STUDIES ON THE MUTANT OF THE MEDAKA, *DX-1*

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ABSTRACT - A new color mutant of the medaka, *Oryzias latipes*, was found in the wild population. The mutant gene *dx-1*, which modifies the gene action of *R* and reduces the carotenoid content in the xanthophore in a homozygous condition, is recessive and autosomal. The *dx-1* alleles are independent of the *b*, *ci* and *r* alleles.

INTRODUCTION

A number of mutant genes have been found in the medaka, *Oryzias latipes* (see Tomita 1975). In this fish, three types of chromatophores, melanophores, xanthophores and leucophores are observable in the skin. The heritable color varieties have been phenotypically classified into brown *(BR)*, blue*(Br)*, orange-red*(bR)*, white*(br)*, variegated orange-red*(B'R)* and variegated white*(B'r)* by Aida (1921). The color interferer gene*(ci)*, which makes the body color pale in a homozygous condition, is recessive, autosomal and independent of the *b* and *r* alleles (Aida unpublished). The *ci* gene in a homozygous condition causes the reduction of deposition of carotenoid, while the number of leucophores increases three to four times that of normal fish (Takeuchi 1969).

A new mutant of bluish brown color was found in the wild population. Its xanthophores exhibited extremely dilute orange-red color. The results of gene analyses of this new mutant will be dealt with in this paper.

MATERIALS AND METHODS

A female of the mutant with bluish brown color was found in a paddy field on Sugashima Island, Mie Prefecture in 1958. Another mutant fish of a similar phenotype was collected again in the same place in 1960. To study the distribution of this mutant in the population, third and fourth collections were carried out in the autumn of 1961 and 1962.

In the present study, the new mutant fish were mated with homozygous fish of the following color types : brown *(BBRR)*, orange-red*(bbRR)*, white*(bbrr)*, and gray*(BBciciRR)*. The methods of care for keeping and breeding the fish followed Yamamoto (1967). As the new mutant gene was recessive, it was designated *dx-1* (dilute xanthophore).

Xanthophores of the mutant contained less orange-red pigment(carotenoid) than those of the wild (brown) type. The mutant with slightly colored xanthophores was easily distinguished from the blue*(Br)* type under the microscope.

To estimate the carotenoid content, whole skins were torn off and immersed in 50 volumes of 100% acetone, until the carotenoid dissolved. The extinction of supernatant was measured with a blue filter(430 mμ) and the carotenoid concentration was calculated from a standard curve of β-carotene. The fish used were 30 to 35 mm in total length. Five fish were used to estimate the carotenoid content in the orange-red type and ten fish of other color types. In each series of experiments, the fish were reared to adulthood outdoors in the same container.

RESULTS

1. Gene analyses

a. Crosses between bluish brown *(Bdx-1R)* and brown *(BBRR)*

The bluish brown*(Bdx-1R)*female was mated with the brown *(BBRR)* male. The F_1 progeny were all brown *(BR)* in a phenotype (25 fish). The characters of the F_2 progeny were brown *(BR)* (64 females and 52 males) and bluish brown *(Bdx-1R)* in phenotypes (14 females and

15 males) in a ratio of 3 : 1 ($\chi^2_{(1)}=1.93$, $p=0.1$-0.25) and the sex ratio was 1 : 1 in each color type ($\chi^2_{(3)}=3.27$, $p=0.25$-0.5). Reciprocal mating was also tested. The progeny were all brown *(BR)* in a phenotype (47 fish). The characters of the F_2 progeny were brown *(BR)* (48 females and 41 males) and bluish brown *(Bdx-1R)* in phenotypes (13 females and 14 males) in a ratio of 3 : 1 ($\chi^2_{(1)}=0.17$, $p=0.5$-0.75). When the bluish brown *(Bdx-1R)* were inbred, the progeny were all bluish brown *(Bdx-1R)* in a phenotype. So it is concluded that the *dx-1* gene is recessive and autosomal. The F_1 brown (heterozygous for *dx-1*) female was mated with the bluish brown *(Bdx-1R)* male. The progeny were brown *(BR)* (111 fish) and bluish brown *(Bdx-1R)* in phenotypes (118 fish) in a ratio of 1 : 1 ($\chi^2_{(1)}=0.21$, $p=0.5$-0.75). Reciprocal mating also gave the same result (brown *(BR)* 98 fish and bluish brown *(Bdx-1R)* 106 fish) ($\chi^2_{(1)}=0.31$, $p=0.5$-0.75).

b. Crosses between bluish brown *(Bdx-1R)* and orange-red *(bbRR)*

The bluish brown *(Bdx-1R)* female was mated with the orange-red *(bbRR)* male. The progeny were all brown *(BR)* in a phenotype (72 fish). The characters of the F_2 progeny were divided into four types, brown *(BR)* (91 fish), bluish brown *(Bdx-1R)* (27 fish), orange-red *(bR)* (24 fish) and new type in phenotypes (8 fish) in a ratio of 9 : 3 : 3 : 1 ($\chi^2_{(3)}=1.37$, $p=0.5$-0.75). The new type showed a dilute orange-red color. When the new type was mated with the bluish brown *(Bdx-1R)*, the progeny were all bluish brown *(Bdx-1R)* in a phenotype. They were *bdx-1R* in a phenotype. Therefore, the *dx-1* alleles are independent of the *b* alleles.

c. Crosses between orange-red *(bbRR)* and dilute orange-red *(bdx-1R)*

The orange-red *(bbRR)* female was mated with the dilute orange-red *(bdx-1R)* male. The F_1 progeny were all orange-red *(bR)* in a phenotype (97 fish). The characters of the F_2 progeny were segregated into orange-red *(bR)* (67 fish), and dilute orange-red *(bdx-1R)* in phenotypes (21 fish) in a ratio of 3 : 1 ($\chi^2_{(1)}=0.09$, $p=0.75$-0.9). The F_1 orange-red (heterozygous for *dx-1*) male was mated with the dilute orange-red *(bdx-1R)* female. The progeny were orange-red *(bR)* (78 fish) and dilute orange-red *(bdx-1R)* in phenotypes (72 fish) in a ratio of 1 : 1 ($\chi^2_{(1)}=0.3$, $p=0.5$-0.75).

d. Crosses between white *(bbrr)* and bluish brown *(Bdx-1R)*

The bluish brown *(Bdx-1R)* male was mated with the white *(bbrr)* female. The F_1 progeny were all brown *(BR)* (63 fish). The F_2 offspring consisted of brown *(BR)* (231 fish), blue *(Br* and *Bdx-1r)* (91 fish), bluish brown *(Bdx-1R)* (73 fish), orange-red *(bR)* (80 fish), dilute orange-red *(bdx-1R)* (23 fish) and white *(br* and *bdx-1r)* in phenotypes (28 fish) in a ratio of 27 : 12 : 9 : 9 : 3 : 4 ($\chi^2_{(5)}=2.05$, $p=0.75$-0.9).

e. Crosses between white *(bbrr)* and dilute orange-red *(bdx-1R)*

The white *(bbrr)* female was mated with the dilute orange-red *(bdx-1R)* male. The progeny were all orange-red *(bR)* in a phenotype (72 fish). The characters of the F_2 progeny were segregated in orange-red *(bR)* (54 fish), dilute orange-red *(bdx-1R)* (19 fish) and white *(br* and *bdx-1r)* in phenotypes (23 fish) in a ratio of 9 : 3 : 4 ($\chi^2_{(2)}=0.09$, $p=0.75$-0.9).

f. Crosses between gray *(BBciciRR)* and bluish brown *(Bdx-1R)*

The gray *(BBciciRR)* female was mated with the bluish brown *(Bdx-1R)* male. The progeny were all brown *(BR)* in a phenotype (43 fish). The characters of the F_2 progeny were brown *(BR)* (139 fish), gray *(BciR)* (39 fish), bluish brown *(Bdx-1R)* (42 fish) and new type in phenotypes (10 fish) in a ratio of 9 : 3 : 3 : 1 ($\chi^2_{(3)}=2.65$, $p=0.25$-0.5). The new type had melanophores and leucophores similar to those of the gray *(BciR)*, but their xanthophores were extremely dilute in color. When the new type was mated with the bluish brown *(Bdx-1R)* and gray *(BciR)*, the offspring were all bluish brown *(Bdx-1R)* in the former and all gray *(BciR)* in the latter. The new type was pale blue *(Bcidx-1R)* in a phenotype. Therefore, the *dx-1* alleles must be independent of the *ci* alleles.

2. Carotenoid content

As carotenoid content in xanthophores was affected to some extent by environmental factors, such as food and background color. The orange-red *(bR)* and the dilute orange-red (*bdx-1R*) types were reared to adult in a container outdoors. The brown (*BR*) and the bluish brown (*Bdx-1R*) were also reared in a container outdoors. The results are shown in Table 1. The *dx-1* gene under the homozygous condition reduces the carotenoid content approximately one eighth to one third.

Table 1 Carotenoid content of different strains of the medaka. The fish were reared in a container outdoors from the larval stage to adult. The body length was 30 to 35 mm. Ten fish (*Bdx-1R*, *BR* and *bdx-1R*) or 5 fish (*bR*) were used in each experiment.

Series	Phenotype	Sex (No. of experiment)	β-carotene(μg) / skin dry weight(mg)
1	*Bdx-1R*	female (3)	1.83±0.18
		male (4)	1.81±0.12
	BR	female (4)	4.41±0.17
		male (3)	3.75±0.26
2	*Bdx-1R*	female (3)	1.57±0.08
		male (3)	1.30±0.17
	BR	female (4)	4.25±0.16
		male (2)	3.75±0.10
3	*bdx-1R*	female (2)	1.54±0.10
		male (2)	1.39±0.05
	bR	female (2)	12.65±0.35
		male (2)	12.68±0.40
4	*bdx-1R*	female (3)	2.12±0.29
		male (3)	2.28±0.12
	bR	female (3)	8.00±0.35
		male (5)	8.03±0.18

3. *Distribution of mutants in fields*

The distribution and the frequency of the mutant in the natural population were studied. Paddy fields in Sugashima were located as steps in narrow spaces isolated from each other. The wild medaka was found in four paddy fields separated by low hills. In one place, no collection was performed because there were only a few fish. In 1961, 790 fish were collected at site 1, 751 fish at site 2 and 532 fish at site 3. In 1962, 498 fish were collected at site 1, 72 fish at site 2 and none at site 3, where paddy fields were dried out. Only at site 1 were bluish-brown (*Bdx-1R*) found 4 fish (1 male and 3 females) in 1961 and 3 fish (1 female and 2 males) in 1962 .

DISCUSSION

In fish, the carotenoid originates in food, since it can not be synthesized. Although the carotenoid content in xanthophores is to some extent affected by environmental factors, it is primarily controlled by genetic factors. The white (*br*) medaka does not deposit carotenoid in its xanthophores even if this substance is present in foods. The edges of the caudal fin turn an intense red color in the brown (*BR*) and orange-red (*bR*) types. In the bluish brown (*Bdx-1R*) and dilute orange-red (*bdx-1R*) types, this feature was generally lacking and the xanthophores on the edges of caudal fin contained less pigment than those of the brown (*BR*) and orange-red (*bR*) types. In the preliminary tests, the numbers of melanophores, xanthophores and leucophores in 0.05mm^2 of dorsal part numbered 17, 44 and 2 in the brown (*BR*) type and 18, 44 and 2 in the bluish brown *(Bdx-1R)* type, respectively. The *dx-1* alleles do not affect the numbers of melanophores, xanthophores and leucophores.

The content of carotenoid in xanthophores of the brown *(BR)* and orange-red *(bR)* types were three to eight times more than those of the bluish brown *(Bdx-1R)* and dilute orange-red *(bdx-1R)* types. The dilution of orange-red color in the bluish brown *(Bdx-1R)* and dilute orange-red *(bdx-1R)* in phenotypes may depend on the decrease of pigment content in each xanthophores.

The carotenoid in xanthophores may be mainly supplied as food from green algae in culture water. The carotenoid content of fish in a phenotype may be variable in different containers in which green algae grows in different degrees. The deposition of carotenoid in xanthophores may be controlled by many processes, including the absorption of pigment in the digestive organ, the stabilization of pigment in the body fluid, changes in permeability at the surface of xanthophores, the reception of pigment in xanthophores and so on. It is not clear what processes control the *dx-1* alleles.

The mutant fish were rarely found in the wild population. The frequency of bluish brown *(Bdx-1R)* was about 0.56% in the wild medaka collected from site 1. If field conditions are constant and mating occurs at random, the *dx-1* gene frequency is 0.75%.

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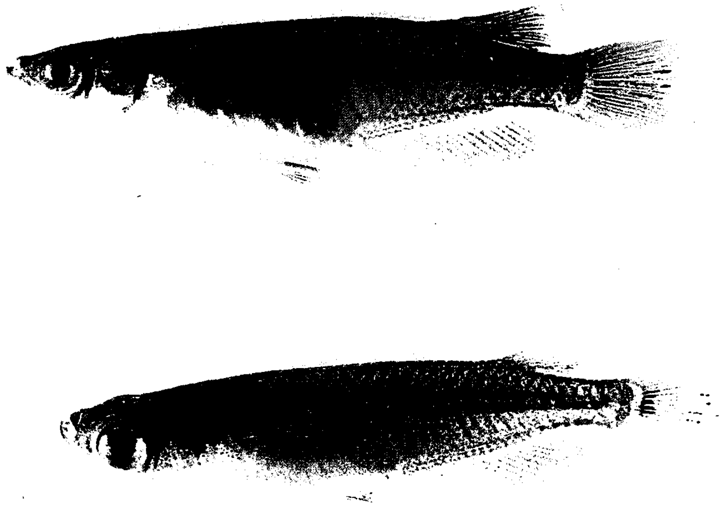

Plate 1 Brown (wild type, *BR*) male (above) and bluish brown *(Bdx-1R)* male (below).