A FURTHER STUDY ON INDUCTION OF FUNCTIONAL SEX
REVERSAL IN GENOTYPIC MALES OF THE MEDAKA
(*ORYZIAS LATIPES*) AND PROGENIES OF
SEX REVERSALS^{1, 2}

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DESPITE a great deal of work on artificial control of gonadal sex differentiation in every class of vertebrates, functional sex reversal induced by administration of sex hormones has rarely been achieved. Complete and functional sex reversal in genetic males under the influence of estrogens (either estrone or stilbestrol) was successful in a cyprinodont fish, the medaka (*Oryzias latipes*). Induced sex reversals of genetic males in mating with normal males produced viable offspring (YAMAMOTO 1953). GALLIEN (1954a,b) demonstrated that functional sex reversal in genetic males of the newt (*Pleurodeles waltlii*) can be induced by estradiol benzoate. GALLIEN (1955, 1956) and CHANG and WITSCHI (1955) succeeded in inducing functional sex reversal in genetic males of the clawed toad (*Xenopus laevis*) by treatment with estradiol. In both the newt and the clawed toad, progenies of sex reversals by normal males were only males, indicating that the female is heterogametic (WZ) and the male homogametic (ZZ). In amphibians and higher vertebrates, however, functional sex reversal from female to male by administration of male sex hormone had not heretofore been achieved. In the medaka, induction of functional sex reversal in genetic females under the influence of methyl testosterone was also successfully accomplished (YAMAMOTO 1958).

In an oviparous cyprinodont, the medaka (*Oryzias latipes*) in which the sex-determining mechanism is XX for female and XY for male, either the gene *R* (orange-red) or *r* (white) is borne by the X or Y chromosomes (AIDA 1921). When a white female (X^rX^r) is mated with a heterozygous orange-red male (X^rY^R), the progeny are white females (X^rX^r) and orange-red males (X^rY^R) in equal numbers. In matings of this sort, white (*r*) fish are females and orange-red (*R*) are males because the gene *R* for a dominant orange-red condition is located only in the Y chromosome. Hence the *r* and *R* can be used as markers of sex genotypes XX and XY.

The father-to-son inheritance is always displayed except in rare exceptional fish in which the *R* gene dissociates itself from the sex-limited inheritance. Since the

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R and r are partially sex-linked, exceptions such as $X^R X^r$ females or $X^r Y^r$ males can be produced by crossing over between the heterosomes. Exceptions such as $X^r X^r$ males or $X^r Y^R$ females may arise by genic imbalance between the sex chromosomes and autosomal sex genes. The total of the exceptions in our stock ($d-rR$ strain) is less than one percent. Hence, in our breed, the r and R can be used as markers of sex genotypes XX and XY with a reliability of more than 99 percent.

It has already been shown that by using offspring of these matings XY individuals can be inverted in their gonadal sex differentiation and that they will develop into complete and functional females by continuous administration *per os* of estrogens (estrone and stilbestrol) for several months after hatching (YAMAMOTO 1953). Induced sex reversals of male genotype ($X^r Y^R$) were mated with normal orange-red males ($X^r Y^R$). A number of the F_1 orange-red sons were individually mated with separate white females ($X^r X^r$) in tests for either $X^r Y^R$ or $Y^R Y^R$ sons. A single F_1 orange-red son among the estrone-sex-reversed females was actually identified as the very $Y^R Y^R$ male which had fathered all 72 male offspring. Induction of sex reversal of the male genotype (XY) by means of estrone was thus conclusively demonstrated by the occurrence of a YY male among F_1 offspring of sex reversals (YAMAMOTO 1955).

The present paper is a continuation of the study of the induction of reversal of gonadal sex differentiation in genetic males (XY) made under the influence of the estrogens, and of the progenies of sex reversals. While in this further experiment, the dosage level of estrone is the same as that used in the previous study (1,250 i.u./g diet), the higher level of stilbestrol in the previous study was lowered in this study to the same level of international unit as estrone. The period of administration for both estrogens in two of the three experimental series of this study was limited to the time between hatching and certain juvenile stages. This was done to ascertain the period for hormone administration sufficient to insure reversal of sex differentiation. Our success in inducing reversal of sex differentiation through use of estrogens led to the conviction that sex reversal of $Y^R Y^R$ zygotes would be induced by the same means. To realize this, sex reversal in genotypic males must be induced in two consecutive generations. Such a purpose was carried out in the present study.

MATERIALS AND METHODS

A genetically analyzed breed ($d-rR$ strain) of the medaka (*Oryzias latipes*) was used as material. Parents were products of mass breeding of heterozygous orange-red males ($X^r Y^R$) and white females ($X^r X^r$). Before time of breeding, a stock census was conducted and all the detected rare exceptions were excluded.

Since the present experiments were run on two or more generations, where the first one or two generations were estrogenized, the following symbols are used to denote the offspring.

N : Normal-fed fish of one generation. White female is $Nr \text{ } \varnothing$ and orange-red male $NR \text{ } \delta$.

E : Estrone-fed fish of one generation. Estronized white female is $Er \text{ } \varnothing$ and estrone-sex-reversed orange-red female $ER \text{ } \varnothing$.

S : Stilbestrol-fed fish of one generation. Stilbestrolized white female is $Sr \text{ } \varnothing$ and stilbestrol sex-reversed orange-red female $SR \text{ } \varnothing$.

$Nr \text{ } \varnothing > N$: Normal-fed offspring produced by mating normal-fed white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is $Nr \text{ } \varnothing > Nr \text{ } \varnothing$ and orange-red son $Nr \text{ } \varnothing > NR \text{ } \delta$.

$Er \text{ } \varnothing > N$: Normal-fed offspring produced by mating estronized white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is $Er \text{ } \varnothing > Nr \text{ } \varnothing$ and orange-red son $Er \text{ } \varnothing > NR \text{ } \delta$.

$ER \text{ } \varnothing > N$: Normal-fed offspring produced by mating estrone-sex-reversed orange-red females (X^rY^R) with normal orange-red males (X^rY^R). White daughter is $ER \text{ } \varnothing > Nr \text{ } \varnothing$ and orange-red son $ER \text{ } \varnothing > NR \text{ } \delta$.

$ER \text{ } \varnothing > E$: Estrone-fed offspring produced by mating estrone-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). Estronized white daughter is $ER \text{ } \varnothing > Er \text{ } \varnothing$ and sex-reversed orange-red daughter $ER \text{ } \varnothing > ER \text{ } \varnothing$.

$Sr \text{ } \varnothing > N$: Normal-fed offspring produced by mating stilbestrolized white females (X^rX^r) with normal orange-red males (X^rY^R). White daughter is $Sr \text{ } \varnothing > Nr \text{ } \varnothing$ and orange-red son $Sr \text{ } \varnothing > NR \text{ } \delta$.

$SR \text{ } \varnothing > N$: Normal-fed offspring produced by mating stilbestrol-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). White daughter is $SR \text{ } \varnothing > Nr \text{ } \varnothing$ and orange-red son $SR \text{ } \varnothing > NR \text{ } \delta$.

$SR \text{ } \varnothing > S$: Stilbestrol-fed offspring produced by mating stilbestrol-sex-reversed females (X^rY^R) with normal orange-red males (X^rY^R). Stilbestrolized white daughter is $SR \text{ } \varnothing > Sr \text{ } \varnothing$ and stilbestrol-sex-reversed daughter $SR \text{ } \varnothing > SR \text{ } \varnothing$.

Other symbols designating exceptions are given in proper places. To assist the reader to visualize experiments, diagrams are introduced into the text (Figures 3 and 4).

Normal diet of fry and juveniles consists of 60 g shrimp powder, 30 g toasted whole barley flour, 6 g yeast preparation and 4 g powdered green tea. As estrogen diet, tablets of commercial preparation were finely ground in a mortar and homogeneously mixed with the normal diet. The dosage level of both estrone and stilbestrol is 1,250 i.u. (estrone 125 μg and stilbestrol 62.5 μg) per gram diet.

Two series of experiments for induction of sex reversal were performed. In one, estrogens were administered to fish *per os* for about seven months after hatching, as in the previous study. Newly hatched fry average about 4.8 mm in total length, varying from 4.5 to 5.4 mm. By the end of the administration period, fish had reached 14–24 mm. It may be remarked that fish are mature when they reach about 28 mm. In the other series of experiments, estrogens were administered to the first generation for 4–6 weeks, and to the second for more than six weeks from the time of hatching, until young reached 8–13 mm and 10–15 mm, respectively. Definite developmental stages were taken as the time for discontinuation of hormone treatment instead of using definite periods of estrogen administration because fish exhibit great variability in growth rate. Fry were reared indoors

under windows in three-liter glass jars during the administration of estrogen diet. Upon reaching either of these definite stages mentioned above, young fish were successively reared outdoors in concrete pools (75 cm square and 18 cm deep) until they reached sexual maturity. The method employed encourages large numbers of full-grown sex reversals because rearing of fish out-of-doors greatly reduces mortality. The young fish under outdoor conditions were fed on the mixture of shrimp powder and toasted whole barley flour with the addition occasionally of live tubificids and water fleas.

The total length measuring from the snout to the end of the caudal fin is given for size of fish. Unless otherwise stated, the sex referred to is the sex phenotype instead of the sex genotype.

RESULTS

1. *Effect of estrogen feeding from hatching up to stage of 14–24 mm and results of testcrosses of estrogenized fish:* Between July 1st and 15th, the daily hatchings were divided into three groups; the first was fed normal diet (N), the second estrone diet (E) and the third stilbestrol diet (S). Thus the total hatchings for fifteen days were of three diet classifications and these diets were successively administered for several months. By the end of this period, fish had reached the stage of 14–24 mm. Thereafter they were reared outdoors until they reached sexual maturity. The experimental result is presented in the upper section of Table 1. Whatever the sex genotype, all the estrogenized fish differentiated into females. It follows that all the estrogen-administered orange-red fish (X^rY^R) had inverted their genetically determined sex and differentiated into females, while control orange-red fish had developed into normal males. Both estrogenized white females and orange-red sex reversals were found to be fertile. Sex reversals of the male genotype (X^rY^R) not only have body configuration and secondary sex characters similar to those of normal females, but they have feminine sex equip-

TABLE 1

Sex reversal in genotypic males (X^rY^R) of the medaka induced by administration of estrogens for seven months (14–24 mm) and progenies of sex reversals of male genotype and estrogenized genotypic females

Symbol of offspring	Parents		Diet of offspring	Period of estrogen feeding	Offspring			
	♀	♂			$r\bar{Q}$	$r\bar{Q}$	$R\bar{Q}$	$R\bar{Q}$
N	X^rX^r	X^rY^R	Normal		5	0	0	8
E	X^rX^r	X^rY^R	Estrone	H→14–24 mm	5	0	7	0
S	X^rX^r	X^rY^R	Stilbestrol	H→14–24 mm	14	0	9	0
$Nr\bar{Q} > N$	X^rX^r	X^rY^R	Normal		9	0	1	15
$Er\bar{Q} > N$	X^rX^r	X^rY^R	Normal		106	0	3	120
$ER\bar{Q} > N$	X^rY^R	X^rY^R	Normal		18	0	1	32
$Sr\bar{Q} > N$	X^rX^r	X^rY^R	Normal		53	0	0	55
$SR\bar{Q} > N$	X^rY^R	X^rY^R	Normal		11	0	0	28

Dosage level of estrogens = 1,250 i.u./g diet. r = White, R = Orange-red, H = Hatching time,
 N = Normal-fed, E = Estrone fed, S = Stilbestrol fed.

ment and capacity. Evidently XY zygotes had developed into functional females under the influence of estrogens. An estrone-sex-reversal female ($ER\ \varnothing$, X^rY^R) and a stilbestrol-sex-reversal female ($SR\ \varnothing$, X^rY^R) are illustrated in Figures 1 and 2, respectively.

Estrogenized white females (X^rX^r) and sex-reversed orange-red females (X^rY^R) were mated with normal orange-red males (X^rY^R). The result is shown in the lower half of Table 1. The estrogenized genotypic females (X^rX^r), either estronized white fish ($Er\ \varnothing$) or stilbestrolized white ones ($Sr\ \varnothing$), in mating with normal orange-red males (X^rY^R) produced white daughters and orange-red sons in approximately 1:1 ratio as is the case of control white females ($Nr\ \varnothing$). Four estrone-sex-reversal $ER\ \varnothing\ \varnothing$ (X^rY^R) and nine stilbestrol-sex-reversal $SR\ \varnothing\ \varnothing$ (X^rY^R) in mating with two and five normal males (X^rY^R), respectively, produced offspring in which orange-red sons preponderate (2–3 times) over white daughters. In the offspring denoted by $ER\ \varnothing\ >N$, the ratio of white daughters to orange-red sons is close to 1:2 ($\chi^2=0.16$, $P=0.70-0.50$) rather than that of expected 1:3 ($\chi^2=3.2$, $P=0.10-0.05$). This is ascribed to the rarity of $Y^R Y^R$ males.

2. *Effect of estrogen-feeding from hatching to 8–13 mm stage:* In another series of experiments, either estrone or stilbestrol was administered to fry from hatching to juvenile stage of 8–13 mm. Thereafter they were reared on normal diet until they became fully grown. The result is shown in the upper section of Table 2. The controls (N) had developed into white females and orange-red males in equal numbers, as expected. Out of 57 estronized fish (E), 26 were white females ($Er\ \varnothing\ \varnothing$) and 31 were orange-red fish of which 28 were females (ER

FIGURE 1.—(Above) Control orange-red male ($NR\ \delta$, X^rY^R); (Below) Functional estrone-sex-reversal ($ER\ \varnothing$) of male genotype (X^rY^R) carrying a cluster of fertilized eggs. $\times 3.2$.

FIGURE 2.—(Above) Control orange-red male (NR ♂, X^rY^R); (Below) Functional stilbestrol-sex-reversal (SR ♀) of male genotype (X^rY^R) carrying a cluster of fertilized eggs. × 3.2.

TABLE 2

Sex reversal in genotypic males of the medaka induced by estrogens in one and two consecutive generations

Symbol of offspring	Parents		Diet of offspring	Period of estrogen feeding	Offspring				
	♀	♂			r♀	r♂	R♀	R(i.s.)	R♂
N	X ^r X ^r	X ^r Y ^R	Normal		24	0	0	0	22
E	X ^r X ^r	X ^r Y ^R	Estrone	H→ 8-13 mm	26	0	28	1	2
S	X ^r X ^r	X ^r Y ^R	Stilbestrol	H→ 8-13 mm	49	0	67	0	0
Er ♀ > N	X ^r X ^r	X ^r Y ^R	Normal		45	0	0	0	55
ER ♀ > N	X ^r X ^r	X ^r Y ^R	Normal		16	1	0	0	52
ER ♀ > E	X ^r Y ^R	X ^r Y ^R	Estrone	H→ 10-15 mm	7	0	24	0	1
Sr ♀ > N	X ^r X ^r	X ^r Y ^R	Normal		6	0	0	0	5
SR ♀ > N	X ^r Y ^R	X ^r Y ^R	Normal		34	2	3	0	86
SR ♀ > S	X ^r Y ^R	X ^r Y ^R	Stilbestrol	H→ 10-15 mm	4	0	5	0	0

The period of administration covers from hatching to certain juvenile stages, as noted above. Dosage level of estrogens = 1,250 i.u./g diet. r = White, R = Orange-red, H = Hatching time, N = Normal fed, E = Estrone-fed, S = Stilbestrol fed.

♀♀), one androintersex and two were males ($ER\delta\delta$). Obviously 90 percent of genotypic males (X^*Y^R) had reversed their sex differentiation and developed into phenotypic females. The single intersex was characterized by having secondary sex characters of both sexes in intermediate state as well as having a hermaphroditic gonad. An account has already been given elsewhere of the gonad of this intersex (YAMAMOTO 1957).

Apparently, regardless of the sex genotype, all the stilbestrolized fish (S) had developed into females. Evidently 100 percent of genotypic males ($n=67$) had differentiated into females under the effect of stilbestrol at the dosage level of 1,250 i.u./g diet. This dosage is one fourth that used in the previous study. At the same international unit, stilbestrol is evidently more effective than estrone. When we compare the effectiveness of stilbestrol and estrone in weight per gram diet, the potency of the former is more than twice that of the latter, since 1 i.u. of estrone is 0.1 μ g while that of stilbestrol is 0.05 μ g.

3. *Progenies of estrogen-treated fish to 8–13 mm and induction of sex-reversal in two consecutive generations:* It should be noted that not only do estrogen-sex-reversals have structural traits appropriate to normal females, but their sexual behavior is the same. Mass matings in separate groups were performed on estrogenized genetic females (15 $Er\varnothing\varnothing$) and estrone-sex-reversals (9 $ER\varnothing\varnothing$) of the male genotype (X^*Y^R) with normal orange-red males (X^*Y^R). Of the stilbestrolized fish, stilbestrolized genotypic females (25 $Sr\varnothing\varnothing$) and stilbestrol-sex-reversals (43 $SR\varnothing\varnothing$) of the male genotype (X^*Y^R) were mated with normal orange-red males (X^*Y^R) in two separate groups. Most of the estrogenized fish were found to be fertile. Offspring of either the estronized white females ($Er\varnothing\varnothing$) or stilbestrolized white females ($Sr\varnothing\varnothing$) were reared on normal diet.

Daily hatchings (July 2–8) of two groups, *viz.*, one of estrone-sex-reversals ($ER\varnothing\varnothing$) mated with normal males and the other of stilbestrol-sex-reversals ($SR\varnothing\varnothing$) with normal males, were used. The first group was divided, one half reared on normal diet designated as $ER\varnothing > N$ (*cf.* Figure 3 and Table 2) and one half on estrone diet denoted as $ER\varnothing > E$ (*cf.* Figure 4 and Table 2). The second group was divided in the same way, one half reared on normal diet designated as $SR\varnothing > N$ and one half on stilbestrol diet denoted as $SR\varnothing > S$. In both groups, the half fed on normal diet ($ER\varnothing > N$ and $SR\varnothing > N$) served as controls for fish estrogenized in two generations ($ER\varnothing > E$ and $SR\varnothing > S$). Fry in the other half of the two groups were reared on their respective hormone diets until reaching 10–15 mm stage. The normally fed offspring ($Er\varnothing > N$) of estronized genotypic females ($Er\varnothing\varnothing$) mated with normal orange-red males (X^*Y^R) were white daughters and orange-red sons in equal numbers. The same was true of normally fed offspring ($Sr\varnothing > N$) produced by stilbestrolized genotypic females ($Sr\varnothing\varnothing$) and normal orange-red males. The normally fed offspring ($ER\varnothing > N$) of estrone-sex-reversal females ($ER\varnothing\varnothing$, X^*Y^R) mated with normal orange-red males (X^*Y^R) (*cf.* Figure 3), were 16 white daughters and 52 orange-red sons with one exceptional white son (lower section of Table 2). The ratio of white daughters to orange-red sons approximates the expected 1:3. The number in this case is, how-

ever, too small to give much significance to agreement with the theoretical expectancy. The normally fed offspring ($SR \varnothing > N$) produced by stilbestrol-sex-reversals ($SR \varnothing \varnothing, X^rY^R$) and normal orange-red males (X^rY^R) comprised 34 white daughters, 86 orange-red sons with two exceptional white males and three

FIGURE 3.—Diagram of induction of sex reversal in genotypic males (X^rY^R) of the medaka by estrone in one generation and normal-fed progenies of sex reversals and of estronized genotypic females (X^rX^r).

FIGURE 4.—Diagram of induction of sex reversal in genotypic males of the medaka by estrone in two consecutive generations.

exceptional orange-red females. The ratio of white daughters to orange-red sons was 1:2.5 instead of the theoretical 1:3.

Estronized offspring ($ER \text{ } \varnothing > E$) produced by mating estrone-sex-reversals ($ER \text{ } \varnothing \text{ } \varnothing$, X^rY^R) with normal orange-red males (X^rY^R) (cf. Figure 4) were seven white daughters and 24 orange-red daughters with one orange-red son. Obviously 24 out of 25 (96 percent) genotypic males (X^rY^R and Y^RY^R) had inverted their sex differentiation and developed into females, with only one genotypic male remaining nonreversed.

Stilbestrolized offspring ($SR \text{ } \varnothing > S$) of stilbestrol-sex-reversals ($SR \text{ } \varnothing \text{ } \varnothing$, X^rY^R) mated with normal orange-red males (X^rY^R) comprised four white daughters and five orange-red daughters. Evidently under the influence of stilbestrol all the genotypic males (X^rY^R and Y^RY^R) had reversed their sex and developed into functional females.

4. *Testcrosses of F_1 orange-red sons of sex-reversals estrogenized for one generation:* Normally fed F_1 orange-red sons, which had been produced by matings of estrogen-sex-reversals (X^rY^R) estrogenized to 8–13 mm stages with normal orange-red males (X^rY^R), were submitted to genetic analysis. Theoretically, we could expect that there would be two kinds of males, the X^rY^R and Y^RY^R , among normally fed F_1 orange-red sons (cf. Figure 3). Because Y^RY^R males cannot be distinguished from the X^rY^R males except by testcrosses, a random selection was made of ten out of 52 normal-fed orange-red sons ($ER \text{ } \varnothing > NR \text{ } \delta$) of estrone-sex-reversals and six normal-fed orange-red F_1 sons ($SR \text{ } \varnothing > NR \text{ } \delta$) of stilbestrol-sex-reversals. These males were singly tested by white females (X^rX^r). Results showing fully grown offspring from 16 matings are given in the upper section of Table 3. The results of all matings that yielded both sexes in equality are consolidated in Table 3. Of ten F_1 orange-red sons ($ER \text{ } \varnothing > NR \text{ } \delta$) of estrone-sex-reversals tested by white females (X^rX^r), nine produced offspring consisting of white females and orange-red males in approximately equal numbers, a total

TABLE 3

Testcrosses of F_1 males of estrogen-sex-reversals in one generation (upper section) and of fish estrogenized in two consecutive generations (lower section)

$\varnothing$	Parents σ	No. of matings	$r\varnothing$	Offspring $r\sigma$ $R\varnothing$		$R\sigma$	Genotype of fish tested
X^rX^r	$ER \text{ } \varnothing > NR \text{ } \delta$ a	9	841	5	10	911	X^rY^R
X^rX^r	$ER \text{ } \varnothing > NR \text{ } \delta$ b	1	0	0	1	354	Y^RY^R
X^rX^r	$SR \text{ } \varnothing > NR \text{ } \delta$	6	594	1	4	711	X^rY^R
$ER \text{ } \varnothing > ER \text{ } \varnothing$	X^rY^R	16	496	7	35	1,012	X^rY^R
X^rX^r	$ER \text{ } \varnothing > ER \text{ } \delta$ *	1	51	0	0	56	X^rY^R
$ER \text{ } \varnothing > Er \text{ } \varnothing$	X^rY^R	5	115	0	2	100	X^rX^r
$SR \text{ } \varnothing > SR \text{ } \varnothing$	X^rY^R	5	55	2	3	106	X^rY^R
$SR \text{ } \varnothing > Sr \text{ } \varnothing$	X^rY^R	1	23	0	0	22	X^rX^r

* $ER \text{ } \varnothing > ER \text{ } \delta$ on the second line in lower section is the single nonreversed orange-red son of estrone-sex-reversed mother (cf. Table 2). r = White, R = Orange-red, N = Normal fed, E = Estrone fed, S = Stilbestrol fed.

of 841 $r\varnothing\varnothing$ and 911 $R\delta\delta$ with five exceptional $r\delta\delta$ and ten exceptional $R\varnothing\varnothing$. Obviously the nine parents tested ($ER\varnothing > NR\delta a$) bred as X^rY^R males. Only one ($ER\varnothing > NR\delta b$) of the ten tested proved to be a Y^RY^R male and this extraordinary fish is seen in Figure 5. He fathered a total of 355 orange-red offspring of which 354 were males and one was an exceptional female. One of these 354 orange-red sons ($ER\varnothing > NR\delta b > NR\delta$) presumably having the genotype (X^rY^R), in mating with white females (X^rX^r) actually sired 20 white females and 24 orange-red males, proving his constitution to be X^rY^R . The single exceptional orange-red daughter ($ER\varnothing > NR\delta b > Nexc.R\varnothing$) in two matings with X^rY^R males, produced 30 white females and 92 orange-red males (or $1r\varnothing:3R\delta$) with one exceptional orange-red female, as shown in the third line in the upper section of Table 4.

TABLE 4

Testcrosses of exceptions among F_2 offspring of estrogen-sex-reversals in one generation (upper section) and of exceptions among estrogen-sex-reversals in two consecutive generations (lower section)

$\varnothing$	Parents	δ	No. of matings	$r\varnothing$	Offspring $r\delta$	$R\varnothing$	$R\delta$	Genotype of exc. fish
X^rX^r	$ER\varnothing > NR\delta a > Nexc.R\varnothing$	$ER\varnothing > NR\delta a > Nexc.r\delta$	3	88	96	0	0	X^rY^r
$ER\varnothing > NR\delta a > Nexc.R\varnothing$		X^rY^R	1	5	0	0	21	X^rY^R
$ER\varnothing > NR\delta b > Nexc.R\varnothing$		X^rY^R	1	30	0	1	92	X^rY^R
$SR\varnothing > NR\delta > Nexc.R\varnothing$		X^rY^R	1	2	0	0	8	X^rY^R
X^rX^r	$ER\varnothing > ER\varnothing > Nexc.R\varnothing$	$ER\varnothing > ER\varnothing > Nexc.r\delta$	1	9	11	0	0	X^rY^r
$ER\varnothing > ER\varnothing > Nexc.R\varnothing$		X^rY^R	2	72	0	70	86	X^rX^R
X^rX^r		$SR\varnothing > SR\varnothing > Nexc.r\delta$	2	71	58	0	0	X^rY^r
$SR\varnothing > SR\varnothing > Nexc.R\varnothing$		X^rY^R	1	3	0	0	8	X^rY^R

r = White, R = Orange-red, N = Normal-fed, E = Estrone fed,
 S = Stilbestrol fed, *exc.* = Exceptional.

FIGURE 5.—The second Y^RY^R male detected among normal-fed F_1 orange-red sons ($ER\varnothing > NR\delta$) of estrone-sex-reversal X^rY^R females mated with normal X^rY^R males. This Y^RY^R male in mating with white females (X^rX^r) fathered 354 orange-red sons and a single orange-red daughter. $\times 3.2$.

Obviously the single daughter of the $Y^R Y^R$ male was of the constitution $X^r Y^R$. This exception might have arisen by genic imbalance between heterosomes and autosomal sex genes.

The single $Y^R Y^R$ male in the present study is the second $Y^R Y^R$ male to have been detected among F_1 offspring of estrogen-sex-reversal females mated with normal males. When the first $Y^R Y^R$ male, previously reported (YAMAMOTO 1955), was mated with white females ($X^r X^r$), all 72 offspring were orange-red males. The first $Y^R Y^R$ male was found to be exceedingly virile in secondary sex characters. The papillar processes on the anal fin are the most prominent feature among the male-positive sex characters. The second $Y^R Y^R$ male, 32 mm when sacrificed, had a total of 310 papillar processes. This number is high as compared with the average of those of ordinary XY males of the same size.

All six normal-fed F_1 sons ($SR \text{ } \varnothing > NR \text{ } \delta$) of stilbestrol-sex-reversals ($SR \text{ } \varnothing$, $X^r Y^R$) by normal $X^r Y^R$ males were proved to be $X^r Y^R$ by the fact that each of them in mating with white females ($X^r X^r$) fathered white females and orange-red males in nearly equality. The consolidated data are given on the third line in upper section of Table 3. The lack of $Y^R Y^R$ males among fish ($SR \text{ } \varnothing > NR \text{ } \delta$) tested is probably due to the inadequate number of individuals in these tests.

5. *Progenies of fish estrogenized for two consecutive generations:* Fish estrogenized for two consecutive generations were subjected to testcrosses. The results are presented in the lower section of Table 3. Twenty-four estrone-sex-reversals ($ER \text{ } \varnothing > ER \text{ } \varnothing$, cf. lower half of Table 2) produced by sex-reversal in two consecutive generations (cf. Figure 4) were singly tested by normal orange-red males ($X^r Y^R$) for the purpose of deciding the genotype of sex-reversals for either $X^r Y^R$ or $Y^R Y^R$. Twenty-one out of 24 $ER \text{ } \varnothing > ER \text{ } \varnothing$ were found to be fertile, five of which died after having produced a few fry which were not reared to adulthood. The other 16 estrone-sex-reversals produced offspring in sufficient numbers to decide their genotype. Since each of 16 matings yielded offspring comprising the same phenotypic classes and having the same ratios, all their offspring are listed together on the first line in the lower section of Table 3. Out of a total of 1,550 offspring, 496 white females, 1,012 orange-red males, seven exceptional white males and 35 exceptional orange-red females were produced. Obviously all 16 estrone-sex reversals in two consecutive generations had the constitution $X^r Y^R$. No estrone-sex-reversal females of the constitution $Y^R Y^R$ were detected. One of the 16 estrone-sex-reversals which had been estrogenized in two consecutive generations and bred as $X^r Y^R$ is illustrated in Figure 6. When $X^r Y^R$ females are mated with $X^r Y^R$ males, we can expect that offspring will consist of white females ($X^r X^r$), ordinary orange-red males ($X^r Y^R$) and extraordinary orange-red males ($Y^R Y^R$) in a ratio of 1:2:1. The observed ratio of white daughters to orange-red sons was 1:2 ($\chi^2=0.13$, $P=0.80-0.70$) instead of the theoretical 1:3 ($\chi^2=50$, $P<0.01$). The discrepancy may be attributable to the rarity of $Y^R Y^R$ males.

A single estronized orange-red male ($ER \text{ } \varnothing > ER \text{ } \delta$, nonreversal, cf. lower section of Table 2) was tested to $X^r X^r$ females and bred as $X^r Y^R$ instead of $Y^R Y^R$ (second line in lower section of Table 3).

FIGURE 6.—Functional sex-reversal female ($ER\varnothing > ER\varnothing$) of male genotype treated with estrone in two consecutive generations. The genotype was proved to be X^rY^R . Note a cluster of fertilized eggs. $\times 3.2$.

Five out of seven estronized white females ($ER\varnothing > Er\varnothing$, X^rX^r , cf. lower section of Table 2) were individually tested by normal orange-red males (X^rY^R). The results from all five matings are consolidated and listed on the third line in lower section of Table 3. The total offspring comprised 115 white daughters and 100 orange-red sons with two exceptional orange-red females.

Five stilbestrol-sex-reversals ($SR\varnothing > SR\varnothing$, cf. lower half of Table 2) produced by sex reversal in two consecutive generations and presumed to have either genotype X^rY^R or $Y^R Y^R$, were singly tested to normal X^rY^R males. Each of the five matings gave white daughters and orange-red sons indicating that all these sex-reversal females had the genotype X^rY^R . The consolidated data are presented on the fourth line in the lower section of Table 3. Two exceptional white sons and three exceptional orange-red daughters were produced in addition to the expected phenotypic classes. In this case, also, the ratio of white daughters to orange-red sons is 1:2 ($\chi^2 = 0.05$, $P = 0.90-0.80$) instead of the expected 1:3 ($\chi^2 = 7.2$, $P < 0.01$). No $Y^R Y^R$ females were detected among females symbolized as $SR\varnothing > SR\varnothing$. Three out of four stilbestrolized white females $SR\varnothing > Sr\varnothing$ (X^rX^r), which were stilbestrolized daughters of stilbestrol-sex-reversal females, were mated to a normal X^rY^R male. As is shown on the fifth column in lower section of Table 3, the offspring comprised white daughters and orange-red sons in a 1:1 ratio, as expected.

6. *Testcrosses of exceptions:* Some exceptions produced among offspring of sex reversals in one and two consecutive generations were subjected to testcrosses. Exceptional white males were singly tested to two or three white X^rX^r females and exceptional orange-red females were individually tested to a normal X^rY^R male. The offspring from 12 successful matings are tabulated in Table 4. A separate record was kept on offspring of each mating. Results of matings yielding offspring in similar proportions of phenotypic classes are consolidated on the same line in Table 4. The progeny of an exceptional orange-red female ($ER\varnothing > NR\delta b > Nexc.R\varnothing$) have already been described. All three exceptional white males ($ER\varnothing > NR\delta a > Nexc.r\delta$) which are exceptions among F_2 offspring of

estrone-sex-reversals in one generation, were found to be X^rY^r arising from crossing over. One exceptional white male ($ER\varnothing > ER\varnothing > Nexc.r\delta$) and two exceptional white males ($SR\varnothing > SR\varnothing > Nexc.r\delta$), the exceptions among offspring of estrogen-sex-reversals in two consecutive generations, also were proved to be crossovers having the constitution X^rY^r .

One exceptional orange-red female ($ER\varnothing > NR\delta > Nexc.R\varnothing$) and one exceptional orange-red female ($SR\varnothing > NR\delta > Nexc.R\varnothing$), among F_2 offspring of estrone and stilbestrol sex reversals in one generation, tested by separate matings with X^rY^R males, produced progenies in which orange-red sons predominated over white daughters. One exceptional orange-red female ($SR\varnothing > SR\varnothing > Nexc.R\varnothing$) showed the same genetic behavior which proved her constitution to be X^rY^R .

Two exceptional orange-red females ($ER\varnothing > ER\varnothing > Nexc.R\varnothing$), the exceptions among offspring of estrone-sex-reversals in two consecutive generations, however, displayed quite a different genetic behavior. In matings singly with normal orange-red males X^rY^R , these two exceptional females produced offspring which comprised white daughters, orange-red daughters and orange-red sons. Consolidated data resulting from the two matings show 72 white daughters, 70 orange-red daughters and 86 orange-red sons, a ratio approaching a 1:1:1 instead of the expected 1:1:2. The sex distribution (142 ♀♀ and 86 ♂♂) is close to a ratio of 2 ♀:1 ♂ ($\chi^2=1.97, P=0.20-0.10$) rather than that of the expected 1 ♀:1 ♂ ($\chi^2=13.8, P<0.01$). Although the observed proportion showed a significant departure from the expected, we can not label these two exceptions other than X^rX^R , i.e., crossovers. A plausible explanation of the discrepancy is given in the eighth paragraph under Discussion. Since only two out of 35 exceptional orange-red females ($ER\varnothing > ER\varnothing > Nexc.R\varnothing$, cf. Table 4) were successfully tested, there is the possibility that X^rY^R females arising from genic imbalance might also be included among nontested fish.

Genetic analyses of exceptions showed that all the exceptional white males had the constitution X^rY^r arising from crossing over between the X^r and Y^R . However, exceptional orange-red females proved to have either the constitution X^rX^R resulting from crossing over or X^rY^R produced by genic imbalance between sex chromosomes and autosomal sex genes.

If exceptions were the result of reciprocal crossing over only, then the number of exceptional white males and orange-red females would be equal. The preponderance of the latter over the former is due to the occurrence of exceptional X^rY^R females in addition to crossover X^rX^R females. Exceptional X^rY^R females might be produced by an accumulation of the feminine genes in autosomes caused by recombination, as was maintained by WINGE (1934) for the guppy (*Lebistes reticulatus*).

DISCUSSION

According to the prevailing view the genetic sex determination is primarily dependent upon the male and female sex genes. The genic balance theory of sex

determination originally proposed by BRIDGES (1922, 1925, 1939) and later modified by WINGE (1934) has merit in explaining normal sex determination in the present fish. Although the sex genes are effective sex determiners, their action appears to be mediated by the sex gene controlled sex inductors (androinductor and gynoiductor). It has not yet been decided whether the steroid hormones are identical to, or allied with natural sex inductors, or if they are entirely different. The problem remains to be answered. In amphibians, WITSCHI (1942, 1955) stresses that natural sex inductors are not steroid hormones and are of protein nature. CHANG (1953), CHANG and WITSCHI (1956) and GALLIEN (1958) support the nonsteroid theory.

Our previous and present studies on the medaka indicate that an effective heterotypic sex hormone administered during larval life overrides the genetic mechanism for sex determination, *viz.*, the genetic male (XY) develops into functional female by estrogen and *vice versa* the genetic female (XX) differentiates into functional male by androgen, methyl testosterone (YAMAMOTO 1958). It can be stated that estrogen has an action of gynoiductor and methyl testosterone can act as an androinductor.

As shown in a previous paper (YAMAMOTO 1953), day-old fry (average length 4.8 mm) of the medaka have an indifferent gonad and their sex differentiation takes place during the larval period. The present study indicates that continuous administration of the estrogens at the dosage level of 1,250 i.u./g diet, beginning with hatching and ending at certain juvenile stages (8–13 or 10–15 mm), *i.e.*, covering the period prior to and passing through the stage of gonadal sex differentiation, is sufficient to induce reversal of sex differentiation in the majority of genetic males and to develop them into functional females. Continuation of administration after 12 mm seems to be superfluous. The result suggests that normally the sex genes play an active role only during a short period preceding the stage of gonadal sex differentiation. Perhaps, the sex genes become active and control the production of natural sex inductors within inductor cells surrounding indifferent gonidia. These natural sex inductors may cause indifferent gonidia to cyto-differentiate into either male or female germ cells.

An effective estrogen, administered during the proper period, may counteract and override the action of the male sex genes or that of the androinductor. Once the indifferent gonad in genetic males has undergone differentiation into ovary under the influence of an administered estrogen, the remaining sex equipment follows the female pattern under the control of endogenous ovarian activity which prevails after hormone administration has been discontinued. The sexual habits are those of the female, also. It is to be noted that the induction of sex reversal is permanent. There is no instance of sex-reversed fish which later turns into the genetically determined sex. On the other hand, we have had a number of sex reversals which produced offspring in two consecutive years.

In a previous study (YAMAMOTO 1955), a single $Y^R Y^R$ male was detected among 20 F_1 sons of estrone-sex-reversals and none among 21 F_1 sons of stilbestrol-sex-reversals. In the present study only one out of ten F_1 sons of estrone-sex-reversals

and none out of six F_1 sons of stilbestrol-sex-reversals was proved to be $Y^R Y^R$ male. When all these data are combined, it follows that only two $Y^R Y^R$ out of 57 F_1 sons ($X^r Y^R \delta \delta + Y^R Y^R \delta \delta$) of sex reversals ($X^r Y^R$) mated to $X^r Y^R$ males, *viz.*, approximately one $Y^R Y^R$ out of every 30 sons are viable instead of the theoretical one out of every three. This shows that only one out of every ten $Y^R Y^R$ zygotes can be viable. A lethal action of some sort seems to be operative in the majority of zygotes having the constitution $Y^R Y^R$. The failure to detect viable $Y^R Y^R$ females among sex reversals ($X^r Y^R \varnothing \varnothing + Y^R Y^R \varnothing \varnothing$) estrogenized in two consecutive generations may be ascribed to the rare occurrence of developing $Y^R Y^R$ zygotes since the number of successful testcrosses was only 21 ($ER \varnothing > ER \varnothing$ plus $5 SR \varnothing > SR \varnothing$).

In normal breeding of white females ($X^r X^r$) with heterozygous orange-red males ($X^R Y^r$) of the medaka, in which was displayed a crisscross inheritance, AIDA (1936) obtained two exceptional white daughters having the genotype $X^r Y^r$ resulting from genic imbalance of sex genes. These exceptional $X^r Y^r$ females were mated with normal $X^R Y^r$ males. The observed proportion of white sons ($X^r Y^r \delta \delta + Y^r Y^r \delta \delta$) to orange-red sons ($X^R Y^r \delta \delta$) to orange-red daughters ($X^R X^r \varnothing \varnothing$) did not depart from that of the theoretical 2:1:1 ratio indicating that the $Y^r Y^r$ zygotes were as viable as $X^r Y^r$ and $X^R Y^r$ males. By testcrosses, he proved that about one half of white sons were $Y^r Y^r$ males. This is in strong contrast to the rarity of viable $Y^R Y^R$ zygotes demonstrated in our previous and present experiments. In this connection it is worth while noting that in the guppy (*Lebistes reticulatus*) WINGE (1934) demonstrated that $Y^{Ma} Y^{Pa}$ (Ma =maculatus, Pa =pauper) were viable and fertile, while WINGE and DITLEVSEN (1938, 1947) showed that $Y^{Ma} Y^{Ma}$ males are lethal. They postulated the presence of a lethal factor closely linked to the Ma gene.

GORDON (1946, 1947) demonstrated that "wild" stocks of the platyfish (*Platypoecilus maculatus*) from Mexico had the genetic mechanism for sex determination in which XX =female and XY =male—just opposite to the established type (WZ =female and ZZ =male) for the common aquarium-bred stock of the same species. GORDON (1951) reported that the platyfish from British Honduras belongs to the latter type (WZ - ZZ). CASTLE (1936) suggested that WZ and ZZ could be expressed as XY and YY , respectively. GORDON (1947) commented that although the W of the "domesticated" stock is not homologous to any X of the platyfish, the Z and the Y are apparently homologous. Whole populations of the female-heterogametic platyfish exist in which ZZ (YY) males represent the normal condition. Our finding that viable $Y^R Y^R$ males are rare in the medaka, where normal male is heterogametic (XY), may be interesting in view of evolution of heterosomes in fishes.

The lethality of the majority of $Y^R Y^R$ males in the medaka cannot be ascribed to their rapid energy consumption, although it might result in a relatively shorter life span. The present study indicates that if $Y^R Y^R$ females were ever viable, they also would be rare in spite of the fact that the female consumes less energy than the male. On the other hand, there is some evidence suggesting the presence of an

inert section in the ordinary Y^R chromosome, which causes lethality of the zygote homozygous for it. It is inferred that both the regular X^r and X^R contain a positive section, essential for viability, in the region corresponding to the inert section of the ordinary Y^R . The rare viable $Y^R Y^R$ male was found to be heterozygous for the inert section, *viz.*, one Y^R having the positive section derived from the X^r by crossing over (unpublished). Incidentally, the assumption of the presence of an inert section in the ordinary Y^R chromosome rationalizes readily the previously mentioned discrepancy between the observed ($2\text{♀}:1\text{♂}$) and expected ($1\text{♀}:1\text{♂}$) sex ratios among offspring of crossover orange-red females denoted by $X^r X_c^R$ mated with normal males ($X^r Y^R$). The recombinant X_c^R is suspected to have received the inert section together with the R from the ordinary Y^R in crossing over occurring in the parents (sex-reversal $X^r Y^R \text{♀}$ and $X^r Y^R \text{♂}$). Hence, the $X_c^R Y^R$ zygotes may be lethal because they are homozygous for the inert section. A detailed account on this problem is reserved until data from current experiments are completed.

The occurrence of WW females in cases of female heterogamety has also been recorded. BREIDER (1942), in breeding the domesticated platyfish (*Platypoecilus maculatus*), found an exceptional WZ male. This exceptional male was presumed to have the constitution $W^{Rsp} Z^{Dr}$, and in a mating with a normal female $W^+ Z^R$, sired 51 daughters and 13 sons. Among the daughters, he claimed to have obtained some WW females. This was highly probable but unfortunately testcrosses for detecting WW females were not carried out. If the WW females had been mated with normal males (ZZ), all female progenies would surely have resulted. BELLAMY and QUEAL (1951), in breeding the same fish, found a number of exceptional WZ males which, in mating with normal WZ females, fathered WW females in addition to ordinary females (WZ) and males (ZZ). Progenies of these WW females by normal males (ZZ) consisted entirely of females. It was noted by them that WW females were less fertile. HUMPHREY (1945) proved experimentally that in ambystomid salamanders (*Siredon mexicanum*, *Ambystoma tigrinum*) WW females were viable and fertile. It is remarkable that HUMPHREY (1948) was able to show that males having the genotype WW, induced by grafting of testis preprimordium on WW embryos, are viable. These results led him to conclude that in these species the W chromosome is not greatly different from the Z except as to the sex-determining factor.

Data on offspring of sex reversals in the present study indicate that the percentage of both exceptional white males and exceptional orange-red females is significantly greater than that in the stock from which experimental fish were derived. This is particularly true with the offspring of sex reversals in two consecutive generations. That seven out of 503 or 1.4 percent of white fish are exceptional males and 35 out of 1,047 or 3.3 percent of orange-red fish are exceptional females, is shown in Table 3, fourth line. The frequency of exceptional fish in these data is 5–6 times that among the stock bred for several generations. In the stock exceptional orange-red females always exceed exceptional white males. The preponderance of exceptions among offspring of sex reversals over those among

the controls and the stock suggests the occurrence of crossing over between the X^r and Y^R chromosomes in both parents, *i.e.*, sex-reversal mothers (X^rY^R) and normal X^rY^R fathers.

SUMMARY

This study is on artificial induction of functional sex reversal in genotypic males (XY) of the medaka (*Oryzias latipes*) made under the influence of estrogens (estrone and stilbestrol) using the offspring of X^rX^r females mated to X^rY^R males (r =white, R =orange-red).

The continuous administration *per os* of either estrone or stilbestrol at the dosage level of 1,250 i.u./g diet, beginning with the time of hatching and ending at certain juvenile stages, covering the period prior to and passing through the stage of the gonadal sex differentiation, is sufficient to induce permanent sex reversal in the majority of genotypic males (X^rY^R). A rare intersex of the male genotype was also induced.

A single Y^RY^R male, the second ever to have been detected in our series of experiments, was detected among ten normal-fed F_1 sons of estrone-sex-reversals by X^rY^R males.

Functional sex reversal in genotypic males by means of estrogens was successful in two consecutive generations. So far, 16 estrone-sex-reversals and five stilbestrol-sex-reversals estrogenized for two consecutive generations and presumably having the genotype either X^rY^R or Y^RY^R , were singly tested to normal X^rY^R males. All those tested were found to have the genotype X^rY^R . No Y^RY^R females were detected, a fact ascribed to the rarity of surviving Y^RY^R zygotes.

The exceptions among offspring of sex reversals were proved to be either cross-overs (X^rY^r males and X^rX^R females) or X^rY^R females arising from genic imbalance of sex genes. The preponderance of exceptions among offspring of sex reversals over those among controls and the stock suggests the occurrence of crossing over between the X^r and Y^R in both parents, *i.e.*, sex-reversed mothers (X^rY^R) and normal X^rY^R fathers.

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LITERATURE CITED

- AIDA, T., 1921 On the inheritance of color in a fresh-water fish *Aplocheilus latipes* Temminck and Schlegel, with special reference to the sex-linked inheritance. *Genetics* **6**: 554–573.
 1936 Sex reversal in *Aplocheilus latipes* and a new explanation of sex differentiation. *Genetics* **21**: 136–156.
 BELLAMY, A. W., and M. L. QUEAL, 1951 Heterosomal inheritance and sex determination in *Platyopocilus maculatus*. *Genetics* **36**: 93–107.

- BREIDER, H., 1942 ZW-Männchen und WW-Weibchen bei *Platyopocilus maculatus*. Biol. Zentr. **62**: 183-195.
- BRIDGES, C. B., 1922 The origin of variation in sexual and sex-limited characters. Am. Naturalist **56**: 51-63.
- 1925 Sex in relation to chromosomes and genes. Am. Naturalist **59**: 127-137.
- 1939 Cytological and genetic basis of sex. Ch. 2, 15-63. Edited by E. ALLEN. *Sex and Internal Secretions*. Williams and Wilkins Co. Baltimore, Maryland.
- CASTLE, W. E., 1936 A simplified explanation of BELLAMY's experiments concerning sex determination in tropical fishes. Proc. Natl. Acad. Sci. U.S. **22**: 679-682.
- CHANG, C. Y., 1953 Parabiosis and gonad transplantation in *Xenopus laevis* Daudin. J. Exptl. Zool. **123**: 1-27.
- CHANG, C. Y., and E. WITSCHI, 1955 Breeding of sex-reversed males of *Xenopus laevis* Daudin. Proc. Soc. Exptl. Biol. Med. **89**: 150-152.
- 1956 Genetic control and hormonal reversal of sex differentiation in *Xenopus*. Proc. Soc. Exptl. Biol. Med. **93**: 140-144.
- GALLIEN, L., 1954a Démonstration de l'homogamétie du sexe mâle chez le triton *Pleurodeles waltlii* Michah. par l'étude de la descendance d'animaux à sexe physiologiques inversé, après un traitement hormonal gynogène (benzoate d'oestradiol). Compt. Rend. acad. sci. (Paris) **238**: 402-404.
- 1954b Inversion expérimentale du sexe, sous l'action des hormones sexuelles, chez le triton *Pleurodeles waltlii* Michah. Analyse de conséquences génétiques. Bull. Biol. France et Belg. **88**: 1-51.
- 1955 Descendance unisexuée d'une femelle de *Xenopus laevis* Daud. Ayant subi, pendant sa phase larvaire, l'action gynogénèse du benzoate d'oestradiol. Compt. Rend. acad. sci. (Paris) **240**: 913-915.
- 1956 Inversion expérimentale du sexe chez un Anoure inférieur *Xenopus laevis* Daudin. Analyse des conséquences génétiques. Bull. Biol. France et Belg. **90**: 163-183.
- 1958 Vue d'ensemble sur l'action des stéroïdes sexuels dans la différenciation du sexe chez les amphibiens. 15th Intern. Congr. Zool., Sect. VII, Paper 37.
- GORDON, M., 1946 Interchanging genetic mechanisms for sex determination. J. Heredity **37**: 307-320.
- 1947 Genetics of *Platyopocilus maculatus*. IV. The sex determining mechanism in two wild populations of the Mexican platyfish. Genetics **32**: 8-17.
- 1951 Genetics of *Platyopocilus maculatus*. V. Heterogametic sex determining mechanism in females of a domesticated stock originally from British Honduras. Zoologica **32**: 127-134.
- 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.
- 1948 Reversal of sex in females of genotype WW in the axolotl (*Siredon* or *Ambystoma mexicanum*) and its bearing upon the role of the Z chromosomes in the development of the testis. J. Exptl. Zool. **109**: 171-185.
- WINGE, Ö., 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. lab. Carlsberg. Sér. physiol. **22**: 203-210.
- 1947 Color inheritance and sex determination in *Lebistes*. Heredity **1**: 65-83.
- WITSCHI, E., 1942 Hormonal regulation of development in lower vertebrates. Cold Spring Harbor Symp. Quant. Biol. **10**: 145-153.
- 1955 On morphogenic capacity of estrogens. J. Clin. Endocrinol. and Metabolism **15**: 647-652.
- YAMAMOTO, T., 1953 Artificially induced sex reversal in genotypic males of the medaka (*Oryzias latipes*). J. Exptl. Zool. **123**: 571-594.

- 1955 Progeny of artificially induced sex reversals of male genotype (XY) in the medaka (*Oryzias latipes*) with special reference to YY-male. *Genetics* **40**: 406-419.
- 1957 Estrone-induced intersex of genetic male in the medaka (*Oryzias latipes*). *J. Fac. Sci. Hokkaido Univ. Ser. VI* **13**: 440-444 (Prof. T. Uchida Jubilee Volume).
- 1958 Artificial induction of functional sex reversal in genotypic females of the medaka (*Oryzias latipes*). *J. Exptl. Zool.* **137**: 227-264.