. Many of those successfully tested were found to be the result of crossing over between the X^r and Y^R chromosomes. Two exceptional orange-red females among F_2 offspring of induced sex-reversals were detected as spontaneous sex-reversals of the male genotype (X^rY^R).

Normal sex-determining mechanism, XX for female and XY for male, is quickly reestablished when induced sex-reversed XY females are crossed with normal XY males. The XX-XY mechanism was also retained in the F_2 and F_3 offspring of artificially induced sex-reversals.

ADDENDUM

After the present manuscript was submitted, there came to my hands L. GALLIEN's brief paper entitled "Demonstration de l'homogamétie du sexe mâle chez le triton *Pleurodeles wallii* Michah. par l'étude de la descendance d'animaux à sexe physiologique inversé, après un traitement hormonal gynogène (benzoate d'oestradiol)." published in *Compt. Rend. Acad. Sci.* 238: 402-404, 1954. He describes having reared larvae of an urodelian amphibian (*Pleurodeles wallii*) in solution of estradiol benzoate and obtained fully grown animals. All estrogenized animals were found to be female. Among these he was able to detect genetic males, acting as females, which in mating with normal males yielded all male offspring. This shows that the sex genotypes of the urodele are WZ for female and ZZ for male. His result indicates that functional sex-reversal from male to female direction can be induced also in Amphibia by the administration of an estrogen without a surgical operation.

LITERATURE CITED

- AIDA, T., 1921 On the inheritance of color in a fresh-water fish *Aplocheilichthys latipes* Temmick and Schlegel, with special reference to the sex-linked inheritance. *Genetics* 6: 554-573.
 1936 Sex reversal in *Aplocheilichthys latipes* and a new explanation of sex differentiation. *Genetics* 21: 136-156.
- AMEMIYA, I., and S. MURAYAMA, 1931 Some remarks on the existence of developing embryos in the body of an oviparous cyprinodont, *Oryzias (Aplocheilichthys) latipes* (Temmick et Schlegel). *Proc. Imp. Acad. (Tokyo)* 7: 176-178.
- BELLAMY, A. W., and M. L. QUEAL, 1951 Heterosomal inheritance and sex determination in *Platyphacellus maculatus*. *Genetics* 36: 93-107.

- BREIDER, H., 1942 ZW-Männchen und WW-Weibchen bei *Platyopocilus maculatus*. Biol. Zbl. **62**: 187-195.
- BRIDGES, C. B., 1916 Non-disjunction as proof of the chromosome theory of heredity. Genetics **1**: 107-163.
- CREW, F. A. E., 1921 Sex-reversal in frogs and toads. A review of the recorded cases of abnormality of the reproductive system and an account of a breeding experiment. J. Genet. **11**: 141-181.
- 1923 Studies in intersexuality. II. Sex reversal in the fowl. Proc. Roy. Soc., London, B. **95**: 256-278.
- DOMM, L. V., 1930 Artificial insemination with motile sperm from ovariectomized fowl. Proc. Soc. Exp. Biol. Med. **28**: 316-318.
- GORDON, M., 1946a Interchanging genetic mechanisms for sex determination. J. Hered. **37**: 307-320.
- 1946b Sexual transformation of a genetically constituted female fish (*Platyopocilus maculatus*) into a functional male (Abstract). Anat. Record. **94**: 6.
- 1947 Genetics of *Platyopocilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. Genetics **32**: 8-17.
- HARMS, J. W., 1926 Beobachtungen über Geschlechtsumwandlungen reifer Tiere und deren F₁ Generation. Zool. Anz. **67**: 67-79.
- HUMPHREY, R. R., 1945 Sex determination in ambystomid salamanders: a study of the progeny of females experimentally converted into males. Am. J. Anat. **76**: 33-66.
- NAGATA, Y., 1934 Gonadectomy in the medaka (*Oryzias latipes*). Zool. Mag. (Tokyo) **46**: 293-294.
- 1936 Transplantation of testis into ovariectomized *Oryzias latipes*. Zool. Mag. (Tokyo) **48**: 102-108.
- OKA, T. B., 1931 On the processes on the fin-rays of the male of *Oryzias latipes* and other sex characters of this fish. J. Fac. Sci. Tokyo Imp. Univ., sec. IV (Zool) **2**: 209-219.
- OKADA, Yô. K., 1952 A biological method of determining the male hormone dissolved in water. Papers from Coord. Comm. Res. Genet. (Tokyo) **3**: 155-159.
- OKADA, Yô. K., and H. YAMASHITA, 1944 Experimental investigation of the manifestation of secondary sexual characters in fish, using the medaka, *Oryzias latipes* (Temnick & Schlegel) as material. J. Fac. Sci. Tokyo Imp. Univ. sec. IV (Zool) **6**: 383-437.
- PONSE, K., 1925 Ponte et développement d'oeufs provenant de l'organe de Bidder d'un crapaud male feminisé. Comp. Rend. Soc. Biol. **92**: 582-583.
- 1926 Changement expérimental du sexe et inter-sexualité chez le crapaud. Compt. Rend. Soc. Phy. Hist. Nat. (Genève) **43**: 19-22.
- 1931 Le problème du sexe et l'évolution de l'organe de Bidder du crapaud. Proc. 2nd Int. Cong. Sex research: 202-210.
- WINGE, Ö., 1930 On the occurrence of XX-males in *Lebistes*, with some remarks on AIDA's so-called "Non-disjunction" males in *Aplocheilus*. J. Genet. **23**: 69-76.
- 1934 The experimental alteration of sex chromosomes into autosomes and *vice versa*, as illustrated by *Lebistes*. Compt. Rend. Trav. Lab. Carlsberg. Sér. Physiol. **21**: 1-49.
- WINGE, Ö., and E. DITLEVSEN, 1938 A lethal gene in the Y chromosome of *Lebistes*. Compt. Rend. Trav. Carlsberg. Sér. Physiol. **22**: 202-210.
- 1947 Colour inheritance and sex determination in *Lebistes*. Heredity **1**: 65-83.
- WITSCHI, E., 1923 Über die genetische Konstitution der Froschwitwer. Biol. Zentr. **43**: 89-96.
- YAMAMOTO, T., 1953 Artificially induced sex-reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exp. Zool. **123**: 571-594.