The Fish Biology Journal MEDAKA, 4: 37–39 (1992)

Studies on the mutant of the medaka, *lf*

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Abstract The color mutant (*lf*) was found and established in the medaka (*Oryzias latipes*). Visible leucophores did not appear throughout life in this mutant. The *lf* gene is autosomal and recessive. The *lf* alleles did not link with other alleles used in this experiment. In test crosses, the *lf* gene was epistatic for other genes concerning the expression of leucophores.

Introduction

The medaka (*Oryzias latipes*) have melanophores, xanthophores and leucophores as chromatophores and iridocytes. The color of leucophores is orange to white in a reflected light and brown in a transparent light. The mutant (*BlfR*) had black melanophores, orange-red xanthophores and metallic colored iridocytes as in the wild fish (*BR*), but had no visible leucophores.

In this paper, gene analyses of this mutant were dealt with special attention to gene interaction.

Materials and methods

A male mutant (*BlfR*) was segregated in 1970 in the progeny of wild medaka collected at Toyokawa, Aichi. The adult *BlfR* fish were brown in body color. The color difference between wild (*BR*) and *BlfR* was difficult to detect by naked eyes. The *BlfR* fish had no visible leucophores throughout the embryo to adult. The *lf* character was checked by microscopic observation of leucophores. As the first leucophores appeared under the brain at embryonic stage (beginning of heart beat), the *lf* mutant was able to detect at this early stage.

The genes used were *b, ci, co, Da, df-1, df-2, di, dm, dx-1, dx-2, fl, fu-6, i, i-3, ml-3, r* and *vc*. These genes are explained briefly as follows. They have been described in some detail (Tomita 1975, 1982, 1984, 1985). The *r* alleles are sex-linked and the other alleles are autosomal. The *Da* gene is incomplete dominant and the other genes are recessive.

b : The *b* alleles (*B* > *b*) control the melanin formation. The *B* gene makes black melanophores. The *b* gene produces colorless melanophores (Aida 1921).

ci : The *ci* gene decreases the number of xanthophores and makes well developed leucophores (Takeuchi, 1969).

co : The *co* gene produces concentrated xanthophores.

Da : The *Da* homozygote has an anal fin in place of the dorsal fin. The *Da* heterozygote has a large dorsal fin (7–14 fin rays).

df-1 : The *df-1* gene makes broken membrane fins at embryonic and larval stage. Adult fish have normal fins.

df-2 : The *df-2* characters are similar to the *df-1* ones.

dm : The *dm* gene makes dispersed melanophores and leucophores.

dx-2 : The *dx-2* gene causes dilute orange-red xanthophores.

fl : The *fl* gene causes a few leucophores.

fu-6 : The *fu-6* gene makes notochord bent wavily at embryonic stages. It makes fused vertebrae and short body in adult fish.

i : The *i* gene makes albino.

i-3 : The *i-3* gene also makes albino.

ml-3 : The *ml-3* gene increases leucophores three times in number on dorsum at embryonic and larval stages.

r : The *r* alleles (*R* > *r*) control deposition of orange-red pigment in xanthophores. The *R* gene makes orange-red xanthophores. The *r* gene makes colorless xanthophores. The *BR* fish is brown (wild type) in body color. The *bR* and *br* fish are orange-red and white in body color (Aida 1921).

vc : The *vc* gene produces variegation caused by the local absence of melanophores and leucophores.

Results

1) Crosses between brown (BR) and brown lacking leucophores (BlfR)

A *BR* female was bred with a *BlfR* male. The F_1 progeny were *BR* (♀ 41 fish, ♂ 48 fish). The F_2 progeny were segregated to *BR* (♀ 57 fish, ♂ 49 fish) and *BlfR* (♀ 17 fish, ♂ 15 fish) in a ratio of 3 : 1 (χ^2=0.24, p=0.75–0.5). The sex ratio was 1 : 1 (χ^2=0.72, p=0.75–0.5). In the χ^2 test for segregation, there was no significant deviation from expectation. The *lf* gene was recessive and autosomal.

2) Crosses between brown lacking leucophores (BlfR) and orange-red (bR)

A *BlfR* female was mated with a *bR* male. The F_1 progeny were *BR* (49 fish). The F_2 progeny were *BR* (92 fish), *BlfR* (32 fish), *bR* (30 fish) and *BlfR* (13 fish) in a ratio of 9:3:3:1 (χ^2=0.73, p=0.9–0.75). The *lf* alleles were independent of the *b* alleles. The *blfR* fish was orange-red in body color and had no visible leucophores.

3) Crosses between gray (BciR) and brown lacking leucophores (BlfR)

The *BciR* fish was gray in body color. It had well developed leucophores and dilute colored xanthophores. A *BciR* female was mated with a *BlfR* male. The F_1 progeny were *BR* (46 fish). The F_2 progeny were divided to *BR* (113 fish), *BciR* (40 fish), *BlfR* (34 fish) and *BcilfR* (12 fish) in a ratio of 9:3:3:1 (χ^2=0.73, p=0.9–0.75). The *lf* alleles were independent of the *ci* alleles. The *BcilfR* fish was bluish gray color.

4) Crosses between brown lacking leucophores (BlfR)and reddish brown (BcoR)

The *BcoR* fish had concentrated xanthophores and body color was reddish brown. A *BlfR* female was mated with a *BcoR* male. The F_1 progeny were *BR* (63 fish). The F_2 progeny consisted of *BR* (82 fish), *BcoR* (35 fish), *BlfR* (30 fish) and *BcolfR* (8 fish) in a ratio of 9:3:3:1 (χ^2=1.87, p=0.75–0.5). The *lf* alleles did not link with the *co* alleles.

5) Crosses between brown having double anal fins (BDaR) and brown lacking leucophores (BlfR)

The *BDaR* fish had an anal fin in place of dorsal fin and it had anal fins at dorsum and ventrum. A *BDaR* female was mated with a *BlfR* male. The F_1 progeny were *BR* (61 fish). As the F_1 progeny were heterozygous for the *Da* gene, they had a large dorsal fin having 7–14 fin rays. The wild fish have the dorsal fin having 6 fin rays. The F_2 progeny consisted of *BR* (143 fish), *BDaR* (36 fish), *BlfR* (43 fish) and *BDalfR* (11 fish) in a ratio of 9:3:3:1 (χ^2=3.87, p=0.5–0.25). The *lf* alleles did not link with the *Da* alleles.

6) Crosses between brown lacking leucophores (BlfR) and brown (Bdf-1R)

In the *Bdf-1R* fish, adult fins were normal in shape, but the membrane structure of the juvenile fins at embryonic and larval stages were broken in various regions. The *df-1* character was able to detect only as the deformity in fin membrane when fish were immature. Therefore, the F_2 segregation ratio was measured at hatching stage. A *BlfR* female was mated with a *Bdf-1R* male. The F_1 progeny were *BR* (64 fish). The F_2 progeny were divided to *BR* (138 fish), *Bdf-1R* (45 fish), *BlfR* (39 fish) and *Bdf-1lfR* (8 fish) in a ratio of 9:3:3:1 (χ^2=4.01, p=0.5–0.25). The *lf* alleles were independent of the *df-1* alleles.

7) Crosses between brown lacking leucophores (BlfR) and brown (Bdf-2R)

The *df-2* gene gave a similar effect to the *df-1* gene. A *BlfR* female was mated with a *Bdf-2R* male. The F_1 progeny were *BR* (64 fish). The F_2 progeny were *BR* (67 fish), *Bdf-2R* (21 fish), *BlfR* (18 fish) and *Bdf-2lfR* (11 fish) in a ratio of 9:3:3:1 (χ^2=4.36, p=0.25–0.1). The *lf* alleles were independent of the *df-2* alleles.

8) Crosses between yellowish orange-red (bdiR) and orange-red lacking leucophores (blfR)

The *bdiR* fish had dispersed xanthophores and the body color was yellowish orange-red. A *bdiR* female was mated with a *BlfR* male. The F_1 progeny were *bR* (67 fish). The F_2 progeny were *bR* (94 fish), *bdiR* (27 fish), *blfR* (26 fish) and *bdilfR* (8 fish) in a ratio of 9:3:3:1 (χ^2=1.22, p=0.75–0.5). The *lf* alleles did not link with the *di* alleles.

9) Crosses between dilute brown (BdlR) and brown lacking leucophores (BlfR)

the *BdlR* fish had dilute brown melanophores. A *BdlR* female was mated with a *BlfR* male. The F_1 progeny were *BR* (57 fish). The F_2 progeny were *BR* (135 fish), *BdlR* (45 fish), *BlfR* (39 fish) and *BdllfR* (8 fish) in a ratio of 9:3:3:1 (χ^2=3.63, p=0.5–0.25). The *lf* alleles were independent of the *dl* alleles.

10) Crosses between dark brown (BdmR) and brown lacking leucophores (BlfR)

The *BdmR* fish had dispersed melanophores and leucophores and the body color was dark brown. A *BdmR* female was mated with a *BlfR* male. The F_1 progeny were *BR* (57 fish). The F_2 progeny were segregated to *BR* (96 fish), *BdmR* (26 fish), *BlfR* (31 fish) and *BdmlfR* (11 fish) in a ratio of 9:3:3:1 (χ^2=0.54, p=0.9–0.75). The *lf* alleles did not link with the *dm* alleles.

11) Crosses between bluish brown (Bdx-2R) and brown lacking leucophores (BlfR)

The *Bdx-2R* fish had dilute orange-red xanthophores. A *Bdx-2R* female was bred with a *BlfR* male. The F_1 progeny were *BR* (74 fish). The F_2 progeny were divided to *BR* (31 fish), *Bdx-2R* (11 fish), *BlfR* (14 fish) and *Bdx-2lfR* (4 fish) in a ratio of 9:3:3:1 (χ^2=0.95, p=0.9–0.75). The *lf* alleles did not link with the *dx-2* alleles.

12) Crosses between orange-red lacking leucophores (blfR) and orange-red having a few leucophores (bflR)

A *blfR* female was mated with a *bflR* male. The F_1 progeny were *bR* (52 fish). The F_2 progeny were *bR* (221 fish), *bflR* (63 fish), and *blfR* (85 fish) in a ratio of 9:3:4 (χ^2=1.57, p=0.75–0.5). The *lf* alleles did not link with the *fl* alleles. The F_2 *blfR* included *blfR* and *bfllfR*.

13) Crosses between brown lacking leucophores (BlfR) and brown having fused vertebrae (Bfu-6R)

The *fu-6* gene caused a notochord bent wavily at embryonic stages, and adult fish had fused vertebrae and short body. The score of F_2 progeny were counted at hatching stage because severe deformity caused low viability. A *BlfR* female was mated with a *Bfu-6R* male. The F_1 progeny were *BR* (63 fish). The F_2 progeny were *BR* (128 fish), *Bfu-6R* (34 fish), *BlfR* (48 fish) and *Bfu-6lfR* (9 fish) in a ratio of 9:3:3:1 (χ^2=3.86, p=0.5–0.25). The *lf* alleles were independent of the *fu-6* alleles.

14) Crosses between orange-red lacking leucophores (blfR) and albino (biR)

A *blfR* female was mated with a *biR* male. The F_1 progeny were *bR* (73 fish). The F_2 progeny consisted of *bR* (133 fish), *biR* (43 fish), *blfR* (44 fish) and *bilfR* (13 fish) in a ratio of 9:3:3:1 (χ^2=0.63, p=0.9–0.75). The *lf* alleles did not link with the *i* alleles.

15) Crosses between brown lacking leucophore (BlfR) and albino (Bi-3R)

A *BlfR* female was mated with a *Bi-3R* male. The F_1 progeny were *BR* (48 fish). The F_2 progeny were segregated to *BR* (107 fish), *Bi-3R* (33 fish), *BlfR* (37 fish) and *Bi-3lfR* (12 fish) in a ratio of 9:3:3:1 (χ^2=0.41, p=0.95–0.9). The *lf* alleles were independent of the *i-3* alleles.

16) Crosses between brown lacking leucophores (BlfR) and orange-red having many leucophores (bml-3R)

The *bml-3R* fish had leucophores about three times as many as those of the wild fish at embryos and lavae. A *BlfR* female was mated with a *bml-3R* male. The F_1 progeny were *BR* (36 fish). The F_2 progeny were divided to *BR* (66 fish), *BlfR* (22 fish), *Bml-3R* (12 fish), *bR* (17 fish), *blfR* (7 fish) and *bml-3R* (3 fish) in a ratio of 27:12:9:9:4:3 (χ^2=7.51, p=0.25–0.1). The *lf* alleles did not link with the *ml-3* alleles. The F_2 *BlfR* included *BlfR* and *Blfml-3R*. The F_2 *blfR* contained *blfR* and *blfml-3R*.

17) Crosses between variegated brown (BRvc) and brown lacking leucophores (BlfR)

The *BRvc* fish were variegated brown caused by the local absence of melanophores and leucophores. A *BRvc* female was mated with a *BlfR* male. The F_1 progeny were *BR* (59 fish). The F_2 progeny were *BR* (104 fish), *BlfR* (40 fish), *BRvc* (33 fish) and *BlfRvc* (11 fish) in a ratio of 9:3:3:1 (χ^2=0.87, p=0.9–0.75). The *lf* alleles were independent of the *vc* alleles.

Discussion

Melanophores, xanthophores and iridocytes (iridophores) are common in many fishes, but leucophores are rare.

The *BlfR* fish had no visible leucophores throughout life. Whether leucophores are absent or leucophores are present but they have no pigment granules (or contain colorless granules) is not known.

In the breeding season, adult males had many leucophores on the edges of caudal, dorsal and anal fins as a secondary sex character. These leucophores disappeared in non breeding season. The *lf* mutant males did not show this secondary sex character.

In this experiment, 16 mutant genes were used for test breeding, and 6 genes are concluded to be most probably related to the expression of leucophores. When the *lf* gene was homozygous, any hybrid between *lf* and other genes had no visible leucophores. The *lf* gene was epistatic for other genes in the expression of leucophores. The *lf* gene may have important roles in manifestation of leucophores.

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