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STUDIES ON THE MUTANTS OF THE MEDAKA, *CO* AND *DI*

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ABSTRACT- In the medaka (*Oryzias latipes),* two mutants concerning the color changes of xanthophores were established in our laboratory. Gene analyses of these mutants were performed. The two mutants, concentrated xanthophore (*co)* and dispersed xanthophore (*di),* are recessive and autosomal. The *co* alleles are linked with the *dx-2* alleles and the *di* alleles may be linked with the *wl* alleles.

INTRODUCTION

In the medaka *(Oryzias Iatipes),* the body color is composed of chromatophores such as melanophores, xanthophores and leucophores, and iridocytes. The chromatophores are effector cells. When the medaka are reared in the white background, the xanthophores are concentrated and disperse in the black background.

The color mutants, concentrated xanthophore *(co)* and dispersed xanthophore *(di),* were established in our laboratory. The former mutant has concentrated xanthophores, even if the fish are reared in the black background, and the latter shows the dispersed xanthophores in the white background.

This paper deals with gene analyses of these mutants with special attention paid to gene interaction.

MATERIAL AND METHOD

A male medaka having concentrated xanthophores was found in a paddy field at the eastern area (Idaka) of Nagoya in 1963. It was reddish brown in body color. The same mutant was found in wild medaka collected in Toyohashi, Aichi Prefecture, in 1983.

Ten medaka having dispersed xanthophores (4 females and 6 males) were found in orange-red fish (about 3,000) of a fish dealer in Yatomi, near Nagoya, in 1963. The body color was a yellowish orange-red. The same medaka were easily found in the orange-red fish stock of other fish dealers. Two wild, male medaka having the dispersed xanthophores were found in a paddy field in Toyokawa, Aichi prefecture, in 1967, and the same female was collected at Nagakute, near Nagoya, in 1967. Their body color was yellowish brown. These dispersed xanthophores were governed by the same mutant gene.

As will be mentioned later in this paper, the concentrated xanthophore and the dispersed xanthophore mutants are recessive, and, to explain briefly, they are expressed by the gene symbols, *co* and *di,* respectively.

The mutant genes used in these experiments were *b, ci, cm, co-2, Da, de, dm, dx-1, dx-2, em fm il-1, il-2, lf, mm, r, rs, Si, sm, vc,* and *wl* (cf. Tomita 1975, 1982).

The remarkable characteristics of these genes are explained briefly as follows.

b : The *b* gene is recessive and autosomal. The *b* alleles control melanin formation. The *B* ($+^{b}$) gene makes black melanophores and the *b* causes colorless melanophores (Aida, 1921).

ci : The *ci* gene is recessive and autosomal. The *ci* type has a decreased number of xanthophores and well-developed leucophores (Takeuchi, 1969)

cm : The *cm* gene, which is recessive and autosomal, produces concentrated melanophores in the body.

co-2 : The *co-2* gene is recessive and autosomal, and makes concentrated xanthophores. The *co-2* type is not distinguished from the *co* type in a phenotype.

Da : The *Da* gene, which is incomplete

dominant and autosomal, makes the anal fin in place of the dorsal fin on dorsum in the homozygous condition. It has two anal fins at ventrum and dorsum symmetrically. In the heterozygous condition, the dorsal fin is large and the fin ray increases from 7 to 14 in number.

dm : The *dm* gene is recessive and autosomal. The *dm* type has dispersed melanophores and leucophores at the adult stage.

dx-1 : The *dx-1* gene, which is recessive and autosomal, dilutes the orange-red color in xanthophores (Tomita, 1984).

dx-2 : The *dx-2* gene, which is recessive and autosomal, has similar effects on xanthophores as the *dx-1* gene. The *dx-2* alleles are not linked with the *dx-1* alleles.

em : The *em* gene, which is recessive and autosomal, makes large dorsal and anal fins, of which fin rays increase in numbers.

fm : The *fm* gene is recessive and autosomal. It makes a small number of melanophores throughont its life.

fs : The *fs* gene, which is recessive and autosomal, produces small dorsal and anal fins caused by the fusion of interneural and interheamal spins respectively.

il-1, il-2 : The *il-1* and *il-2* genes are polymeric and autosomal. They are the cause of fewer iridocytes on gill covers, and in the skin. The gill covers are transparent and a red, blood color is seen through them.

lf : The *lf* gene is recessive and autosomal. The leucophores are not found throughout its life in the homozygous condition.

mm : The *mm* gene, which is recessive and autosomal, makes punctate melanophores and leucophores in parts of variegation.

r : The *r* gene is recessive and sex-linked. It produces colorless xanthophores. The R($+^{r}$) gene makes orange-red xanthophores.

rs : The *rs* gene is recessive and autosomal. It causes small (reduced) scales of an irregular shape.

Si : The *Si* gene is dominant and autosomal. This character is the result of a defect of iridocyte spots, of which a pair lies on the brain membrane behind the eye balls.

sm : The *sm* gene is recessive and autosomal. The melanophores of this mutant show a slow response in color changes.

vc : The *vc* gene, which is recessive and autosomal, shows variegation caused by the distribution of melanophores and leucophore (presence or absence).

wl : The *wl* gene is recessive autosomal. This mutant has white leucophores, while normal leucophores are often yellowish at the larval stage.

RESULTS

I) *Physiological color changes*

When the wild medaka were reared in the white background, the melanophores and xanthophores concentrated to a punctate state and the leucophores dispersed. The melanophores and xanthophores were dispersed and the leucophores were concentrated when the medaka were reared in the white backhgound. The color changes of melanophores and leucophores were clear phenomena, but the xanthophores showed obscure changes.

The xanthophores and melanophores in isolated skin on scales were dispersed in M/7.5 NaCl and concentrated in M/7.5 KCl and 10^{-5} M adrenaline solution. The leucophores were dispersed in M/7.5 KCl and 10^{-5} M adrenaline solution and concentrated in M/7.5 NaCl.

a) *co* type

When the reddish-brown *(BcoR)* were reared in the black background, the xanthophores remained in a concentrated state, the melaonphores dispersed and the leucophores were concentrated. In the white background, the melaonphores were concentrated, the leucophores dispersed and the xanthophores were in a concentrated state. In the *co* type, the melanophores and leucophores showed normal color changes, but the xanthophores remained in a concentrated state in both the black and white backgrounds.

The xanthophores in isolated skin of the *co* type did not disperse fully in M/7.5 NaCl.

b) *di* type

The yellowish-brown *(BdiR)* xanthophores did not concentrate to a punctate state after long adaptation to the white background. The

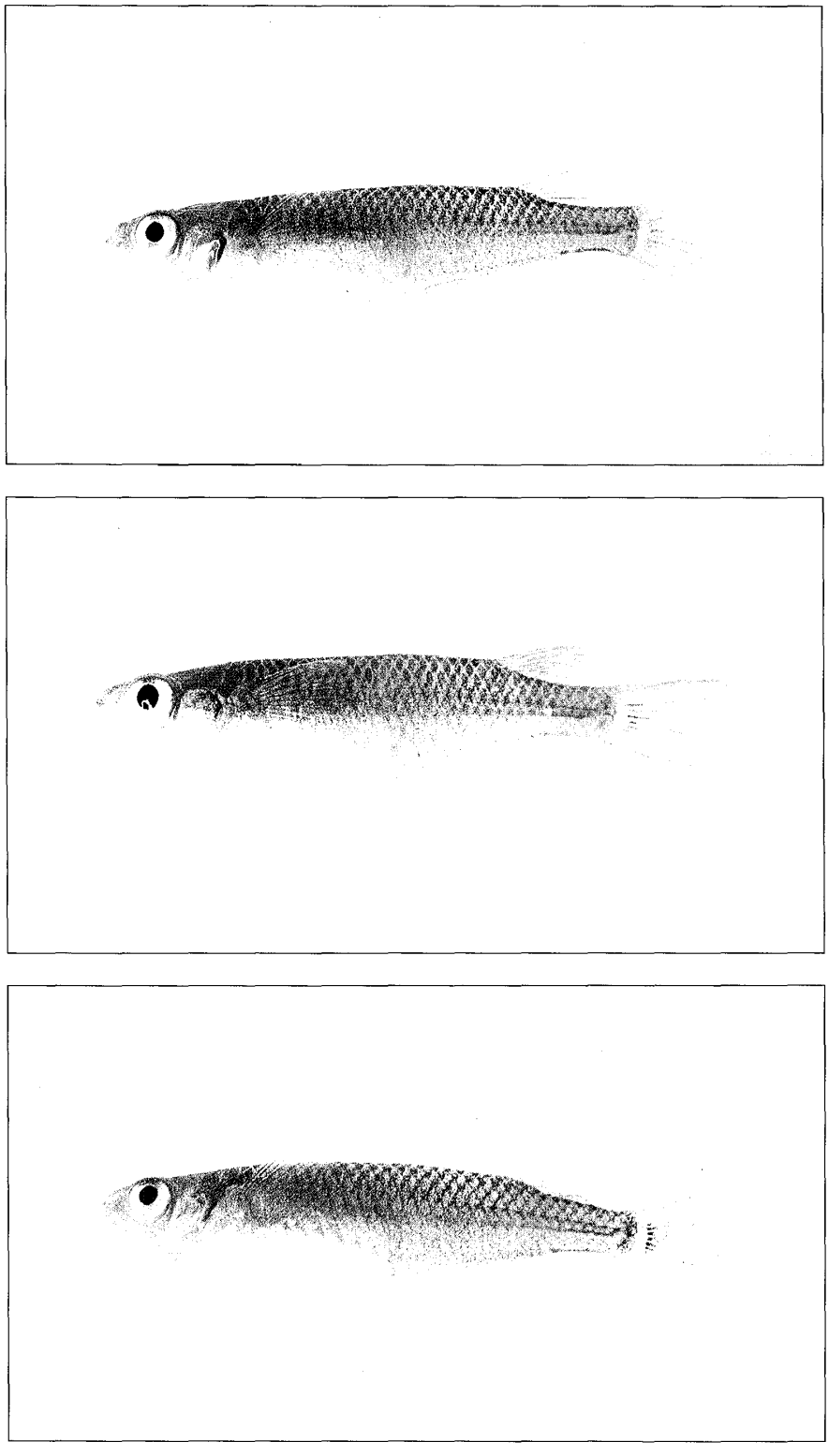

Plate 1 Brown (wild type, *BR*) male (above), reddish-brown *(BcoR)* male (middle) and yellowish-brown *(BdiR)* male (below).

dispersed xanthophores were not affected by the injection of adrenaline solution (0.05 ml of 0.01%) to body cavity, while the xanthophores of the wild type *(BR)* were concentrated. The melaonphores and leucophores of the *BdiR* type showed a normal response of color changes. The xanthophores in the isolated skin of the *BdiR* type did not concentrate to punctate in the M/7.5 KCl and 10 M adrenaline solution, while normal xanthophores were concentrated in these solutions. The melaonphores and leucophores in the isolated skin of the *BdiR* type showed normal color changes.

II) *Gene analyses*

1) Crosses between brown*(BBRR)* and reddish-brown*(BcoR)*

The brown female*(BBRR)* was mated with the reddish-brown male*(BcoR)*. The F_1 progeny were all brown*(BR)* (118 fish). In the F_2 progeny, the segregation ratio of brown*(BR)* (153 fish) to reddish-brown*(BcoR)* (53 fish) was **1 : 1**(χ^2 =0.05, p=0.95-0.90). The sex ratio of the F_2 progeny was 1 : 1 in each of the color types (brown female 70 and male 83, and reddish-brown female 28 and male 25). The F_1 brown females (heterozygous for *co*) were mated with the reddish-brown males (*BcoR*). The progeny were brown *(BR)* (64 fish) and reddish-brown *(BcoR)* (72 fish) in a ratio of 1 : 1. The results indicated that the *co* gene is recessive and autosomal.

2) Crosses between orange-red *(bbRR)* and reddish-brown *(BcoR)*

The orange-red female *(bbRR)* was mated with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (80 fish). The F_2 progeny consisted of brown *(BR)* (60 fish), orange-red *(bR)* (22 fish), reddish-brown *(BcoR)* (27 fish) and reddish orange-red *(bcoR)* (6 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =1.97, p=0.75-0.50). The *co* alleles are independent of the *b* alleles. From the results of the χ^2 test for segregation, there was no significant deviation from what was expected.

3) Crosses between white *(bbrr)* and reddish-brown *(BcoR)*

The white female *(bbrr)* was bred with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (116 fish). The F_2 progeny were segregated into brown *(BR)* (131 fish), blue *(Br)* (60 fish), orange-red *(bR)* (33 fish), reddish-brown *(BcoR)* (39 fish), reddish orange-red *(bcoR)* (12 fish) and white *(br)* (14) in a modified trihybirid ratio of 27 : 12 : 9 : 9 : 3 : 4 (χ^2 =3.76, p=0.75-0.50). The blue type contained *Br* and *Bcor* and the white type cortained *br* and *bcor*. As the *co* character was detectable in the presence of the gene *R* while the *Br* and *br* were not distinguished from the *Bcor* and *bcor*, respectively.

4) Crosses between reddish-brown *(BcoR)* and gray *(BBciciRR)*

The reddish-brown female *(BcoR)* was bred with the gray male *(BBciciRR)*. The F_1 progeny were all brown *(BR)* (78 fish). The F_2 progeny were brown *(BR)* (118 fish), reddish-brown *(BcoR)* (33 fish), gray *(BciR)* (28 fish) and reddish-gray *(BcicoR)* (8 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =4.24, p=0.25-0.10). The *co* alleles were not linked with the *ci* alleles.

5) Crosses between blond *(BBcmcmRR)* and reddish-brown *(BcoR)*

The blond female *(BBcmcmRR)* was bred with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (81 fish). The F_2 progeny were segregated into brown *(BR)* (101 fish), blond *(BcmR)* (35 fish), reddish-brown *(BcoR)* (32 fish) and reddish-blond *(BcmcoP)* (9 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =0.54, p=0.95-0.90). The *co* alleles were independent of the *cm* alleles. The reddish-blond *(BcmcoR)* had the concentrated melanophores and xanthophores.

6) Crosses between reddish-brown *(BcoR)* and reddish-brown-2 *(BBco-2co-2RR)*

The reddish-brown female *(BcoR)* was bred with the reddhsh-brown male *(BBco-2co-2RR)*. The F_1 progeny were all brown *(BR)* (48 fish). The F_2 progeny were divided into brown *(BR)* (98 fish) and reddish-brown *(BcoR, Bco-2R,*and *Bcoco-2R)* (71 fish) in a modified trihybrid ratio of 9 : 7 (χ^2 =0.24, p=0.75-0.50). The reddish-brown *(BcoR)* were not distinguishable from the reddish-brown-2 *(Bco-2R)* in the phenotype. The *co* alleles were not linked with the *co-2* alleles.

7) Crosses between reddish-brown *(BcoR)* and brown-having-double anal fins *(BBDaDaRR)*

The reddish-brown female *(BcoR)* was bred wish a brown were having double anal fins

(BBDaDaRR). The F_1 progeny were all brown having a large dorsal fin (7-14 fin rays) *(BR* heterozygous for *Da)* (90 fish). The F_2 progeny were brown *(BR)* (109 fish), reddish-brown *(BcoR)* (36 fish), brown (double anal fins) *(BDaR)* (29 fish) and reddish-brown (double andal fins) *(BcoDaR)* (11 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =0.85, p=0.90-0.75). The F_2 brown and reddish-brown contained the fish having a large dorsal fin (7-14 fin rays). The *co* alleles were independent of the *Da* alleles.

8) Crosses between reddish-brown *(BcoR)* and orange-reddish brown *(BBdedeRR)*

The reddish-brown female *(BcoR)* was bred with the orange-reddish brown male *(BBdedeRR).* The F_1 progeny were all brown *(BR)* (65 fish). The F_2 progeny were grouped into brown *(BR)* (66 fish), reddish-brown *(BcoR)* (19 fish), orange-reddish brown *(BdeR)* (19 fish) and reddish-orange brown *(BcodeR)* (7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =0.50, p=0.95-0.9). The *co* alleles were not linked with the *de* alleles.

9) Crosses between reddish-brown *(BcoR)* and dark brown *(BBdmdmRR)*

The reddish-brown female *(BcoR)* was mated wish the dark brown male *(BBdmdmRR).* The F_1 progeny were all brown *(BR)* (66 fish). The F_2 progeny were brown *(BR)* (188 fish), reddish-brown *(BcoR)* (62 fish), dark brown *(BdmR)* (59 fish) and reddish-dark brown *(BcodmR)* (11 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =4.55, p=0.25-0.10). The reddish-dark brown *(BcodmR)* had the concentrated xanthophores and dispersed melanophores and leucophores. The *co* alleles were not linked with the *dm* alleles.

10) Crosses between reddish-brown *(BcoR)* and bluish-brown *(BBdx-1dx-1RR)*

The reddish-brown female *(BcoR)* was mated with the bluish-brown male *(BBdx-ldx-lRR).* The F_1 progeny were all brown *(BR)* (91 fish). The F_2 progeny were segregated into brown *(BR)* (205 fish), reddish-brown *(BcoR)* (68 fish), bluish-brown *(Bdx-lR)* (71 fish) and reddish-blue brown *(Bcodx-lR)* (18 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =1.08, p=0.75-0.50). The *co* alleles were not linked with the *dx-l* alleles.

11) Crosses between reddish-brown *(BcoR)* and bluish-brown-2 *(BBdx-2dx-2RR)*

The reddish-brown female *(BcoR)* was mated with the bluish-brown-2 male *(BBdx-2dx-2RR).* The F_1 progeny were all brown *(BR)* (63 fish). The F_2 progeny were grouped into brown *(BR)* (62 fish), reddish-brown *(BcoR)* (34 fish) and bluish-brown *(Bdx-2R)* (28 fish) in a ratio of 2 : 1 : 1 (χ^2 =3.60, p=0.25-0.10).

In the second case, the F_1 progeny were all brown *(BR)* (65 fish). The F_2 progeny were brown *(BR)* (77 fish), reddish-brown *(BcoR)* (44 fish) and bluish-brown *(Bdx-2R)* (38 fish) in a ratio of 2 : 1 : 1 (χ^2 =0.58, p=0.75-0.50).

In the third case, the F_1 progeny were all brown *(BR)* (72 fish). The F_2 progeny were brown *(BR)* (42 fish), reddish-brown *(BcoR)* (33 fish) bluish-brown *(Bdx2R)* (26 fish) in a ratio of 2 : 1 : 1 (χ^2 =3.83, p=0.25-0.10).

These results showed that the *co* alleles were linked with the *dx-2* alleles.

The four reddish-brown males of the F_2 progeny were mated with the bluish-brown females *(BBdx-2dx-2RR)* in each pair. The progeny of one reddish-brown male were brown *(BR)* and bluish-brown *(Bdx-2R)* in a ratio of 1 : 1. This male was *B/B,codx-2/co+,R/R* in the genotype. The progeny of the three males were only brown. Their genotype was *B/B, co+/co,R/R.* The six bluish-brown males of F_2 progeny were mated with reddish-brown females *(BBcocoRR)* in the same manner. These six males were *B/B,+dx-2/+dx-2,R/R.* The six bluish-brown females of the F_2 progeny were tested with the reddish-brown males. The three bluish-brown famales were *B/B,codx-2/+dx-2,R/R.* The other three bluish-brown females were *B/B,+dx-2/+dx-2, R/R.* The four reddish-brown females of the F_2 progeny were also tested with the bluish-brown *(BBdx-2dx-2RR).* Only one female was *B/B, codx-2/co+,R/R.* The other three females were *B/B,co+/co+,R/R.*

The reddish-brown female *(BB,codx-2/co+ RR)* was mated with the reddish-brown male *(B/B,codx-2/co+,R/R).* The progney were reddish-brown *(BcoR)* and reddish-blue brown *(Bcodx-2R)* in a ratio of 3 : 1. The double recessive *B/B,codx-2/codx-2,R/R* was established. The brown female *(BBRR)* was mated with the reddish-blue brown male *(B/B, codx-2/codx-2,R/R).* Their progeny were *B/B,++/codx-2,R/R* in the genotype, and the their body color was brown.

12) Recombination frequency between *co* alleles and *dx-2* alleles

To measure the recombination frequency, the reddish-blue brown female *(B/B,codx-2/codx-2, R/R)* was mated with the brown male *(B/B,++/codx-2,R/R)*. The F_1 progeny were brown *(BR)*, reddish-blue brown *(Bcodx-2R)*, reddish-brown *(BcoR)* and bluish-brown *(Bdx-2R)*. The reddish-brown *(BcoR)* and bluish-brown *(Bdx-2R)* were recombinant. These results are shown in Table I. The recombination frequency was 12.9±0.9.

In reciprocal crosses, the brown female *(B/B,++/codx-2,R/R)* was mated with the reddish-blue brown male *(B/B,codx-2/codx-2, R/R)*. The results are shown in Table II. The recombination frequency was 12.2±1.1.

These results showed that the recombination frequency in females is the same ratio as in the males.

Table 1 Progeny of crosses between the reddish-blue brown females *(B/B,codx-2/codx-2,R/R)* and the brown males *(B/B,++/codx-2,R/R)*

series	progeny(phenotype)				
	BR	*BcoR*	*Bdx-2*	*Bcodx-2R*	total
1	75	8	7	78	168
2	52	6	5	56	119
3	60	8	6	54	128
4	62	13	15	59	149
5	62	7	5	52	126
6	46	16	13	51	126
7	142	14	13	117	286
8	84	18	15	71	188
9	38	5	2	27	72
	621	95	81	565	1362

Table 2 Progeny of crosses between the brown females *(B/B,++/codx-2,R/R)* and the reddish-blue brown male *(B/B,codx-2/codx-2,R/R)*

series	progeny(phenotype)				
	BR	*BcoR*	*Bdx-2R*	*Bcodx-2R*	total
1	43	5	3	33	84
2	61	10	9	54	134
3	72	8	6	83	169
4	48	8	4	41	101
5	31	5	4	36	76
6	109	18	19	97	243
	364	54	45	344	807

13) Crosses between reddish-brown *(BcoR)* and orange-reddish brown *(BBfmfmRR)*

The reddish-brown female *(BcoR)* was mated with the orange-reddish brown *(BBfmfmRR)*. The F_1 progeny were all brown *(BR)* (41 fish). The F_2 progeny were divided into brown *(BR)* (164 fish), reddish-brown *(BcoR)* (53 fish), orange-reddish brown *(BfmR)* (48 fish) and reddish-orange brown *(BcofmR)* (11 fish) in a ratio of 9 : 3 : 3 : 1(χ^2 =3.06, p=0.50-0.25). The *co* alleles were independent of the *fm* alleles.

14) Crosses between brown-having-small anal fin *(BBfsfsRR)* and reddish-brown *(BcoR)*

The brown-having-small anal fin female *(BBfsfsRR)* was bred with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (36 fish). The F_2 progeny were divided into brown *(BR)* (119 fish), reddish-brown *(BcoR)* (35 fish), brown (small anal fin) *(BfsR)* (39 fish) and reddish-brown (small anal fin) *(BcofsR)* (13 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 = 0.93, p=0.90-0.75). The *co* alleles were not linked with the *fs* alleles.

15) Crosses between brown-lacking-iridiocyte *(BBil-lil-lil-2ll-2RR)* and reddish-brown *(BcoR)*

The brown-lacking-iridocyte female *(BBil-1il-1il-2il-2RR)* was mated with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (47 fish). The F_2 progeny were segregated into brown *(BR)* (96 fish), reddish-brown *(BcoR)* (36 fish), brown (iridocyte) *(Bil-1il-2R)* (7 fish) and reddish-brown (iridocyte) *(Bcoil-1il-2R)* (2 fish) in a modified trihybrid ratio of 48 : 12 : 3 : 1 (χ^2 =4.36, p=0.25-0.10). The *co* alleles were not linked with the *il-1* and *il-2* alleles.

16) Crosses between brown-lacking-leucophore *(BBlflfRR)* and reddish-brown *(BcoR)*

The brown-lacking-leucophore female *(BBlflfRR)* was bred with the reddish-brown male *(BcoR)*. The F_1 were all brown *(BR)* (63 fish). The F_2 progeny were segregated into brown *(BR)* (82 fish), reddish-brown *(BcoR)* (35 fish), brown(lecophore) *(BlfR)* (30 fish) and reddish-brown(leucophore)*(BcolfR)* (8 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =1.45, p=0.75-0.50). The *co* alleles were not linked with the *lf* alleles.

17) Crosses between variegated brown *(BBmmmmRR)* and reddish-brown *(BcoR)*

The variegated brown female *(BBmmmmRR)* was mated with the reddish-brown male *(BcoR)*. The F_1 progeny were all brown *(BR)* (57 fish). The F_2 progeny were divided into brown *(BR)* (32 fish), variegated brown *(BmmR)* (15 fish), reddish-brown *(BcoR)* (17 fish) and reddish varegated brown *(BcommR)* (4 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 = 2.53, p=0.50-0.25). The *co* alleles were independent of the *mm* allels.

18) Crosses between reddish-brown *(BcoR)* and brown-having-reduced scales *(BBRRrsrs)*

The reddish-brown female *(BcoR)* was mated with the brown-having-reduced scale male *(BBRRrsrs)*. The F_1 progeny were all brown *(BR)* (116 fish). The F_2 progeny were segregated into brown *(BR)* (164 fish), reddish-brown *(BcoR)* (50 fish), brown (reduced scales) *(BRrs)* (55 fish) and reddish-brown (reduced scales) *(BcoRrs)* (12 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =2.20, p=0.75-0.50). The *co* alleles were not linked with the *rs* alleles.

19) Crosses between reddish-orange-red *(bcoR)* and brown-lacking-iridocyte spots *(BBRRSiSi)*

The reddish orange-red female *(bcoR)* was bred with the brown male lacking-iridocyte spot *(BBRRSiSi)*. The F_1 progeny were all brown-lacking-iridocyte spots *(BRSi)* (45 fish). The F_2 progeny were segregated into brown (iridocyte spots) *(BRSi)* (141 fish), reddish-brown (iridiocyte spots) (BcoRSi) (41 fish), brown *(BR)* (39 fish),orange-red (iridiocyte spots) *(bRSi)* (47 fish), reddish brown *(BcoR)* (18 fish), orange-red *(bR)* (17 fish), reddish-orange-red (iridiocyte spots) *(bcoRSi)* (14 fish) and reddish orange-red *(bcoR)* (3 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =3.32, p=0.90-0.75). The *co* alleles were independent of the *Si* alleles.

20) Crosses between reddish-brown *(BcoR)* and brown having-slow-responsive melanophore *(BBRRsmsm)*

The reddish-brown female *(BcoR)* was mated wish the brown male-having-slow-responsive melanophore *(BBRRsmsm)*. The F_1 progeny were all brown *(BR)* (46 fish). The F_2 progeny were brown *(BR)* (85 fish), reddish-brown *(BcoR)* (24 fish), brown (slow reponsive) *(BRsm)* (28 fish) and reddish-brown (slow responsive) *(BcoRsm)* (9 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 = 0.46, p=0.95-0.90). The *co* alleles were not linked

with the *sm* alleles.

21) Crosses between reddish-brown *(BcoR)* and variegated brown *(BBRRvcvc)*

The reddish-brown female *(BcoR)* was bred with the variegated brown male *(BBRRvcvc)*. The F_1 progeny were all brown *(BR)* (56 fish). The F_2 progeny were divided to brown *(BR)* (98 fish), reddish-brown *(BcoR)* (43 fish), variegated brown *(BRvc)* (41 fish) and brown *(BcoR)* (43 fish), variegated brown *(BRvc)* (41 fish) and reddish variegated brown *(BcoRvc)* (12 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =2.90, p=0.50-0.25). The *co* alleles were not linked with the *vc* alleles.

22) Crosses between brown *(BBRR)* and yellowish-brown *(BdiR)*

The brown female *(BBRR)* was bred with the yellow-brown male *(BdiR)*. The F_1 progeny were all brown *(BR)* (82 fish). The F_2 progeny were divided into brown *(BR)* (90 female and 89 male fish) and yellowish-brown *(BdiR)* (29 female and 32 male fish) in a ratio of 3 : 1 (χ^2 = 0.31, p=0.75-0.50). The sex ratio of the F_2 progeny was 1 : 1. The *di* gene is recessive and autosomal.

23) Crosses between brown *(BBRR)* and yellowish orange-red *(bdiR)*

The brown female *(BBRR)* was mated with the yellowish orange-red male *(bdiR)*. The F_1 were all brown *(BR)* (51 fish). The F_2 progeny were divided into brown *(BR)* (124 fish), yellowish-brown *(BdiR)* (37 fish), orange-red *(bR)* (34 fish), yellowish orange-red *(bdiR)* (8 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =2.98, p=0.50-0.25). The *di* alleles are not linked with the *b* alleles.

24) Crosses between yellowish orange-red *(bdiiR)* and gray *(BBciciRR)*

The yellowish orange-red female *(bdiR)* was mated with the gray male *(BBciciRR)*. The F_1 progeny were all brown *(BR)* (62 fish). The F_2 progeny were segregated into brown *(BR)* (119 fish), yellowish-brown *(BdiR)* (35 fish), gray *(BciR)* (35 fish), orange-red *(bR)* (29 fish), yellowish orange-red *(bdiR)* (10 fish) cream *(bciR)* (8 fish) and yellowish-cream *(bcidip)* (4 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 = 5.09, p = 0.75-0.50). The *di* alleles were independent of the *ci* alleles.

25) Crosses between blond *(BBcmcmRR)* and yellowish-brown *(BdiR)*

The blond female *(BBcmcmRR)* was bred with the yellowish-brown male *(BdiR)*. The F_1 progeny were all brown *(BR)* (43 fish). The F_2 progeny were brown *(BR)* (93 fish), blond *(BcmR)* (33 fish), yellowish-brown *(BdiR)* (58 fish) and yellowish-blond *(BcmdiR)* (11 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =1.03, P=075-0.50). The yellowish-blond *(BcmdiR)* had the concentrated melanophores and the dispersed xanthophores. The *di* alleles were not linked with the *cm* alleles.

26) Crosses between yellowish orange-red *(bdiR)* and reddish-brown *(BBcocoRR)*

The yellowish-red female *(bdiR)* was bred with the reddish-brown male *(BBcocoRR)*. The F_1 progeny were all brown *(BR)* (30 fish). The F_2 progeny were grouped into brown *(BR)* (152 fish), yellowish-brown *(BdiR)* (57 fish), reddish-brown *(BcoR)* (69 fish), orange-red *(bR)* (53 fish), bright reddish-brown *(BcodiR)* (18 fish), yellowish orange-red *(bdiR)* (18 fish), reddish orange-red *(bcoR)* (15 fish) and bright reddish orange-red *(bcodiR)* (5 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =5.68, p=0.75-0.50). The *di* alleles were not linked with the *co* alleles.

27) Crosses between yellowish-brown *(BdiR)* and reddish-brown-2 *(BBco-2co-2RR)*

The yellowish-brown female *(BdiR)* was mated with the reddish-brown-2 male *(BBco-2co-2RR)*. The F_1 progeny were all brown *(BR)* (92 fish). The F_2 progeny were segregeted into brown *(BR)* (69 fish), yellowish-brown *(BdiR)* (24 fish), reddish-brown-2 *(Bco-2R)* (30 fish) and bright reddish-brown-2 *(Bco-2diR)* (8 fish) in ratio of 9 : 3 : 3 : 1 (χ^2 =1.35, p=0.75-0.50). The *di* alleles were independent from the *co-2* alleles.

28) Crosses between yellowish orange-red *(bdiR)* and brown having-double anal fins *(BBDaDaRR)*

The yellowish orange-red female *(bdiR)* was bred with the brown male-having-double anal fish *(BBDaDaRR)*. The F_1 progeny were all brown *(BR)* (38 fish). The F_2 progeny were divided into brown *(BR)* (127 fish), yellowish-brown *(BdiR)* (42 fish), brown (double anal fins) *(BDaR)* (45 fish), orange-red *(bR)* (33 fish), yellowish-brown (double anal fins) *(BDadiR)* (15 fish), yellowish-orange-red *(bdiR)* (7 fish), orange-red (double anal fins) *(bDaR)* (10 fish)

and yellowish orange-red (double anal fins) *(bDadiR)* (3 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =6.83, p=0.50-0.25). The brown, yellowish-brown, orange-red and yellowish orange-red of the F_2 progeny contained the fish having large dorsal fins (7-14 fin rays) (heterozygous for *Da*). The *di*·alleles were not linked with the *Da* alleles.

29) Crosses between yellowish-brown *(BdiR)* and orange-reddish brown *(BBdedeRR)*

The yellowish-brown female *(BdiR)* was bred with the orange-reddish brown male *(BBdedeRR)*. The F_1 progeny were all brown *(BR)* (71 fish). The F_2 progeny were segregated into brown *(BR)* (116 fish), yellowish-brown *(BdiR)* (36 fish), orange-reddish brown *(BdeR)* (26 fish) and yellowish-orange brown *(BdediR)* (10 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =3.83, p=0.50-0.25). The *di* alleles were not linked with the *de* alleles.

30) Crosses between dark brown *(BBdmdmRR)* and yellowish-brown *(BdiR)*

The dark brown female *(BBdmdmRR)* was bred with the yellowish-brown male *(BdiR)*. The F_1 progeny were all brown *(BR)* (69 fish). The F_2 progeny were brown *(BR)* (74 fish), dark brown *(BdmR)* (21 fish), yellowish-brown *(BdiR)* (26 fish) and yellowish-dark brown *(BdidmR)* (7 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 = 0.75, p=0.90-0.75). The yellowish-dark brown *(BdidmR)* had dispersed melanophores, xanthophores and leucophores. The *di* alleles were indendent from the *dm* alleles.

31) Crosses between yellowish orange-red *(bdiR)* and bluish-brown *(BBdx-1dx-1RR)*

The yellowish orange-red female *(bdiR)* was bred with the bluish-male *(BBdx-1dx-1RR)*. The F_1 progeny were all brown *(BR)* (56 fish). The F_2 progeny were brown *(BR)* (117 fish), bluish brown *(Bdx-1R)* (35 fish), yellowish-brown *(BdiR)* (32 fish), orange-red *(bR)* (33 fish), yellowish-blue brown *(Bdidx-1R)* (13 fish), yellowish orange-red *(bdiR)* (6 fish), diluted orange-red *(bdx-1R)* (7 fish) and diluted yellowish orange-red *(bdidx-1R)* (2 fish) in ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =7.12, p=0.50-0.25). The *di* alleles were not linked with the *dx-1* alleles.

32) Crosses between yellowish orange-red *(bdiR)* and bluish-brown-2 *(BBdx-2dx-2RR)*

The yellowish orange-red female *(bdiR)* was bred with the bluish-brown-2 male *(BBdx-2dx-2RR)*. The F_1 progeny were all brown *(BR)* (106 fish). The F_2 progeny were divided into brown *(BR)* (42 fish), yellowish-brown-2 *(Bdx-2R)* (16 fish), orange-red *(bR)* (10 fish), yellowish-blue brown *(Bdidx-2R)* (5 fish), yellowish orange-red *(bdiR)* (1 fish), diluted orange-red *(bdx-2R)* (2 fish) and yellowish diluted orange-red *(cdidx-2R)* (1 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =5.56, p=0.50-0.25). The *di* alleles were independent from the *dx-2* alleles.

33) Crosses between brown having-small anal fin *(BBfsfsRR)* and yellowish orange-red *(bdiR)*

The brown female having-small anal fin *(BBfsfsRR)* was bred with the yellowish orange-red male *(bdiR)*. The F_1 progeny were all brown *(BR)* (28 fish). The F_2 progeny were segregated into brown *(BR)* (127 fish), brown (small anal fin) *(BfsR)* (46 fish), yellowish-brown *(BdiR)* (36 fish), orange-red *(bR)* (52 fish), yellowish-brown (small anal fin) *(Bdidx-2)* (16 fish), orange-red (small anal fin) *(bfsR)* (18 fish), orange-red *(bdiR)* (20 fish) and yellowish orange-red (small anal fin) *(bdifsR)* (8 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =8.39, p=0.50-0.25). The *di* alleles were independent from the *fs* alleles.

34) Crosses between yellowish orange-red *(bdiR)* and orange-red lacking-leucophore *(bblflfRR)*

The yellowish orange-red female *(bdiR)* was bred to the orange-red male lacking-leucophore *(bblflfRR)*. The F_1 progeny were all orange-red *(bR)* (67 fish). The F_2 progeny were divided into orange-red *(bR)* (94 fish), yellowish orange-red *(bdiR)* (27 fish), orange-red (leucophore) *(blfR)* (26 fish) and yellowish orange-red (leucophore) *(bdilfR)* (8 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =1.24, p=0.75-0.50). The *di* alleles were not linked with the *lf* alleles.

35) Crosses between variegated brown *(BBmmmmRR)* and yellowish-brown *(BdiR)*

The variegated brown female *(BBmmmmRR)* was mated with the yellowish-brown male *(BdiR)*. The F_1 progeny were all brown *(BR)* (45 fish). The F_2 progeny were segregated into brown *(BR)* (54 fish), yellowish-brown *(BdiR)* (19 fish), varigated brown

(BmmR) (19 fish), yellowish-variegated brown *(BdimmR)* (5 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 = 0.82, p=0.90-0.75). The *di* alleles were not linked with the *mm* alleles.

36) Crosses between yellowish orange-red *(bdiR)* and brown-having-reduced scales *(BBRRrsrs)*

The yellowish orange-red female *(bdiR)* was bred with the brown male-having-reduced scales *(BBRRrsrs)*. The F_1 progeny were all brown *(BR)* (61 fish). The F_2 progeny were brown *(BR)* (93 fish), yellowish brown *(BdiR)* (36 fish), brown (reduced scales) *(BRrs)* (30 fish), orange-red *(bR)* (25 fish), yellowish-brown (reduced scales) *(BdiRrs)* (11 fish), yellowish orange-red *(bdiR)* (4 fish), orange-red (reduced scales) *(bRrs)* (3 fish) and yellowish orange-red (reduced scales) *(bdiRrs)* (2 fish) in a ratio of 27 : 9 : 9 : 9 : 3 : 3 : 3 : 1 (χ^2 =10.82, p=0.25-0.10). The *di* alleles were not linked with the *rs* alleles.

37) Crosses between yellowish orange-red *(bdiR)* and brown having-slow-responsive melanophore *(BBRRsmsm)*

The yellowish orange-red female *(bdiR)* was bred with the brown male having-slow-responsive melanophore *(BBRRsmsm)*. The F_1 progeny were all brown *(BR)* (139 fish). The F_2 progeny were segregated into brown *(BR)* (80 fish), yellowish-brown *(BdiR)* (30 fish), brown (slow responsive) *(BRsm)* (30 fish), orange-red *(bR* and *bRsm)* (38 fish), yellowish orange-red *(bdiR* and *bdiRsm)* (12 fish) and yellowish-brown (slow responsive) *(BdiRsm)* (8 fish) in a modified trihybrid ratio of 27 : 9 : 9 : 12 : 3 : 4 (χ^2 =0.89, p=0.97-0.95). The *di* alleles were independent from the *sm* alleles.

38) Crosses between yellowish-brown *(BdiR)* and variegated brown *(BBRRvcvc)*

The yellowish-brown female *(BdiR)* was mated with the variegated brown male *(BBRRvcvc)*. The F_1 progeny were all brown *(BR)* (68 fish). The F_2 progeny were divided into brown *(BR)* (219 fish), yellowish-brown *(BdiR)* (61 fish), variegated brown *(BRvc)* (57 fish) and yellowish-variegated brown *(BdiRvc)* (14 fish) in a ratio of 9 : 3 : 3 : 1 (χ^2 =6.78, p=0.10-0.50). The *di* alles were not linked with the *vc* alleles.

39) Crosses between orange-red having-white leucophore *(bbRRwlwl)* and yellowish orange-red *(bdiR)*

The orange-red female having-white leucophores *(bbRRwlwl)* was bred with the yellowish orange-red male *(bdiR)*. The F_1 progeny were all orange-red *(bR)* (93 fish). The F_2 progeny were segregated into orange-red *(bR)* (133 fish), orange-red (white leucophore) *(bRwl)* (57 fish) and yellowish orange-red *(bdiR)* (56 fish) in a ratio of 2 : 1 : 1 (χ^2 =1.62, p=0.50-0.25). In another case, the F_1 progeny were all orange-red *(bR)* (68 fish). The F_2 progeny were orange-red *(bR)* (137 fish), orange-red (white leucophore) *(bRwl)* (75 fish) and yellowish orange-red *(bdiR)* (80 fish) in a ratio of 2 : 1 : 1 (χ_2 =1.23, p=0.75-0.50). These results showed that the *di* alleles may be linked with the *wl* alleles.

DISCUSSION

The physiological color changes of the xanthophores are complicated processes that remain unknown. In the reddish-brown *(BcoR)* type, the melanophores and leucophores showd normal color change, but the xanthophores were concentrated in the condition in which nomal xanthophores disperse. As for possible explanations as to why the xanthophores of this type did not disperse, one may be that the xanthophores did not differentiate into the dendritic form with their shape being small and round, another may be that the structure of the xanthophore is the same as that of the wild type, but the migratory ability of pigment granules was lost.

The xanthophores of the bright reddish orange-red *(bcodiR)* type were obscure in cellular boundary and the red pigment granules scattered in the xanthophores. The red pigment granules were not found in the xanthophores of the *co* type and the *di* type. They may be produced by the interactions of the *co* and *di* genes.

The xanthophores of the *co* type developed into a concentrated state at the late embryonic stage, while those of the wild type were difficult to detect before hatching. In the *di* type, the dispersed xanthophores appeared after hatching.

In the madaka, the first autosomal linkage (the *ci* and *i* alleles) was found by Yamamoto and Oikawa (1973). The linkage between the *co* and *dx-2* alleles was in the second case. The *di* and *wl* alleles were the third linkage, though the recombination frequency was not measured.

Yamamoto (1961,1964) showed that a crossover between the X^r and Y^R in sex-reversal females was 5 times as large as in heterogametic X^r Y^R males. He went on to discuss that possible cause for sex differences in crossover was the difference of internal conditioning between ovocytes and spermatocytes.

In the linkage between the *co* and *dx-2* alleles. the recombination frequency in females was the same ratio as of that in males. The recombination frequency of autosome was not different between female and male.

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