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PRELIMINARY EXPERIMENTS ON SEX CONTROL IN TROUT : PRODUCTION OF STERILE FISHES AND SIMULTANEOUS SELF-FERTILIZABLE HERMAPHRODITES

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SUMMARY

Steroid hormones are administered in food to young fishes during gonad morphogenesis (1 to 6 months) in order to induce preferentially artificial sex reversal to either the male or the female side. The results which follow are evaluated after two years, when most of the fishes reach sexual maturity :

1. — *Estrone treatment* (30 to 120 µg per gram of pellets)

54 p. 100 of females
10 p. 100 of males only
6 p. 100 of sterile fishes
30 p. 100 of hermaphrodites. The gonads present a complex picture with distinct male and female parts. Testicular and ovarian parts are sometimes simultaneously mature, then allowing experimental self-fertilization using artificial insemination.

2. — *Methyltestosterone treatment* (15 to 60 µg per gram of pellets)

18 p. 100 of females
58 p. 100 of males
12 p. 100 of sterile fishes
12 p. 100 of hermaphrodites

These results demonstrate the lability of the sexual phenotype in trout and open interesting possibilities for sex control. Moreover, the ability to produce hermaphrodite trouts giving self-fertilized eggs by artificial insemination, is a good tool for genetic purposes.

INTRODUCTION

In certain experimental conditions, sexual phenotype may be reversed opposite to genotype in some gonochoric teleost fishes (T. YAMAMOTO, 1969). The best way up to now of obtaining this result has been to administer exogenous steroid sex hormones in the food of fry before normal differentiation of the gonad begins. Thus, YAMAMOTO working on Medaka, *Oryzias latipes* (1953, 1958), and then on the goldfish, *Carassius auratus* (T. YAMAMOTO and T. KAJISHIMA, 1968) obtained the first complete sex reversal either to the male or female side, under the respective action of methyltestosterone and estrone. The reversed fishes were functional, producing progeny characteristic of their genotype (T. YAMAMOTO, 1955, 1959). With the same method, other species have been reversed to the male side under methyltestosterone action. These include the guppy, *Poecilia reticulata* (DZWILLO, 1962), *Tilapia mossambica* (CLEMENS and INSLEE, 1968) and *Tilapia nilotica* (JALABERT *et al.*, 1974).

In spite of these achievements, numerous unsuccessful attempts in this direction were made on other species (review by T. YAMAMOTO, 1969). PADOA (1937, 1939) working on *Salmo gairdnerii* and ASHBY (1957, 1965) on *Salmo trutta* tested the action of sex steroids on sexual differentiation in the trout. Unfortunately, the animals were not kept until sexual maturity, and, furthermore, the tendencies noted were not encouraging as regards obtention of sex reversal.

In referring, however, to experimental conditions which led to positive results in other families, there seem to be two possible reasons for failure ; one is an unsuitable method of administering the steroids (introduced into water in alcoholic solution), and the other is treating the fry after sexual differentiation had already begun or had even completed.

Thus, in the present study, we tried to choose the best conditions for success by treating fry as early as possible with steroids incorporated into the food and chosen for their efficiency in the successful cases reported up to now (methyltestosterone and estrone).

MATERIAL AND METHODS

Rainbow trout (*Salmo gairdnerii*) fry used for the experiment originated from a lot of eggs fertilized, incubated and hatched simultaneously in the same conditions. At the end of yolk sack resorption (28 days after hatching at 6-8°C), and when active feeding behavior appeared, they were separated into 6 experimental lots of 500 and one control lot of 2 000 fry (fig. 1), placed at the same temperature and fed.

Steroids destined for experimental lots were first put into oil (Soybean + Cod liver) representing 5 p. 100 in the food pellets to make the following doses :

Estrone : 30, 60, 120 µg per gram of food (lots E_1, E_2, E_3) ;
Methyltestosterone : 15, 30, 60 µg per gram of food (lots T_1, T_2, T_3).

The food lots were given *ad libitum* separately to the different batches of fry for 5 months (or until 6 months after hatching). At that age, the fry weighed 5 to 7 grams and totaled about 2 400 degrees × days since fertilization. Each lot was then systematically reduced to 200 fishes and was fed, up to 2 years, with the control food containing no steroids.

All animals were sacrificed at 2 years in the period of sexual maturity (December) in order to determine the sex according to the nature of the gonads, and independently of presence of secondary sex characters (« beak », coloring, genital papilla), which were strongly affected by the treatments and sometimes expressed tendencies opposite to the nature of the gonads. Immature gonads, or those supposed sterile, were fixed in Bouin-Hollande, embedded in paraffin, cut and stained with Regaud's hematoxyline, Orange G and aniline blue for histological determination. Mature gametes produced by gonads of mature fishes were tested for their fertilizing ability by artificial insemination, according to the standard procedure already described (PETIT *et al.*, 1973).

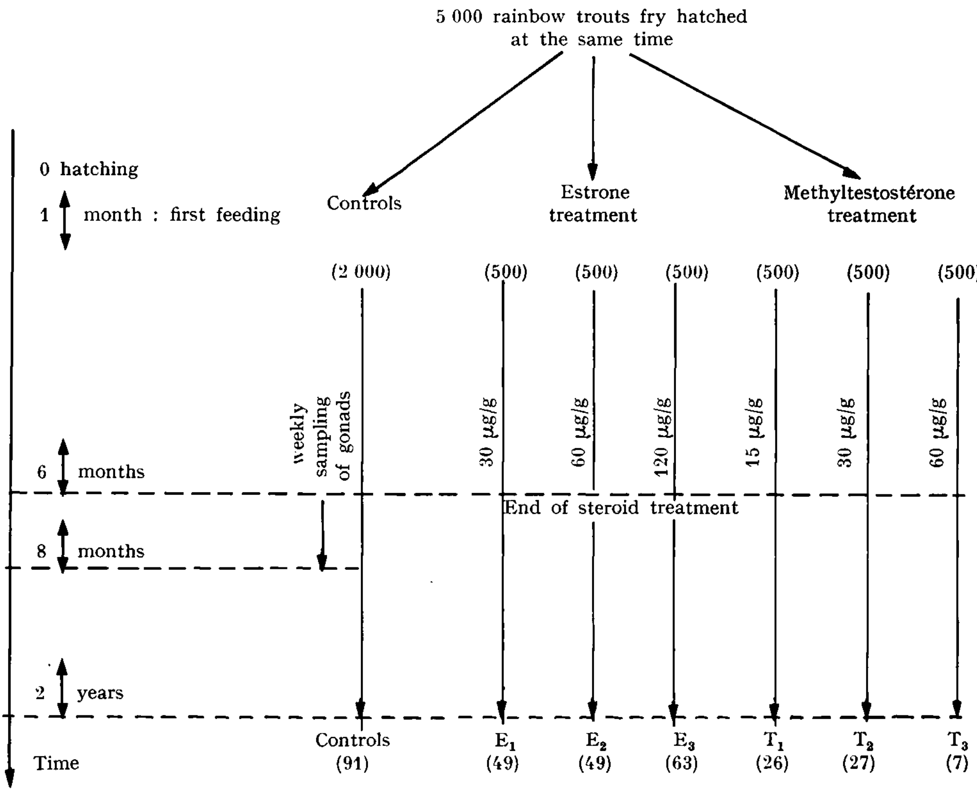


FIG. 1. — *Experimental protocol*

The small number of individuals remaining in some lots at 2 years (fig. 1) is the result of purely accidental mortality, such as water cuts due to the precariousness of breeding tanks. Thus, because of the heterogeneity of the lots at the end of the experiment, growth performance, as well as eventual differential mortality resulting from the treatments, could not be compared.

RESULTS

Controls

The sex ratio in 2 year-old control fishes does not differ significantly from 1/1 with well-differentiated phenotypes and no case of intersexuality in the 91 individuals observed.

Estrone lots (table 1)

Besides an abnormal distribution of gonochoric male and female individuals, we observed a high proportion of hermaphrodites or steriles animals. Thus, lot E_1 is composed of 49 p. 100 of females, only 10.2 p. 100 of males and 34.7 p. 100 of hermaphrodites having gonads with functional ovarian and testicular parts, more or less overlapping, mature or immature simultaneously or not, and 6.1 p. 100 of sterile animals having an undifferentiated filiform gonad which, on histological examination, was found to contain no germ cells. Results obtained in the three lots, E_1, E_2, E_3, are rather comparable (table 1). This would seem to indicate that effect of the estrone dose given in the food does not matter much in the range chosen.

TABLE I

Distribution of different sex phenotypes in 2 year-old rainbow trout given estrone in the food from 1 to 6 months

		E_1 (49) (%)	E_2 (49) (%)	E_3 (63) (%)	Controls (91) (%)
♂	mature	10.2	8.2	7.9	52
	immature	—	2.0	1.6	
♀	mature	14.3	10.2	30.2	48
	immature	34.7	38.8	31.7	
⚥	♂ and ♀ mature	12.2	4.1	14.3	—
	[♂ mature, ♀ immature]	18.4	26.5	12.7	—
	♂ and ♀ immature	4.1	—	—	—
0	undifferenciated sterile	6.1	10.2	1.6	

In fact, the classification adopted (males, females, hermaphrodites, steriles) has an artifical character in the sense that there are all possible intermediaries between normal males and normal females, as regards secondary sex characters and gonad structure. Only the females (an average of 54 p. 100 for the 3 lots, E_1, E_2, E_3, table 3) were apparently normal. On the other hand, there were very few really normal males (table 3 : 9.9 p. 100) which had a complete genital apparatus permitting sperm ejaculation through the genital papilla. The other males presented more or less pronounced abnormalities, such as an incomplete (fig. 2) or totally absent (fig. 3) deferent duct and testicles which were multilobed (fig. 2) or arranged in series. One of the testicles might even be absent (fig. 3). Moreover, non-emission of sperm due to absence of the deferent duct caused testicular hypertrophy which was sometimes considerable due to hydration phenomena which accompany spermiation.

TABLE 2

Distribution of different sex phenotypes in 2 year-old rainbow trout given methyltestosterone in the food from 1 to 6 months

		T_1 (26)	T_2 (27)	T_3 (7)	$T_1 + T_2 + T_3$ (60) (%)	Controls (91) (%)
♂	mature	13	14	3	50	52
	immature	3	1	1	8.3	
♀	mature	—	—	—	—	48
	immature	1	8	2	18.3	
⚥	♂ and ♀ mature	—	—	—	—	
	♂ mature ♀ immature	3	1	1	8.3	
	♂ and ♀ immature	1	1	—	3.4	
0	undifferenciated sterile	5	2	—	11.7	

As concerns « hermaphrodite » gonads, we observed all the intermediaries between an almost completely male gonad with only some nodes of ovarian tissue (fig. 4) and an almost entirely female gonad with only some testicular nodes (fig. 5). In no case, however, could the male and female gametes both be emitted, even when they were mature at the same time. When the genital papilla was of the female type, ovocytes ovulated in the abdominal cavity could be laid, but the spermatozoa could not be ejaculated due to absence of the deferent duct. On the other hand, when testicular zones were connected to a male type genital papilla by a deferent duct spermatozoa could be ejaculated but ovocytes were released and kept in the abdominal cavity. It was very easy, however, to recover both types of gametes by biopsy in order to cross-fertilize or self-fertilize. In both cases, eggs thus fertilized showed normal embryonic development.

TABLE 3 (recapitulation)

Comparison of the percentage of different sex phenotypes in 2 year-old rainbow trout given either estrone or methyltestosterone from 1 to 6 months

Phenotype	Estrone (161)	Methyltestosterone (60)	Controls (91)
♂	9.9	58.3	52
♀	54.0	18.3	48
⚥	30.5	11.7	—
0	5.6	11.7	—

PLANCHE I

Fig. 2

Abnormal testicles with few modifications from an estrone-lot male : note plurilobed testicles and incomplete deferent ducts (arrows).

Testicules anormaux peu modifiés d'un mâle d'un lot traité à l'œstrone. Remarquer les testicules plurilobés et les canaux déférents incomplets (flèches).

Fig. 3

Abnormal, extensively modified testicle of an estrone-lot male : the right filiform gonad is sterile (arrows). The single plurilobed testicle (t) has no deferent duct.

Testicule anormal, profondément modifié d'un mâle d'un lot traité à l'œstrone : la gonade droite filiforme est stérile (flèches) ; l'unique testicule, plurilobé (t) est dépourvu de canal déférent.

Fig. 4

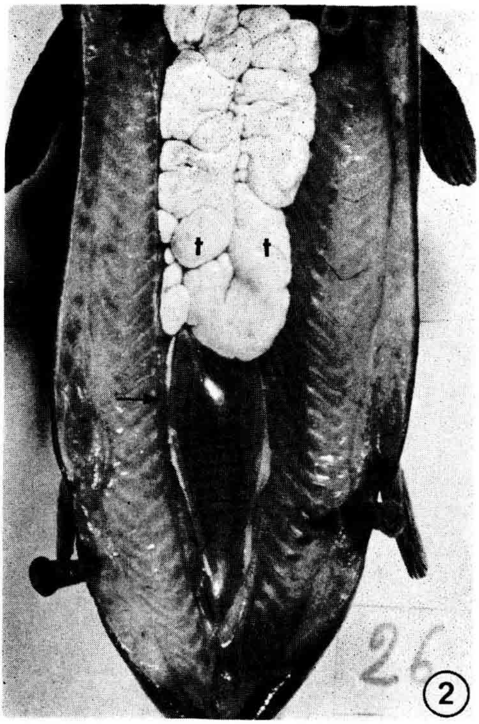
Gonads of an hermaphrodite trout from the estrone lots : testicle parts (t) arranged in series are separated by immature ovarian tissue (o) with young ovocytes apparent.

Gonades d'une truite hermaphrodite des lots traités à l'œstrone : les parties testiculaires (t) disposées en chapelet sont séparées par du tissu ovarien immature (o) avec de jeunes ovocytes apparents.

Fig. 5

Left gonad of an hermaphrodite trout from the estrone lots : the submature ovarian part (o) is predominant, while the testicular mature parts are only represented by some nodes (t) ; the right filiform gonad (arrows) is sterile.

Gonade gauche d'une Truite hermaphrodite des lots traités à l'œstrone : la partie ovarienne (o), submature, est prédominante, alors que les parties testiculaires, matures, ne sont représentées que par quelques ilôts (t) ; la gonade droite filiforme (flèches) est stérile.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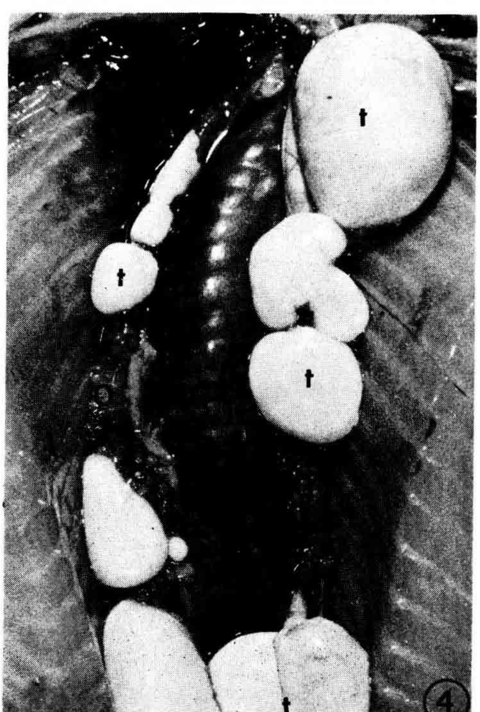
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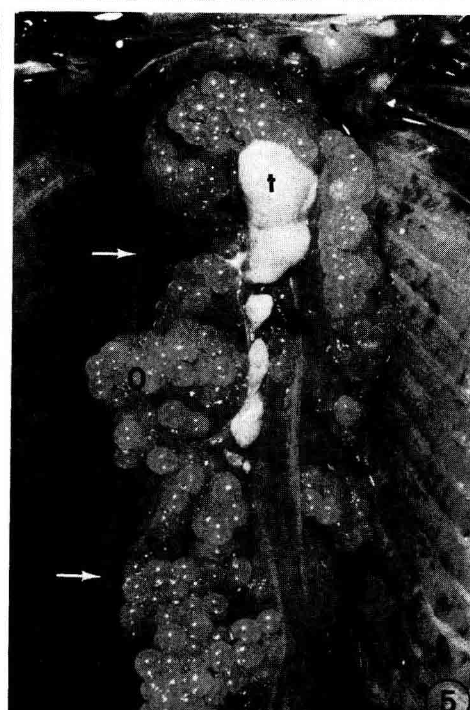
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Methyltestosterone lots (table 2)

Because of the small number of 2 year-old animals remaining in each lot due to rearing accidents, we did not take the dose into consideration, and we re-grouped the 3 lots. « Normal » males predominated ; some of these were still immature (8.3 p. 100). All females (18.3 p. 100) were still immature at 2 years. There were fewer hermaphrodites observed than in the estrone lots, and they were not simultaneously mature at that age. In all cases, maturation of ovaries or ovarian parts seemed to be delayed. Finally, the proportion of sterile animals was higher (11.7 p. 100) than in the estrone-treated fishes.

DISCUSSION

Comparison of the results obtained respectively with estrone and methyltestosterone (table 3) shows that the two steroids do not have an absolutely symmetrical effect. In general, it seems that estrone does not affect deeply female gonad morphogenesis (54 p. 100 of normal females) and methyltestosterone male gonad morphogenesis (58.3 p. 100 of males). But, while estrone gives only a few sterile animals and enhances the appearance of hermaphrodites (30.5 p. 100) having testicular zones which do not mature particularly late, methyltestosterone seems to induce fewer hermaphrodites (18.3 p. 100) having late ovarian zone maturity and to favorize the production of sterile fishes (11.7 p. 100).

Although true sex inversion did not clearly appear, the results obtained open astonishing experimental possibilities, such as the production of simultaneous self-fertilizable hermaphrodites in a gonochoric species or the production of sterile animals. Consequently, methods of deviating gonad morphogenesis in the trout should be more exactly defined so that the sex type in demand for intensive production, selection or experimentation can be produced as needed ; this means a maximum number of females or sterile animals or hermaphrodites. A considerable field of experimentations still remains :

We have only tried two steroids, estrone and methyltestosterone, on the trout, referring to prior results on sexual reversal obtained on fishes belonging to different families, such as *Oryzias latipes* (Cyprinodontiform), *Carassius auratus* (Cyprinid), *Tilapia mossambica* and *Tilapia nilotica* (Cichlid). However, T. YAMAMOTO (1969) found that various other estrogen or androgen steroids give the same result in *Oryzias latipes* as estrone and methyltestosterone with greater efficiency in some cases. Moreover, the efficiency of these steroids as artificial inducers of sexual differentiation certainly varies, depending on the species ; for instance, E. HACKMANN (1974) found a paradoxal effect of methyltestosterone in some cichlid fishes ; thus, the possibility that the exogenous steroids may act by an artificial indirect way on the gonad to stimulate the synthesis of another natural and specific inducer, possibly steroid, must not be underestimated. Such a possibility was well shown by WENINGER and ZEIS (1973) in Chick embryonnal testis where exogenous dihydrotestosterone which shows a feminizing action induces estrogen synthesis.

As YAMAMOTO (1969) emphasizes, the length of the treatment in relation to the period of gonad morphogenesis is very important. Morphogenesis is related both to the external factor of temperature (which should be strictly controlled during the treatment so that it can be repeated) and to genetic characteristics of the strain. Thus, the contradictory results of MRSIC (1930) and PADOA (1937, 1939) on the period and mode of gonad differentiation in the rainbow trout, *Salmo gairdnerii*, probably come from differences both in rearing temperature and in the genetical characteristics of two geographically different strains.

CONCLUSION

The unexpected results obtained are at least as important as the primary objective which was to induce complete sex reversion ; producing self-fertilizable hermaphrodite trout could furnish geneticists with a working tool permitting rapid production of highly homozygous strains.

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RÉSUMÉ

ESSAIS PRÉLIMINAIRES SUR LE CONTRÔLE DU SEXE CHEZ LA TRUITE : PRODUCTION D'ANIMAUX STÉRILES ET D'HERMAPHRODITES SIMULTANÉS AUTOFÉCONDABLES

L'administration d'hormones stéroïdes dans l'alimentation des alevins pendant la période de morphogenèse des gonades (1 à 6 mois) en vue d'orienter le sex ratio préférentiellement dans le sens mâle ou femelle, a permis d'observer lors de la première maturité sexuelle, à 2 ans :

1. — *Traitement par l'œstrone* (30 à 120 μg par gramme d'aliment)

54 p. 100 de femelles
10 p. 100 de mâles seulement
6 p. 100 d'animaux stériles
30 p. 100 d'animaux hermaphrodites dont les gonades complexes présentaient des zones mâles et femelles distinctes, souvent simultanément matures. Celles-ci ont permis de réaliser des autofécondations par insémination artificielle.

2. — *Traitement par la méthyltestostérone* (15 à 60 μg par gramme d'aliment)

18 p. 100 de femelles
58 p. 100 de mâles
12 p. 100 d'animaux stériles
12 p. 100 d'animaux hermaphrodites.

Ces résultats démontrent la labilité du phénotype sexuel de la Truite et ouvrent d'intéressantes perspectives quant à la possibilité, par la modulation de l'intensité, de la durée et de la nature du traitement, de contrôler le sex ratio et de produire éventuellement des animaux stériles. Enfin la possibilité de produire des animaux hermaphrodites simultanés autofécondables peut fournir un outil intéressant en génétique.

REFERENCES

ASHBY K. R., 1957. The effect of steroid hormones on the brown trout (*Salmo trutta* L.) during the period of gonadal differentiation. *J. Embryol. exp. Morph.*, **5**, 225-249.

ASHBY K. R., 1965. The effect of steroid hormones on the development of the reproductive system of *Salmo trutta* L. when administered at the commencement of spermatogenetic activity in the testes. *Riv. Biol.*, **58**, 139-169.

CLEMENS H. P., INSLEE T., 1968. The production of unisexual broods by *Tilapia mossambica* sex-reversed with methyltestosterone. *Trans. Amer. Fish. Soc.*, **97**, 18-21.

DZWILLO M., 1962. Uber künstliche Erzeugung funktionellee Männchen weiblichen Genotypus bei *Lebistes reticulatus*. *Biol. Zentr.*, **81**, 575-584.

HACKMANN E., 1974. Einfluss von Androgenen auf die Geschlechtsdifferenzierung verschiedenen Cichliden (Teleostei). *Gen. Comp. Endocr.*, **24**, 44-52.

JALABERT B., MOREAU J., PLANQUETTE P., BILLARD R., 1974. Déterminisme du sexe chez *Tilapia macrochir* et *Tilapia nilotica* : action de la méthyltestostérone dans l'alimentation des alevins sur la différenciation sexuelle ; obtention de mâles « inversés » fonctionnels et proportion des sexes dans la descendance. *Ann. Biol. anim. Bioch. Biophys.*, **14**, 729-739.

PADOA E., 1937. Differenziazone e inversione sessuale (feminizzazione) di avanoti di Trota (*Salmo irideus*) trattati con ormone follicolare. *Monit. Zool. Ital.*, **48**, 195-203.

PADOA E., 1939. Observations ultérieures sur la différenciation du sexe, normale et modifiée par l'administration d'hormone folliculaire chez la Truite iridée (*Salmo irideus*). *Bio-Morphosis.*, **1**, 337-354.

PETIT J., JALABERT B., CHEVASSUS B., BILLARD R., 1973. L'insémination artificielle de la Truite. I. Effets du taux de dilution, du pH et de la pression osmotique du dilueur sur la fécondation. *Ann. Hydrobiol.*, **4**, 201-210.

MRSIC W., 1930. Uber das Auftreten intermediärer Stadien bei der Geschlechtsdifferenzierung der Forelle. *Arch. Entwicklungsmech. Organ.*, **123**, 301-332.

WENIGER J.-P., ZEIS A., 1973. Induction de la production d'œstrogènes dans le testicule embryonnaire du Poulet par la dihydrotestostérone. *Biochimie*, **55**, 1163-1164.

YAMAMOTO T., 1953. Artificially induced sex reversal in genotypic males of the medaka (*Oryzias latipes*). *J. Exp. Zool.*, **123**, 571-594.

YAMAMOTO T., 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.

YAMAMOTO T., 1958. Artificial induction of functional sex reversal in genotypic females of the medaka (*Oryzias latipes*). *J. Exp. Zool.*, **137**, 227-262.

YAMAMOTO T., 1959. A further study of induction of functional sex reversal in genotypic males of the medaka (*Oryzias latipes*) and progenies of sex-reversals. *Genetics*, **44**, 739-757.

YAMAMOTO T., 1969. Sex differentiation, in HOAR and RANDALL *Fish Physiology*, vol. 3, p. 203-211, Academic Press, New York/London.

YAMAMOTO T., KAJISHIMA T., 1968. Sex-hormonic induction of reversal of sex differentiation in the goldfish and evidence for its male heterogamety. *J. Exp. Zool.*, **168**, 215-222.