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Sex-Linked Inheritance of the *If* Locus in the Medaka Fish (*Oryzias latipes*)

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ABSTRACT—The *If* (leucophore free) locus was previously reported autosomal recessive in the medaka fish (*Oryzias latipes*). However, extensive linkage analyses in this study using various strains revealed that the *If* locus was closely sex-linked. The recombination frequency between *If* and the male determining factor (*y*) was 2.2% (10 recombinants out of 464 progeny). Because the *If*/*If* homozygous fish do not have visible leucophores, they are distinguishable from wild type in early developmental stages. In the Qurt strain with heterozygous sex chromosomes (X^{If}/X^{If} in females and X^{If}/Y^{+} in males), we can predict sex of each embryo on second day after fertilization. The strain should be a very useful material for studying sex determination or differentiation mechanisms in the medaka fish.

INTRODUCTION

In the guppy (*Poecilia reticulata*) and platy fish (*Xiphophorus maculatus*) , the presence of sex-linked coloration loci was demonstrated (Winge, 1922; Schartl, 1988). Such sex-linked body color mutations are considered to be very important not only for producing sexual selections in the fish (Houde and Endler, 1990) but also for starting degenerations of Y chromosomes in the evolution of sex determining mechanisms (Rice, 1987). Also in the medaka fish (*Oryzias latipes*), inheritance of a sex-linked color locus *r* (colorless xanthophore) has been intensively demonstrated (Aida, 1921; Yamamoto, 1964). In our previous study (Wada *et al*., 1995), we constructed a genetic map which consisted of more than 200 markers (RAPDs, allozyme loci and visible color loci), and in consequence, a pigment pattern locus *If* (leucophore free) was suggested to be sex chromosomal. In this study, further linkage analysis on the *If* locus was carried out in five independent backcrosses using five medaka strains to confirm the sex-linked inheritance. A precise map distance between the *If* locus and the male determining factor (*y*) was determined. Furthermore, the phenotypes of the *If* locus in early developmental stages of embryos were observed under a microscope. This second sex-linked coloration locus in the medaka fish is very important because it is a powerful genetic marker which allows us to distinguish genetic sex of each embryo at early developmental stages.

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MATERIALS AND METHODS

Strains

The *If* locus was found and isolated by Tomita in the offspring of the wild medaka population collected at Toyokawa, Aichi prefecture, Japan in 1970 and first demonstrated as an autosomal locus (Tomita, 1992). Homozygotes of the *If* (*If*/*If*) have no leucophores throughout the life. In wild-type homozygotes (+/+) and heterozygotes (+/*If*), the yellow colored leucophores appear beneath the brain at stage 25 and then develop on the body surface at stage 26 (Tomita, 1992; Iwamatsu, 1994). Table 1 shows the strains used in this experiment. The strains HNI (genotype for the *If* locus: +/+; Hyodo-Taguchi, 1980) and Sabae (+/+; Shima *et al*., 1985) were derived from the northern Japanese population of the medaka fish while the others, Hd-rR (+/+; Hyodo-Taguchi, 1980) and AA2 (*If*/*If*; Shimada and Shima, submitted) were from the southern population. The two populations were well demonstrated to be genetically much different (Sakaizumi *et al*., 1983) though the hybrids and backcross progeny among the populations can be obtained (Naruse and Shima, 1989). The Qurt strain was newly established from a domesticated stock of the southern population for this study. The derived stock was originally maintained to obtain a tester strain for the specific locus test system in the medaka fish (Shima and Shimada, 1991). One male with genotype *b*/*b*, *If*/+, *gu*/+, and *r*/*r* and a female with *b*/*b*, *If*/*If*, *gu*/*gu*, and *r*/*r* were selected from the mass-mated stock and mated (*b*, colorless melanophore and *gu*, guanineless are autosomal recessive body color mutations; Shima and Shimada, 1991). A male progeny with *b*/*b*, *If*/+, *gu*/*gu*, and *r*/*r* was selected and backcrossed with the female-parent. Preliminary results showed that the males had leucophores but the females did not have leucophores in the population (genotypes were considered to be *b*/*b*, *If*/*If*, *gu*/*gu*, and *r*/*r* in females and *b*/*b*, *If*/+, *gu*/*gu*, and *r*/*r* in males). The stock thus obtained was named Qurt and used in this study.

Genetic crosses

Following crosses were carried out in this experiment; Cross 1: two AA2 females were mated with two (AA2 female X HNI male) F_1 males, Cross 2: an AA2 female was mated with a (AA2 female X Sabae male) F_1 male, Cross 3: an AA2 female was mated with a

Table 1. The medaka strains used in the present study

Strain	Genotypes of the *If* locus	Derived population
HNI	+/+	northern
Sabae	+/+	northern
Hd-rR	+/+	southern
AA2	*If*/*If*	southern
Qurt	♀, *If*/*If*; ♂, *If*/+	southern

The strains HNI, Hd-rR and AA2 are inbred strains while others are maintained as closed colonies. The genotypes for other visible loci were omitted for simplicity.

(AA2 female X Hd-rR male) F_1 male, Cross 4: two Qurt females were mated with two Qurt males, Cross 5: an AA2 female was mated with a (HNI female X AA2 male) F_1 male. The fish used for the five crosses were kept in plastic cages (34 × 17 × 13cm) in aged tap water at 27 ± 2°C under 14-hr light/10-hr dark photo period cycle. The fish were fed with TetraMin (Tetra Werke, FRG).

Photomicroscopy

The phenotypes of embryos were photographed by transmitting light and epi-illuminating fluorescent light using an Olympus microscope, BX50 with a BX-FLA fluorescence system (using a cube set, U-MWU).

RESULTS

Closely sex-linked inheritance of the *If* locus in the five independent crosses

Table 2 summarizes the results of the crosses. In the five independent crosses, a total of 238 males and 226 females was obtained. The sex ratio was reflected the Mendelian 1:1 ratio (χ^2 value was 0.31, $0.5 < P < 0.6$). The phenotypes for the *If* locus of the progeny were 250 wild types (supposed to be heterozygotes, *If*/+) and 214 mutant types (supposed to be homozygotes, *If*/*If*). Thus the *If* locus was also reflected the Mendelian 1:1 segregation ratio (χ^2 value was 2.79, $0.10 < P < 0.15$). In all of the five crosses, the *If* locus was closely sex-linked and there was not significant difference in the recombination frequency among the crosses (the recombination frequencies were ranging from 0 to 3.8% and the maximum χ^2 value was 3.08, $0.3 < P < 0.4$). Altogether, 10 recombinants (five males and five females) were obtained in 464 backcross progeny. These recombinants were fertile and produced progeny normally. The average recombination frequency between the *If* locus and the male determining factor (*y*) was 2.2% (90% upper and lower confidence limits were 3.6 and 1.3%, respectively).

Expression of leucophores in early developmental stages

Figure 1 shows the photographs of embryos of the Hd-rR strain which carries wild type alleles for the *If* locus homozygously (+/+). The leucophores were not visible in a 1-day embryo at the late neural stage by a transmitting illumination (Fig. 1a) nor by an epi-illumination fluorescent condition (Fig. 1b). However, in a 2-day embryo when the heart started beating, the leucophores were observable beneath the brain by a transmitting illumination (Fig. 1c) as well as by an epi-illumination fluorescence condition (Fig. 1d). In a 4-day embryo, when retinal pigmentation and optic vesicle formation were evident, the leucophores were more easily observable (Fig. 1e, f). Figure 2 shows the photographs of embryos of the Qurt strain. The half of the embryos have wild type leucophores (the genotype was supposed to be *If*/+), although the rest do not have visible leucophores at all (the genotype was supposed to be *If*/*If*). Most of the phenotypically wild type embryos developed to males while the mutant type embryos developed to females as shown in the experiment of genetic crosses.

DISCUSSION

The sex-linked inheritance of the *If* locus

The *If* locus was firstly demonstrated as an autosomal locus (Tomita, 1992), however, in the present study, the closely sex-linked inheritance was demonstrated in all of the five crosses. We observed recombinations between sex chromosomes derived from different strains and populations. In Cross 1, X chromosomes from the AA2 strain (abbreviated to AA2-X) and Y chromosome from the HNI strain (HNI-Y); Cross 2, AA2-X and Sabae-Y; Cross 3, AA2-X and Hd-rR-Y; Cross 4, Qurt-X and Qurt-Y; and Cross 5, HNI-X and AA2-Y. Although the HNI and Sabae strains were genetically much different from the other stains, the backcross progeny were obtained with normal 1:1 sex ratio. A total of 130 males and 137 females was obtained in Cross 1, 2 and 5 (χ^2 value was 0.18, $0.6 < P < 0.7$). In the crosses using southern strains, 108 males

Table 2. The recombination frequencies between the *If* and *y* loci in the 5 independent backcrosses

Cross	Numbers of progeny with *If* phenotypes		Recombinants /progeny	% Recombination frequency (90% confidence UL/LL)
	wild type	mutant type		
1 AA2 × (AA2 × HNI)	47♂	32♀	0/79	0 (3.3/0)
2 AA2 × (AA2 × Sabae)	26♂	33♀/1♂	1/60	1.7 (7.1/0.37)
3 AA2 × (AA2 × Hd-rR)	51♂/2♀	50♀/2♂	4/105	3.8 (8.2/1.7)
4 Qurt × Qurt	54♂	37♀/1♂	1/92	1.1 (4.7/0.24)
5 AA2 × (HNI × AA2)	69♀/1♂	55♂/3♀	4/128	3.1 (6.8/1.4)
Total			10/464	2.2 (3.6/1.3)

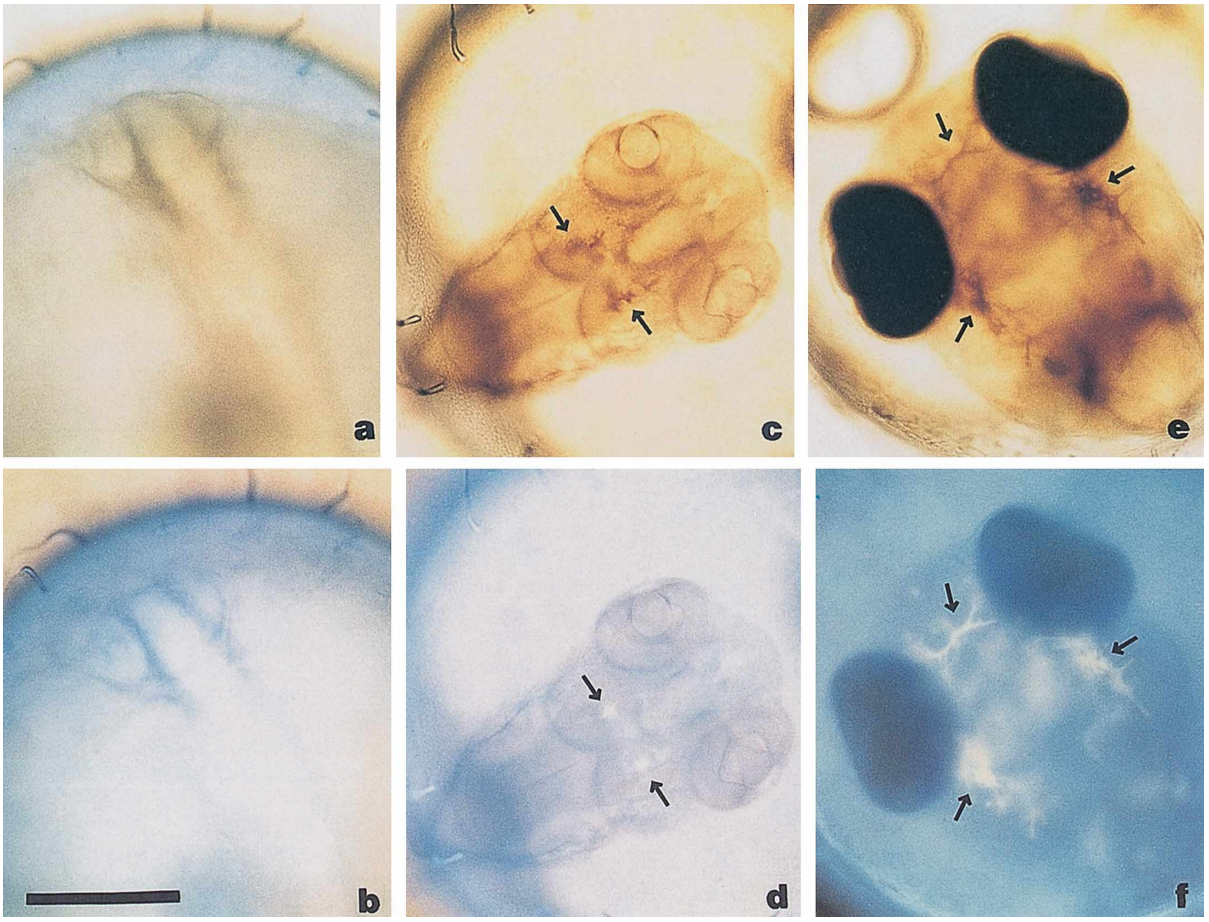

Fig. 1. Photographs of embryos of the Hd-rR strain (wild type +/+). (**a**,**b**) 1-day embryo (26 hr after fertilization, 27°C), (**c**,**d**) 2-day embryo (50 hr), (**e**,**f**) 4-day embryo (74 hr). (**a**,**c**,**e**) photographed by transmitting illumination, (**b**,**d**,**f**) photographed by epi-illuminating fluorescent light. Arrows show leucophores. Scale bar: 500 μm.

and 89 females were obtained (Cross 3 and 4, χ^2 value was 1.83, $0.15 < P < 0.17$). These results indicated that all the strains used in this study shared the same sex determination system (i.e., the same sex chromosome).

Expression of the leucophores in wild type and mutant type embryos were the same as originally described (Tomita, 1992). In the Tomita's experiment, an exceptional autosomal inheritance might have been detected. Such inheritance could be explained by chromosomal rearrangements (translocation) or high recombinations between sex chromosomes (pseudoautosomal inheritance).

Prediction of sex of each embryo in early developmental stages by using the expression of leucophores

As shown in the photographs, expression of wild type leucophores began on two days after fertilization, while in the mutant *lf*/*lf* homozygotes, visible leucophores were not observed at all. Although a question still remained to be addressed whether the pigment cells were colorless or the cells themselves were absent, the *lf* locus should be a very useful genetic marker for prediction of sex in each embryo because it is very easy to distinguish *lf*/*lf* phenotype from *lf*/+ phenotype. For example, in the Qurt strain where the Y chromosomes carry the wild type *lf* allele while the X chromosomes carry the mutant type *lf* alleles, males (X^{lf}/Y^{+}) always have leucophores but females (X^{lf}/X^{lf}) do not have leucophores as far as X and Y chromosomes do not recombine. Because the recombination frequency between *lf* and *y* was 2.2%, we can predict sex of each embryo with approximately 97% confidence in this strain. In the medaka fish, differentiation of gonads begins in 5-day embryos just before hatching (Satoh and Egami, 1972). Because the phenotypic expression of the *lf* locus is much earlier than the gonadal differentiation, this genetic marker is a powerful tool for studying sex determination or sex differentiation mechanisms in the medaka fish.

The first sex-linked color locus demonstrated in the medaka was the *r* locus (Aida, 1921). The recombination frequency between *r* and *y* was estimated to be 0.2% (Yamamoto, 1964). Experiments using double recessive mutant strains to determine gene orders for the *r*, *lf* and *y* loci are now under way.

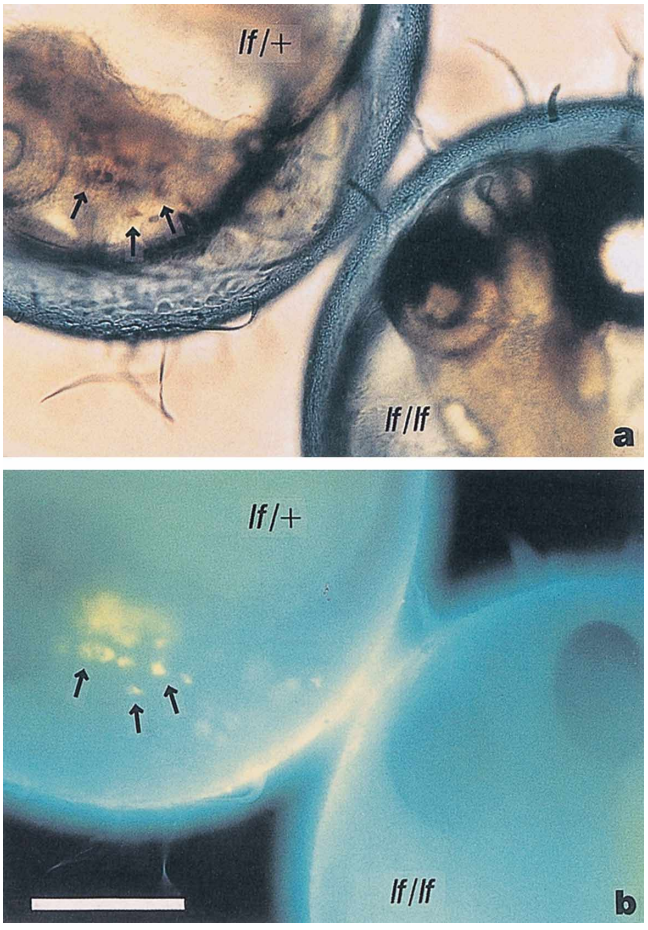

Fig. 2. Photographs of 2-day (30 hr) embryos of the Qurt strain; the heterozygous *lf*/+ fish on the left and the mutant *lf*/*lf* fish on the right. (**a**) photographed by transmitting light, (**b**) photographed for autofluorescence. Leucophores (arrows) are present in the heterozygous *lf*/+ fish but absent from the mutant homozygous *lf*/*lf* fish. Scale bar: 500 μm.

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