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Estrone-Induced Intersex of Genetic Male in the Medaka, *Oryzias latipes*

By

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(With 2 Text-figures)

I

In the medaka (*Oryzias latipes*), an oviparous cyprinodont, where sex determining mechanism is XX for female and XY for male, the sex-linked color genes *R* (orange-red) and *r* (white) are resident in the X and Y chromosomes or both (Aida 1921). When the white female (X^rX^r) is mated with the 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 this type of mating, white (*r*) offspring are females and orange-red (*R*) are males, i.e., the father-to-son inheritance is revealed.

Through the use of such mating type, it has been shown that complete and functional sex-reversal in genetic males (XY) can be achieved by administration of estrone at the dosage level of 1,250 i. u. ($125 \mu\text{g}$)/g-diet continuously from hatching time to the age of several months (Yamamoto 1953). No intersexes were produced in that experiment. Sex-reversed orange-red females (X^rY^R) were mated with normal orange-red males (X^rY^R) and progeny testings were made on the F_1 orange-red sons. A single F_1 orange-red son was identified as actually a Y^RY^R male which had fathered all male offspring, thus furnishing crucial evidence of induced sex-reversal (Yamamoto 1955).

Thereafter some series of further studies along this line were performed in which administration of estrogen was limited to the period between hatching time and certain juvenile stages after which treated fish were reared to adulthood on normal diet. An account covering all these series of experiments will be given elsewhere. The present paper deals only with an intersex produced through administration of estrone in one series of experiments.

II

Materials used were sixth generation offspring of mass-breeding of heterozygous orange-red males (X^rY^R) repeatedly mated with white females (X^rX^r). In one series of experiments performed in 1952, daily hatchings were taken from July 1 to 15 and successively reared on estrone diet (1,250 i.u. = $125 \mu\text{g}$ /g-diet) for a period of from 4 to 6 weeks until they reached the juvenile stage of 8–13 mm

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(caudal included). Thereafter they were reared outdoors on normal diet until they reached full growth in 1953. Of 46 matured controls, 24 were white females and 22 orange-red males. Out of 57 estronized fish, 26 were white females ($X'X'$) and 31 were orange-red fish ($X'Y^R$). Of the latter, 28 were females, one was intersex and two were males. Obviously 90 percent of genetic males ($X'Y^R$) having been treated with estrone in larval life, had reversed their sex-differentiation and developed into females almost all of which were proved to be fertile in separate matings.

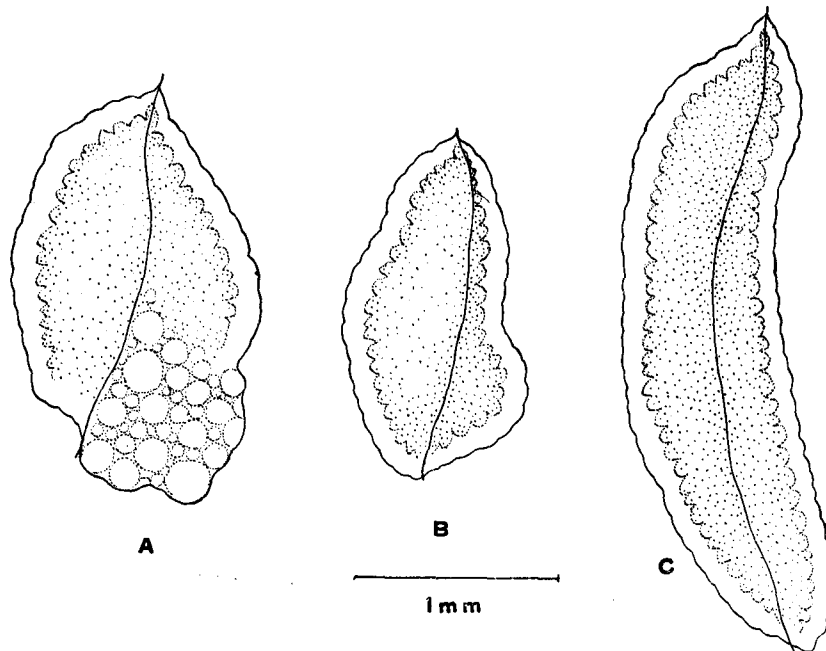


Fig. 1. A, Hermaphroditic gonad of an intersex of the male genotype ($X'Y^R$). 26 mm. Ventral view. B, Testis of estronized non-reversal male ($X'Y^R$). 29 mm. Ventral view. C, Testis of normal (control) male. 29 mm. Ventral view.

The testes of the two estronized non-reversal males ($X'Y^R$) were examined on autopsy. The testis of the estronized genetic males (Fig. 1, B) is a foreshortened and laterally distended organ instead of the normal elongated gland (Fig. 1, C). Although it is somewhat atypical in form and size, the serial sections disclose that the essential structure is the same as that of normal testis and that it contains only male germ cells, no oocytes being found.

It is obvious that the single intersex which appeared is of the male genetic

constitution X^rY^R and can be classified as a male intersex. When it was found to be intersex and autopsied, it measured 26 mm in total length. It had male-positive as well as female-positive secondary sexual characters both of which were intermediate between the two sexes. In over-all appearance it is a foreshortened gland, the testicular tissue being cephalad, the ovarian caudad (Fig. 1, A). Careful observation reveals that the entire right side (shown on the left hand in the figure) and the anterior region of the left side are composed almost entirely of testicular tissue while the posterior end of the left side (right in the figure) consists of ovarian tissue. This condition is found to be generally true upon serial sections (Fig. 2). The hermaphroditic gonad is covered by a thin peritoneal membrane. Generally speaking, the male and female germ cells are found sectorially, no intermingling of both cells existing except in the middle region where some ovocytes are seen among prevailing testicular elements.



Fig. 2. Longitudinal section of hermaphroditic gonad of an intersex of the male genotype (X^rY^R). Anterior end is on the left-hand side. $\times 60$

The testicular region in the hermaphroditic gonad has the same structure as that of the normal testis. The core of the testicular region contains the intra-testicular sperm ducts. Many spherical acini (cysts) lie between the surface and the sperm ducts. At the very periphery all the acini consist of spermatogonia. The latter stages (spermatocytes and spermatids) are found toward the center. Acini containing mature spermatozoa are found close to the center of the core. The sperm ducts are full of ripe spermatozoa. Mature spermatozoa are also found in the lumen of the ovarian region. Obviously they must have been produced in anterior testicular region. Ovocytes in various stages of development are lodged in the hermaphroditic gonad. It is worth while noting that ovocytes located among testicular acini are always small and that the ovarian region contains larger ovocytes as well as the small ones. The chromatin of larger ovocytes collects into numerous nucleoli peripherally arranged along the nuclear membrane

in the germinal vesicle. Each ovocyte is surrounded by a number of follicle cells. The large ovocytes are in the process of yolk formation and are invested by the egg membrane on which villi are already formed. The largest of these ovocytes measures 130μ in diameter with a germinal vesicle of 85μ in diameter. Thus these ovocytes may be regarded as true ovocytes because we cannot find any difference between them and those of normal females. In this connection it is to be noted that the ovary of complete sex-reversals of the male genotype (X^YR) is the same in form and structure as that of normal females.

III

In this hermaphroditic gonad, the anterior part is prevailing testicular and the posterior region is chiefly ovarian. This situation is interesting in connection with the regional difference in developmental rate of the gonad. As has been pointed out previously (Yamamoto 1953), there is an antero-posterior gradient in rate of differentiation. The germ cells in anterior region differentiate earlier. In the genetic male, germ cells in posterior region may remain indifferent for a certain period after those in anterior region have differentiated into male germ cells. It may be postulated that posterior region of juvenile gonad of genetic males is more sensitive to the feminizing effect of estrone than anterior region.

There are varying degrees of stability in sexuality among fishes. The prevailing view that fishes are labile in sex-differentiation seems to be derived from occasional instances of the intersex in "undifferentiated" species of which the medaka is not one. The strain of *Oryzias latipes* used in our experiments is a "differentiated" race, *i.e.*, the male shows no signs of a temporary ovary-like state in juvenile gonad. In the medaka (a differentiated gonochorist) genuine intersex either occurring naturally or produced experimentally, seems to be rare. There seems to be some difficulty for the hermaphroditic condition to remain through adulthood in such a differentiated race as the present form because of the competitive interaction between ovarian and testicular tissues.

In normal *Oryzias*, sexing can be safely made by examining secondary sex characters under a low power microscope using chloretone as an anesthetic. Intersex can be detected by the presence of both male-positive and female-positive sex characters (*cf.* Yamamoto and Suzuki 1955) in intermediate state. No single sporadic intersex, however, has ever been detected among the approximately 4,000 specimens of our stock which have been handled individually during the past ten years.

For an explanation of "testis-ova", either sporadically found (Oka 1931) or experimentally induced (Okada 1943, 1944) in adult males of the medaka, the reader is asked to refer to Egami (1956).

Summary

A case of true intersex of the genetic male (XY) in the medaka (*Oryzias latipes*) induced by administration of estrone during larval life is reported. The intersex has secondary sex characters intermediate between both sexes as well as a hermaphroditic gonad. The anterior region of the gonad is composed of testicular elements while one side of the posterior region consists of ovarian components.

This paper is dedicated to Prof. T. Uchida in celebration of his 60th birthday anniversary.

Literature

- Aida, T. 1921. Genetics 6 : 553-573.
Egami, N. 1956. Annot. Zool. Japon. 29 : 11-18.
Oka, T. B. 1931. Jour. Fac. Sci. Imp. Univ. Tokyo sec. IV (Zool.) 2 : 219-224.
Okada, Yô K. 1943. Proc. Imp. Acad. Tokyo 19 : 501-504.
——— 1944. Proc. Imp. Acad. Tokyo 20 : 244-250.
Yamamoto, T. 1953. Jour. Exp. Zool. 123 : 571-594.
——— 1955. Genetics 40 : 406-419.
Yamamoto, T. and H. Suzuki 1955. Embryologia 2 : 133-144.
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