THE PROBLEM OF VIABILITY OF YY ZYGOTES IN THE MEDAKA, ORYZIAS LATIPES

TOKI-O YAMAMOTO

Biological Institute, Faculty of Science, Nagoya University, Nagoya, Japan

Received February 5, 1964

SUCCESSFUL induction of complete reversal in sex differentiation in the medaka (*Oryzias latipes*), where the normal sex-chromosome mechanism is XX for female and XY for male, makes it possible to attack a number of problems otherwise difficult to approach. The difference of the allosomal recombination values between estrone-induced heterogametic females (XY) and normal XY males is one example (YAMAMOTO 1961). The viability of YY zygotes is another challenging problem. Exceptional fishes that have arisen by genic outbalance between allosomal and autosomal sex-genes can be and have been used for such a purpose. For instance, WINGE (1934) and WINGE and DITLEVSEN (1938, 1947) in the guppy (*Lebistes reticulatus*) demonstrated viability of $Y^{Ma}Y^{Pa}$ (Ma = Maculatus, Pa = Paupaus) versus lethality of $Y^{Ma}Y^{Ma}$. AIDA (1936) showed that Y^rY^r males in *Aplocheilus* (now *Oryzias*) *latipes* are viable. They used exceptional XY females, which sporadically appeared in their breeds. The appearance of such exceptions, however, is often too infrequent and sporadic to be used satisfactorily to attack the problem under consideration. AIDA for instance, used only two exceptional XY females, which were inappropriately called sex-reversals.

Artificial control of sex differentiation as advanced in Oryzias (YAMAMOTO, 1953, 1955, 1959a,b) renders it possible to obtain estrogen-induced XY females in abundance, thus offering sufficient material to approach the problem of viability of YY zygotes. In *Oryzias latipes*, either the gene R (orange-red) or its allele r (white) is borne by the X or Y chromosome (AIDA 1921). Our success in inducing complete reversal in sex differentiation in genetic males (X^rY^R) under the influence of estrogens (estrone or stilbestrol) renders it possible to obtain an Y^RY^R male by mating estrogen-induced X^rY^R females with normal X^rY^R males. Although the viable Y^RY^R genotype was well demonstrated in the experiments reported (YAMAMOTO 1955, 1959a) the actual proportion of X^rY^R to Y^RY^R genotypes deviated seriously from the expected 2:1 ratio. Of 57 F_1 orange-red sons (X^rY^R and Y^RY^R) singly tested by white females (X^rX^r), only two proved to be Y^RY^R, *viz.* only one in 28.5 sons instead of the theoretical one in three. The rarity of viable Y^RY^R males is beyond question. This is in strong contrast to AIDA's (1936) finding that Y^rY^r males, produced by mating sporadic X^rY^r females with normal X^rY^r males, are as viable as X^rY^r males. The discrepancy is cogently rationalized on the basis of the inductive reasoning presented in the ensuing pages. In short, both of us are correct. The scarcity of surviving Y^RY^R zygotes is reflected by failure to detect Y^RY^R animals among induced females having the male genotypes X^rY^R and Y^RY^R (Yamamoto 1959a). A lethal action of some sort seems to be operative in the majority of the zygotes having the constitution Y^RY^R. In contrast to the rarity of Y^RY^R males, the majority of Y^RY^r males are found to be viable (Yamamoto 1961)—a most unexpected finding, to which we shall refer later. As a matter of fact, it was possible to invert the sex differentiation of Y^RY^r zygotes and actually to detect them as functional females (Yamamoto 1963).

To interpret the rarity of viable Y^RY^R zygotes relative to Y^RY^r, a number of possibilities are considered in the Discussion. Among them, the hypothesis that I have been led to adopt is the presence of an inert segment (−) in the regular Y^R chromosome, which in the duplex condition results in nonviability, and the presence of a "viability" section (+) at the corresponding region in the X chromosome. It is true that our results could be interpreted as well on the basis of the presence of a recessive lethal factor (*l*) in the regular Y^R chromosome. The asymmetry of crossing over occurring in X^rY^R and X^RY^r males (Aida 1930) favors the inert-section hypothesis rather than a single lethal factor, since a point lethal does not, as a rule, affect the frequency of crossing over.

Our hypothesis that an inert section (−) is present in the ordinary Y^R chromosome (*c.f.* Figure 1) and that crossing over occurs between the $X^{r,+}$ and $Y^{R,-}$ involving the section (−) not only interprets previous observations plainly but rationalizes all the data which show serious deviations from the expectation (Yamamoto 1959a, p. 754). In short, viable Y^RY^R zygotes are considered to be $Y^{R,-}Y_c^{R,+}$, where $Y_c^{R,+}$ stands for a recombinant Y^R having the (+) derived from the $X^{r,+}$.

The present paper offers the various lines of verification of the hypothesis referred to already. Our experiments and considerations provide us with a new insight into the constitution of the sex chromosomes. A tentative linkage map of the sex chromosomes is presented in this paper (Figure 1). A more detailed one will be given in a paper that follows. If our hypothesis be correct, we would expect the following three corollaries: (1) the F_1 orange-red sons from X^rX^r ♀ × Y^RY^R ♂ to comprise the two genotypes, $X^{r,+}Y^{R,-}$ and $X^{r,+}Y_c^{R,+}$ (2) non viability of the majority of $X_c^RY^R$ males ($X_c^{R,-}Y^{R,-}$), where X_c^R is a recombinant X^R and (3) viability of Y^rY^r zygotes, which would be of the constitution $Y^{r,+}Y^{r,+}$. All these consequences of the hypothesis are actually found to be true. The presence of the inert section (−) in a region of the regular Y chromosome, therefore, has become more than just a hypothesis.

From the evolutional viewpoint, the present study may be of some interest in demonstrating the fact that possibly the medaka represents a transitional stage in the deterioration of the Y chromosome in the animal kingdom. A preliminary report has appeared in Abstract form (Yamamoto 1963b).

MATERIALS AND METHODS

The materials used are color varieties of the medaka, an oviparous cyprinodont fish. The genealogy of the d-*rR* strain, where females are X^rX^r (white) and males are X^rY^R (orange-red) was described previously (Yamamoto 1961).

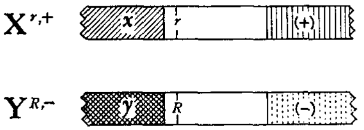

FIGURE 1.—The constitution of regular X^r and Y^R chromosomes. x and y are differential segments, the white region including the r locus represents the homologous segment, and (+), (—) are "viability" and inert segments, respectively.

Since the time of AIDA (1921), it has been known that white fish appearing among non-pedigreed stocks of dealers are usually females of the genotype X^rX^r. In our laboratory, exceptional white males of the constitution X^rY^r have appeared as crossovers between the X^r and Y^R both in the normal breed and in progenies of induced sex-reversals. Hence, we were able to establish the white strain (d-*rr*), where females are X^rX^r and males are X^rY^r. We have also pure orange-red strain, with X^RX^R females and X^RY^R males. Heterozygous X^RY^r males are obtained by crossing X^RX^R females with X^rY^r males. These strains were used either for induction of reversal in sex differentiation or as testers of genotypes. The experimental design, rearing methods, and administration of hormones were as described previously (YAMAMOTO 1961, 1963a). To avoid confusion in terminology, sex does not refer to sex-chromosome combinations (XX or XY) but to phenotypic expressions, a male being defined as an individual with the testis and a female, the ovary.

RESULTS

(1) *Genetic analysis of F_1 sons of* Y^RY^R *male:* If our hypothesis is correct, it is legitimate to suppose that surviving Y^RY^R males would be different from non-viable Y^RY^R zygotes in their genetic constitution, the former would be $Y^{R,-}Y^{R,+}_{c}$ and the latter $Y^{R,-}Y^{R,-}$. We would then expect the F_1 of the $Y^{R,-}Y^{R,+}_{c}$ male by $X^{r,+}X^{r,+}$ females to comprise two genotypes, $X^{r,+}Y^{R,-}$ and $X^{r,+}Y^{R,+}_{c}$. It is impossible to distinguish the two genotypes by testing with normal $X^{r,+}X^{r,+}$ females, but the estrogen-induced X^rY^R female (presumably $X^{r,+}Y^{R,-}$) used as tester would provide information about their genotypes.

To follow up this expectation, 12 orange-red sons (X^rY^R) of the Y^RY^R male (the second Y^RY^R reported in 1959) by normal females (X^rX^r) were selected at random and mated in 1957 singly to estrone-induced females of male genotype (X^rY^R, E*R* females 1956). Of 12 matings, seven produced offspring in a reasonable numbers (Table 1). Of seven male parents, four fathered offspring in a ratio of 1*r*♀ : 2*R*♂ rather than the expected 1*r*♀ : 3*R*♂, and the remaining two sired to conform to a 1*r*♀ : 3*R*♂ ratio rather than 1*r*♀ : 2*R*♂. The remaining parent (starred in Table 1) produced offspring which are in agreement with both 1*r*♀ : 2*R*♂ and 1*r*♀ : 3*R*♂ expectations, but closer to the former. It appears that matings of five out of seven probably were induced $X^{r,+}Y^{R,-}$♀ × $X^{r,+}Y^{R,-}$♂, and the remaining two were induced $X^{r,+}Y^{R,-}$♀ × $X^{r,+}Y^{R,+}_{c}$♂. Among the offspring of the former, the genotype $Y^{R,-}Y^{R,-}$ is considered to be lacking, although rare $Y^{R,-}Y^{R,+}_{c}$ could be present.

These results can be explained if we assume that the viable Y^RY^R males had the constitution $Y^{R,-}Y^{R,+}_{c}$, where the (+) has been transferred from the $X^{r,+}$ by crossing over. The ratio of $X^{r,+}Y^{R,-}$ (5) to $X^{r,+}Y^{R,+}_{c}$ (2) agrees statistically

TABLE 1

Testcrosses of F_1 orange-red sons (X^rY^R) from X^rX^r ♀ × Y^RY^R ♂, by esterone-induced females (ER ♀ ♀, X^rY^R 1956)

Parents		Offspring				χ^2 for 1 : 3	χ^2 for 1 : 2	Probable matings
Female	Male	r♀	r♂	R♀	R♂			
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-2	56	1	1	108	7.3	0.05	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},-$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-5*	22	1	0	51	1.0	0.3	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},-$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-7	70	1	0	150	5.5	0.2	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},-$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-8	101	2	2	195	13.1	0.1	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},-$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-9	42	0	0	92	2.9	0.2	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},-$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-4	41	0	1	131	0.1	7.0	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},+$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-6	14	0	0	43	0.0	2.0	$(X^{r},+Y^{R},-) \times X^{r},+Y^{R},+$
Total		346	5	4	770			

Data are arranged according to two categories of progenies instead of experimental number. Numeral following hyphen is fish number. r = white, R = orange-red.

* Referred to in text.

with the expected 1:1 ratio. Although the F_1 sons (X^rY^R) of the viable Y^RY^R male are phenotypically alike, they are of the two different types with respect to the nature of their offspring, in agreement with our hypothesis.

(2) *Curious genetic behavior of crossover* $X^rX^R_c$ *females:* Another evidence for the presence of an inert section in the regular Y^R chromosome comes from a curious genetic behavior of crossover $X^rX^R_c$ females, where X^R_c stands for the X^R chromosome, the R of which is known to have been derived from the Y^R by crossing over. On several occasions, I have met with the fact that a crossover $X^rX^R_c$ female in mating with a normal X^rY^R male produced offspring in the ratio 1r♀ : 1R♀ : 1R♂ instead of the theoretical 1r♀ : 1R♀ : 2R♂. This fact is particularly evident when enough offspring are produced for statistical tests of significance. In a previous paper (YAMAMOTO 1959a, p. 751), I commented that "Although the observed proportion showed a significant departure from the ex-

TABLE 2

Analyses of exceptions appearing from $E(X^rY^R)$♀ × X^rY^R♂ < Y^RY^R (cf. Table I), suggesting lethality of $X^R_cY^R$ (X^R_c denotes recombinant X^R). r = white, R = orange-red

Grand parents		Parents		Offspring				Genotype of exceptions
Female	Male	Female	Male	r♀	r♂	R♀	R♂	
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-2	X^rX^r	exc.r♂	44	61	0	0	X^rY^r
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-5	X^rX^r	exc.r♂	41	38	0	0	X^rY^r
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-8	X^rX^r	exc.r♂	20	20	0	0	X^rY^r
Total				105	119	0	0	χ^2 for 1:1=0.9
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-2	exc.R♀	X^rY^R	97	0	90	82	$X^rX^R_c$
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-8	exc.R♀	X^rY^R	11	0	6	11	$X^rX^R_c$
Total				108	0	96	93	χ^2 for 1:1:2=43*

* χ^2 for 1:1:1=1.3, P=0.5.

pected, we can not label these exceptions other than X^rX^R, *viz.* crossovers." Further data relevant to this curious fact were given earlier (YAMAMOTO 1961 p. 173). Now we can conclude convincingly that it is the $X_c^{R,-}Y^{R,-}$ class that is lacking among progeny of crossover $X^{r,+}X_c^{R,-}$ females × $X^{r,+}Y^{R,-}$ males.

The present section gives additional results confirming this fact. Among 1,125 offspring of estrone-induced X^rY^R ♀ ♀ × F_1 sons (X^rY^R) of Y^RY^R, five exceptional white males and four exceptional orange-red daughters appeared (Table 1). Of these exceptions three white males and two orange-red females were successfully submitted to progeny tests. The results are given in Table 2. All the three white exceptions bred as crossover X^rY^r males when tested by X^rX^r females. The two exceptional orange-red daughters ($X^rX_c^R$) yielded offspring comprising a 1*r* ♀ : 1*R* ♀ : 1*R* ♂ ratio on testing with X^rY^R males. The results do not agree with a 1:1:2 ratio ($\chi^2 = 43$). The result is quite consistent with the previous findings.

Hitherto, we have used the X^rY^R males as a tester for exceptional orange-red females. Table 3 gives results obtained by testing these females with white males (X^rY^r). In this type of mating, four classes are expected to occur in the ratio of 1*r* ♀ : 1*r* ♂ : 1*R* ♀ : 1*R* ♂. In the first row of Table 3, is given the result of an exceptional orange-red female tested by an X^rY^r male. This exception is the same fish that was previously tested by an X^rY^R (*c.f.* Table 2) and produced offspring in a 1*r* ♀ : 1*R* ♀ : 1*R* ♂ ratio instead of the theoretical 1:1:2. The same fish when tested by an X^rY^r male produced offspring in an expected 1:1:1:1 ratio. The deviation is barely significant. The other two exceptions shown in Table 3 are exceptional orange-red females appeared among progenies of estrone-induced X^rY^R females mated with methyltestosterone-induced X^rX^r males. These were previously tested by X^rY^R males and produced offspring in a 1*r* ♀ : 1*R* ♀ : 1*R* ♂ ratio instead of the expected 1:1:2 ratio (*cf.* YAMAMOTO 1961). These same fishes produced offspring in an expected 1*r* ♀ : 1*r* ♂ : 1*R* ♀ : 1*R* ♂ ratio in matings with X^rY^r males. The pooled data agree with expectation (P = 0.3—0.2). All these results indicate that the majority of the $X_c^RY^R$ genotype is inviable in contrast to the viability of most of the $X_c^RY^r$. On the basis of our hypothesis, the former has the genotype $X_c^{R,-}Y^{R,-}$ and the latter $X_c^{R,-}Y^{r,+}$.

TABLE 3

Progenies of exceptional orange-red (R) *females tested by white males* (X^rY^r), *demonstrating viability of* $X_c^RY^r$ *versus lethality of* $X_c^RY^R$, *where* X_c^R *denotes a recombinant* X^R

Grandparents		Parents		Offspring				Genotype of exc.*R* ♀	χ^2 for 1:1:1:1
Female	Male	Female	Male	*r* ♀	*r* ♂	*R* ♀	*R* ♂		
$E(X^rY^R)$ ♀	$Y^RY^R > F_1$ ♂-2	exc.*R* ♀	X^rY^r	7	17	9	17	$X^rX_c^R$	6.4
$E(X^rY^R)$ ♀	$M(X^rX^r)$ ♂-9	exc.*R* ♀	X^rY^r	12	13	14	13	$X^rX_c^R$	1.5
$E(X^rY^R)$ ♀	$M(X^rX^r)$ ♂-10	exc.*R* ♀	X^rY^r	20	24	19	23	$X^rX_c^R$	0.8
	Total			39	54	42	53		3.7*

$E(X^rY^R)$ is estrone-induced female and $M(X^rX^r)$ methyltestosterone-induced male (*cf.* YAMAMOTO 1961). *r* = white, *R* = orange-red.
* P = 0.3.

Induction of reversal in sex differentiation of genetic males (X^rY^R) has been accomplished with estradiol (YAMAMOTO and MATSUDA 1963). Among offspring of estradiol-induced X^rY^R females mated with normal X^rY^R males, two exceptional daughters appeared among 141 orange-red (*R*) fish. These are denoted as Ed *R*♀♀ > exc.*R*♀-1 and Ed *R*♀♀ > exc.*R*♀-2. Each of them was mated successively with an X^rY^R male and an X^rY^r male in the same year, and their offspring are listed in Table 4. When the tester is an X^rY^R, numbers of segregated classes depart seriously from the expectation of 1*r*♀ : 1*R*♀ : 2*R*♂, but show fair agreement with 1*r*♀ : 1*R*♀ : 1*R*♂, suggesting that the $X^{R,-}_{c}$-$Y^{R,-}$ genotype is nonviable, because both chromosomes possess inert segments. On the other hand, the same exceptions on testing with the X^rY^r male, yielded offspring in an expected 1*r*♀ : 1*r*♂ : 1*R*♀ : 1*R*♂ ratio, indicating that the $X^{R,-}_{c}$-$Y^{r,+}$ genotype is viable.

Until now, nonviability of the genotype $X^R_cY^R$ has been inferred from the numerical relations of the ratios of the phenotypic classes segregating. To prove the actuality of this supposition, the F_1 orange-red sons ($X^rY^R + X^R_cY^R$) from the mating $X^rX^R_c$ ♀ × X^rY^R ♂ are submitted to progeny tests in order to ascertain whether or not the genotype $X^R_cY^R$ could be detected. Two series of such testcrosses are presented in Tables 5 and 6. The exceptional paternal grandmother in Table 5 is an exception appearing among the F_1 of estrone-induced X^rY^R females and methyltestosterone-induced X^rX^r males, and she is known to be a crossover $X^rX^R_c$ (YAMAMOTO 1961). Two paternal grandmothers ($X^rX^R_c$-1 and $X^rX^R_c$-2) shown in Table 6 are exceptions that appeared among offspring of estradiol-induced X^rY^R females (*cf.* Table 4). If the genotype $X^R_cY^R$ were viable we would expect a $1X^rY^R$ ♂ : $1X^R_cY^R$ ♂ ratio among males tested. Of 26 males tested all proved to be X^rY^R, and no $X^R_cY^R$ was detected. Therefore, it seems inevitable to suppose that the majority of $X^R_cY^R$ would be nonviable since they were duplex for the (−) segment. There is, however, a possibility of the occurrence of surviving $X^{R,-}_{c}$-$Y^{R,+}_{c}$ males, where Y_c stands for a recombinant. Since both $X^{R,-}_{c}$ and $Y^{R,+}_{c}$ could only be produced by crossing over between the $X^{r,+}$ and $Y^{R,-}$ the $X^{R,-}_{c}$-$Y^{R,+}_{c}$ combination would be extremely rare.

TABLE 4

Testcrosses of two exceptional orange-red daughters from estradiol-induced Ed(X^rY^R) ♀ × normal X^rY^R ♂

Parents		Offspring				Genotype of exc.*R*♀	χ^2 and P for ratio
Female	Male	*r*♀	*r*♂	*R*♀	*R*♂		
Ed(X^rY^R) ♀ > exc.*R*♀-1	X^rY^R	27	0	25	25	$X^rX^R_c$	1:1:2=9.6, P=0.01
							1:1:1=0.1, P=0.9
Ed(X^rY^R) ♀ > exc.*R*♀-1	X^rY^r	56	45	64	57	$X^rX^R_c$	1:1:1:1=3.3, P=0.3
Ed(X^rY^R) ♀ > exc.*R*♀-2	X^rY^R	25	0	32	28	$X^rX^R_c$	1:1:2=11, P=0.01
							1:1:1=0.9, P=0.6
Ed(X^rY^R) ♀ > exc.*R*♀-2	X^rY^r	41	40	44	27	$X^rX^R_c$	1:1:1:1=4.5, P=0.2

Each of two exceptions is tested separately and successively by X^rY^R and X^rY^r males. *r*=white, *R*=orange-red, X^R=Recombinant X^R.

TABLE 5

Testcrosses of orange-red sons from exceptional $X^rX^R_c$ ♀ × X^rY^R ♂ *in test for either* X^rY^R *or* $X^R_cY^R$

Parents		Offspring				Genotype of P-♂ tested
Female	Male	r♀	r♂	R♀	R♂	
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-1	48	0	1	53	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-2	50	0	0	52	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-3	24	0	0	35	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-4	88	0	0	82	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-5	42	0	0	25*	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-6	8	0	0	15	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-7	27	0	0	20	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-8	33	0	0	45	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-9	81	0	0	100	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-10	45	0	0	65*	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-11	100	0	0	131*	X^rY^R
X^rX^r	$X^rX^R_c \times X^rY^R > R$♂-12	27	0	0	22	X^rY^R
	Total	573	0	1	645*	

The exceptional paternal grandmother ($X^rX^R_c$) used is an exception appeared among F_1 of estrone-induced X^rY^R♀ × methyltestosterone-induced X^rX^r♂-10 and is known to be a crossover $X^rX^R_c$ (*cf.* Tables 8 and 9 in the previous paper by YAMAMOTO 1961). r = white, R = orange-red, X^R_c = recombinant X^R.

* Deviation from 1r♀ :1R♂ is significant. Other crosses are in agreement with 1:1.

Several repetitions of the two kinds of matings, $X^rX^R_c$ ♀ × X^rY^R ♂ and $X^rX^R_c$ ♀ × X^rY^r ♂, showed absence of $X^R_cY^R$ sons in the former, and presence of $X^R_cY^r$ sons in the latter, conforming to our hypothesis.

(3) *Experimental production of white* Y^rY^r *males:* A previous series of experiments made it clear that viable Y^RY^R zygotes are rare whereas Y^RY^r are not rare, and are as viable as normal X^rY^R zygotes (YAMAMOTO 1955, 1959a, 1961). This leads us to inquire whether Y^rY^r males in our strain are viable.

A diagram of the experiment is given in Figure 2. By administration of estrone (50 μg/g diet) during larval stage, 37 X^rY^r females were obtained. Of these, 15 were mated with 12 normal white males (X^rY^r). The result is given in Table 7. The segregation for white daughters and white males agrees statistically with an expectation of 1:3.

Twelve out of 58 white sons (starred in Table 7), presumably either X^rY^r or Y^rY^r, were individually tested by normal white females X^rX^r (Table 8). Of 12 males tested, 10 sired white daughters and white sons in a 1:1 ratio, demonstrating their genotype to be X^rY^r. The remaining two fathered only males, proving their sex genotype to be Y^rY^r.

In this type of mating, the probability of picking a Y^rY^r son is 0.33 if all Y^rY^r survive. The observed ratio of 10:2 does not deviate significantly from the theoretical 8:4. Thus, both numerical relations of the ratio of phenotypic classes segregating among the F_1 of estrone-induced X^rY^r ♀ × X^rY^r ♂, and the ratio of the genotypes X^rY^r to Y^rY^r actually detected by testcrosses, demonstrate the

TABLE 6

Testcrosses of orange-red sons from exceptional $X^rX^R_c$♀ × normal X^rY^R♂ in test for either X^rY^R or $X^R_cY^R$

Parents		Offspring				Genotype of P-♂ tested
Female	Male	r♀	r♂	R♀	R♂	
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-1	11	0	0	14	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-2	32	0	0	39	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-3	28	0	0	27	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-4	36	0	0	31	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-5	29	0	0	33	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-6	19	0	0	28	X^rY^R
X^rX^r	$X^rX^R_c$-1 × X^rY^R > F_1R♂-7	10	0	0	10	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-1	12	0	0	14	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-2	15	0	0	16	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-3	23	0	1	18	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-4	24	0	0	26	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-5	15	0	0	20	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-6	3	0	0	13*	X^rY^R
X^rX^r	$X^rX^R_c$-2 × X^rY^R > F_1R♂-7	19	0	0	17	X^rY^R
	Total	276	0	1	306	

Two exceptional paternal grandmothers ($X^rX^R_c$-1 and $X^rX^R_c$-2) used are exceptions that appeared among offspring of estradiol-induced X^rY^R♀. r = white, R = orange-red, X^R_c = recombinant X^R.

* $\chi^2 = 5.1$, $P = 0.02$ for deviation from 1r♀:1R♂. In no other cross was the deviation significant.

viability of Y^rY^r males. The majority of Y^rY^r zygotes are considered to be viable because their constitution is $Y^{r,+}Y^{r,+}$ on our hypothesis.

It is appropriate here to recall that AIDA (1936) detected in the medaka that

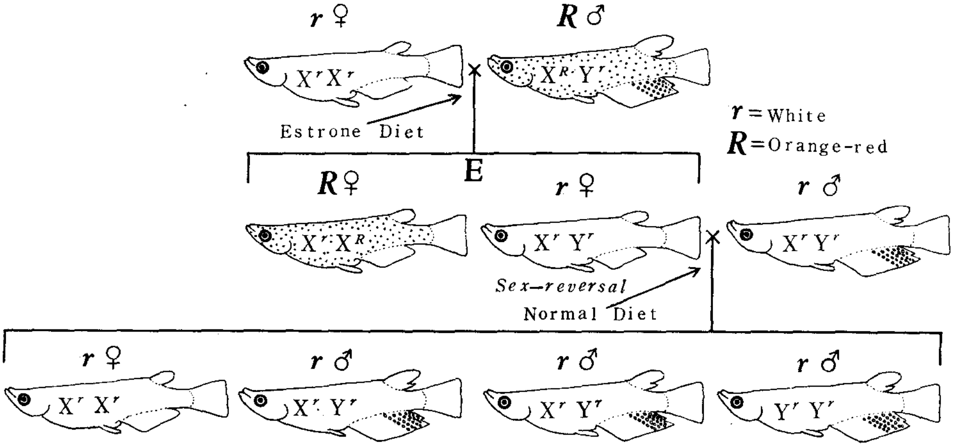

FIGURE 2.—A diagram illustrting experimental production of Y^rY^r males.

TABLE 7

Offspring produced by presumed estrone-induced white females (*E*r ♀♀) *of male genotype* (X^rY^r) *with normal white males* (X^rY^r). r=*white*

Parents		Offspring		Genotype of $E_{50}(r)$ ♀♀	Agreement with 1r♀:3r♂
Females (15)	Males (12)	r♀	r♂		
$E_{50}(r)$ ♀♀	X^rY^r	24	58*	X^rY^r	$\chi^2=0.8$, $P=0.4$

TABLE 8

*Testcross of estrone-induced E*r♀♀ × *normal* X^rY^r♂♂ > F_1r♂♂ (*starred in Table 8*), *presumably either* X^rY^r *or* Y^rY^r. *Data are arranged according to categories of progenies instead of experimental number of matings.* r=*white*

Parents		Offspring		Genotype of P-♂ tested
Female	Male	r♀	r♂	
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-1	33	25	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-2	64	71	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-3	41	40	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-5	31	22	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-6	24	27	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-7	7	7	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-9	15	13	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-10	79	81	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-11	28	25	X^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-12	49	50	X^rY^r
	Total	371	361	
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-4	0	7	Y^rY^r
X^rX^r	E(r)♀♀ × X^rY^r♂♂ > r♂-8	0	24	Y^rY^r
	Total	0	31	

None of the first ten deviate significantly from 1r♀:1r♂.

Y^rY^r males among the F_1 of normal X^rY^r males mated with exceptional X^rY^r females appeared sporadically as the result of genic imbalance between allosomal and autosomal sex-genes. His result is confirmed herein by induced X^rY^r females mated with normal X^rY^r males, and is rationalized on the basis of the foregoing hypothesis.

DISCUSSION

Since the time of Bridges (1916) the YY zygote in Drosophila, arising from nondisjunction, has been known to be invariably nonviable. This is because the Y in Drospohila is mostly, if not entirely, inert. The detection of two surviving Y^RY^R males (Yamamoto 1955, 1959a) in our fish has been taken by some authors as evidence of the fact that the Y chromosome may be so slightly different from the X chromosome that the YY zygotes have genes essential for viability. It is true that the sex chromosomes have a homologous section including the *r* locus, and the Y chromosome may contain even its normal allele *R*. So that it is quite conceivable that the homologous section may comprise other major genes. To account for the rarity of viable Y^RY^R zygotes, one might be tempted to consider that they would be "weak," and that only a few relatively stronger ones might survive, even though the constitution of the two Y^R's might be the same.

There is an array of possibilities which would cause "weakness" of Y^RY^R zygotes. Among these, two deserve special consideration: (1) the life span of the extraordinary male is shorter because his metabolism may be greater than that of ordinary XY male; (2) the maternal Y^R becomes detrimental in some way under the influence of administred estrogen.

The first can be ruled out because the numerical ratio of phenotypic classes in the F_1 offspring of estrogen-induced X^rY^R females by X^rY^R males deviates seriously from the expected 1*r*♀ : 3*R*♂ even if they are scored in a subadult stage. Crucial evidence against the first alternative comes from the failure to detect estrogen-induced Y^RY^R females among estrogen-induced (X^rY^R and Y^RY^R) females which were estronized in two consecutive generations (Yamamoto 1959a). Theoretically, we would expect that one-third of these induced females of male genotype would have the constitution Y^RY^R. Of 21 fish tested, all were induced X^rY^R females, and no induced Y^RY^R female was detected. This experiment implies that if induced Y^RY^R females were ever viable, they would be rare. The majority of Y^RY^R zygotes seem to be nonviable even if they are induced to become females, and this is so in spite of the fact that the female consumes less energy.

The second possibility can be excluded because, as shown previously (Yamamoto 1961), Y^RY^r males are by no means rare, but are as viable as normal X^rY^R males in contrast to the scarcity of Y^RY^R males. In both Y^RY^r and Y^RY^R males, one Y^R chromosome of the two Y's is derived from estrone-induced X^rY^R mothers; the Y^r and the other Y^R come from normal X^rY^r and normal X^rY^R fathers, respectively. Had administered estrogen something to do with the potentiality of the Y^R chromosome, the effect would have been the same in both Y^RY^r and Y^RY^R males. Further evidence for viability of the majority of Y^RY^r zygotes is provided by success in detecting estrone-induced Y^RY^r females (Yamamoto 1963a). Induced Y^RY^r and X^rY^R females were detected in statistical agreement with an expected 1:1 ratio among estrone-induced (X^rY^R and Y^RY^r) females, and in disagreement with the expectation based on the second possibility. Further evidence against this supposition is provided by the demonstrated viability of the majority of Y^rY^r males. As a matter of fact, the two rare surviving Y^RY^R males showed no decline in either vigor or fecundity. The same is true of Y^RY^r and Y^rY^r males, which are not rare. Hence, the viability of YY zygotes cannot be ascribed to general "weakness" of YY as such, but can be explained on the basis of a constitutional difference among the Y chromosomes.

The hypothesis that we adopt is the presence of an inert section (—) in the ordinary Y chromosome and a "viability" segment at the corresponding region in the regular X^r and X^R chromosomes. The linear order of sections and locus in the regular X^r is (x)—r—(+) and in the ordinary Y^R it is (y)—R—(—), where the (x) and (y) stand for the nonhomologous differential segments in the X and Y. The majority of Y^RY^R are considered to be nonviable, because the inert section is duplex ($Y^{R,-}Y^{R,-}$). Only a few recombinant $Y^{R,-}Y_c^{R,+}$ zygotes are viable, where Y_c denotes the recombinant Y, the (+) having been derived from $X^{r,+}$ by crossing over. By an inert section, we mean a region which contains few or no major genes essential for viability. It is possible that minor polygenes or modifiers may be present. Mather (1944) demonstrated in Drosophila that although heterochromatin contains few or no major genes, it is polygenically active.

Our results can be interpreted also on the assumption of a recessive lethal factor (l) in the regular Y chromosome, instead of the inert section (—). However, the fact that recombination values are asymmetrical between X^r and Y^R and between X^R and Y^r (Aida 1930) favors the view that some structural difference exists between the X and Y, besides the sex-differential sections. It has been generally accepted that a single lethal factor does not, as a rule, affect the frequency of crossing over. Thus, the recombination value between $X^{r,+}$ and $Y^{R,l}$ should be the same as that between either $X^{r,l}$ and $Y^{r,+}$ or the $X^{R,+}$ and $Y^{r,l}$, where l stands for a lethal factor and + for its normal allele.

The problem of viability of YY zygotes is considered on the basis of an inert segment in the ordinary Y^R chromosome and of a viability (positive) segment in the corresponding section of its mate (regular X^r). For our symbols the ordinary X^R and X^r are $X^{R,+}$ and $X^{r,+}$, while the regular Y^R and Y^r are $Y^{R,-}$ and $Y^{r,+}$, respectively. At this point it is necessary to refer to the origin of the Y^r chromosome in our fish. We have as yet no instance at all of the Y^r

chromosome having arisen from the Y^R chromosome by mutation. The Y^r chromosome in both AIDA's and our breeds is known to have originated through transfer of the *r* from the X^r chromosome by crossing over. Hence, the Y^r is a recombinant in reality. It is natural to infer that the *r* gene together with the (+) section must have transferred from the $X^{r,+}$ to the Y by crossing over, resulting in the formation of the $Y^{r,+}$ chromosome. From this we can plainly understand why the Y^r chromosome contains the viability segment (+) whereas the regular Y^R involves an inert section (—).

While we can detect crossovers occurring between the differential segment (*x*) and the *r* locus by their color phenotypes and sex, crossovers occurring in the region delimited by the *r* and (+) segment cannot be identified by any visible traits. They can only be detected by the sex-ratio among the progeny of suitable testcrosses.

Previous data and the experiments presented here lead us to construct a provisional linkage map (Figure 1). The recombination value delimited by the differential segment and the *r* locus in the normal male (X^rY^R) has been estimated already to be 0.2 ± 0.01, and it is 1.0 ± 0.4 crossover unit in the induced X^rY^R female (YAMAMOTO 1961). A full account on the determination of the linear order and the estimation of recombination between the *r* locus and the (+) segment will be dealt with in another paper. This map may not only account for the scarcity of surviving Y^RY^R zygotes and viability of the majority of Y^RY^r ($Y^{R,-}Y^{r,+}$) and Y^rY^r ($Y^{r,+}Y^{r,+}$) zygotes, but can explain the lethality of recombinant $X^R_cY^R$ ($X^{R,-}_cY^{R,-}$) males and viability of $X^R_cY^r$ ($X^{R,-}_cY^{r,+}$) males.

A particular interesting aspect of the map is that it can be related to the difference between crossover values of certain genes (dominant in effect) from the Y to the X and *vice versa*, as reported in Lebistes and Oryzias. No solution of this problem has hitherto been offered. WINGE's data (1927) with Lebistes indicated that the gene *El* (Elongatus) is transferred from the X to the Y in 1 out of 74 cases (1.4 percent), and from the Y to the X in 4 out of 68 cases (5.9 percent). WINGE, however, did not attach any significance to the difference, since the numbers are small. ELOFF (1932), on repeating WINGE's experiment, showed a slightly higher frequency of crossing over from the Y to the X than the reverse. AIDA (1930) carried out an extensive experiment to ascertain if the frequency of crossing over of *R* from the Y to the X and *vice versa* is the same. In order to estimate the frequency of transfer of the *R* from the Y to the X, a heterozygous orange-red male (X^rY^R) was mated with homozygous females (X^rX^r), and the frequency was found to be approximately 1:300 (0.3 percent), which closely approaches our value (0.2 ± 0.01). To determine the frequency of transfer of *R* from X to Y, a heterozygous orange-red male (X^RY^r) was mated to homozygous white females (X^rX^r), and the frequency was found to be 1:1200 (0.08 percent). In calculating these values, all the so-called "nondisjunctional males and females," which were later (AIDA 1936) interpreted as exceptions that had arisen from genic imbalance of sex-genes, were excluded. His data indicate that the frequency of crossing over between the X^r and Y^R is fourfold that between the X^R and the Y^r. He questioned whether the difference is because of a dissimilarity in structure of the X and Y chromosomes or because of the activity of some gene or genes situated in one of the allosomes. Although the question remained unsolved at that time, he called attention to a tendency of the Y chromosome to lose the dominant gene more frequently and to regain it less frequently, and stated that if this difference had occurred also in other animals, perhaps he had found a plausible reason for the so-called almost empty Y chromosome of Drosophila, where crossing over might have taken place between the X and Y chromosomes so often that the Y chromosome had gradually lost its dominant genes, to eventually become almost empty.

In working on the problem of viability of YY zygotes, several lines of evidence leave little doubt that there is the dissimilarity of the X and Y chromosomes aside from the nonhomologous differential segments. The regular X and Y chromosomes are found to consist of at least three major regions: differential, homologous and the (+),(—) segments. The linkage map presented in Figure 1 may offer a clue for interpreting the asymmetry of crossing over of the *R* in the two directions. In $X^{r,+}Y^{R,-}$, there are two pairs of dissimilar segments at both ends of the homologous section containing the *r* locus. In $X^{R,+}Y^{r,+}$, there is only one pair of dissimilar segments at one end, the rest being homologous. Chiasma formation may be more liable to occur in the case where the X and the Y have dissimilar sections at both ends than in the case where they are dissimilar at one end only.

The teleost fishes represent the largest class of the vertebrates, with the greatest diversity in sexuality as well as in the sex-determining mechanism. The class includes all the known sex-types, from strict hermaphroditism through intermediate sex-types (protandric and protogynic) to genuine gonochorism. Sex-types may be correlated with the grade of the differentiation of the sex chromosomes. In strict and basic hermaphrodites, sex chromosomes may not yet be differentiated. Even in gonochorists, there are such species as the swordtail, *Xiphophorus helleri*, in which the sex chromosomes have not yet been differentiated. The situation is well reflected by the well-known instability of sexuality in this fish (*cf.* ESSENBERG 1926). Broadly speaking, fishes seem to be at the verge of differentiation of the sex chromosomes. Certain teleosts, however, possess sex chromosomes, as proved by sex-linked inheritance, although until recently cytological evidence has not been sufficient to substantiate the presence of heteromorphic sex chromosomes. NOGUSA (1955, 1957), however, first succeeded in demonstrating the X-Y pair in males of the two species of teleosts, *Mogurunda obscula* (Gobiidae) and *Cottus pollux* (Cottidae).

Male heterogamety (XX = female, XY = male) as normal to the medaka, was first established by AIDA (1921). The guppy, *Lebistes reticulatus*, is also normally XX in the female and XY in the male (WINGE 1922a,b). The same is true of the platyfish, *Platypoecilus variatus* (KOSSWIG 1935; BELLAMY 1936). On the contrary, the "domesticated" races of the platyfish, *P. maculatus*, are female heterogametic (WZ = female, ZZ = male) (BELLAMY 1922, 1928; GORDON 1927; FRASER and GORDON 1929; KOSSWIG 1938). GORDON (1946, 1947, 1951) found that "wild races" of this species collected from certain Mexican river systems are male heterogametic (XY) whereas he found that a wild race from British Honduras is female heterogametic (WZ), the same as the domesticated races. The domesticated races of the platyfish are believed to have derived from the latter region of Central America. So far, however, the interpretation is based on genetic experiments without being substantiated cytologically. Recently elaborated techniques make it possible to identify the sex chromosomes in *Oryzias latipes*. DR. K. MORI and I (not yet published), using normal XY and androgen-induced XX males, have succeeded in demonstrating convincingly a heteromorphic pair of sex chromosomes, a large X and a smaller Y. Thus with our material, a XX-XY chromosome mechanism for normal sex determination has been established by both genetic and cytologic evidence. Embryologically, it belongs to "differentiated" species (YAMAMOTO 1953). So our material seems to be relatively stable in sexuality among fishes, if not so stable as in higher vertebrates.

WINGE (1934) in Lebistes postulated the presence of a superior male gene in the Y chromosome. Appearance of a fertile XXY male in Oryzias (YAMAMOTO 1963a) indicates that the Y chromosome has a male determining property, to an extent, even if not so strong as in mammals. In this respect, the normal sex-determining mechanism of the medaka plainly conforms with the mammalian system.

WINGE and DITLEVSEN (1938) assumed the presence of a lethal factor in the Y^{Ma} chromosome of the guppy, which in the duplex condition ($Y^{Ma}Y^{Ma}$) kills the fish. On the contrary, the genotype $Y^{Ma}Y^{Pa}$ is viable. According to them, a lethal factor (*l*) in the Y^{Ma} is either closely linked to the *Ma* locus or *Ma* gene itself. In view of the asymmetry in crossing over between allosomes, as already mentioned, it is highly desirable to clarify whether or not their result can be accommodated by our scheme. The presence of a single lethal factor would hardly be expected to affect the mode of crossing over. In our case, the inert section (—) in the regular Y^R is found to be in the region different from that containing the *R* gene. It is of interest that normal orange-red males (X^RY^R or X^rY^R) are usually protected by the X chromosome through the presence of the (+) segment in it, which counteracts the detrimental effect of the inert section (—) in the ordinary Y^R.

In this sense, the (+) segment is referred to as a "viability" section which must include genes essential for viability. In the medaka, the regular Y still retains the homologous section containing *R* or *r* and perhaps other genes, in addition to the (—) segment. From the evolutional viewpoint, it is interesting to find that even among fishes there is such a species as the medaka in which the Y chromosome already shows a sign of deterioration, perhaps representing a transitional stage in the gradual deterioration of the Y chromosome in the animal kingdom.

SUMMARY

In the medaka, an oviparous cyprinodont, where the normal sex-chromosome mechanism is XX= ♀ and XY= ♂, either the gene *R* (orange-red) or its allele *r* (white) is borne by either the X or the Y. Artificial control of sex differentiation by heterotypic sex hormones, makes it possible to attack problems otherwise difficult to approach.

When induced X^rY^R females are mated with normal X^rY^R or X^rY^r males, Y^RY^R zygotes rarely survive, whereas Y^RY^r zygotes are common. The hypothesis is formulated that an inert section $(-)$ is present in the regular Y^R, and a "viability" segment $(+)$ in the ordinary X^r and X^R. The linear order in the regular X^r is (x)—*r*—$(+)$, and in the ordinary Y^R it is (y)—*R*—$(-)$ where (x) and (y) stand for the nonhomologous differential segments. Most Y^RY^R zygotes are nonviable because the $(-)$ is in the duplex condition. A few viable Y^RY^R males are considered to be crossovers of constitution $Y^{R,-}Y_c^{R,+}$, where $Y_c^{R,+}$ stands for a recombinant having $(+)$ derived from the $X^{r,+}$. Most Y^RY^r zygotes are viable because their constitution is $Y^{R,-}Y^{r,+}$. The Y^r is known to have arisen by crossing over between the X^r and the Y^R, so a concomitant transfer of both *r* and $(+)$ is possible.

Further verifications of the hypothesis are: (1) the F_1 sons from X^rX^r ♀ × Y^RY^R ♂ comprise two genotypes $X^{r,+}Y^{R,-}$ and $X^{r,+}Y_c^{R,+}$; (2) $X_c^RY^R(X_c^{R,-}Y^{R,-})$ are not viable, but both X^RY^R $(X^{R,+}Y^{R,-})$ and $X_c^RY^r$ $(X_c^{R,-}Y^{r,+})$ are viable. (X_c^R symbolizes a recombinant); (3) Y^rY^r $(Y^{r,+}Y^{r,+})$ are viable.

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