The Fish Biology Journal MEDAKA, **4**: 31–35 (1992)

Studies on the mutants of the medaka, *cm* and *dm*

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Abstract Two body color mutants (*cm* and *dm*) were found and established in the medaka (*Oryzias latipes*). The *cm* and *dm* genes are autosomal and recessive. The *BcmR* fish had concentrated melanophores, and the body color was blond. The *dm* gene caused dispersed melanophores and leucophores. The *BdmR* fish was dark brown in body color. The *cm* and *dm* genes did not link with other genes used in test crosses. Some gene analyses were done.

Introduction

There are melanophores, xanthophores and leucophores as chromatophores, and iridocytes as pigment cells in the medaka (*Oryzias latipes*). Chromatophores are effector cells and show physiological color changes. Two mutants (*cm* and *dm*) concerning the expression of melanophores and leucophores were found and established. The *cm* mutant caused concentrated melanophores and the *dm* mutant made dispersed melanophores and leucophores. These mutants did not show clear physiological color changes.

This paper deals with gene analyses and phenotypic expression of these mutants.

Materials and methods

A *cm* female (blond color) was found in paddy fields at Idaka, Nagoya at 1961. Most melanophores except those on fins were concentrated and did not disperse on the black background, while wild fish became black color as melanophores dispersed. Melanophores on fins were smaller than ones of wild fish and showed normal color changes.

A *dm* female (dark brown color) was found in paddy fields at Tsukude, Minamishidara, Aichi in 1962. The *dm* fish was dark brown on the white background, as wild fish became pale color. The processes of melanophores were more slender than ones of wild fish.

The following genes were used for gene analyses (*cf.* 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 produces black melanophores and the *b* gene makes colorless melanophores.

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

co : The *co* gene makes concentrated xanthophores.

Da : The *Da* fish has an anal fin in the place of the dorsal fin. It has anal fins at both dorsum and ventrum. The *Da* heterozygote has a large dorsal fin having 7–14 fin rays. The wild fish has a dorsal fin having 6 fin rays.

de : The *de* gene decreases the number of melanophores.

di : the *di* gene induces dispersed xanthophores.

dx-1 : The *dx-1* gene dilutes orange-red color in xanthophores.

dx-2 : The *dx-2* gene has similar effects on xanthophores to those of the *dx-1* gene.

fl : The *fl* gene makes a small number of leucophores.

fs : The *fs* gene causes small anal and dorsal fins because of fusions of some interhemal and interneural spines.

lf : The *lf* gene makes no visible leucophores.

mm : The *mm* gene makes punctuate melanophores and leucophores in pale color regions of variegation.

rs : The *rs* gene causes small scales having irregular shapes.

rs-3 : The *rs-3* gene causes the lack of scales except for a few large ones.

sm : The *sm* gene makes melanophores slowly responding in color changes.

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

Results

The following gene analyses were made.

1) Crosses between blond (BcmR) and brown (BR)

A *BcmR* female was mated with a *BR* male. The F_1 progeny were all *BR* (147 fish). The F_2 progeny were *BR* (♀66 fish, ♂55 fish) and *BcmR* (♀23 fish, ♂20 fish) in a ratio of 3 : 1 (χ^2=1.01, p=0.5–0.25). The sex ratio of them was 1 : 1 (χ^2= 1.18, p= 0.5–0.25). The *cm* gene is recessive and autosomal.

2) Crosses between blond (BcmR) and orange-red (bR)

A *BcmR* female was bred with a *bR* male. The F_1 progeny were all *BR* (114 fish). The F_2 progeny consisted of *BR* (199 fish), *BcmR* (64 fish) and bR (79 fish) in a ratio of 9 : 3 : 4 (χ^2=0.72, p= 0.75–0.50). The F_2 *bR* included both *bR* and *bcmR*, because the *bcmR* was orange-red in body color and was not distinguished from the *bR*. From the χ^2 test for segregation, there was no significant deviation from expectation. The *cm* alleles did not link with the *b* alleles.

3) Crosses between blond (BcmR) and reddish brown (BcoR)

The *BcoR* fish had concentrated xanthophores and the body color was reddish brown. A *BcmR* female was mated with a *BcoR* male. The F_1 progeny were all *BR* (81 fish). The F_2 progeny divided to *BR* (101 fish), *BcmR* (35 fish), *BcoR* (32 fish) and *BcmcoR* (9 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.37, p=0.9–0.75). The *cm* alleles did not link with the *co* alleles. The *BcmcoR* fish had concentrated melanophores and xanthophores and the body color was reddish blond.

4) Crosses between blond (BcmR) and brown having double anal fins (BDaR).

A *BcmR* female was mated with a *BDaR* male. The F_1 progeny were *BR* (18 fish). The F_1 *BR* was heterozygous for *Da* and they had large dorsal fin having 7–14 fin rays. The F_2 progeny consisted of *BR* (154 fish), *BcmR* (52 fish), *BDaR* (48 fish) and *BcmDaR* (8 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=4.37, p=0.25–0.1). The *cm* alleles are independent of the *Da* alleles.

5) Crosses between orange-reddish brown (BdeR) and blond (BcmR)

The *BdeR* fish had a decreased number of melanophores, and red color was remarkable at head and caudal fin regions. A *BdeR* female was mated with a *BcmR* male. The F_1 progeny were all *BR* (63 fish). The F_2 progeny were *BR* (71 fish), *BcmR* (32 fish), *BdeR* (27 fish) and *BcmdeR* (7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=2.38, p=0.5–0.25). The *cm* alleles did not link with the *de* alleles. The *BcmdeR* fish had a small number of melanophores small in size except on fins, and the body color was orange-red except dark colored fins.

6) Crosses between blond (BcmR) and yellowish orange-red (bdiR)

The *bdiR* fish had dispersed xanthophores which showed no physiological color change. A *BcmR* female was bred with a *bdiR* male. The F_1 progeny were all *BR* (84 fish). The F_2 progeny were segregated to *BR* (108 fish), *BcmR* (35 fish), *BdiR* (31 fish), *bR* (36 fish), *BcmdiR* (7 fish) and *bdiR* (9 fish) in a ratio of 27 : 9 : 9 : 12 : 3 : 4 (χ^2= 6.47, p=0.1–0.05). The F_2 *bR* included *bR* and *bcmR*, and the F_2 *bdiR* contained *bdiR* and *bcmdiR*. The *cm* alleles are independent of the *di* alleles.

7) Crosses between blond (BcmR) and bluish brown (Bdx-1R)

The *Bdx-1R* fish had dilute orange-red xanthophores and were bluish brown in body color. A *BcmR* female was mated with a *Bdx-1R* male. The F_1 progeny were all *BR* (61 fish). The F_2 progeny consisted of *BR* (73 fish), *BcmR* (21 fish), *Bdx-1R* (30 fish) and *Bcmdx-1R* (4 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=3.81, p=0.5–0.25). The *cm* alleles are independent of the *dx-1* alleles. The *Bcmdx-1R* fish was bluish blond.

8) Crosses between blond (BcmR) and brown having small anal fin (BfsR)

The *BfsR* fish had small anal and dorsal fins because some interhemal and interneural spins were fused and the bases of fins were short. A *BcmR* female was bred with a *BfsR* male. The F_1 progeny were all *BR* (34 fish). The F_2 progeny were divided to *BR* (125 fish), *BcmR* (42 fish), *BfsR* (38 fish) and *BcmfsR* (12 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.36, p=0.95–0.9). The *cm* alleles did not link with the *fs* alleles. The *BcmfsR* fish were blond in body color and had small anal and dorsal fins.

9) Crosses between variegated brown (BmmR) and blond (BcmR)

The *BmmR* fish showed dark and pale color variegation. There were non-dendritic melanophores and leucophores in pale regions. A *BmmR* female was bred with a *BcmR* male. The F_1 progeny were all *BR* (53 fish). The F_2 progeny were divided to *BR* (63 fish), *BcmR* (26 fish), and *BmmR* (16 fish) in a ratio of 9 : 4 : 3 (χ^2=0.96, p=0.9–0.75). The F_2 *BcmR* included *BcmR* and *BcmmmR*.

10) Crosses between blond (BcmR) and brown having small irregular scales (BRrs)

A *BcmR* female was mated with a *BRrs* male. The F_1 progeny were all *BR* (80 fish). The F_2 progeny were segregated to *BR* (164 fish), *BcmR* (55 fish), *BRrs* (45 fish) and *BcmRrs* (15 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=1.74, p=0.9–0.75). The *cm* alleles did not link with the *rs* alleles.

11) Crosses between brown having slow response melanophores (BRsm) and blond (BcmR)

The *BRsm* fish were brown in body color and their melanophores concentrated slowly in the white background. A *BRsm* female was bred with a *BcmR* male. The F_1 progeny were all *BR* (45 fish). The F_2 progeny were divided to *BR* (165 fish), *BcmR* (53 fish), *BRsm* (52 fish) and *BcmRsm* (15 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.60, p=0.9–0.75). The *cm* alleles are independent of the *sm* alleles.

12) Crosses between brown (BR) and dark brown (BdmR)

A *BR* female was mated with a *BdmR* male. The F_1 progeny were all *BR* (57 fish). The F_2 progeny were *BR* (189 fish) and *BdmR* (61 fish) in a ratio of 3 : 1 (χ^2=0.04, p=0.9–0.75). The *dm* gene is recessive.

13) Crosses between dark brown (BdmR) and orange-red (bR)

The *BdmR* female was mated with the *bR* male. The F_1 progeny were *BR* (♀ 95 fish, ♂ 81 fish). The F_2 progeny were segregated to *BR* (♀ 58 fish, ♂ 52 fish), *BdmR* (♀ 17 fish, ♂ 22 fish), *bR* (♀ 19 fish, ♂ 23 fish) and *bdmR* (♀ 5 fish, ♂ 7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.30, p=0.95–0.9). The sex ratio was 1 : 1 (χ^2=0.12, p=0.75–0.5). The *dm* alleles are independent of the *b* alleles and are autosomal. The *bdmR* fish had dispersed leucophores.

14) Crosses between blond (BcmR) and dark brown (BdmR)

A *BcmR* female was bred with a *BdmR* male. The F_1 progeny were all *BR* (52 fish). The F_2 progeny were *BR* (192 fish), *BcmR* (62 fish), *BdmR* (50 fish) and *BcmdmR* (22 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=1.97, p=0.75–0.5). The *dm* alleles are independent of the *cm* alleles. The *BcmdmR* fish had intermediate melanophores in form and size between ones of *BcmR* and *BdmR*. Their leucophores were dispersed (*dm* type).

15) Crosses between reddish brown (BcoR) and dark brown (BdmR)

A *BcoR* female was bred with a *BdmR* male. The F_1 progeny were *BR* (66 fish). The F_2 progeny were *BR* (188 fish), *BcoR* (58 fish), *BdmR* (62 fish) and *BcodmR* (11 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=3.75, p=0.5–0.25). The *dm* alleles did not link with the *co* alleles. The *BcodmR* fish had dispersed melanophores and leucophores and concentrated xanthophores.

16) Crosses between dark brown (BdmR) and brown having double anal fins (BDaR)

A *BdmR* female was bred with a *BDaR* male. The F_1 progeny were *BR* (71 fish). The F_1 *BR* fish had large dorsal fin having 7–14 fin rays, as the *Da* gene was heterozygous. The F_2 progeny consisted of *BR* (158 fish), *BDaR* (41 fish), *BdmR* (53 fish) and *BDadmR* (7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=7.58, p=0.1–0.05). The *dm* alleles did not link with the *Da* alleles.

17) Crosses between dark brown (BdmR) and orange-reddish brown (BdeR)

A *BdmR* female was mated with a *BdeR* male. The F_1 progeny were *BR* (71 fish). The F_2 progeny consisted of *BR* (83 fish), *BdeR* (30 fish), *BdmR* (26 fish) and *BdedmR* (7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.81, p=0.9–0.75). The *dm* alleles are independent of the *de* alleles.

18) Crosses between dark brown (BdmR) and yellowish orange-red (bdiR)

A *BdmR* female was bred with a *bdiR* male. The F_1 progeny were *BR* (92 fish). The F_2 progeny were divided to *BR* (84 fish), *BdiR* (29 fish), *BdmR* (29 fish), *bR* (31 fish), *bdiR* (12 fish) and *BdidmR* (7 fish) in a ratio of 27 : 9 : 9 : 12 : 4 : 3 (χ^2=1.48, p=0.75–0.5). The *dm* alleles did not link with the *di* alleles. The F_1 *bR* included *bR* and *bdmR*, and *bdiR* did *bdiR* and *bdidmR*. The *BdidmR* fish had dispersed melanophores, xanthophores and leucophores.

19) Crosses between dark brown (BdmR) and bluish brown (Bdx-1R)

A *BdmR* female was mated with a *Bdx-1R* male. The F_1 progeny were *BR* (49 fish). The F_2 progeny were *BR* (168 fish), *BdmR* (61 fish), *Bdx-1R* (46 fish) and *Bdmdx-1R* (14 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=3.02, p=0.5–0.25). The *dm* alleles did not link with the *dx-1* alleles.

20) Crosses between bluish brown (Bdx-2R)and dark brown (BdmR)

A *Bdx-2R* female was mated with a *BdmR* male. The F_1 progeny were *BR* (91 fish). The F_2 progeny consisted of *BR* (51 fish), *BdmR* (17 fish), *Bdx-2R* (14 fish) and *Bdmdx-2R* (5 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.80, p=0.9–0.75). The *dm* alleles are independent of the *dx-2* alleles.

21) Crosses between brown having a few leucophores (BflR) and dark brown (BdmR)

A *BflR* female was bred with a *BdmR* male. The F_1 progeny were *BR* (57 fish). The F_2 progeny were *BR* (211 fish), *BdmR* (82 fish), *BflR* (70 fish) and *BdmflR* (30 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2= 2.67, p=0.5–0.25). The *dm* alleles did not link with the *dx-2* alleles.

22) Crosses between brown having small anal fin (BfsR) and dark brown (BdmR)

A *BfsR* female was bred with a *BdmR* male. The F_1 progeny were *BR* (36 fish). The F_2 progeny were *BR* (98 fish), *BdmR* (28 fish), *BfsR* (38 fish) and *BdmfsR* (9 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2= 1.95, p=0.75–0.5). The *dm* alleles did not link with the *fs* alleles.

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

The *BlfR* fish had no visible leucophores throughout life. A *BdmR* female was bred 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 *dm* alleles were independent of the *lf* alleles.

24) Crosses between variegated brown (BmmR) and dark brown (BdmR)

A *BmmR* female was mated with a *BdmR* male. The F_1 progeny were *BR* (65 fish). The F_2 progeny were divided to *BR* (105 fish), *BdmR* (34 fish), *BmmR* (35 fish) and *BdmmmR* (10 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=1.65, p=0.75–0.5). The *dm* alleles were independent of the *mm* alleles.

25) Crosses between dark brown (BdmR) and brown having small irregular scales (BRrs)

A *BdmR* female was bred with a *BRrs* male. The F_1 progeny were *BR* (39 fish). The F_2 progeny were *BR* (132 fish), *BdmR* (38 fish), *BRrs* (34 fish) and *BdmRrs* (13 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2= 2.08, p=0.75–0.5). The *dm* alleles did not link with the *rs* alleles.

26) Crosses between dark brown (BdmR) and brown having a few scales (BRrs-3)

The *BRrs-3* fish had a few large scales along the bases of dorsal, anal and caudal fins, and most scales were absent. A *BdmR* female was mated with a *BRrs-3* male. The F_1 progeny were *BR* (46 fish). The F_2 progeny were segregated to *BR* (68 fish). *BdmR* (20 fish), *BRrs-3* (18 fish) and *BdmRrs-3* (3 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=3.27, p=0.5–0.25). The *dm* alleles are independent of the *rs-3* alleles.

27) Crosses between brown having slow response melanophores (BRsm) and dark brown (BdmR)

A *BRsm* female was bred with a *BdmR* male. The F_1 progeny were all *BR* (57 fish). The F_2 were *BR* (83 fish), *BdmR* (30 fish), and *BRsm* (25 fish) in a ratio of 9 : 4 : 3 (χ^2=0.22, p=0.9–0.75). The *dm* alleles did not link with the *sm* alleles.

28) Crosses between dark brown (BdmR) and variegated brown (BRvc)

The *BRvc* fish were variegated brown caused by the local absence of melanophores and leucophores. A *BdmR* female was mated with a *BRvc* male. The F_1 progeny were *BR* (42 fish). The F_2 progeny consisted of *BR* (50 fish), *BdmR* (17 fish), *BRvc* (20 fish) and *BdmRvc* (6 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2=0.50, p=0.9–0.75). The *dm* alleles are independent of the *vc* alleles.

Discussion

When the wild medaka (*BR*) reared in the white background, the body color becomes pale brown due to the concentration melanophores. In the black background, the body color changes to dark brown since melanophores disperses to assume a dendritic form. Melanophores in isolated skins disperse in 1/7.5M NaCl and concentrate in 1/7.5M KCl. On the contrary, leucophores concentrate in 1/7.5M NaCl and disperse in 1/7.5M KCl.

The *BcmR* fish exhibited no change of the body color in the white and black backgrounds, but the color of fins changed to pale and dark according to the backgrounds. In isolated skins, most melanophores remained in a punctate form in 1/7.5M NaCl. Xanthophores and leucophores showed normal color changes.

Melanophores and leucophores of the *BdmR* fish dispersed in the white and black backgrounds. After long adaptation to the white background (for a few days), the form of melanophores became slightly stellate but did not concentrate to a punctuate form. In isolated skins, melanophores did not concentrate in 1/7.5M KCl. Xanthophores showed normal color changes. At embryonic and larval stages, melanophores were dispersed and inert in color change. Leucophores exhibited normal response. At about 10 mm body length, inert leucophores appeared on dorsum among normal ones. Inert leucophores increased in number and gradually replaced normal ones.

In double recessive, *BcmdmR*, melanophores exhibited an intermediate character between *BcmR* and *BdmR*. Melanophores were smaller in size

than ones of the *BdmR* fish, and larger than ones of the *BcmR*. They were incompletely dispersed (stellate), but did not concentrate completely. Leucophores were dispersed like in the *BdmR* fish. Melanophores of *BcmdmR* were made by interaction of the genes *cm* and *dm*.

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