

## Polymorphism in Lactate Dehydrogenase of Skeletal Muscle Associated With YY Sex Chromosomes in Medaka (*Oryzias latipes*) (36995)

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It is well known that in vertebrates lactate dehydrogenase (LDH; E.C.1.1.1.27) has five isozymic forms resulting from random association of two different subunits (1). Synthesis of these two subunits is controlled by two separate genes linked with autosomes (2, 3).

The natural sex chromosomal constitution of medaka, the Japanese killifish (*Oryzias latipes*), is XX in females and XY in males, but it is possible to produce animals with YY sex chromosomes in the d-rr strain, using techniques devised by Yamamoto (4); estrogenic treatment changes XY fry to females which can then be bred with XY males. Males with two Y sex chromosomes tend to differ from XY males in survival when fed sex hormones (5) and in competition to mate with females (6). In a further comparison of YY with XY and XX fish, we have investigated isozyme patterns of LDH in muscle, since the pattern of LDH isozymes is genetically controlled and LDH is important in glycolysis.

**Materials and Methods.** Skeletal muscle was obtained from the body wall caudal to the urogenital vent. LDH isozymes were separated by disc-electrophoresis as described by Ornstein and Davis (7), except for use of a 6.5% gel in the running gel. Fifteen microliters of muscle homogenate (equal parts of tissue and 30% sucrose solution W/V) were charged directly on the stacking gel and the isozyme patterns were developed by a constant current (3mA/column). The gel was stained for the demonstration of LDH isozymes by utilizing a Nitro-BT method (8).

**Results.** LDH zymograms of muscle from mature fish of different genotypes are shown in Fig. 1 and represented diagrammatically in Fig. 2. The eye of an XX female was

used as the standard to determine the relative site of each LDH isozyme.

In skeletal muscle of YY males, three types of LDH zymograms were found (Figs. 1 and 2). In type-I, four isozymes were always demonstrated which can be designated as A, D, E, and 5. A slight activity of isozyme F was often present. In type-II, isozyme D, E, F, and 5 were detected. In type-III, six isozymes were found and identified as B, 4, C, E, F, and 5. Mixtures of homogenates from two fish with different isozyme patterns were made to determine the relative sites of bands (Figs. 3 and 4).

In skeletal muscle of XX females or XY males, there are none of the three types of polymorphism demonstrated in YY males (Table I). Instead, XX females and XY males invariably had three isozymes, namely 5 and its two sub-bands E and F, and often had a slight activity of isozyme 4 (Figs. 1 and 2). There was no significant difference between XX females and XY males (Figs. 1 and 2).

In YY males, the three types of zymograms (types-I, -II, and -III) were never observed in hybrid patterns and, in breeding studies,

TABLE I. In Skeletal Muscle of Medaka There Are 3 Distinctive Types (-I, -II, or -III) of LDH Zymograms in the YY Genotype (Male) and Another Type Common to XY (Male) and XX (Female) Genotypes.

Sex chromosomes	No. of fish with zymogram type				Total no. of fish
	-I	-II	-III	XX-XY	
YY ♂	25	27	10	0	62
XY ♂	0	0	0	20	20
XX ♀	0	0	0	21	21

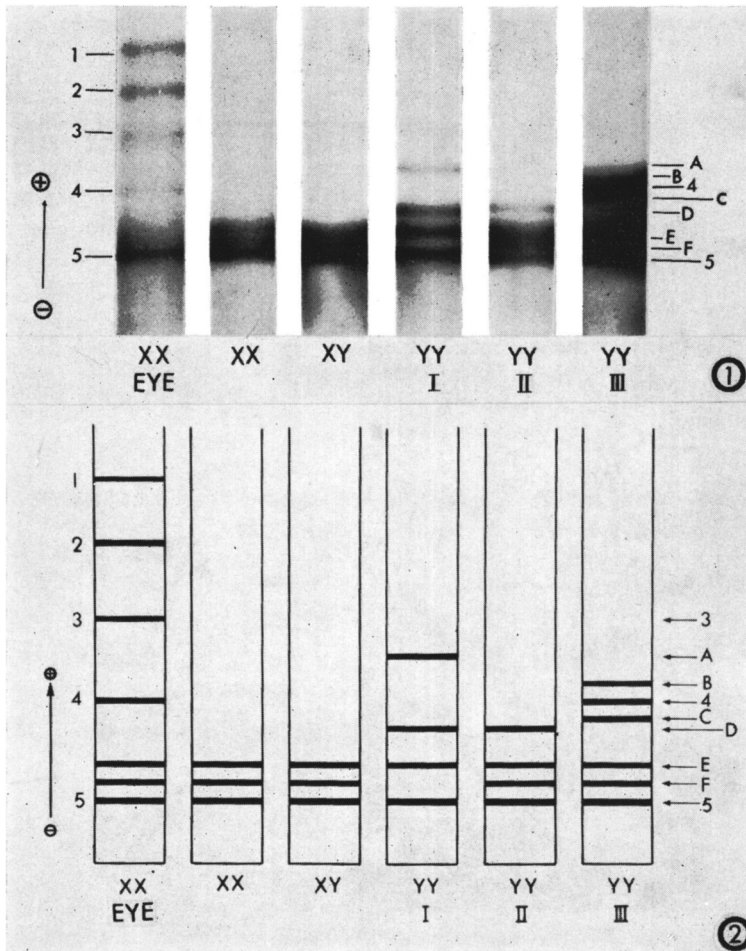


FIG. 1. LDH isozymes of medaka. XX-EYE is the standard pattern from the eyeball of XX females. The other patterns are derived from skeletal muscle. In YY the LDH zymograms of skeletal muscle are distinctive and are designated types-I, -II, or -III. Note the absence of these 3 distinctive types in zymograms of the eyeball of XX females (XX-EYE), and of skeletal muscle of XX females (XX) or XY males (XY).

FIG. 2. Schematic representation of zymograms in Fig. 1.

always segregated among the progeny (Table II). A single YY (type-III) male was mated with a YY (type-I) female and with an XX female (Fig. 5). Offspring of the mating of the two YY parents were YY sons which showed segregation of types-I and -III, whereas all offspring of the mating of the YY father with the XX mother had the XX-XY type of zymograms.

The type-I pattern of LDH in a YY female (Fig. 5) and other evidence (Table III) show that the YY genotype and not androgenic stimulation is responsible for the dis-

tinctive quality of LDH patterns in YY fish. Types-I or -II of LDH isozyme pattern were present not only in other adult female YY but also in immature and castrated YY males. Androgenic treatment, in amounts that grew flourishing male secondary sex characters in castrated males, did not produce YY patterns of LDH in XX or XY genotypes.

Isozymes A, B, C, and D were found to be specific to skeletal muscle of YY medaka, since they were missing in other organs of the YY male (Fig. 6).

*Discussion.* The three types of polymor-

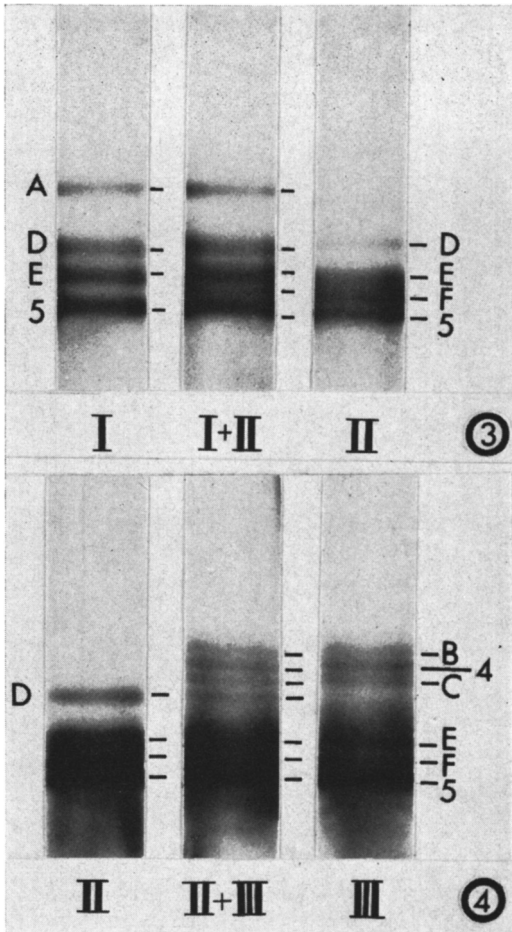


FIG. 3. Mixtures made of homogenized muscle of YY fish with LDH types-I and -II.

FIG. 4. Mixtures made of homogenized muscle of YY fish with LDH types-II and -III.

phisms of LDH from skeletal muscle, now reported for YY fish, are not necessarily all that may occur in the YY genotype. The YY fish under study were descendants of YY breeders which have been in this laboratory for 7 years. Ancestors of the present generations showed greater interindividual variation in several known regards including the incidence of hypogonadism and of sterility during adolescence.

Polymorphism of LDH does not seem to characterize skeletal muscle of the YY genotype of all species, since we did not observe this in any of five adult fish of each of YY, XY, and XX genotypes of *Xiphophorus*

*maculatus*, which were kindly provided by Dr. Klaus Kallman (9).

LDH-5 of muscle has been reported to be accompanied by two sub-bands which show LDH activity (10) in medaka with orange-red body color, and we have obtained similar results in white XX and XY medaka. Therefore, LDH-5 and two sub-bands designated as E and F are independent of the gene for orange-red color of medaka which is located on the sex chromosomes (11).

TABLE II. Number of Males with Each Type of LDH Zymogram of Skeletal Muscle in YY Progeny of 3 Different Pairs of Parents.

Parents	No. of YY sons with each zymogram type				Total no. of fish
	-I	-II	-III	XX-XY	
Pair no. 1					
YY ♀	1	10	1	0	12
YY ♂					
Pair no. 2					
YY ♀	8	2	1	0	11
YY ♂					
Pair no. 3					
YY ♀	4	4	0	0	8
YY ♂					

It was noted that LDH-5 and two sub-bands E and F appear in types-II and -III patterns from muscle of the YY as well as XX genotype. This shows that LDH-5 and sub-bands E and F are not specifically dependent upon just the X or just the Y chromosome.

In striking contrast to LDH-5 and its two sub-bands, the occurrence of the four isozymes

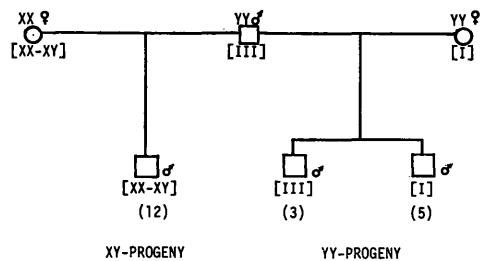


FIG. 5. LDH zymograms (in brackets) of XY and YY sons sired by the same YY father. Numbers in parentheses are numbers of fish examined.

TABLE III. Relationship of Types -I and -II of LDH of the YY Genotype *Per Se* Rather than to Endocrine Status. These Distinctive Types Were Not Produced by Androgenic Stimulation, and Were Not Prevented by Estrogenic Stimulation Or by Lack of Sex Hormones.

Genotype	Status	No. of fish	Type of LDH			
			XX-XY	-I	-II	-III
XX	Mature ♂ (sex-reversed) (had androgenic treatment as fry)	1	1			
XX	Adult ♀ (during treatment with testosterone propionate <sup>b</sup> )	3	3			
XY	Adult ♂ (during treatment with testosterone propionate <sup>b</sup> )	1	1			
YY	Mature ♀ (sex-reversed) (had estrogenic treatment as fry)	3			3	
YY	Immature ♂ (2 days old)	50 <sup>a</sup>		50 <sup>a</sup>		
YY	Castrate adult ♂ (operated as adolescent)	3		1	2	

<sup>a</sup> 50 fry were analyzed as a group.

<sup>b</sup> As subcutaneous pellet that stimulates supranormal male secondary characters in females or in castrate males.

A, B, C, and D seems to be related to the double Y chromosomal constitution, since these four isozymes are found only in the YY genotype. This evidence indicates the presence of at least one additional type of subunit in the YY genotype.

It has been reported that in fish, synthesis of LDH subunits is controlled by autosomal genes which have co-dominancy (12, 13), but the three YY-specific types of LDH were segregated in all of 31 YY progeny produced by three pairs of parents, with no offspring show-

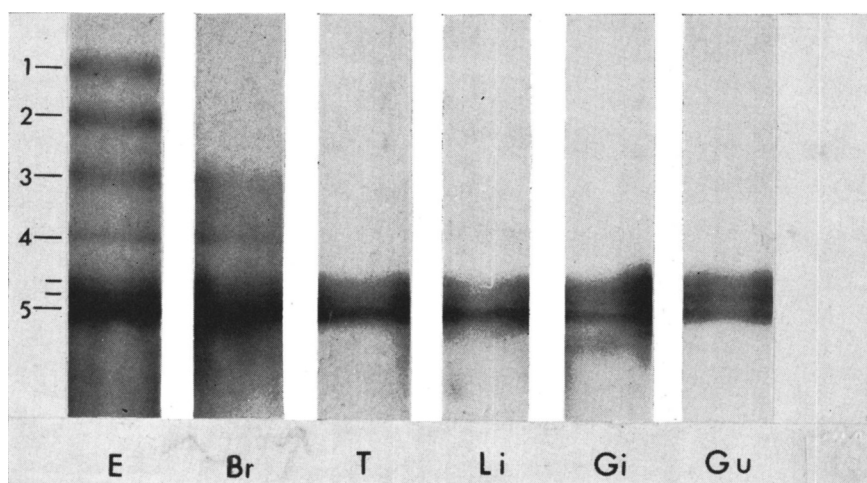


FIG. 6. Zymograms of various organs of a YY male show that the 4 isozymes (A, B, C, and D) are specific to skeletal muscle in YY medaka. E (eye); Br (brain); T (testis); Li (liver); Gi (gill); Gu (gut).

ing hybridization of types-I, -II, or -III.

Other evidence also strongly suggests that the synthesis of specific LDH isozymes (A, B, C, and D) in YY fish is controlled by the gene(s) on sex chromosomes. Notably, in progeny of the same YY father, XY sons lacked the YY-specific patterns of their YY brothers and YY father.

In LDH isozyme patterns the difference of YY from either XY or XX may be due to one or more regulator genes (for the synthesis of subunits of LDH) located on an X chromosome and absent in the YY genotype.

Interindividual polymorphism of LDH isozyme patterns in YY medaka may be due to differences in genetic elements of individual Y chromosomes. We believe that the present data suggest a third genetic function of Y chromosomes in medaka (the control of polymorphism of LDH isozymes) in addition to two functions previously observed (differentiation of the testis and orange-red pigment).

*Summary.* In skeletal muscle of medaka, 3 distinctive patterns of LDH isozymes were found only in animals with YY sex chromosomes. These patterns were characterized by additional isozymes quite different from the single pattern that characterized both XX and XY genotypes. It is suggested that the X chromosome may have one or more regu-

lator genes for synthesis of subunits of LDH.

The interindividual variation of LDH zymograms in the YY genotype of medaka (*Oryzias latipes*) may be due to differences in genetic elements of individual Y chromosomes and seems to establish a third genetic function of Y chromosomes in medaka.

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