LINKAGE BETWEEN ALBINO GENE (*i*) AND COLOR INTERFERER (*ci*) IN THE MEDAKA, *ORYZIAS LATIPES*

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In the previous paper (Yamamoto 1969), the mode of inheritance of albinism in the medaka, *Oryzias latipes*, was reported. The pink-eyed albino (*i*) was found to be a simple autosomal Mendelian recessive to its normal allele ($+$ ^{*i*}) and non-allelic with heretofore known color genes including the *b*-series (*B*, *B'*, *b*) controlling melanin formation, and is semi-lethal in the larval stage. The F_2 segregation from $i/i; b/b \times +^i/+^i; B/B$, observed in advanced embryos was the 9:3:4 for $+^iB$, $+^ib$ and albinos (*i*, *B* and *i*, *b*), characteristic for albinism segregating by recessive epistasis. The *B*-carrying albino hybrids were actually detected among F_2 albinos. The albino gene manifests itself pleiotropic effects. In homozygotes, it not only inhibits melanin formation almost completely, but partially suppresses orange-red pigmentation governed by the sex-linked *R* or *R*^{*d*} gene. Importance of the normal allele ($+$ ^{*i*}) for manifestation of other color genes was stressed.

There is another color gene, the color interferer (*ci*), in the medaka, which has not been touched upon in the previous paper. This color modifier studied by the late Aida (unpublished) is also non-allelic with all known color genes, leads in the homozygote to partial suppression of autosomal *B* governing melanophores and the sex-linked *R* (and *R*^{*d*}) responsible for orange-red color in xanthophores. The suppression to the latter is stronger than to the former, so that *ci*, *B*, *R* appears gray while $+^{ci}$, *B*, *R* looks brown. According to Takeuchi (1969) the number of leucophores in *ci*-homozygotes is greatly outnumbered as compared with $+^{ci}$ -fish.

The present paper provides evidence of linkage between the albino (*i*) and the color interferer (*ci*). Special effort has been made to breed fish with genotypes appropriate to detect recombinants neglecting semi-lethal albinos. Using appropriate mating types, the recombinants between the two loci were observed.

MATERIALS AND METHODS

In 1961, about two dozens of pink-eyed albino medaka were segregated by Tomita of our laboratory from fish obtained from a fish culturalist at Yatomi near Nagoya.

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Some of them were supplied to us by his courtesy.

They appeared from normal eyed pale orange-red parents ($+^i, b, -$), where $-$ represents any one of the sex-linked alleles (R, R^d, r) responsible for a carotenoid pigmentation in xanthophores. Evidently they appeared from heterozygotes.

Since the time of Aida (1921) only R and r have been known in the r -alleles. Tomita (unpublished) found that there is an intermediate R^d gene responsible for dilute orange-red pigmentation, effect of which is recessive to R and dominant to r . Phenotype of $+^i, b, R^d$ is dilute orange-red.

The X chromosome of the original albinos had carried either r or R^d while the Y chromosome was usually borne by R with one exception (albino ♂ No. 4 reported in 1969) which had Y^{R^d} . Albino parents used in early period of the present study were two original ones $A\varphi^3, A\varphi^5$ and one from pure stock ($A\varphi^1 \times A\sigma^2$) φ^1 designated (A^1A^2) φ^1 . The genotype of $A\varphi^3$ and (A^1A^2) φ^1 was $i/i; b/b; X^r/X^{R^d}$ and that of $A\varphi^5$ was $i/i; b/b; X^r/X^r$. In the present study, these and their descendants were used as materials.

Segregations of albinos can not be scored on adults because of high mortality in the larval stage. No deficiency of albinos, however, can be observed in embryonic stage. So that genetic segregations were scored on advanced embryonic stage. Stages after pectoral-fin movements are preferable.

In 1951, Eiji Nakabori, an employee of Kyoto University collected a pair of gray fish among a population of the wild (brown, B, R) medaka at northern outskirts of Kyoto. He presented the fish to the late Aida for genetic studies. According to Aida's oral communication to senior writer (T. Y.) in 1955, he performed two matings, *viz.* gray $\times$ white (b, r) and variegated white (B', r) $\times$ gray. F_1 hybrids of both cases were all brown (B, R). Segregations in F_2 were of a trihybrid type. Evidently Aida found that the new mutant gene behaved as a Mendelian autosomal recessive and non-allelic to and independent from color genes known by that time (B and R).

He designated it as *ci* (color interferer) because its effect in homozygotes was to suppress manifestation of R intensively and B less significantly. He intended to read a paper entitled 'On the newly discovered white in the medaka' before the the 148th meeting of Kyoto Genetic Conversion held on Dec. 4th in 1954. However, a disease prevented him to attend it. So that his result was unpublished eventually.

Before his death in 1957, he asked to the senior writer (T. Y.) and Takeuchi (Oka-yama) to keep the strain and gave them some gray fish. The gray fish used in the present study belong to the Nagoya colony established since 1955 from the fish received from Aida.

Following Aida, Takeuchi (1969) studied genetic behavior of *ci*-gene carefully. In F_2 of a mating of white ($+^{ci}/+^{ci}; b/b; r/r$) $\times$ gray ($ci/ci; B/B; R/R$), eight color phenotypes appeared: brown ($+^{ci}, B, R$), gray (ci, B, R), blue ($+^{ci}, B, r$), pale blue (ci, B, r), orange-red ($+^{ci}, b, R$), cream (ci, b, R), white ($+^{ci}, b, r$) and milky (ci, b, r). Milky fish used as testers of recombinants were those received from him.

RESULTS

1. F_1 of albino (*A*) $\times$ gray (*G*)

Two matings between albino and gray were made independently: the mating type of the one designating $A\varphi^3 \times G\sigma^1$ were established later to be ii , $+^{ci}+^{ci}$; bb ; $X^rX^{R^d} \times +^i+i^i$; $cici$; BB ; X^RY^R and the other denoted $A\varphi^5 \times G\sigma^2$ ii ; $+^{ci}+^{ci}$; bb ; $X^rX^r \times +^i+i^i$; $cici$; BB ; X^RY^R .

F_1 adults produced (66 $\varphi\varphi$, 60 $\sigma\sigma$ in the former and 33 $\varphi\varphi$, 30 $\sigma\sigma$ in the later) were all normal eyed wild type (brown, $+^i$, $+^{ci}$, B , R).

2. F_2 advanced embryos of albino (*A*) $\times$ gray (*G*) scored without distinguishing $+^i$, $+^{ci}$, B and $+^i$, ci , B

As has been described previously (Yamamoto 1969), three types of advanced embryos, viz. dark ($+^i$, B), light ($+^i$, b) and albino (i , $B+i$, b) in a ratio 9:3:4 can be observed in F_2 of albino (ii ; bb) $\times$ brown ($+^i+i^i$; BB). The dark is characterized by having pigmented eyes and embryonic melanophores, the light by pigmented eyes with scarcely visible melanophores whereas the albino by non-pigmented eyes and no melanophores.

It is natural that in F_2 advanced embryos of albino (ii , $+^{ci}+^{ci}$; bb) $\times$ gray ($+^i+i^i$; $cici$; BB), also, only three kinds of phenotypes, viz. 'dark' ($+^i$, $+^{ci}$, B and $+^i$, ci , B), 'light' ($+^i$, $+^{ci}$, b and $+^i$, ci , b) and 'albinos' (i , $-$, $-$) appear if we score without distinguishing difference between $+^i$, $+^{ci}$, B and $+^i$, ci , B , where $-$ represents any one of respective alleles. At the first stage of the present study, the two phenotypes of $+^i$, $+^{ci}$, B and $+^i$, ci , B in embryos were considered to be impossible to discriminate. In Table 1, F_2 advanced embryos designated $(A^3G^1)F_1\varphi^1 \times (A^3G^1)F_1\sigma^1$, superscripts being fish number, are presented. 'Dark', 'light' and 'albinos' were observed to segregate in 9:3:4 ratio. It may be noticed that even in advanced embryos, the r -alleles (r , R^d , R) do not manifest themselves. They can gradually be distinguishable after hatching.

3. Backcross advanced embryos

Backcross (FR₁) advanced embryos, designated albino (A^1A^2) $\varphi^1 \times$ brown (A^3G^1) $F_1\sigma^2$, are shown in Table 2. 'Dark' ($+^i$, $+^{ci}$, B), 'light' ($+^i$, $+^{ci}$, b) and 'albino' (i , $-$, $-$) were observed in 1:1:2 ratio, instead of the ordinary 1:1:1:1. It is noted that no ci -embryos appeared since the original albino was homozygous for $+^{ci}$.

Table 1. F_2 advanced embryos from a mating of an albino (A) φ^3 (ii ; $+^{ci}+^{ci}$; bb ; $X^rX^{R^d}$) and a gray (G) σ^1 ($+^i+i^i$; $cici$; BB ; X^RY^R), designated $(A^3G^1)F_1\varphi^1 \times (A^3G^1)F_1\sigma^1$. Superscripts denote fish number. Hyphen ($-$) represents any one of respective alleles

F_2 embryos:	'Dark' $+^i$, $+^{ci}$, B and $+^i$, ci , B	'Light' $+^i$, $+^{ci}$, b and $+^i$, ci , b	'Albino' i , $-$, $-$	Total
Observed;	501	165	237	903
Expected:	508	169	226	903
Ratio:	(9	: 3	: 4)	
Significance: $\chi^2_{(2)}=0.72$, $P=0.7$				

Table 2. Backcross (FR₁) advanced embryos from a mating of an albino (A¹A²)F₁♀¹ (*ii*; +^{*ci*}+^{*ci*}; *bb*; X^{*r*}X^{*Rd*})×a brown (A³G¹)F₁♂²(*i*+^{*i*}; +^{*ci*}*ci*; *bB*; X^{*Rd*}Y^{*R*}). Super-scripts denote fish number. Hyphen (–) represents any one of respective alleles

FR ₁ embryos:	'Dark' + ^{<i>i</i>} , + ^{<i>ci</i>} , <i>B</i>	'Light' + ^{<i>i</i>} , + ^{<i>ci</i>} , <i>b</i>	'Albino' <i>i</i> , –, –	Total
Observed:	121	151	288	560
Expected:	140	140	280	560
Ratio:	(1 : 1 : 2)			
Significance:	χ ² ₍₂₎ =3.62, P=0.2–0.1			

4. F₂ adults from albino (A) × gray (G)

Three color phenotypes of F₂ embryos shown in Table 1 were reared separately. The result is shown in Table 3. The 'dark' embryos developed into brown (+^{*i*}, +^{*ci*}, *B*, *R*), gray (+^{*i*}, *ci*, *B*, *R*), blue (+^{*i*}, +^{*ci*}, *B*, *r*) and pale blue (+^{*i*}, *ci*, *B*, *r*) hybrids; 'light' ones into orange-red (+^{*i*}, +^{*ci*}, *b*, *R*), cream (+^{*i*}, *ci*, *b*, *R*), white (+^{*i*}, +^{*ci*}, *b*, *r*) and milky (+^{*i*}, *ci*, *b*, *r*) and 'albino' ones into pink-eyed albinos (*i*, –, –, –), where – stands for any one of the respective alleles.

Since the genotype of sex chromosomes of (A)♀³ was X^{*r*}X^{*Rd*} and that of (G)♂¹ was X^{*R*}Y^{*R*}, that of (A³G¹)F₁ females must have been either X^{*r*}X^{*R*} or X^{*r*}X^{*Rd*} and that of (A³G¹)F₁ males either X^{*r*}X^{*R*} or X^{*Rd*}Y^{*R*}. Since no *Rd* females appeared in this cross, the mating type of F₁♀¹×F₁♂¹, where superscripts represent fish number, must have been *i*+^{*i*}; +^{*ci*}*ci*; *bB*; X^{*r*}X^{*R*}×*i*+^{*i*}; +^{*ci*}*ci*; *bB*; X^{*r*}Y^{*R*}.

Noteworthy point is that survival rate of albinos is extremely low. So that albinos were necessarily neglected from computation of F₂ adult classes.

If we had been dealing with independent segregation of *i* and *ci*, expected ratio of classes would have been; brown (+^{*i*}, +^{*ci*}, *B*, *R*) 81: gray (+^{*i*}, *ci*, *B*, *R*) 27: blue (+^{*i*}, +^{*ci*}, *B*, *r*) 27: orange-red (+^{*i*}, +^{*ci*}, *b*, *R*) 27: pale blue (+^{*i*}, *ci*, *B*, *r*) 9: cream (+^{*i*}, *ci*, *b*, *R*) 9: white (+^{*i*}, +^{*ci*}, *b*, *r*) 9: milky (+^{*i*}, *ci*, *b*, *r*) 3, totalling 192 and other 64 (*i*, –, –, –) being albinos out of 256 (16×16) tetrahybrid zygotic combinations, where – represents any one of respective alleles. If, on the other hand, the two were linked, the expected ratio of classes would be 18:9:6:6:3:3:2:1, respectively, totalling 48 and other 16 (*i*, –, –, –) being albinos out of 64 (8×8) trihybrid combinations. The χ² test evidently suggests that the two are linked. In Table 4, observed and expected ratios of +^{*i*}, +^{*ci*} to +^{*i*}, *ci* in separate color phenotypes are listed. On the basis of independent segregations, the expected ratio would have been 3:1. In the case of linkage, it would be 2:1. Observed ratios approach 2:1 rather than 3:1.

5. Backcross adults

Backcross (FR₁) embryos listed in Table 2 were reared together to reach adults. The result is shown in Table 5. No albinos appeared. This might be the result of a strong selection against semi-lethal albinos. Since female parent was homozygous for +^{*ci*}, no *ci*-fish appeared.

Table 3. F₂ adults developed from (A³G¹)F₁ $\frac{1}{2}$ F₁×(A³G¹)F₁ $\frac{1}{2}$ $\frac{1}{2}$. Of four possible mating types, this cross was established to be F₁ⁱ+ⁱ; +ⁱci; bB; X^RX^R×F₁ⁱ+ⁱ; +ⁱci; bB; X^RY^R. Hyphen (—) represents any one of respective alleles

'Dark' embryos (501)	Brown + ⁱ , + ^{ci} , B, R	Gray + ⁱ , ci, B, R	Blue + ⁱ , ci, B, r	Pale blue + ⁱ , + ^{ci} , B, r	Total	χ ² and P	% Survival
Observed:	97	40	27	14			(178/501)×100=36
Expected 1 (Ind.):	102.5	34.2	34.2	11.4			
Ratio:	(81 : 27	:	27 :	9)			
Expected 2 (Link.):	91.0	45.6	30.4	15.2			
Ratio:	(18 : 9	:	6 :	3)			
(Cont'd) 'Light' embryos (165)	Orange-red + ⁱ , + ^{ci} , b, R	Cream + ⁱ , ci, b, R	White + ⁱ , + ^{ci} , b, r	Milky + ⁱ , ci, b, r			
(Cont'd) Observed:	35	16	8	6	243		(65/165)×100=39
Expected 1 (Ind.):	34.2	11.4	11.4	3.8	243.1	χ ² ₍₇₎ =16.34	
Ratio:	(27 :	9 :	2 :	3)		P=0.02	
Expected 2 (Link.)	30.4	15.2	10.1	5.1	243	χ ² ₍₇₎ =2.74	
Ratio:	(6 :	3 :	2 :	1)		P=0.90	
(Cont'd) 'Albino' embryos (237)	Albino i, —, —						
(Cont'd) Observed:	6						(6/237)×100=2.5

Table 4. Observed and expected ratios of $+^i, +^{ci}$ to $+^ici$ types in F_2 adult data presented in Table 3

Exp. 1 (Ind.): Ratio expected on independence
Exp. (Link.): Ratio expected on linkage

$+^i, +^{ci}$		$+^i, ci$		Ratios		
				Obs.	Expet. 1 (Ind.)	Expet. 2 (Link.)
Brown	$(+^i, +^{ci}, B, R)$	Gray	$(+^i, ci, B, R)$			
	97		40	2.4 : 1	3 : 1	2 : 1
Blue	$(+^i, +^{ci}, B, r)$	Pale blue	$(+^i, ci, B, r)$			
	27		14	1.9 : 1	3 : 1	2 : 1
Orange-red	$(+^i, +^{ci}, b, R)$	Cream	$(+^i, ci, b, R)$			
	35		16	2.2 : 1	3 : 1	2 : 1
White	$(+^i, +^{ci}, b, r)$	Milky	$(+^i, ci, b, r)$			
	8		6	1.3 : 1	3 : 1	2 : 1
Total	167		76	2.2 : 1	3 : 1	2 : 1

Table 5. Backcross (FR_1) adults developed from $(A^1A^2)\varnothing^1(ii; +^{ci}+^{ci}; bb; X^rX^{Rd}) \times A^3G^1F_1\sigma^2(i+^i; +^{ci}ci; bB; X^{Rd}*Y^R)$.

FR_1 adults:	Brown $+^i, +^{ci}, B, R$	Dilute brown $+^i, +^{ci}, B, R^d$	Orange-red $+^i, +^{ci}, b, R$	Dilute orange-red $+^i, +^{ci}, b, R^{dd}$	Albino $i, -, -, -$	Total
Observed:	$\varnothing\varnothing$ 1**	18	0	18	0	
	$\sigma\sigma$ 19	0	23	2†	0	
Total:	20	18	23	20	0	
Expected:	20.25	20.25	20.25	20.25	—	
Ratio:	(1 : 1	: 1	: 1	: 1)		
Significance:	$\chi^2_{(3)}=0.62, P=0.9-0.8$					

*: Established genotype. (—) represents any one of respective alleles.
**, †: Exceptions. See text.

There are only four color phenotypes, *viz.*, brown ($+^i, +^{ci}, B, R$), dilute brown ($+^i, +^{ci}, B, R^d$), orange-red ($+^i, +^{ci}, b, R$), dilute orange-red ($+^i, +^{ci}, b, R^d$), while blue ($+^i, +^{ci}, B, r$) and white ($+^i, +^{ci}, b, r$) are lacking. It may be noticed, also, that all fish with R are males and all fish with R^d are female with exceptions of one brown female and two dilute orange-red males. Here, we are dealing with a sex-linked inheritance. Since the genotype of female parent was known to be $ii; +^{ci}+^{ci}; bb; X^rX^{Rd}$, the genotype of male parent is established from these results as $i+^i; +^{ci}ci; bB; X^{Rd}Y^R$. Exceptions appeared are considered to be the result of crossing over between the X^{Rd} and Y^R in the male parent, *i.e.*, the exceptional brown female is either $X^rX_c^{Rd}$ or $X^{Rd}X_c^R$ and two exceptional males are either $X^rY_c^{Rd}$ or $X^RY_c^{Rd}$ in sex-chromosome constitution, where crossover chromosomes are denoted with a subscript c .

6. F_2 advanced embryos discriminating $+^i, +^{ci}, B$ and $+^i, ci, B$

Later, a phenotypic difference between the $+^i, +^{ci}, B$ and the $+^i, ci, B$ embryos was found at advanced stage (pectoral-fin movement). The $+^i, ci, B$ embryos were found

Fig. 1. Photograph of four phenotypes in F_2 advanced embryos. A: Dark ($+^i, +^{ci}, B$), B: Pale dark ($+^i, ci, B$), C: Light ($+^i, -, b$), D: Albino ($i, -, -$), where $-$ represents any one of respective alleles.

to have melanophores smaller in size than those of the $+^i, +^{ci}, B$ ones, although number of melanophores of both types seemed to be the same. So that four phenotypes; $+^i, +^{ci}, B$; $+^i, ci, B$; $+^i, -, b$; and $i, -, -$; can be observed in advanced embryos, where $-$ represents any one of respective alleles. They may be referred to dark, pale dark, light and albino, respectively. They are characterized by the following traits. Dark ($+^i, +^{ci}, B$): eyes are pigmented and deeply pigmented corolla type melanophores on the head are large and contact each other, so that they cannot be counted in the expanded condition. Pale dark ($+^i, ci, B$): eyes are pigmented and deeply pigmented corolla type melanophores on the head are smaller, so that they are countable even in the expanded condition. Light ($+^i, -, b$): eyes are pigmented and faintly pigmented corolla type melanophores on the head are scarcely visible. Albino ($i, -, -$): eyes are non-pigmented and no melanophores in all parts of the body.

These four types of advanced embryos are shown in Fig. 1. At this point, it may be remarked that melanophores in the $+^i, -, b$ adults are almost colorless, so that they cannot be seen in their "expanded" state. In their embryos, however, melanophores are faintly pigmented and scarcely observable. Separate cultures of dark ($+^i, +^{ci}, B$) and pale dark ($+^i, ci, B$) embryos show that the former invariably develop into brown and the latter into gray.

In Table 6, lumped data of (A^3G^1) F_2 and (A^2G^2) F_2 advanced embryos discriminating four phenotypes are listed. If the *i* and *ci* had been independent, the expected ratio

Table 6. Detection of linkage between *i* and *ci* in F_2 advanced embryos from two matings, designated $(A^3G^1)F_2$ and $(A^5G^2)F_2$, of albino (*ii*, $+^{ci}+^{ci}$; *bb*) $\times$ gray ($+^i+^i$; *cici*; *BB*). Hyphen (—) represents any one of respective alleles

	'Dark' $+^i, +^{ci}, B$	'Pale Dark' $+^i, ci, B$	'Light' $+^i, -, b$	'Albino' <i>i</i> , —, —	Total
Observed:	840	426	424	644	2,334
Expected 1:	984.7	328.2	437.6	583.5	2,334
(Independent)	(27 : 9 : 12 : 12)				
Significance: $\chi^2_{(3)}=63.7$, $P=0.001$					
Expected 2:	875.3	437.6	437.6	583.5	2,334
(Linked)	(6 : 3 : 3 : 4)				
Significance: $\chi^2_{(2)}=1.04$, $P=0.8-0.7$					

Table 7. Detection of linkage between *i* and *ci* in advanced embryos from single back-cross with respect to *B, b* genes, viz. $(A^3G^1)F_2$ white ($+^i$; $+^{ci}ci$; *bb*) $\times$ (A^3G^1) F_1 brown ($+^i$; $+^{ci}ci$; *Bb*). Hyphen (—) represents any one of respective alleles

	'Dark' $+^i, +^{ci}, B$	'Pale Dark' $+^i, ci, B$	'Light' $+^i, -, b$	'Albino' <i>i</i> , —, —	Total
Observed:	167	85	235	185	672
Expected 1:	189	63	252	168	672
(Independent)	(9 : 3 : 12 : 8)				
Significance: $\chi^2_{(3)}=13.2$, $P=0.01-0.001$					
Expected 2:	168	84	252	168	672
(Linked)	(2 : 1 : 3 : 2)				
Significance: $\chi^2_{(3)}=2.3$, $P=0.5-0.3$					

would have been a 27:9:12:16 for $+^i, +^{ci}, B$; $+^i, ci, B$; $+^i, -, b$; and *i*, —, —, where — represents any one of respective alleles. If, on the other hand, the two are linked, the ratio would be a 6:3:3:4. The chi square test for significance strongly suggests the linkage hypothesis. The two loci were linked in the repulsion phase in original albinos ($i+^{ci}$) and the gray stock ($+^ici$).

7. Advanced embryos of F_2 white $\times F_1$ brown

In order to obtain a further evidence of linkage, advanced embryos of single back-cross with respect to *B, b* genes, viz., F_2 white ($+^i$; $+^{ci}ci$; *bb*) $\times F_1$ brown ($+^i$; $+^{ci}ci$; *Bb*) were scored distinguishing four phenotypes. The results are shown in Table 7.

If the *i* and *ci* had been independent, the expected ratio would have been a 9:3:12:8 for $+^i, +^{ci}, B$; $+^i, ci, B$; $+^i, -, b$; and albino (*i*, —, —). If, on the other hand, they are linked, the expected ratio would be a 2:1:3:2. The $\chi^2_{(3)}$ test for significance indicates that the two are linked.

8. Detection of recombinant fish with single *i, ci*-chromosome

Some hundreds of $(A^5G^2)F_2$ albino fry were reared to adults. Owing to a high mortality at the larval stage, only about some dozens reached to adulthood.

From these, eleven males were chosen at random, numbered 1 to 11 (designated by

superscripts) and submitted test crosses by mating single with milky females ($+^i, ci/+^i, ci; b/b; X^r/X^r$). Of these only six crosses listed in Table 8 successfully produced reasonable numbers of offspring, others either yielded only a few fry or died before reproducing. Crosses can be classified into four categories in segregating kinds of phenotypes.

Crosses 1, 7 and 10 belong to one category in producing only orange-red ($+^i, +^{ci}, b, R$) sons and daughters informing that the male parents were of the genotype $i+^{ci}/i+^{ci}; b/b; X^R Y^R$. Data of these crosses are lumped together in Table 8.

Cross 2 yielded hybrids of four color phenotypes, i.e., brown ($+^i, +^{ci}, B, R$), blue ($+^i, +^{ci}, B, r$), orange-red ($+^i, +^{ci}, b, R$) and white ($+^i, +^{ci}, b, r$). This cross manifests itself a typical Y-linked inheritance with respect to *R*, viz., all the hybrids with *R* are males and all ones with *r* are females. From this fact, it seems that the genotype of cross 2 male was $i+^{ci}/i+^{ci}; b/B; X^r/Y^R$. If this were the case, the expected ratio would be a 1:1:1:1 for brown ($+^i, +^{ci}, B, R$), blue ($+^i, ci, B, r$), orange-red ($+^i, +^{ci}, b, R$) and white ($+^i, +^{ci}, b, r$). The deviation from this ratio might be the result of selection against light-colored varieties ($+^i, +^{ci}, b, R$ and $+^i, +^{ci}, b, r$).

Cross 3 produced orange-red ($+^i, +^{ci}, b, R$) and cream ($+^i, ci, b, R$) hybrids. Both sexes are included in the two color phenotypes. From appearance of the unexpected cream fish in this mating, it appears that cross 3 male may be accepted as a fish with the genotype $i+^{ci}/ici; b/b; X^R/Y^R$, i.e., a fish with a single recombinant *i, ci*-chromosome linked in coupling phase. This *ici*-chromosome must have produced by crossing over between $i+^{ci}$ and $+^ici$ chromosomes linked in repulsion phase.

Cross 5 produced brown ($+^i, +^{ci}, B, R$), gray ($+^i, ci, B, R$), blue ($+^i, +^{ci}, B, r$) and pale blue ($+^i, ci, B, r$), hybrids in approximately 1:1:1:1 ratio. All had *B* and hybrids with *R* were always males and those with *r* always females, i.e., a Y-linked inheritance is apparent. These facts appear to indicate that cross 5 male had the genotype $i, +^{ci}/i, ci; B/B; X^r/Y^R$, viz., a fish having single *ici*-chromosome, also.

For the purpose of obtaining recombination value between the *i* and *ci* loci, the following matings were made to obtain appropriate genotypes.

9. Production of *i, ci*-homozygotes

Cream daughters ($+^i, ci/i, ci; b/b; X^r/X^R$) and cream sons ($+^i, ci/i, ci; b/b; X^r/Y^R$) from cross 3 were crossed *en masse* in order to obtain *ici*-homozygotes. Theoretically, this mating type is expected to produce cream ($+^i, ci, b, R$), milky ($+^i, ci, b, r$) and albino (i, ci, b, R plus i, ci, b, r) in a 9:3:4 ratio. Since the purpose of this mating was to obtain *ici*-albinos as many as possible and not to score segregation ratios, hundreds of just hatched albino fry were separated from non-albino ones and were raised to adulthood. Although survival rate of albinos was very low, we were able to obtain some dozens of them. From the type of mating, it is clear that albino females are either (a) $i, ci/i, ci; b/b; X^r/X^r$ or (b) $i, ci/i, ci; b/b; X^r/X^R$, the former is albino clear milky and the latter albino tinged with cream. The two genotypes can, however, be established with certainty by testing with either white ($+^i, +^{ci}, b, r$) or milky ($+^i, ci, b, r$) male. The female used in cross 1 in Table 9 was found to be (a) and that used in cross 2 in Table 9 was (b). Cross 1 male was a (A^5G^2) $F_1 \hat{\sigma}$.

Table 8. Detection of two males, (A^5G^2) F_2^3 and (A^5G^2) F_2^5 , with a recombinant *ici*-chromosome among (A^5G^2) F_2 albino males. Superscripts denote fish number. Hyphen (—) represents any one of respective alleles. Data of crosses 1, 7 and 10 were lumped together since they produced only orange-red fish. So that their parental males must have the same genotype described below.

Cross No.	Parent		Offspring							Established genotype of P-♂
	Milky ♀ $+^i ci, b, r$	F ₂ albino ♂ $i-, -, -$	Brown $+^i+^ci, B, R$	Gray $+^i ci, B, R$	Blue $+^i+^ci, B, r$	Pale blue $+^i ci, B, r$	Orange-red $+^i+^ci, b, R$	Cream $+^i ci, b, R$	White $+^i+^ci, b, r$	
1	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ⁵ G ²)F ₂ ¹								
7	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ⁵ G ²)F ₂ ⁷	0	0	0	0	51 (♀♀, ♂♂)	0	0	0
10	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ₅ G ²)F ₂ ¹⁰								
2	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ⁵ G ²)F ₂ ²	30 (♂♂)	0	45 (♀♀)	0	9 (♂♂)	0	15 (♀♀)	0
3	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ⁶ G ²)F ₂ ³	0	0	0	0	12 (♀♀, ♂♂)	10* (♀♀, ♂♂)	0	0
5	$+^i, ci/+^i, ci;$ $b/b; X^r/X^r$	(A ⁵ G ²)F ₂ ⁵	12 (♂♂)	20 (♂♂)	22 (♀♀)	14 (♀♀)	0	0	0	0

* Genotype of ♀♀: $+^i, ci/i, ci; b/b; X^r/X^R$, that of ♂♂: $+^i, ci/i, ci; b/b; X^r/Y^R$

Table 9. Detection of adult $+^i+^{ci}$ -recombinants from cross of $i, ci/i, ci \times i, +^{ci}/+^i, ci$. Hyphen (—) represents any one of respective alleles. Semi-lethal albinos were not scored.

Cross No.	Parents		Offspring			Total
	$i, ci/i, ci$ -Albino ♀ $b/b; X^r/X^r$	$i, +^{ci}, +^i, ci$ -Brown ♂ $b/B; X^r/Y^R$	Albino $i, -, -, -$	Gray $+^i, ci, B, R$	Brown $+^i, +^{ci}, B, R$	
1	$i, ci/i, ci; b/b; X^r/X^r$ (a)	$i, +^{ci}/+^i, ci; b/B; X^r/Y^R$	—*	96	4	100
2	$i, ci/i, ci; b/b; X^r/X^r$ (b)	$i, +^{ci}/+^i, ci; B/B; X^R/Y^R$	—	61	2	63
3	$i, ci/i, ci; B/B; X^R/X^R$ (c)	$i, +^{ci}/+^i, ci; B/B; X^R/Y^R$	—	155	9	164
Total				312	15	327

* In cross 1, besides albinos, pale blue ($+^i, ci, B, r$), and cream ($+^i, ci, b, R$) and milky ($+^i, ci, b, r$) hybrids were also neglected.

10. Production of brown males with genotypes $+^i, ci/i, +^{ci}; B/B; X^R Y^R$

Cross 5 male ($i+^{ci}/ici; B/B; X^r/Y^R$) was mated with a gray female ($+^i ci/+^i ci; B/B; X^R/X^R$) of our stock. F_1 offspring were 48 brown (♀♀, ♂♂) and 46 gray (♀♀, ♂♂) hybrids.

From the mating type, it is obvious that F_1 brown females and males are of the genotypes $i, +^{ci}/+^i, ci; B/B; X^r X^R$ and $i, +^{ci}/+^i, ci; B/B; X^R/Y^R$, respectively. Of these, two brown males were used in cross 2 and cross 3 in Table 9.

F_1 gray hybrids (♀♀: $+^i, ci/i, ci; B/B; X^r X^R$, ♂♂: $+^i, ci/i, ci; B/B; X^R Y^R$) were bred *inter se* to obtain *ici*-homozygotes carrying *B* and *R* (♀♀: $i, ci/i, ci; B/B; X^- X^R$, ♂♂: $i, ci/i, ci; B/B; X^- Y^R$), where — represents either *r* or *R*. Of a few F_2 $i, ci/i, ci$ -albino survived to adults, a female (c) used in cross 3 in Table 9 was of the genotype $i, ci/i, ci; B/B; X^R X^R$ by previous testing by a white male ($+^i, +^{ci}/+^i, +^{ci}; b/b; X^r Y^r$).

11. Linkage intensity between *i* and *ci*

Having obtained appropriate genotypes, we were able to detect recombinants between the loci of the *i* and *ci* neglecting albinos from which no information can be available. Three crosses for this purpose were made (Table 9).

From the genotypes of cross 1, we would expect to obtain gray ($+^i, ci, B, R$), pale blue ($+^i, ci, B, r$), cream ($+^i, ci, b, R$), milky ($+^i, ci, b, r$) as non-recombinants in a 1:1:1:1 ratio neglecting albinos, if all these phenotypes were equally survived. Brown ($+^i, +^{ci}, B, R$), blue ($+^i, +^{ci}, B, r$), orange-red ($+^i, +^{ci}, b, R$) and white ($+^i, +^{ci}, b, r$) would appear as observable recombinants. Of these color phenotypes, brown and gray fish are most strong and other phenotypes are more or less weak. So that only gray (non-recombinants) and brown (recombinants) were scored neglecting other phenotypes besides albinos. Efficiency in detecting recombinants in cross 1 was low. In crosses 2 and 3, efficiency was high since all observable non-recombinants are gray and recombinants are brown.

From the results listed in Table 9, the recombinant fraction (*p*) between the loci of the *i* and *ci* was observed to be $15/327=0.046$ with S.E. of $\sqrt{p(1-p)/327}=0.012$. Thus, the recombination value was found to be 4.6 ± 1.2 percent.

DISCUSSION

Albinism appeared in several fishes including the medaka (*Oryzias latipes*) has been known to be the simple Mendelian recessive character. However, recently it was found that albinism appeared in the globe-eyed goldfish (demekin), *Carassius auratus*, is not a simple Mendelian recessive but is the double recessive of the two independently assorting autosomal genes, since non-albino and albino types appear in the 15:1 ratio in the F_2 generation like duplicate genes. When scored on advanced embryos and larvae, the dark (normal), the light and albino types appear in the 12:3:1 ratio, related in action of dominant epistasis. This mode of inheritance may be called as dominant epistasis akin to duplicate genes (Yamamoto, 1973). It is interesting to find two kinds of inheritance of albinism in fishes.

In the medaka, original albinos have appeared in the light-colored race, $b, -$, where b stands for scanty melanin formation in melanophores and $-$ for any one of the r -alleles (R, R^d, r) controlling a carotenoid deposition in xanthophores. Since albinos are semi-lethal, the F_2 segregation cannot be scored on adults. Albino embryos, on the other hand, seem to be all viable. In the F_2 advanced embryos from the cross original albino (i, b) $\times$ non-albino light fish ($+^i, b$), non-albino and albino types appear in the 3:1 ratio.

In F_2 advanced embryos from the dihybrid cross of albino ($i/i; b/b$) $\times$ wild brown type ($+^i/+^i; B/B$), where B stands for full melanin formation in melanophores in non-albinos and b for its recessive allele, dark ($+^i, B$), light ($+^i, b$), and albino (i, B and i, b) types appear in the 9:3:4 ratio, related in action of recessive epistasis. B - and b bearing albinos were actually detected by testing albinos by $+^i/+^i; b/b$ fish (Yamamoto 1939).

The albino gene (ab) in the Mexican blind cave fish (*Anoptichthys astyana*) is also a simple Mendelian recessive to the normal ($+^{ab}$) gene (Sadoglu 1957). Sadoglu and McKee (1969) found a second gene, brown (bw) which reduces pigment in both eyes and body. This is recessive to black ($+^{bw}$) and is non-allelic with albino gene (ab). However, both ab, bw and $ab, +^{bw}$ represents albinos. Thus, in the F_2 of bw -albino ($ab/ab; bw/bw$) $\times$ wild type black ($+^{ab}/+^{ab}; +^{bw}/+^{bw}$), observed segregation ratio was the 9:3:4 for black ($+^{ab}, +^{bw}$), brown ($+^{ab}, bw$) and albinos ($ab, +^{bw}$ and ab, bw) as in the case of advanced F_2 embryos of medaka for dark ($+^i, B$), light ($+^i, b$) and albinos (i, B and i, b) as reported earlier. The bw gene in the former somewhat resembles the b in the latter. However, whereas eye-pigmentation in the $+^{ab}, +^{bw}$ (black) and $+^{ab}, bw$ (brown) besides body color is different in the former, eye-pigmentation of the $+^i, B$ and the $+^i, b$ in the latter are not different in gross appearance in both embryos and adults. Melanophores in the $+^i, B$, on the other hand, are fully pigmented while the $+^i, b$ possesses almost colorless melanophores in non-albino adults.

The genetic interaction characterized by the 9:3:4 F_2 segregation is appropriately termed as recessive epistasis (*cf.* Darlington and Mather 1931; Fasoulas 1971). Fasoulas's symbolism for this non-allelic interaction is $a > B, b$ in general symbols.

The senior author (T.Y.) thinks that it is his duty to mention briefly the unpublished result obtained by the late Aida. In 1955, he personally communicated to me that F_1 of gray ($cici, BB, RR$) $\times$ white ($+^{ci}+^{ci}, bb, rr$) were all wild type brown ($ci+^{ci}, Bb, Rr$).

In F_2 , he observed 100 brown+^{ci}, *B*, *R*, 60 (gray *ci*, *B*, *R*; blue+^{ci}, *B*, *r*; pale blue *ci*, *B*, *r*), 26 orange-red+^{ci}, *b*, *R* and 23 'white' (cream *ci*, *b*, *R*; white+^{ci}, *b*, *r*; milky *ci*, *b*, *r*) totalling 209, whereas expected ratio would be 27:21 (9+9+3):9:7(3+3+1), respectively. According to him 26 orange-red to 23 'white' is approximately 9:7. Phenotypes included by parentheses were scored together by him without discriminating each other, so that cream, white and milky were all called by him as 'white'. He told the senior author that there was a certain 'white' hybrids which produced orange-red (+^{ci}, *b*, *R*) fish in mating with the white (+^{ci}, *b*, *r*). It seems that such 'white' might be the cream (*ci*, *b*, *R*). In this connection, it may also be stated that he called the phenotype of *ci*, *B*, *R* as 'pale blue'. Actually, however, the phenotype looks gray. In addition, to avoid confusion with *ci*, *B*, *r* (pale blue), it is preferable to call *ci*, *B*, *R* as gray.

From the earlier data (Table 2) on the F_2 advanced embryos from the cross albino $\times$ gray no information was available on the linkage because phenotypes of +ⁱ, +^{ci}, *B* and +ⁱ, *ci*, *B* were not discriminated. However, the resulting F_2 adults (Table 3) gave the first evidence of linkage. Thus, the original albinos had the genotype *i*, +^{ci}/*i*, +^{ci}, *b*/*b*, -/-, where - represents any one of the *r*-alleles (*R*, *R*^d, *r*), and gray had +ⁱ, *ci*/+ⁱ, *ci*; *B*/*B*, *R*/*R*, i.e., the *i* and *ci* being linked in repulsion phase. Hence, we were dealing with a trihybrid inheritance instead of a tetrahybrid one.

Later, it was found that phenotypes of +ⁱ, +^{ci}, *B* and +ⁱ, *ci*, *B* in advance embryos are discernible as shown in Fig. 1. Data on the advanced F_2 advanced embryos (Table 6) clearly show that the two loci are linked. Having obtained evidence of linkage, next step was to obtain the recombination value between the *i* and *ci* loci. Reliable recombination value cannot be obtained by the maximum likelihood method using data of Table 6, because no information can be available in the albino class. So that we made special efforts to obtain it by observing non-albino recombinants by using appropriate mating types.

Of six F_2 albino males singly mated with milky females (Table 8), two (crosses 3 and 5) yielded +ⁱ, +^{ci} and +ⁱ, *ci* offspring in a 1:1 ratio. This shows that their parental albino males are of the genotype *i*, +^{ci}/*i*, *ci*, viz. fish with a single recombinant chromosome in which *i* and *ci* genes are linked in coupling phase.

Obviously, the genotype of the cream daughters in cross 3 (Table 8) would be +ⁱ, *ci*/*i*, *ci*; *b*/*b*; *X*^r/*X*^R and that of sons +ⁱ, *ci*/*i*, *ci*; *b*/*b*; *X*^r/*Y*^R. These fish were used to produce the *i*, *ci*-homozygotes necessary to detect recombinants. It was desirable to use appropriate genotypes as mates to the *i*, *ci*-females in order to detect recombinants among strong non-albino classes.

Having bred males with proper genotypes for this purpose, +ⁱ, +^{ci}-brown fish were detected as recombinants among non-recombinant gray fish in crosses *i*, *ci*/*i*, *ci* $\times$ +ⁱ/*i*, +^{ci}/*ci* fish (Table 9). The observed recombination value 4.6 ± 1.2 seems to be reliable since it is based on gray and brown fish, both of which are known to be strong.

Although sex-linkage has been known in the medaka, *Oryzias latipes* (Aida 1921), the guppy, *Poecilia reticulata* (Winge 1922, 1927), the platyfish, *Xiphophorus maculatus* (Bellamy 1922, 1928; Fraser and Gordon 1929; Gordon 1927, 1941, 1942, 1948; Kosswig 1932), no autosomal linkage has been reported heretofore. The present study is the first instance of autosomal linkage in teleost fishes.

SUMMARY

Evidence of linkage between the loci of albino gene (*i*) and color interferer (*ci*), was given by the data scored on both advanced embryos and adults of hybrids. The two were linked in the repulsion phase, i.e., $i, +^{ci}$ in original albinos and $+^i, ci$ in the *ci*-stock.

Two recombinant fish in which one chromosome is linked in the coupling phase (*i, ci*) were detected out of eleven albino males among F_2 of $i, +^{ci}/i, +^{ci} \times +^i, ci/+^i, ci$. Using these fish as progenitors, *ici*-homozygotes were bred. Employing appropriate mating types, recombinants ($+^i, +^{ci}$) were observed in three matings of $i, ci/i, ci \times i, +^{ci}/+^i, ci$. The recombination value was found to be 4.6 ± 1.2 percent.

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